Mechanism-based PK/PD modeling of selective serotonin reuptake inhibitors

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Als je doet wat je leuk vindt,
hoef je nooit te werken

Mahatma Ghandi

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SECTION 1

General Introduction

Chapter 1

Scope and Outline of the Investigations

1.1 Background

A reduced activity of serotonergic neurotransmission is a well-known characteristic in the pathogenesis of depression (Coppen, 1967;Owens and Nemeroff, 1994). Not surprisingly, Selective Serotonin Reuptake Inhibitors (SSRIs) constitute the first line of treatment in depressive disorders (Ables and Baughman, III, 2003;Isaac, 1999). SSRIs selectively and powerfully block the serotonin transporter (SERT) and thereby the reuptake of serotonin (5-hydroxytryptamine, 5-HT) in the presynaptic nerve terminal, resulting in increased extracellular 5-HT levels and ultimately enhancement of serotonergic neurotransmission (Bel and Artigas, 1992;Fuller, 1994). The pharmacodynamics of SSRIs in depressive disorders are complex. Although SSRIs rapidly inhibit the reuptake of 5-HT, maximal antidepressant effects are only observed after weeks of chronic treatment, indicating that long-term adaptive changes are important for therapeutic efficacy (Bel and Artigas, 1996;Bosker et al., 1995;Benmansour et al., 1999).

Over the years, several animal models have been developed that can detect specific behavioral changes that are sensitive to the effects of antidepressants. Specifically, the effects of SSRIs have been extensively investigated in a variety of behavioral pharmacological tests, such as the forced swim (Kelliher et al., 2003), the tail suspension (Teste et al., 1993) and the learned helplessness test (Takamori et al., 2001). Analysis of the relationship between the pharmacokinetics (PK) and pharmacodynamics (PD) in these animal models could provide novel insights in the mechanisms of the time dependencies in the PD of SSRIs and other psychotropic drugs. Yet, very few studies have addressed the PK/PD correlations of SSRIs and other psychotropic drugs in behavioral animal models (Della Paschoa et al., 1998;Jonker et al., 2003;Vis et al., 2001).

Potentially complicating factors in PK/PD modeling in behavioral pharmacology are a) the interference of blood sampling with the measured PD effect, b) the availability of only sparse PK and/or PD data and c) the fact that often the pharmacodynamic endpoints are non-continuous. The complexities can in part be overcome by application of a mixed effects modeling approach.

Population PK/PD modeling is based on nonlinear mixed effects analysis and characterizes the pharmacokinetics and concentration-effect relationships in populations rather than in individual subjects (Hashimoto and Sheiner, 1991;Sheiner and Ludden, 1992). Meanwhile, PK/PD models have been successfully applied to continuous and non-continuous measures of drug effect, for both direct (Karlsson et

al., 1995;Minto et al., 1997b;Schnider et al., 1996) and indirect (Bouillon et al., 1996;Minto et al., 1997a) PD models.

In recent years, progress has been made in the field of mechanism-based PK/PD modeling. The objective of mechanism-based PK/PD modeling is to understand, in a strictly quantitative manner, the mechanisms that determine the time-course of the intensity of the drug effect *in vivo*. A pertinent feature of mechanism-based PK/PD models is that they contain specific expressions to describe processes on the causal path between drug administration and response, such as the distribution of the drug to the target site, the binding to the target, the activation of the target and homeostatic feedback (Danhof et al., 2005). The development of mechanism-based PK/PD models relies on biomarkers, which characterize quantitatively the processes on the causal path between drug administration and response (Biomarkers Definitions Working Group, 2001;Rolan, 1997;Colburn and Lee, 2003).

1.2 Scope and Outline of the Thesis

The objective of the studies described in this thesis was to explore the PK/PD correlations of fluvoxamine, as a prototype for SSRIs. In the investigations, a spectrum of different biomarkers is used, each reflecting a specific process on the causal path between drug administration and response. The information on the different biomarkers is integrated in PK/PD models for the various effects.

In **SECTION 1** of this thesis the various aspects of PK/PD modeling of SSRIs are introduced. **Chapter 2** describes the diagnosis, cause and treatment of depression. In addition, the importance of serotonin in depression and the pertinent characteristics of SSRIs are described with special references to fluvoxamine. In **Chapter 3**, the complexity to study depression in animal behavioral models is outlined. The concept of mechanism-based modeling is introduced in **Chapter 4**, with special reference to SSRIs and in relation to the investigations presented in this thesis.

In **SECTION 2** the various investigational steps are described. **Chapter 5** describes the development and evaluation of a population PK model for fluvoxamine in rat plasma. To this end, information on the PK of fluvoxamine obtained in six separate studies was simultaneously analyzed. It is shown that on the basis of this model the full concentration *versus* times profile of fluvoxamine in individual rats can be described on the basis of information from sparse data. This is important, since blood sampling readily interferes with pharmacodynamic observations in behavioral models. The model enables full characterization of the plasma concentration *versus* time profile on the basis of sparse blood concentrations. The utility of the model in

animal behavioral PK/PD studies is illustrated by simulation of the PK/PD correlation of fluvoxamine for the effects on rapid eye movement (REM) sleep using a sparse PK sampling design. By using the pertinent information from the population PK model, individual PK profiles and the PK/PD correlation could be adequately described. In **Chapter 6,** a physiological PK model is proposed for estimation of the brain distribution of fluvoxamine in the rat. The model is able to predict the time course of the fluvoxamine concentration in brain ECF of the frontal cortex and brain tissue on the basis of fluvoxamine concentrations in plasma. The developed physiological brain distribution model constitutes a basis for precise characterization of the PK/PD correlation of fluvoxamine by taking into account the non-linearity in brain distribution. **Chapter 7** describes the PK/PD correlation for the occupancy of fluvoxamine to the serotonin transporter (SERT) in the rat frontal cortex, which is an important intermediary step in the PD of SSRIs. Fluvoxamine SERT occupancy could be directly related to fluvoxamine concentrations in plasma, brain ECF and brain tissue that could adequately describe observed fluvoxamine SERT occupancy. The proposed PK/PD model constitutes a useful basis for characterization and prediction of the time-course of *in vivo* SERT occupancy in behavioral studies with SSRIs. **Chapter 8** describes the development of a mechanistic PK/PD model that characterizes and predicts the time-course of the effects of fluvoxamine on median microdialysate levels of 5-HT and its major metabolite (5-hydroxyindoleacetic acid, 5-HIAA) in the rat frontal cortex. Differential equations were derived for description of the various processes occurring at serotonergic neurotransmission. However, it was not possible to simultaneously analyze observed 5-HT and 5-HIAA levels in all individual animals, probably as a result of the high inter-individual variability, which could not be defined with this model. The developed PK/PD model was able to describe the relationship between the population PK of fluvoxamine in plasma and observed effects on median 5-HT and 5-HIAA levels and the proposed model is the first step in modeling these types of neurotransmission processes. In **Chapter 9,** a categorical PK/PD model is proposed for the effects of fluvoxamine on behavioral effects induced by administration of *para*-chloroamphetamine (PCA). Since PCA produces its biochemical and behavioral effects only after uptake into serotonergic neurons via SERT, its effects are inhibited by SSRIs. Although only one behavioral observation per animal can be obtained and the readout of the behavioral test is non-continuous, the relationship between fluvoxamine plasma concentration and the effects of fluvoxamine on PCA-induced behavioral effects could be successfully described. In the final experimental **Chapter 10**, preliminary studies on the PK/PD

correlation of the effects of fluvoxamine on REM sleep in the rat were conducted. Fluvoxamine showed a dose-dependent inhibition of the onset of the increase in REM sleep. In the PD model, the effects of fluvoxamine on REM sleep were characterized by an indirect response model, which was controlled by a REM sleep generation pulse function accounting for the changes in REM sleep. The administered fluvoxamine dose was related to the onset of the increase in REM sleep, which is of clinical relevance and of high interest within a translational medicine paradigm in drug development. This investigation is a first step towards comprehensive PK/PD modeling of the effect of SSRIs on sleep-wake cycle following acute and chronic administration.

In **SECTION 3**, the approach for mechanism-based PK/PD modeling of SSRIs is discussed and all the results of the various investigations are summarized and conclusions are drawn (**Chapter 11**).

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Chapter 2

Selective Serotonin Reuptake Inhibitors (SSRIs) in Depression

2.1 Depression

2.1.1 Diagnosis of Depression

Depression consists of a variety of physical and psychiatric symptoms that seriously dampen the basic activity of humans (Murtagh, 1992). Currently, major depressive disorder, which is the most widely studied form of depression, has a lifetime prevalence of 10-25% for woman and 5-12 % for men (American Psychiatric Association, 2000). According to the World Health Organization (WHO), depression is expected to be the leading cause of disability and the second leading cause of premature death worldwide by the year 2020, surpassed only by cardiovascular disease (Michaud et al., 2001).

The depressed patient can experience many symptoms, both physical and mental. The diagnostic criteria for major depression are a depressed mood plus a minimum of five SIGECAPS symptoms (sleep disturbance, interest reduction, guilt feelings or thoughts of worthlessness, energy changes/fatigue, concentration decrease, appetite/weight disturbance, psychomotor disturbances and suicidal thoughts), which must be present for at least two weeks and must cause distress or decreased function (Carlat, 1998).

2.1.2 Causes of Depression

The cause of depression is not well known. The understanding of the pathogenesis of depression has improved on the basis of an accumulated number of different risk factors (Brown and Harris, 1978;Akiskal, 1985;Aneshensel and Stone, 1982), including genetic factors (Strocke, 2002), brain chemistry and psychosocial and environmental factors (Murtagh, 1992;Blehar and Oren, 1997). Deficient transmission of the neurotransmitters serotonin (5-hydroxytryptamine, 5-HT), noradrenaline (NA) and dopamine (DA) has been implicated to be important in the pathogenesis of depression (Goodwin and Post, 1983;Vetulani and Nalepa, 2000).

2.1.3 Treatment of Depression

There are a variety of treatments of depression. Moreover, there are many antidepressants with different mechanisms of action. The treatment for depression can be divided into biological and psychological treatments (Kennedy et al., 2001). There are also other therapies like electroconvulsive therapy (ECT) (Rifkin, 1988;Kraus and Chandarana, 1997), light therapy (Pjrek et al., 2005), sleep deprivation (Wirz-Justice and Van den Hoofdakker, 1999) and psychosurgery, of which this latter is a very rare option for some chronically ill, debilitated depressed

patients (Ballantine, Jr. et al., 1987;Lovett and Shaw, 1987). Psychological treatments can be given alone or in combination with adequate biological treatments (Frank et al., 2000;Ravindran et al., 1999;Keller et al., 2000).

There are many antidepressant drugs that are classified according to their chemical structures, pharmacological properties and function (Briley and Moret, 1993;Sanchez and Hyttel, 1999). From their introduction in the mid-1950's, tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs) have been commonly used for the treatment of depression, despite their poor tolerability and risk profile caused by their broad mechanisms of action (Andrews and Nemeroff, 1994;Pacher and Kecskemeti, 2004). After the introduction of the Selective Serotonin Reuptake Inhibitors (SSRIs, Chapter 2), these drugs became the first-line treatment owing to their efficacy, improved tolerability and safety profile compared to the conventional antidepressants (Lieberman et al., 2005;Thase, 2003;Masand and Gupta, 1999). In the US, SSRIs account for more than 80% of all prescriptions for the treatment of depression (Hirschfeld, 2001). When the treatment of antidepressants is stopped, various symptoms unrelated to depressive syndromes may appear. For instance nausea, dizziness, anxiety, fatigue and dry mouth are well known withdrawal effects. Therefore, if antidepressant therapy is stopped, the dosage should be tapered gradually to avoid these discontinuation symptoms (Haddad, 1998;Kennedy et al., 2001;Paykel, 2001). Generally, the rate of response to an antidepressant is about 60% and close to 80% if therapy with a second drug is tried after an initial antidepressant drug failure (Joffe et al., 1996;Moller et al., 1994). A pertinent feature of the treatment with antidepressants is the delay and gradual onset of the clinical effect (Baldwin, 2001).

2.1.4 Serotonin in Depression

As mentioned, the actual basis for the therapeutic action of antidepressant drugs has never been completely determined and there has been considerable conjecture as to what it might be. However, reduced activity of serotonergic neurotransmission is a well-known characteristic in the pathogenesis of depression (Coppen, 1967;Owens and Nemeroff, 1994) and the focus of the research described in this thesis will be on serotonergic neurotransmission and the related antidepressant drugs, the SSRIs. 5-HT (Figure 1) is a small amine molecule that is synthesized from tryptophan, one of the essential amino acids (Petty et al., 1996).

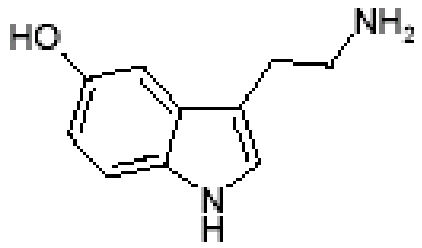

Figure 1. *Chemical structure of serotonin (5-hydroxytryptamine, 5-HT).*

5-HT was discovered over 50 years ago, since then it has been the topic of intense research activities. This has led to the discovery of a range of potential drug targets: the receptors, the metabolizing and synthetic enzymes and the reuptake sites involved in serotonergic neurotransmission (Lieberman et al., 1998). Since its function as a neurotransmitter in the brain was demonstrated (Dahlstrom and Fuxe, 1964), a large proportion of the research to exploit 5-HT pharmacology for therapeutic benefit has focused on its functions on the Central Nervous System (CNS). However, even today, the involvement of various 5-HT receptor subtypes in depression, and in the action of antidepressant drugs, is still far from clear (Moret and Briley, 2000). The pharmacology of 5-HT in the CNS is very complex, since it can exert its action via multiple ways through many different receptors. The 5-HT neurons originate in the midline (raphe) region of the brain stem in a relatively circumscribed area, but they send projections to most parts of the brain and 5-HT is therefore implicated in many functions of the CNS. There are two main serotonergic pathways in the brain: the ascending projections from the medial and dorsal raphe, and the descending projections from the caudal raphe into the spinal cord (Nutt et al., 1999) (Figure 2).

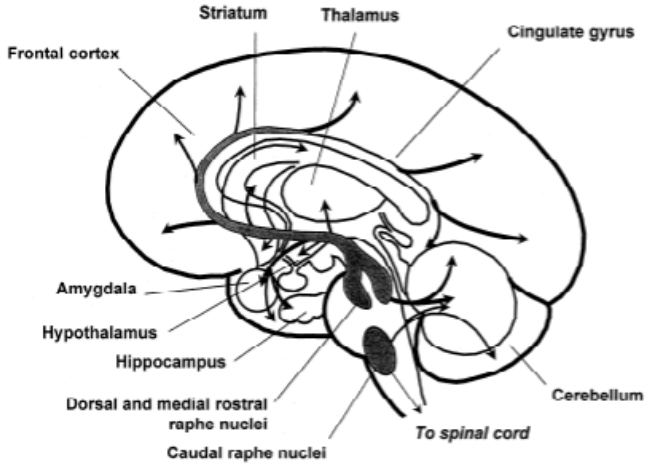

Figure 2. *Ascending and descending serotonergic pathways in the human brain (Nutt et al., 1999).*

The ascending projections are very diverse, to areas such as the frontal cortex, striatum, thalamus, amygdala, hypothalamus and hippocampus. Serotonergic projections have been shown to have important regulatory functions for mood (Golden et al., 1990), movements (Jacobs, 1991), appetite (Fernstrom and Wurtman, 1971), sexual behavior (Gorzalka et al., 1990), and sleep (Jouvet, 1967;Azmitia and Whitaker-Azmitia, 1991). 5-HT is known to interact with other neurotransmitter systems such as dopaminergic systems (Lieberman et al., 1998;Barnes and Sharp, 1999).

2.2 Selective Serotonin Reuptake Inhibitors (SSRIs)

2.2.1 Mechanism of Action of SSRIs

The real boost for the 5-HT hypothesis of depression was the discovery of high clinical efficacy of antidepressants that selectively block the 5-HT reuptake, with negligible effect on noradrenergic system, the SSRIs. The interest in SSRIs was aroused in mid 1970s. The SSRIs are the result of rational research to find drugs that were as effective as the TCAs, which inhibit the reuptake of both NA and 5-HT, but with fewer safety and tolerability problems. They were all designed to inhibit the serotonin reuptake transporter (SERT), with minimal effects on other receptors. As a result, SSRIs do not have the adverse effects typically associated with TCAs. The proposed mechanism action of SSRIs is depicted in Figure 3.

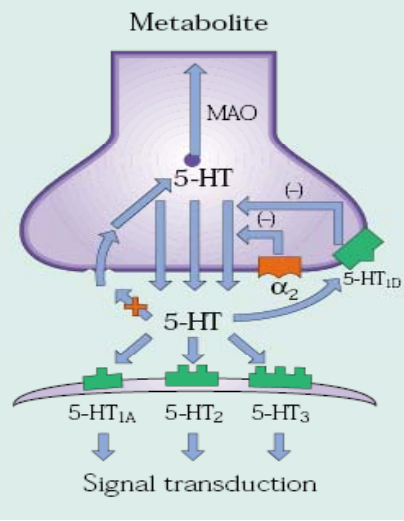

Figure 3. *Serotonin (5-HT) is synthesized in the presynaptic cell of serotonergic neurons. After its release into the synaptic cleft it can bind to several receptors on the postsynaptic membrane as well as on (auto)receptors on the presynaptic membrane. 5-HT can also be transported back into the presynaptic cell via the 5-HT reuptake transporter (SERT). SSRIs can inhibit this reuptake of 5-HT (red cross), thereby enhancing 5-HT concentrations available in the synaptic cleft, which is believed to be responsible for the therapeutic effect of SSRIs.*

SSRIs selectively and powerfully block the SERT and thereby the reuptake of 5-HT in the presynaptic nerve terminal, resulting in increased extracellular 5-HT levels available in the synaptic cleft. 5-HT can interact on a multitude of postsynaptic 5-HT receptors; molecular biological research has identified at least seven distinct families of 5-HT receptors, each of which consist of several subtypes (Lucki, 1996;Peroutka, 1995). Levels of extracellular 5-HT available in the synaptic cleft are immediately increased by inhibition of SERT (Fuller, 1994;Bel and Artigas, 1992). Nevertheless, serotonergic neurotransmission is decreased as a result by decrease in the firing rate activity of the serotonergic neurons by the activation of several 5-HT (auto)receptors and reduction of 5-HT release from the terminals. However, numerous studies have shown that after approximately 2 weeks of chronic SSRI treatment, these autoreceptors (somatodendritic 5-HT_{1A} autoreceptors (Bosker et al., 1994;Invernizzi et al., 1994;Artigas et al., 1996), terminal 5-HT_{1B} receptors (Bosker et al., 1995a;Bosker et al., 1995b) and somatodendritic 5-HT_{1D} receptors (Sprouse et al., 1997;Starkey and Skingle, 1994)) become desensitized (Blier and de Montigny, 1987). Consequently, reduction of the auto-inhibitory processes results in increased release of 5-HT from nerve terminals and therefore potentiation of overall serotonergic neurotransmission, which has been hypothesized to underlie the therapeutic effects of SSRIs, which develop slowly over a period of several weeks (Fuller, 1994;Baumann, 1996a). Hence, the pharmacodynamics of SSRIs in depressive in disorders are complex. Although SSRIs rapidly inhibit the reuptake of 5-HT, maximal antidepressant effects are only observed after weeks of chronic treatment, indicating that long-term adaptive changes are important for therapeutic efficacy (Bel and Artigas, 1996;Bosker et al., 1995a;Benmansour et al., 1999). However, the results of the various studies are not all in agreement with each other and even now many questions remain to be answered (Bosker et al, 1995a;Bosker et al., 1995b;Moret and Briley, 1996).

2.2.2 Selective Serotonin Reuptake Inhibitors (SSRIs)

The approved SSRIs (citalopram (and escitalopram), fluoxetine, fluvoxamine, paroxetine and sertraline, Figure 4) are structurally unrelated and differ in their selectivity, receptor binding and pharmacokinetic properties, but they do have similar antidepressant efficacy, similar mechanism of action and similar side effect profile (Preskorn, 1997;Hiemke and Hartter, 2000;Goodnick and Goldstein, 1998;Baumann, 1996a).

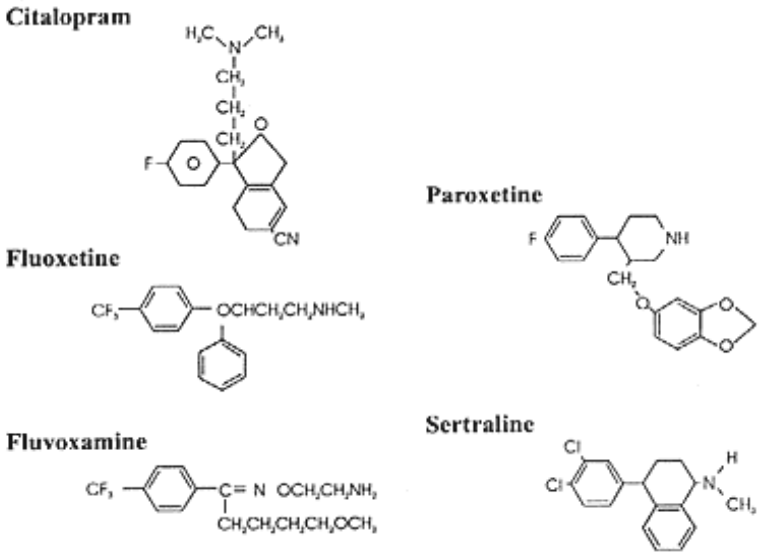

Figure 4. *The chemical structures of the SSRIs: citalopram, fluoxetine, fluvoxamine, paroxetine and sertraline.*

SSRIs are all potent 5-HT reuptake inhibitors *in vitro* as well as *in vivo* (Sanchez and Hyttel, 1999). An important property is that SSRIs possess minimal affinity for cholinergic, α-adrenergic, histaminergic, dopaminergic (DA), GABAergic and (except for paroxetine) muscarinic receptors (Hyttel, 1994;Masand and Gupta, 1999;Goodnick and Goldstein, 1998) (Table 1).

Table 1. *In-vitro potencies of the SSRIs on the inhibition of the reuptake of the neurotransmitters serotonin (5-HT), noradrenaline (NA) and dopamine (DA) (Goodnick and Goldstein, 1998b).*

	IC_{50} values (nM)		
SSRI	5-HT	NA	DA
citalopram	1.8	6100	40000
fluoxetine	6.8	370	5000
fluvoxamine	3.8	620	42000
paroxetine	0.29	81	5100
sertraline	0.19	160	48

Because of their selectivity of action, SSRIs lack many of the side effects associated with other antidepressants, like cardiac, sedative or anticholinergic effects. However, specific SSRIs may also have some σ_1-affinity (fluvoxamine), anticholinergic and noradrenergic properties (paroxetine), 5-HT_{2C} effects (fluoxetine), dopamine activation (sertraline and fluoxetine) and histaminergic affinity (citalopram) (Carrasco

and Sandner, 2005).
SSRIs are also prescribed for the treatment of several disorders other than depression, such as obsessive-compulsive disorder (OCD) (Goodman et al., 1989;Leonard, 1997), bulimia (Kaye et al., 1998;Walsh, 1991), alcoholism (Fawcett et al., 1987;Lemberger et al., 1985), pain syndromes (Aragona et al., 2005;Shimodozono et al., 2002) and anxiety disorders (Zohar and Westenberg, 2000;den Boer et al., 1995).

2.2.3 Fluvoxamine

Fluvoxamine was introduced as the first SSRI in Great Britain in 1983 (DeVane and Gill, 1997). Various reviews describe the clinical pharmacokinetic properties of fluvoxamine (van Harten, 1995;DeVane and Gill, 1997;Claassen et al., 1977;Claassen, 1983) as well as several other SSRIs (Preskorn, 1997;Hiemke and Hartter, 2000;Goodnick and Goldstein, 1998;Baumann, 1996b). Fluvoxamine has little or no effect on other monoamine re-uptake mechanisms or monoamine neuronal function and has low affinity for other neurotransmitter receptors (Claassen, 1983) (Table 1). Fluvoxamine possesses important differences compared to the other SSRIs. In brief, fluvoxamine is the only SSRI that is no racemic drug and appears to lack pharmacologically active metabolites. Fluvoxamine is unlikely to cause pharmacokinetic drug-drug interactions mediated by a plasma protein binding displacement mechanism, since plasma protein binding is low (77%). Fluvoxamine has affinity for several cytochrome P450 (CYP) enzymes in the liver (for CYP1A2, CYP3A4, CYP2C19, CYP2D6 and CYP1A1, with decreasing potency). It is extensively metabolized in the liver, with 11 metabolites, although these have no clinically relevant effect at the neural sites (Preskorn, 1996). The proposed metabolic pathways of fluvoxamine in man are similar to those proposed for rats (Ruijten et al., 1984). Plasma fluvoxamine concentrations are not proportional to the administered dose (nonlinear pharmacokinetics), probably caused by inhibition of CYP isoenzymes responsible for metabolism (Preskorn, 1996) and therefore a decrease of the metabolic clearance (autoinhibition of metabolism). Studies in rats indicate its rapid and wide distribution to most organs (Benfield and Ward, 1986). Most of a dose of fluvoxamine is excreted in urine (van Harten, 1995). No clear relationship between plasma concentrations and clinical effects has been observed for fluvoxamine or other SSRIs. Among the SSRIs, fluvoxamine has the highest affinity for the σ1-receptors, which may confer some specific clinical characteristics on this drug (Narita et al., 1996). Although clinical information of fluvoxamine and other SSRIs are widely

described, preclinical information of these compounds is very limited (Narita et al., 1996).

Fluvoxamine has been used as prototype SSRI compound in all investigations of the current thesis because of its most simple PK properties compared to other SSRIs, in particular, the absence of active metabolites.

2.3 Future Perspectives for Treatment of Depression

After introduction of SSRIs extensive research on the development of new antidepressants has been performed. This includes research on drugs with similar neurochemical mechanisms (e.g. selective and reversible monoamine oxidase inhibitors (e.g. moclobemid (Fitton et al., 1992), selective noradrenaline reuptake inhibitors (e.g. reboxetine (Dostert et al., 1997), dual noradrenaline and serotonin reuptake inhibitors (e.g. venlafaxin (Hardy et al., 2002)), but also drugs with distinct neurochemical mechanisms (e.g. mirtazapine (Anttila and Leinonen, 2001), tianeptine (Mennini et al., 1987), lithium (Jope, 1999) and truly novel concepts (e.g. modulation of dopamine (Corrigan et al., 2000), neuropeptide (e.g. substance P (Adell, 2004) and glutamate receptors (Paul and Skolnick, 2003), and modulation of the mechanisms beyond the receptors (e.g. intracellular messenger systems (Gould and Manji, 2002)). Although this research is very promising, it is not clear whether these compounds are more efficacious or rapidly acting than the SSRIs up to now. Even now, up to 30% of depressive patients do not respond to current available antidepressant therapy and the heterogeneous nature of depression makes it unlikely that the perfect antidepressant will ever be realized.

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Chapter 3

Animal Behavioral Models for the Study of Depression

3.1 Animal Behavioral Models

3.1.1 Use of Behavioral Animal Models for Depression-like Behavior

Obviously, for any disease the only perfect model is the human disease itself and the 'perfect animal model' does not exist. Yet, animal models are useful tools to investigate drugs. When developing animal models of disease, researchers try to develop syndromes in animals which resemble the syndromes observed in humans in order to study selected aspects of human psychopathology (McKinney, Jr. and Bunney, Jr., 1969). Animal models of human disease serve many different purposes and their utility is critically dependent upon the explicit purpose of the model (Willner, 1984). Many animal models have been demonstrated to be useful in elucidating various aspects of the neurobiology of depression and anxiety, including the neuropharmacological mechanisms mediating the effects of antidepressant treatments (Rodgers, 1997;Willner and Mitchell, 2002;Gambarana et al., 2001) as well as support in the development of novel and more effective treatments (Hyde et al., 1993;Le Fichoux et al., 1998;McDonald et al., 1999). Animal models of depression generally derive from genetics, genomics, developmental manipulations, and brain lesioning (Willner and Mitchell, 2002). Clinically, occurrences of major depressive disorder are frequently precipitated by exposure to severe acute stress or chronic low-grade stress (Kessler, 1997). Similarly, animal models of depression are typically generated by exposure to various types of animal stressors, resulting in behavioral changes indicative for aspects of depression, which could typically be reversed with antidepressant drugs (Willner, 1984;Willner, 1990;Geyer and Markou, 1995). However, many of the more recently developed models are not based on stress exposure, but on long-term manipulations that could be better considered as modeling a predisposition to depression, rather than a depressive response to a precipitating event (Willner and Mitchell, 2002).

3.1.2 Complexity to Study Depression in Animal Behavioral Models

As described in Chapter 2, depression is a heterogeneous disorder with symptoms displayed at the psychological, behavioral and physiological level, which leads to additional difficulty in attempting to mimic the disorder in the laboratory animal. Indeed, many of the human symptoms of depression (such as recurring thoughts of death or suicide or having excessive thoughts of guilt) cannot be modeled in the laboratory animal. Consequently, one will never know whether a laboratory animal indeed is 'depressed' (Cryan et al., 2002;Cryan and Mombereau, 2004). Unlike other diseases where the pathophysiology is better characterized such as diabetes or

Parkinson's disease, the underlying pathophysiology of depression is still unresolved, which further enhances the difficulties faced in modeling depression in laboratory animals. In addition, without a convincing and accepted pathophysiological explanation for depression in humans, depression could very well represent the final common pathway of various different disorders of brain function. There are no symptoms or clinical features that are pathognomonic for depression. Assumed 'core' characteristics of depression, like absence of the capacity to experience pleasure (anhedonia), can also present as a common clinical feature in substance misuse (Uslaner et al., 1999) and schizophrenia (Loas et al., 1996). Indeed, many of the diagnostic features of depression, as described in Chapter 2, can be observed within other disorders. In spite of the various difficulties associated with studying depression in the laboratory animal, numerous attempts have been made to create animal models of depression, or at least of the symptoms of depression (Figure 1).

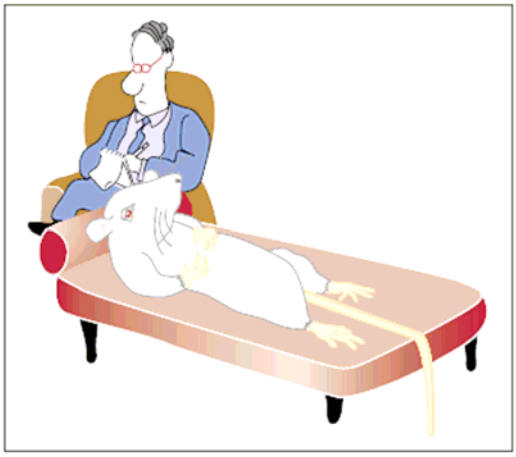

Figure 1. *Although it is impossible to mimic major depressive disorders completely in the laboratory animal, various animal behavioral models have been developed that are sensitive to the effects of antidepressants (adapted from Cryan et al., 2002).*

Clearly, it is most desirable that paradigms can detect depressive-like behavior in addition to antidepressant-like behaviors. However, unlike anxiety-related behaviors, where anxiety can be provoked acutely by a variety of pharmacological (e.g. *m*-chlorophenylpiperazine (*m*-CPP); *β*-carboline; flumazinal; lactate; cholecystokinin (CCK) type B receptor agonists (tetragastrin (CCK-4) and pentagastrin (CCK-5); doxapram) or stressful situations (e.g. brightly lit, elevated environments; placing near scent of predator)) (Shekhar et al., 2001;Blanchard et al., 2003;Sullivan et al., 2003), it is difficult to acutely provoke depression in animals and humans.

3.2 Animal Behavioral Models of Antidepressant-like Activity

In spite of the various problems associated with the analysis of depression in the laboratory, various animal behavioral models of antidepressant-like activity have been developed which are able to detect the antidepressant-like potential of novel compounds in preclinical research. Although attempts to assess the theoretical rationale of animal models are limited by the lack of clear theories on the depressive state in humans, a number of generalizations are possible. Most of the animal models of depression are based on responses to stressors of various kinds, and usually justified by reference to the role of stressful life events in the etiology of depression (Brown and Harris, 1978, 1988; Lloyd, 1980). Instead of anthropomorphizing the human condition, investigators have developed paradigms that can detect specific behavioral differences (clear-cut behavioral outputs) that are sensitive to the effects of antidepressants (both pharmacological and non-pharmacological). However, also models of depression have been used, which were based on primate separation experiments in attempts to model the entire syndrome of depression. Furthermore, various models have been developed to investigate whether manipulations, be they pharmacological, lesion-based, environmental or genetic, can selectively modify the behavior of mice in a manner that can be interpreted as altering depression or antidepressant-like behavior (Cryan et al., 2002;Cryan and Mombereau, 2004). Since the various animal behavioral models of depression and/or anxiety are well described in various reviews (Cryan et al., 2002;Cryan and Mombereau, 2004;Willner and Mitchell, 2002), they will not be discussed in detail in the current thesis.

3.2.1 Commonly Used Animal Models of Depressive-like Behavior

An extensive overview of animal models sensitive to the effects of antidepressant agents is given in (Cryan and Mombereau, 2004). The forced swim test (FST) is the most widely used animal model for assessing antidepressant activity (Cryan et al., 2002;Porsolt et al., 1977). In Table 1, the commonly used animal models sensitive to the effects of antidepressants in preclinical research are depicted.

Table 1. *Widely used rodent models sensitive to the effects of antidepressants (adapted from Cryan et al., 2002).*

Animal model	Ease of use	Reliability	Specificity	Applicable to mice	Comments
Forced swim test	High	High	High[b]	Yes	Sensitive to acute antidepressant treatments; does not reliably detect SSRIs
Modified forced swim test	High	High	High[b]	?	Sensitive to acute antidepressant treatments; differentiates antidepressants from different classes including SSRIs
Tail suspension test	High	High	High[b]	Yes	Sensitive to acute antidepressant treatments; certain strains climb their tail
Olfactory bulbectomy	Medium	High	High	Yes	Behavioral effects evident only following chronic treatment; mechanism of action poorly understood
Learned helplessness	Medium	Medium	High	Yes	Sensitive to short-term antidepressant treatments; ethical restrictions in some countries
DRL-72	Medium	Medium	Medium	?	Sensitive to short-term antidepressant treatments
Neonatal clomipramine[c]	Medium	Medium	?	Yes	Only limited testing of antidepressants have been conducted
Prenatal stress	Medium	?	?	Yes	Only limited testing of antidepressants have been conducted
Chronic mild stress	Low	Low	High	Yes	Reliability has been questioned repeatedly; behavioral effect evident only following chronic treatment
Resident intruder	Low	?	Medium	?	Distinguishable behavioral effects only following chronic treatment; requires further validation in other laboratories
Drug-withdrawal-induced changes in ICSS	Low	High	Medium	Yes	Requires further validation; cannot assess baseline strain differences easily

[a]Abbreviations: DRL-72, differential reinforcement of low-rate 72 second schedule; ICSS, intracranial self-stimulation; SSRI, selective 5-HT reuptake inhibitor.
[b]Stimulants can be screened out by complementary locomotor activity studies.
[c]Clomipramine is a nonselective 5-HT reuptake inhibitor.

The commonly used animal models are diverse and were originally developed based on the behavioral results of stress, drug, lesion or genetic manipulations. Many of these animal models have undergone improvements to keep pace with the continuing advances in the development of drugs with an increasingly wide range of pharmacological actions.

Subsidiary symptoms of depression that can be modeled in laboratory animals include psychomotor changes, fatigue or loss of energy (which might be modeled as decreased persistence), and disturbances of sleep or food intake. In addition to the symptoms of depression, there are also a number of physiological markers described, which are associated with depression (while not in every case showing specificity for depression relative to other psychiatric disorders). The best-established markers are increased activity in the hypothalamic-pituitary-adrenal (HPA) axis (Holsboer, 2001), abnormalities of sleep architecture (of which a decrease in latency to enter the first period of rapid eye movement (REM) sleep is the best established) (Kupfer and Thase, 1983) and a variety of immunological markers (Leonard, 2001).

Interestingly, it is becoming clear that a number of interventions known to be involved in the susceptibility or induction of major depression in humans induce a depression-like effect in models such as the FST or tail suspension test (TST). These manipulations include a genetic predisposition (El Yacoubi et al., 2003;Vaugeois et al., 1996), exposure to early life stressors (Papaioannou et al., 2002), chronic stress (Solberg et al., 1999;Alcaro et al., 2002;Tannenbaum et al., 2002), prenatal stress (Alonso et al., 2000), being in the postpartum state (Alonso et al., 2000;Galea et al.,

2001), immunological activation (Alonso et al., 2000;Makino et al., 1998;Yamano et al., 2000), maternal deprivation (Matthews and Robbins, 2003;Ladd et al., 1996;Pryce and Feldon, 2003), or deprivation of dietary tryptophan (Alonso et al., 2000;Blokland et al., 2002). In addition, withdrawal from morphine, amphetamine and phencyclidine, which in humans has been associated with depressive-like behavior, has been shown to increase immobility in the FST in rats and mice (Alonso, et al., 2000;Kokkinidis et al., 1986;Anraku et al., 2001;Noda et al., 2000;Cryan et al., 2003) and to affect intercranial self-stimulation (ICSS) (Alonso et al., 2000;Cryan et al., 2003), which further supports the use of this parameter to detect depression-like behavior and indicating the etiological (study of the cause of a disease) validity of these paradigms (Geyer and Markou, 1995).

Obviously, behavioral, neurochemical and genetic analysis of the effects of antidepressants will be most relevant in 'depressed' animals in addition to that in 'normal' healthy animals. Therefore, the search for mice having a depression-related phenotype, which possess a genetically altered expression of a specific protein (a receptor, transporter, enzyme or signal transduction protein) should be continued (Cryan et al., 2002;Cryan and Mombereau, 2004). Up to date, there are about 40 different strains of mice with a phenotype that has been interpreted as being related to depression or antidepressant action, such as 5-$HT_{1A/1B}$ receptor knockout (Ramboz et al., 1998;Mayorga et al., 2001), $\alpha_{2A/2C}$ adrenoceptor knockout (Schramm et al., 2001;Sallinen et al., 1999) and serotonin transporter (SERT) knockout (Holmes et al., 2002;Li et al., 1999). In many of these mice strains, predictable phenotypes in depression models have been generated, largely because there is a known association between the specific targeted gene and either depression pathology and/or antidepressant action. Analysis of genetically modified mice represents an important and still growing strategy in elucidating new mechanistic approaches for developing novel treatments for various medical disorders including depression. Nonetheless, it will take perspicacious analysis at the behavioral, genetic, physiological and neurochemical levels to improve the understanding and confirm whether one has indeed found a mouse with alterations relevant to depression endophenotypes.

3.2.2 Validity of Animal Behavioral Models

Generally, the validity of a model refers to the extent to which a model is useful for a given objective. The validity of an animal behavioral model of depression is generally assessed by evaluating the predictive (Chapter 3.2.2.1), construct (Chapter 3.2.2.2) and face validity (Chapter 3.2.2.3). Many reviews have focused on various

definitions with regard to which type of criteria is or is not the most useful for using animal models (McKinney, 2001;McKinney, Jr. and Bunney, Jr., 1969). Some reviewers have advocated the primacy of one of the approaches (predictive validity: Geyer and Markou, 1995; face validity: Weiss and Kilts, 1998; construct validity: Sarter and Bruno, 2002). In principle, construct validity is the most important one, but in practice, however, the construct validity of animal models of depression is difficult to determine. Therefore it is more favorable to have a balanced approach in which the validity of an animal model is evaluated only after considering all three sources of evidence.

3.2.2.1Predictive Validity

Predictive validity is the ability of a model to correctly identify effective antidepressant treatments. The concept of predictive validity therefore implies that manipulations known to influence the pathological condition should have similar effects in the model (Willner and Mitchell, 2002). In practice, the predictive validity of animal models of depression is determined largely by their response to antidepressant drugs. In the narrow sense in which this term is usually employed in behavioral pharmacology, a valid test should be sensitive and specific: a model of depression should respond to effective antidepressant treatments ('true positive' effects) and should fail to respond to ineffective agents ('true negative' effects). Moreover, a positive response should occur at behaviorally selective doses that are within or close to the clinical range and should be demonstrable with a range of structurally diverse compounds. However, it should be recognized that no animal model has 100% prediction rate, although some complex experimental paradigms have approached this level of predictive ability. Part of the problem lies not so much with the animal behavioral model but more with the fact that there are several grey areas in clinical literature where it is not known whether certain drugs (e.g. anticholinergics) possess antidepressant activity or not.

3.2.2.2Construct Validity

Construct validity refers to the accuracy with which the model replicates the key abnormalities or phenomena under study within the clinical condition (Willner and Mitchell, 2002). Hence, construct validity implies that the model has a sound theoretical rationale and it validates whether the model can be related to a scientific theory on the human condition. Theories of depression that could be used to evaluate the construct validity of animal models could relate to neurobiological mechanisms,

etiology (study of the cause of a disease), or psychological mechanisms (Geyer and Markou, 1995). However, any evolution of animal models of depression is intrinsically limited by the rudimentary state of theories of the pathology of depression. Indeed, there is little in the extensive literature describing neurochemical abnormalities or biochemical markers reportedly associated with depression that can be usefully employed to provide a theoretical standard against which to validate animal models. Even the question of whether the level of activity in monoaminergic systems is elevated or decreased in depression remains controversial (Bosker et al., 1995a;Bosker et al., 1995b;Moret and Briley, 1996). In psychopharmacology, construct validity usually is derived by relating the effect of drugs in a model to changes in anatomical and endocrine of molecular mechanisms in the nervous system. Construct validity then refers strongly to brain mechanism.

Although attempts to assess the theoretical rationale of animal models are limited by the lack of clear theories on depression in humans, a number of generalizations are possible, as described in Chapter 3.2. Many animal models of depression are based on responses to various stressors such as restraint or inescapable shock stress and usually justified by reference to the role of stressful life events in the etiology of depression. Nevertheless, some models such as the FST and TST address the behaviors so-called perfectly normal adaptive response but not the mechanism of depression.

3.2.2.3Face Validity

Face validity refers to a phenomenological similarity between the behavior exhibited by the animal model and the symptoms of the disorder being modeled (Willner and Mitchell, 2002). Hence, to assess face validity, the extent of similarity between the model and the disorder is examined. Not all symptoms of a psychiatric condition carry equal weight and for an animal model to be valid, a resemblance to the clinically defined core symptoms of the disorder carries more weight than a resemblance to any subsidiary symptoms (Abramson et al., 1978). However, as mentioned in Chapter 3.1.2, not all human symptoms of depression can be modeled in laboratory animals (Cryan et al., 2002;Willner, 1984). Nevertheless, most behavioral models possess a reasonable degree of face validity, i.e. the responses displayed (whether learned or innate) appear analogous to human reactions under comparable circumstances.

3.2.2.4Other Validity Issues

As described in Chapter 3.2.2.2, theories of depression that could be used to evaluate the construct validity of animal models must relate to neurobiological mechanisms, etiology ("etiological validity"), or psychological mechanisms (Geyer and Markou, 1995). It is known that a variety of different factors are implicated in the etiology of depression: a) 'psychological' factors including undesirable life events, chronic mild stress, adverse childhood experiences and personality traits such as introversion and impulsiveness; b) 'biological' factors including genetic influences and c) a variety of physical illnesses and medications (Akiskal, 1985;Akiskal, 1986). However, for most of these factors, there is little theoretical understanding of the processes by which they influence the physiological processes underlying mood. In certain cases, the immediate precipitant of a depression may be clearly identified, like in seasonal affective disorder (SAD) and post-partum depression. The pathogenesis of depression is better understood as the result of an accumulation of a number of different risk factors (Akiskal, 1985;Aneshensel and Stone, 1982). This point has been largely overlooked in the construction of animal models of depression, which in general have assumed a single causal factor, although also models of depression have been used which were based on primate separation experiments which attempted to model the entire syndrome of depression. It could also be more useful to model single endophenotypic differences (i.e. one clear-cut behavioral output) relevant to the disease state as opposed to a syndrome (Geyer and Markou, 1995).

Discriminant validity (Geyer and Markou, 1995) is a further consideration, which is the extent to which the evidence points to depression, as distinct from a different or a non-specific psychiatric disorder (Willner and Mitchell, 2002). A positive response to antidepressants is insufficient to define an animal model of depression, because this alone does not confer discriminative validity. Some antidepressants are active in some animal models of anxiety and panic (Bodnoff et al., 1988;Fontana et al., 1989) and actually are increasingly observed as drugs in various types of anxiety (den Boer et al., 2002). The extent of predictivity of a given model would also take into account that non-antidepressant psychotropic agents such as antipsychotics, antiepileptic agents and anxiolytic medications should be inactive in the model. However, it is becoming clear that in psychiatric practice in certain situations, such agents may offer some therapeutic benefits in patients suffering from depression, although in many cases as adjunct treatments in combination with standard antidepressant treatments (Manning, 2003). Further, a valid model should also be sensitive to

nonpharmacological treatments for depression such as electroconvulsive shock therapy, sleep deprivation and transcranial magnetic stimulation.

3.2.3 Considerations

Strain differences

In many animal behavioral models, substantial strain differences have been observed (Lucki et al., 2001;Bai et al., 2001;Ripoll et al., 2003). In a recent investigation of 11 different strains of mice in the FST, there was a tenfold difference in baseline immobility scores between strains (Lucki et al., 2001). To discriminate genetic influences on 'depressive like' behavior, several investigators have undertaken selective breeding programs of animals based on the individual responsiveness in animal models for depression, including animals susceptible to learned helplessness (Vollmayr et al., 2001), high and low FST responders (Weiss and Kilts, 1998) and animals bred for spontaneous high or low immobility scores in the tail-suspension test (Vaugeois et al., 1996).

Species differences

There are distinct species differences in the primary targets of certain psychotropic agents, which make translation or prediction of effects from rat or mouse to human difficult. For example, 5-HT_{1B} receptors are receptors relevant to antidepressant action whereby important structural differences between the human and the rat and/or mouse interfere with the ability to predict pharmacological effects across species (Bonaventure et al., 1999;Rupniak et al., 2001). The report of the humanization of the mouse 5-HT_{1B} receptor using a 'knock-in' approach of the human receptor gene demonstrates a novel strategy that can be used to overcome species differences in antidepressant targets (Bonaventure et al., 1999).

Reliability

The reproducibility of the observed behavioral effects between laboratories is an important consideration (Crabbe et al., 1999;Wahlsten et al., 2003). It was demonstrated that even when every possible attempt is made to standardize the experimental conditions at various behavioral laboratories, inter-laboratory differences in these studies are still present (Crabbe et al., 1999;Wahlsten et al., 2003).

The FST is the most widely used animal model for assessing antidepressant activity (Cryan et al., 2002;Porsolt et al., 1977). However, under the standardized conditions

proposed in the initial descriptions of the test SSRIs, these most widely prescribed drugs today are not detected, whereas under a modified design (using an increased water depth and investigating both active and passive behaviors) also SSRIs show antidepressant-like behaviors (Lucki, 1997;Cryan and Lucki, 2000b;Cryan and Lucki, 2000a).

Gender differences

The occurrence of major depression is higher among women than men (Weissman and Klerman, 1977) and such gender differences extend to the presentation, the course of the illness and the treatment response (Kornstein, 1997). Therefore, it is somewhat contradictory that most animal studies related to depression models are performed only in male animals. The reason for this probably includes the fact that male animals are easier to use, as one does not have to take into account for stages within the estrus cycle and hence there is the perception of less variability (Palanza, 2001;Blanchard and Glick, 1995). To date there are only few studies investigating gender effects in animal models of depression and there has been somewhat conflicting evidence with some models showing increased depressive-like behavior in female animals (Stock et al., 2000;Stock et al., 2001) or no effect (David et al., 2001). However, the majority of studies in various animal models observed that females are actually less susceptible to the effects of the stress behave as if they had been administered antidepressants compared to males (Alonso et al., 1991;Alonso et al., 1999;Caldarone et al., 2000;Brotto et al., 2000;Steenbergen et al., 1990;Gorzalka et al., 1998;Caldarone et al., 2003).

Acute versus chronic testing

Although the he pharmacological effects of antidepressants occur soon after acute administration, the clinically relevant antidepressant effects may take up to 2 or 3 weeks (Gelenberg and Chesen, 2000) and become stronger over a period of at least 6 weeks (Frazer and Benmansour, 2002). Therefore, to be valid, the animal model must respond to chronic treatment. Furthermore, the response in the animal model must be maintained until termination of antidepressant treatment, since tolerance does not occur to the clinical effects of antidepressants. Some of the animal models indeed show enhanced effects after chronic administration. This is true, for example, for the rat and mouse FST. Furthermore, tests such as learned helplessness and olfactory bulbectomy procedures require repeated administration of the test drug to

demonstrate a selective effect of antidepressants. On this basis, it may be argued that these tests better reflect the clinical activity or potential activity of the test substance.

3.2.4 Future Direction

Traditionally, antidepressant compounds have been detected in animal models, which were based on a known pharmacology. Models such as the FST, olfactory bulbectomy and learned helplessness, which were validated originally for detection of TCAs and MAOIs, should be able to detect related compounds. It remains unclear as to whether currently used animal models can systematically detect antidepressant agents with non-monoamine mechanisms of action. However, antidepressant-like activity has been demonstrated by such paradigms for compounds whose mechanisms of action are primarily through corticotropin-releasing factor (Harro et al., 2001;Mansbach et al., 1997), neuropeptide Y (Stogner and Holmes, 2000), glucocorticoid (Healy et al., 1999), glutamate (Li et al., 2001) and substance P (Rupniak et al., 2001). Furthermore, neurochemical and behavioral analysis of the effects of novel compounds will be most relevant in 'depressed' animals in addition to 'normal' healthy animals. In addition, analysis of the genetically modified mice with predictable phenotypes in depression models should be continued (Chapter 3.2.1). The further validation of models such as those involving prenatal stress, maternal deprivation, neonatal clomipramine administration or amphetamine withdrawal might provide the appropriate substrates for such analyses. Knockout strategies have provided new possibilities for selection of drug targets in depression. The future will also bring the more systematic use of genechip microarray analysis in animal models of depression, which enables the powerful comparison of changes in gene expression in various animal models before, during and after antidepressant treatment. Such genetic approaches in rodent paradigms might provide novel targets for antidepressants that might translate therapeutically to the human condition. It is clear that current animal models should be refined continuously or new models developed to reveal the therapeutic potential of a broad range of compounds. Such improvements will enhance the utility of the animal models for both the detection of novel targets for antidepressant activity and contributing to a better understanding of the underlying pathophysiology of depression.

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Chapter 4

Mechanism-based PK/PD Modeling of SSRIs

4.1 Introduction

After administration of a drug various processes can occur, resulting in the eventual effects of the drug. Pharmacokinetics (PK) may be defined as "what the body does to a drug", whereas pharmacodynamics (PD) may be defined as "what the drug does to the body". The relationships between the input of a drug (dose), the pharmacokinetics, the pharmacodynamics and resulting effects are illustrated in Figure 1.

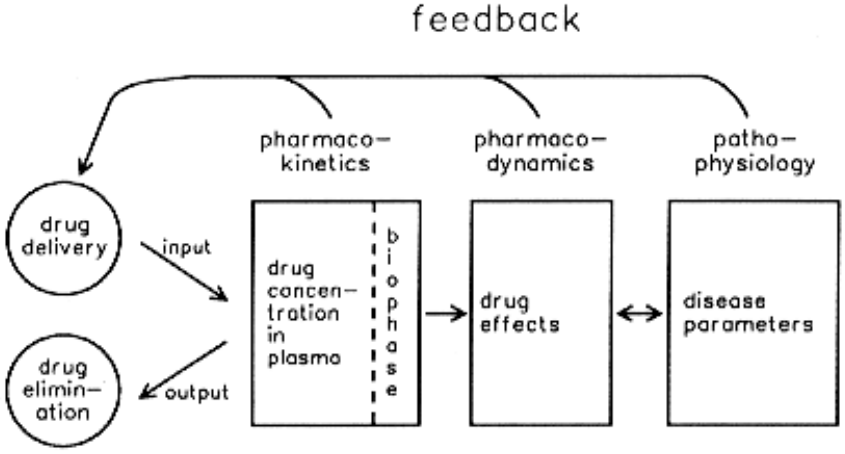

Figure 1. *Schematic illustration of the interrelationships between drug delivery and input (dose), pharmacokinetics, pharmacodynamics and effect (adapted from Breimer and Danhof, 1997).*

4.1.1 Pharmacokinetics (PK)

Pharmacokinetics (PK) is a term used to describe the process controlling drug concentration at any time after one or more doses. The (plasma) concentration *versus* time course of a certain drug is determined by the pharmacokinetic processes of absorption (when the route of administration is not systemic), distribution (including distribution to the site of action), metabolism and excretion (Perez-Urizar et al., 2000), which could be affected e.g. by incomplete absorption or saturability in transport.

Ideally, concentrations should be measured at the site where the interaction with the respective biological receptor system takes place. However, in most experiments, the concentration in the near environment of the target cannot be determined. Since the concentration *versus* time profile of the drug determines the concentration *versus* time profile of the drug at the site of action, plasma PK is an important determinant for the time course of the effect.

4.1.2 Pharmacodynamics (PD)

Pharmacodynamics (PD) is a term used to describe the relationship between (plasma) concentrations of the drug (and/or metabolites) and the magnitude of the pharmacologic effect (e.g., blood pressure, heart rate) (Perez-Urizar et al., 2000). Pharmacodynamics consider the processes that determine the drug response including binding to the receptor, receptor activation and signal transduction processes leading to the ultimate effect.

4.2 Pharmacokinetic/Pharmacodynamic (PK/PD) Modeling

For a long time, the pharmacological areas of pharmacokinetics and pharmacodynamics has been considered as separate disciplines, in which PK was limited to the description of concentration *versus* time courses of a certain drug in different body fluids and PD to the characterization of the intensity of effects resulting from drug concentrations at the assumed effects site. However, the information provided by both of these disciplines is limited if this is regarded independently. PK/PD modeling actually builds the bridge between these two classical disciplines of pharmacology. It is a mathematical description, linking the change in concentration over time (PK) to the change in the pharmacological effect intensity over time (PD). The primary objective of PK/PD modeling is to identify the key properties of a drug *in vivo*, which allows the characterization and prediction of the time course of drug effects under physiological and pathological conditions (with respect to both intensity and duration) (Breimer and Danhof, 1997). Although PK/PD models are always a simplification of real physiological processes, they can be purely descriptive and empirical by simply combining the observed time courses of concentration and effect, neglecting the underlying physiological mechanisms involved, or might be mechanism-based, recognizing the known physiological events involved in the elaboration of the observed effect (Chapter 4.2.2).

4.2.1 Pharmacokinetic and Pharmacodynamic Models

Initial PK/PD models focused on the characterization of rapidly reversible direct drug effects. These models consist of three components: 1) a PK model, characterizing the time course of drug (and possible active metabolites) concentrations in blood or plasma, 2) a PD model, characterizing the relationship between concentration of the drug (and possible active metabolites) and effect and 3) a link model, to account for the delay of effect in relation to drug (and possible active metabolites) concentration,

which is often observed (effect compartment model, indirect response models) (Breimer and Danhof, 1997).

Various PK models can be used to describe the concentration *versus* time profiles, like non-parametric, compartmental and physiologically based (PBPK) PK models. Non-parametric models give an empirical description of the time course of drug concentration in terms of the maximum concentration (C_{max}) and corresponding peak time (T_{max}) and area under the concentration *versus* time curve (AUC). Compartmental models provide a continuous description of the concentration *versus* time profiles of the drug in a particular body fluid, which are particularly useful for extrapolation within individual subjects and/or single species. PBPK models contain specific expressions for physiological processes, such as blood flows to specific organs, binding to plasma proteins and/or tissue components and liver enzyme activity. PBPK models have been found particularly useful for interspecies extrapolation and to explain changes in pharmacokinetics.

In many reviews, the various PD models have been described and will therefore not be discussed in detail in this thesis (Bellissant et al., 1998;Csajka and Verotta, 2006;Holford and Sheiner, 1981;Holford and Sheiner, 1982;Meibohm and Derendorf, 1997;Perez-Urizar et al., 2000). In contrast to PK models, PD models are basically independent of time and describe the equilibrium (time-independent) relationship between concentration and effect. Dependent on the mechanisms involved, a PD model may consist of one or several transduction and response elements that express the finally observed effect directly or via multiple intermediary response steps (Derendorf and Meibohm, 1999). The PD model can take a far wider range of forms since there are numerous outcome measures that may be considered (Holford and Sheiner, 1981), such as continuous responses (Rowland and Tozer, 1995, (Holford and Sheiner, 1981)) but also categorical and 'time-to-event' data, which are frequently collected in clinical trials (Sheiner et al., 1997).

Under steady state conditions, relatively simple models such as fixed-effect, linear, log-linear can be used to characterize the PK/PD relationship. The classic and most commonly used PD model under these conditions is the sigmoid E_{max} model, which is an empirical function for describing non-linear concentration-effect relationships.

This model is mathematically expressed by the Hill equation (Hill, 1910) according to:

$$E = E_0 + \frac{E_{\max} \cdot C^n}{EC_{50}{}^n + C^n} \qquad (1)$$

in which E is the effect, E_0 is the no-drug response, E_{max} is the maximal effect (intrinsic activity), C is the concentration of the drug and/or metabolite in plasma and/or at the effect site, EC_{50} is the concentration of the drug and/or metabolite that result in 50% of the maximal effect (potency) and n is the Hill factor expressing the sigmoidicity of the relation. (Holford and Sheiner, 1982;Meibohm and Derendorf, 1997).

Under nonsteady-state conditions as well as when time-dependent changes in PD parameters are present, basic PK/PD models are unable to explain the observed time course of drug effect. Mechanisms such as disequilibrium between biophase and plasma compartment concentrations (Derendorf and Meibohm, 1999;Meibohm and Derendorf, 1997;Sheiner et al., 1979), appearance of active metabolites (Derendorf and Meibohm, 1999;Meibohm and Derendorf, 1997;Mandema et al., 1992;Lee et al., 1999), indirect mechanisms of action (Derendorf and Meibohm, 1999;Meibohm and Derendorf, 1997;Dayneka et al., 1993;Jusko and Ko, 1994), sensitization and tolerance (Derendorf and Meibohm, 1999;Meibohm and Derendorf, 1997;Bauer et al., 1997;Ragueneau et al., 1998) can explain the frequently observed dissociation between time courses of concentration and effect (effect compartment, acute tolerance, sensitization, indirect response models). The effect compartment model accounts for the delay observed in the pharmacological response by postulating an extra PK compartment, in which the concentration can be related to the effect. Indirect response models (inhibition or stimulation of k_{in} or k_{out}) are based on drug effects (inhibition or stimulation) on the factors controlling either the input or the dissipation of drug response (Csajka and Verotta, 2006;Derendorf and Meibohm, 1999;Mager et al., 2003;Meibohm and Derendorf, 1997).

In the past decade considerable progress has been made in the field of integrated PK/PD modeling, as becomes clear from the considerably increased number of publications on PK/PD in recent clinical pharmacological and pharmaceutical journals, both on the more theoretical modeling aspects and on application. This progress has resulted from a number of improvements in the determination of plasma concentrations of drugs (improved analytical methods) and in effect measurement (progress in non-invasive methods allowing repeated measurements to be performed), in the continued construction and refinement of PD models based on underlying physiological mechanisms, in the advances in computer hardware and software as well as increased regulatory and academic interest. An increased use of PK/PD modeling concepts in drug development has repeatedly been promoted by industry as well as academia and regulatory authorities (Vozeh et al., 1996;Peck et al.,

1994;Kroboth et al., 1991), which has resulted in the current Guidance for Industry on Exposure-Response Relationships: Study Design, Data Analysis, and Regulatory Applications from the Food and Drug Administration (http://www.fda.gov/cder/guidance/index.htm). Specific applications include 1) drug candidate selection and lead optimization, 2) optimization of the dosing and delivery profile of new and existing drugs, 3) design and evaluation of phase I-IV clinical trials and 4) individualization of the dosing in special patient groups. However, despite the continuous increase in the number of publications, still a large number of papers present kinetic and dynamic data without studying the relationships between them. This probably results from the lack of specific and easy to use software and an insufficient knowledge among pharmacologists of the methodology of PK/PD studies.

4.2.2 Mechanism-based PK/PD Modeling

In recent years, PK/PD modeling has developed from an empirical and descriptive approach into a scientific discipline based on the (patho)physiological mechanisms behind PK/PD relationships that can be applied at all stages of drug development. Mechanism-based PK/PD models differ from empirical descriptive models in that they contain specific expressions to characterize, in a strictly quantitative manner, processes on the causal path between drug administration and response, such as the distribution of the drug to the target site, the binding to the target site, the activation of the target site and homeostatic feedback (Danhof et al., 2005). In mechanism-based models, the mechanisms contributing to the drug action *in vivo* are tried to be understood and included in the model. In a strict sense, mechanism-based PK/PD modeling refers to the characterization in a strict quantitative manner of the functioning of the integral biological system *in* vivo and therefore is identical to biological systems analysis. Mechanism-based models are based on the principles of receptor theory and dynamical systems analysis and feature a separation between a drug-specific part, characterizing the drug receptor interaction in terms of *in vivo* affinity (i.e., how well the drug binds to the receptor) and intrinsic efficacy (i.e., to what extent the drug can activate the receptor) and a biological system specific part (e.g. receptor density and the efficiency of receptor-effector coupling), characterizing the *in vivo* stimulus-response relationship (Van der Graaf and Danhof, 1997). The explicit distinction between drug specific parameters and biological system specific parameters is crucial to the prediction of *in vivo* drug effects. The development of mechanism-based PK/PD models relies on biomarkers (Chapter 4.3), which

characterize quantitatively the processes on the causal path between drug administration and response (Rolan, 1997;Colburn and Lee, 2003).

The specific expressions for characterization of processes on the causal path between drug administration and effect include (Figure 2): 1) target site distribution (distribution between plasma and the target site in peripheral tissues), 2) target binding and activation, 3) pharmacodynamic interactions, 4) transduction and 5) influence of *in vivo* homeostatic feedback mechanisms. Ultimately, mechanism-based PK/PD models might also consider the effects on 6) disease processes and disease progression (Danhof et al., 2005).

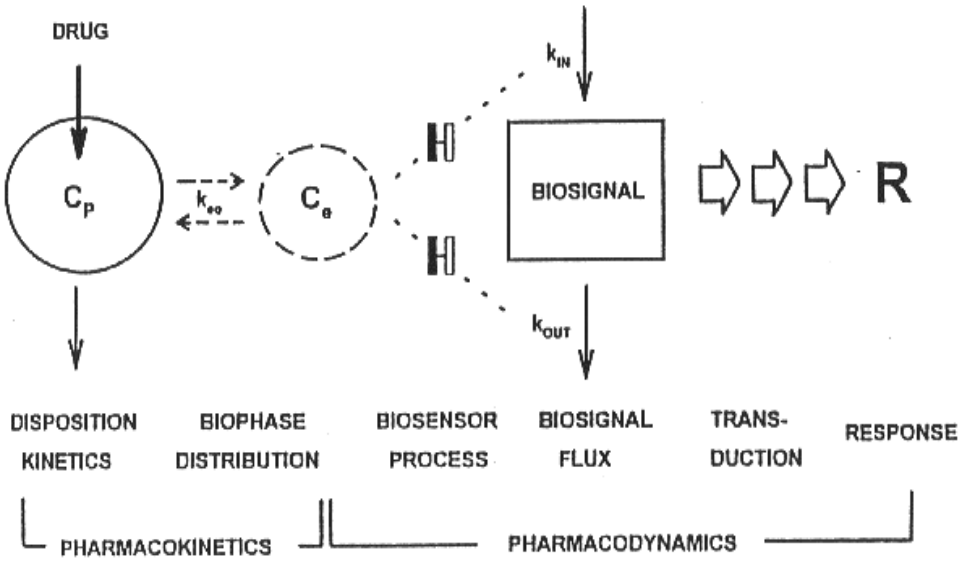

Figure 2. *Schematic illustration of the pharmacokinetic-pharmacodynamic processes that determine the relationship between the administered dose and resulting effect. These determinants include distribution (rate constant, k_{eo}) to a biophase, inhibition or stimulation of the production (k_{in}) or removal (k_{out}) of a mediator biosignal and transduction of the response (R). In mechanism-based PK/PD modeling, the various mechanisms are taken into account (Jusko et al., 1995).*

Target Site Distribution In PK/PD modeling, biophase equilibration is usually characterized on the basis of an effect compartment model, on the assumption that at steady state, the drug concentration in the biophase is identical to the (free) plasma concentration (Sheiner et al., 1979). However, this assumption is not always valid since the target site equilibrium of drugs depends on the target site delivery (blood flow, plasma protein binding) and on the target site distribution (filtration, diffusion, functionality of transporters). Particularly for relatively large hydrophilic molecules and for compounds that are substrates for specific transporters, target site distribution is restricted. This is particularly important for drugs with an intracellular target (i.e., cytostatic drugs) or drugs that act in tissues that are protected by specific barriers

(i.e., the central nervous system). This underscores the need for measures of target site exposure (drug and metabolite concentration) as biomarkers in mechanism-based PK/PD modeling, for example by intracerebral microdialysis to obtain information on the target site exposure in the central nervous system (Bouw et al., 2001).

Target Binding and Activation A limitation of the Hill equation (1) is that it provides only limited insight into the underlying factors that determine the shape and the location of the concentration-effect curve. Specifically, the potency (i.e., EC_{50}) and intrinsic activity (i.e., E_{max}) of a drug are functions of drug-specific (i.e., receptor affinity and intrinsic efficacy) and system-specific properties (i.e., receptor density and the function relating receptor occupancy to pharmacological effect). Classical receptor theory explicitly separates drug-specific properties and system-specific properties as determinants of the drug concentration-effect relationship (Black and Leff, 1983) and therefore constitutes a theoretical basis for the prediction of this relationship. Not surprisingly, receptor theory is increasingly applied in mechanism-based PK/PD modeling to explain and predict (variability in) *in vivo* drug concentration-effect relationships (Van der Graaf and Danhof, 1997).

Pharmacodynamic Interactions Modeling of pharmacodynamic interactions is an integral part of mechanism-based PK/PD modeling. Specifically, the modeling of pharmacodynamic interactions is not limited to situations in which two drugs are given in combination or where a drug is converted into an (inter)active metabolite. Modeling of pharmacodynamic interactions is also relevant when a) a drug interacts with an endogenous ligand, b) a drug interacts at multiple targets, thereby activating multiple pathways, and c) when homeostatic feedback occurs. Moreover, modeling of the influence of baseline effects is also based on theoretical concepts of interaction modeling. Over the years, a number of articles have been published on the theoretical aspects of pharmacodynamic drug-drug interactions in terms of synergy and antagonism (Berenbaum, 1989;Koizumi et al., 1993;Gessner, 1995;Carter, Jr., 1995;Greco et al., 1995). In theoretical terms, modeling of pharmacodynamic interactions concerns the prediction of combined drug effects. In this context, synergy occurs when the combined effect is larger than is expected under the assumption of additivity (‘no interaction’) of the two effects separately. In contrast, antagonism occurs when the combined drug effect is smaller.

Transduction Transduction refers to the processes of the translation of the receptor activation into the ultimate pharmacological response. Specifically, the binding of a drug to a biological target initiates a cascade of biochemical and/or electrophysiological events resulting in the observable biological response. For most

receptors (i.e., G-protein-coupled receptors), phospholipases (i.e., 1,4,5-inositol triphosphate, diacylglycerol) and nucleotide cyclases (i.e., cAMP) serve as second messengers. For other receptors (i.e., glucocorticoid receptors), transduction is mediated through an interaction with DNA, thus regulating the expression of second messengers, proteins, or enzymes. For some drugs like antidepressants, the (molecular) mechanisms underlying transduction are complex and still poorly understood.

Homeostatic Feedback There is a growing need for methods to describe and predict complex pharmacological effect *versus* time profiles. Such complex profiles may be observed when drug exposure leads to tolerance/sensitization or when homeostatic feedback mechanisms are operative (Wakelkamp et al., 1996). Attempts to model physiological counterregulatory mechanisms have resulted in a series of advanced models describing complex behavior (Bauer et al., 1997;Abelo et al., 2000;Mandema and Wada, 1995;Zuideveld et al., 2001).

Disease Processes At present there is a growing interest in the modeling of disease processes and disease progression (Chan and Holford, 2001;Holford and Peace, 1992;Post et al., 2005), which are particularly important for drugs that interact in a highly specific manner with the disease process and that may have no direct observable effects in healthy subjects. Furthermore, application of disease progression analysis is imperative when drug treatment is specifically intended to modify disease progression. The same applies to investigations, which aim to demonstrate the absence of an adverse effect of symptomatic drug treatment on disease progression. The modeling of disease progresses closely follows the modeling of transduction and homeostatic feedback mechanisms, as it is based on the principles of dynamical systems analysis. The modeling of disease progression however, adds a new dimension of time-variant changes in the status of the biological system to the discipline of mechanism-based PK/PD modeling.

Not surprisingly, there is a clear trend toward the development of mechanism-based PK/PD models, which have much improved properties for extrapolation and prediction. This concerns the extrapolation and prediction 1) from *in vitro* bioassays to *in vivo* concentration-effect relationships, 2) from *in vivo* animal studies to humans, 3) from healthy volunteers to patients, and 4) the prediction of intra- and inter-individual variability in drug response (Danhof et al., 2005).

Because mechanism-based models are aimed at describing the system in great detail and investigations of the system should be thorough, they are generally more complex and based on a number of assumptions.

4.2.3 Population Modeling

An important problem in drug therapy is the variability of response among individuals in a population. Numerous studies have demonstrated a contribution of kinetic and dynamic variabilities to the overall variability in clinical response (Rowland, 1985). Population PK/PD modeling is based on nonlinear mixed effects analysis and has been introduced by Sheiner and colleagues to characterize the pharmacokinetics and concentration-effect relationships in populations rather than in individuals (Hashimoto and Sheiner, 1991;Sheiner and Ludden, 1992). PK and/or PD models are used to describe typical trends (population means) and individual profiles. In these models, the parameters of each individual are modeled in terms of both fixed and random effects and therefore it is a 'mixed' effects model. Fixed effects account for large intra- and inter-individual differences in the value of parameter and covariates. Random effects are applied to account for unexplained intra- and inter-individual variability. The concepts and application of population PK/PD modeling have been described in various reviews (Hashimoto and Sheiner, 1991;Sheiner and Ludden, 1992;Vozeh et al., 1996;Yuh et al., 1994). A population PK and PD approach offers the advantage over conventional data analysis in that it is possible to obtain integrated information on the PK and PD, which can include sparse, unbalanced as well as dense data obtained from subjects or a combination. In addition, possible sources that significantly influence the response can be explored and included in the model by a covariate analysis (Williams and Ette, 2000;Sun et al., 1999).

Population PK/PD models have been successfully applied to continuous and non-continuous measures of drug effect, for both direct (Karlsson et al., 1995;Minto et al., 1997b) and indirect (Bouillon et al., 1996;Minto et al., 1997a) PD models. In the meantime population PK/PD modeling has been successfully applied to numerous drugs (Karlsson et al., 1998;Mentre et al., 1998). So far however, limited information is available on the PK/PD correlations of psychotropic drugs in behavioral paradigms.

4.2.4 Complexity and Requirements for Animal Behavioral PK/PD Investigations

Although studies have been reported about the application of PK/PD modeling in animal behavioral studies (Vis et al., 2001;Jonker et al., 2003;Della Paschoa et al., 1998), the number of reported studies is limited. This is caused by the complicated requirements for such a PK/PD study. Ideal requirements for (animal) PK/PD studies include that these studies are well validated, the analysis of the time course of the

effect is feasible within individual animals (repeated measurement), the behavioral measure is constant in time (reproducible), no extensive training is required and the collection of blood samples is feasible. Although currently PK/PD studies do not comply with all of these characteristics either, for animal behavioral studies are even more complicated to meet these requirements. For example, the PK and PD of a compound cannot always be measured simultaneously, since blood sampling may interfere with the behavioral readout. Even when chronically implanted cannulas are used, possible undesirable effects of blood sampling cannot be ignored (Geldof et al, unpublished observations; Bender et al., unpublished observations). The limitations of blood sampling in behavioral investigations could be overcome by application of the population approach as described in Chapter 4.2.3. Application of population PK modeling offer unique possibilities for this problem, including a) sparse sampling during the experiment, b) sparse sampling after the effect measurements, c) PK study at a different occasion and d) PK study in a separate group of animals, in which covariate analysis is important. Therefore, PK sampling designs should be optimized by use of trial simulation (as is performed in clinical trial design (Gieschke and Steimer, 2000)) to obtain indications of the minimal number of blood samples required, optimal sparse sampling design and optimal sampling times for precise description of the PK.

As described in **Chapter 3**, there is no specific animal model of depression (Cryan et al., 2002). Nevertheless, many animal models have been developed that can detect specific behavioral changes that are sensitive to the effects of antidepressants. The effects of SSRIs have been extensively investigated in a variety of behavioral pharmacological tests, such as the forced swim (Kelliher et al., 2003), the tail suspension (Teste et al., 1993) and the learned helplessness test (Takamori et al., 2001). Despite their widespread use and numerous preclinical and clinical investigations, very few studies have addressed the PK/PD correlations of SSRIs. This is remarkable since the PK/PD correlations of SSRIs appear to be rather complex. This is specified by the fact that although SSRIs rapidly inhibit the reuptake of 5-HT, maximal antidepressant effects are only observed after weeks of chronic treatment, indicating that long-term adaptive changes are important for therapeutic efficacy (Chapter 2). Therefore, additional requirements for animal models of antidepressants include that the behavioral measure should ideally be responsive to all SSRIs and display a change in effect after chronic administration.

4.3 Biomarkers

4.3.1 Biomarkers in Mechanism-based PK/PD Modeling

There is an increasing interest in the use of biomarkers in drug development as reflected in the various review publications and commentaries that have recently appeared on this subject (Biomarkers Definitions Working Group, 2001;Colburn and Lee, 2003;Lesko and Atkinson, Jr., 2001;Rolan, 1997). A discussion on the use of biomarkers in the context of biological systems analysis aiming at the prediction of drug effects is described by Danhof and colleagues (Danhof et al., 2005).

Within the context of mechanism-based PK/PD modeling, a biomarker is defined as a measure that characterizes, in a strictly quantitative manner, a process, which is on the causal path between drug administration and effect (Danhof et al., 2005). The concept of mechanism-based PK/PD modeling brings an entirely new dimension to the prediction of drug response because it constitutes a basis for better understanding of the biological system of interest providing a basis for extrapolation and prediction. This is important in drug discovery and development, particularly in relation to extrapolation and prediction within the trajectory from target identification (*in vitro* and in *in vivo* animal studies) to proof of concept (in phase 2 clinical trials). Specifically, it is proposed that within this paradigm, biomarkers can also be of value in the development of drugs acting at novel targets, where the link with clinical efficiency has not yet been demonstrated.

In the paper, Danhof and colleagues proposed a new classification of biomarkers (Danhof et al., 2005), in which each biomarker reflects a specific aspect of the mechanism of action of a drug *in vivo*. The new classification system distinguishes seven types of biomarkers: type 0, genotype/phenotype determining drug response; type 1, concentration of drug and/or drug metabolite; type 2, molecular target occupancy; type 3, molecular target activation; type 4, physiological measures; type 5, pathophysiological measures; and type 6, clinical ratings. The proposed division into seven levels is logical in mechanistic terms, in the sense that it reflects the major intermediate steps in pharmacodynamics, although it is not always necessary to obtain information on each of the intermediate steps.

Within the proposed classification, a type 0 biomarker refers to genotype and/or phenotype as a determinant of drug response. This may be related to either a factor in the disposition of the drug that determines the target exposure (i.e., the expression of a specific enzyme or transporter) or a factor determining the response directly (i.e., the expression of a specific receptor). A type 1 biomarker refers to the concentration of the drug and/or a drug metabolite. Drug concentrations in blood or plasma are

probably the most widely used biomarkers in drug development. Ultimately, however, one is specifically interested in the (free) target site concentration. Because for most drugs the target site is located in peripheral tissue, free target concentrations may not be readily accessible, although novel technologies, such as including microdialysis, may offer new opportunities for quantification of drug concentrations at the target site (Bouw et al., 2001). A type 2 biomarker refers to the target occupancy. Drug effects may occur at different degrees of target occupancy and information on the relationship between target occupancy and response is therefore important for the prediction of *in vivo* concentration-effect relationships (Black and Leff, 1983;Van der Graaf and Danhof, 1997) and for the understanding of intra- and inter-individual variability, in strictly quantitative terms, in drug response (Garrido et al., 2000). For certain drugs, information on the degree of target occupancy can be obtained on the basis of *ex vivo* bioassays (Snoeck et al., 1998). Alternatively, novel imaging techniques (i.e., positron emission tomography scanning) improve the assessment of *in vivo* target occupancy, if a suitable and specific ligand is available (Nordstrom et al., 1993). A type 3 biomarker refers to quantification of the target site activation. According to receptor theory, this target site activation is determined by the intrinsic efficacy of the drug in combination with the level of receptor expression in the target tissue (Black and Leff, 1983;Van der Graaf and Danhof, 1997). It has been demonstrated that differences in target site activation can be an important determinant of tissue selectivity of drug action (Van der Graaf et al., 1997). A type 4 biomarker refers to physiological measures in the integral biological system. An important feature of type 4 biomarkers is that the biomarker response is often influenced by *in vivo* homeostatic control mechanisms. Type 5 biomarkers are parameters that characterize in quantitative manner the disease processes. Examples are biomarkers characterizing inflammatory processes or type 2 diabetes mellitus. Finally, type 6 biomarkers are clinical scales. In a strict sense, these biomarkers can be regarded as clinical endpoints rather than biomarkers *per se*.

As was outlined above, mechanism-based PK/PD models contain specific expressions for processes on the causal path between drug administration and response and typically have much improved properties for extrapolation and prediction. Biomarkers constitute a basis for the characterization, in a strictly quantitative manner, of processes on the causal path between drug administration and response. This allows in principle the development of cascading models in which the effect of drugs from one process in the chain of events to the next can be described. This is important because the relationships between the various processes depend solely on

the functioning of the biological system and are therefore independent of the drug. Mechanism-based PK/PD modeling constitutes, therefore, the scientific basis for prediction of the ultimate clinical effects of novel drugs based on a biomarker response. Furthermore, studies on biomarker responses can provide the basis for the understanding, in mechanistic terms, and the prediction of variability in drug response. Ultimately, after proper validation, such biomarkers might be used in clinical practice in the individualization of drug treatment. Thus, biomarkers are invaluable for the development and application of mechanism-based PK/PD modeling in drug development. At the same time, however, mechanism-based PK/PD modeling constitutes also a scientific basis for the selection, evaluation, and validation of biomarkers, particularly in nonclinical investigations (Visser et al., 2003).

4.3.2 Cascade of Biomarkers used in this Thesis

As described in Chapter 1, the objective of the studies described in this thesis was to explore the PK/PD correlations of fluvoxamine, as a prototype for SSRIs. In the investigations, a spectrum of different biomarkers (Chapter 4.3.1) is used, each reflecting a specific process on the causal path between drug administration and response. The investigations described in the present thesis are illustrated in Figure 3.

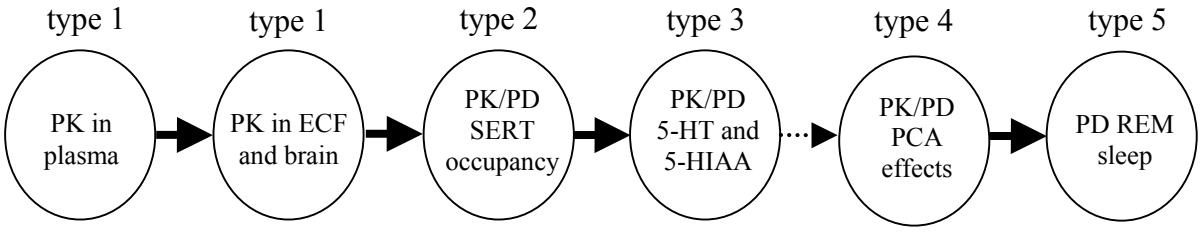

Figure 3. *Schematic illustration of the various biomarkers used in the present thesis, each reflecting a specific aspect of the mechanism of action of fluvoxamine as a prototype for SSRIs.*

These investigations include the development of a population PK model for fluvoxamine in plasma (type 1 biomarker according to (Danhof et al., 2005)), the development of a PK model for the transport of fluvoxamine across the blood-brain barrier in brain ECF of the frontal cortex and brain tissue (type 1), development of a PK/PD model for the effect of fluvoxamine on the occupancy to SERT (type 2), development of a PK/PD model for the effect of fluvoxamine on brain 5-HT and 5-HIAA levels (type 3). Then the development of a categorical PK/PD model for the effect of fluvoxamine on PCA (*para*-chloroamphetamine) induced behavioral effects as a kind of intermediary biomarker (type 4) and ultimately the development of a PD

model for the effect of fluvoxamine on rapid eye movement (REM) sleep (type 5). The information on the different biomarkers was integrated in PK/PD models for the various effects.

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SECTION 2

Investigation Steps

Chapter 5

Population Pharmacokinetic Model of Fluvoxamine in Rats: Utility for Application in Animal Behavioral Studies

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Abstract

The limitations of blood sampling in pharmacokinetic (PK)/pharmacodynamic (PD) studies in behavioral animal models could in part be overcome by a mixed effects modeling approach. This analysis characterizes and evaluates the population PK of fluvoxamine in rat plasma using nonlinear mixed effects modeling. The model is assessed for its utility in animal behavioral PK/PD studies.

In six studies with a different experimental setup, study site and/or sampling design, rats received an intravenous infusion of 1, 3.7 or 7.3 mg/kg fluvoxamine.

A population three-compartment PK model adequately described the fluvoxamine plasma concentrations. Body weight was included as a covariate and mean population PK parameters for *CL*, *V1*, *V2*, *Q2*, *V3* and *Q3* were 25.1 ml/min, 256 ml, 721 ml, 30.3 ml/min, 136 ml and 1.0 ml/min, respectively. Inter-individual variability was identified on *CL* (39.5%), *V1* (43.5%), *V2* (50.1%) and *Q2* (25.7%). A predictive check and bootstrap analysis confirmed the predictive ability, model stability and precision of the parameter estimates. Body weight was identified as a significant covariate of the inter-compartmental clearance *Q2*. The pharmacokinetics was independent of factors such as dose, surgery (for instrumentation) and study site. The utility of the model in animal behavioral studies was demonstrated in a PK/PD analysis of the effects on REM sleep in which a sparse PK sampling design was used. By using the pertinent information from the population PK model, individual PK profiles and the PK/PD correlation could be adequately described.

Introduction

A reduced activity of serotonergic neurotransmission has been postulated for the pathogenesis of depression (Coppen, 1967;Owens and Nemeroff, 1994). Selective Serotonin Reuptake Inhibitors (SSRIs) are presently the first-line treatment for depression. SSRIs selectively and powerfully block the serotonin transporter (SERT) and thereby the reuptake of serotonin (5-HT) in the presynaptic nerve terminal, resulting in increased extracellular 5-HT levels and enhancement of serotonergic neurotransmission (Bel and Artigas, 1992;Fuller, 1994). Although SSRIs rapidly inhibit 5-HT uptake, maximal antidepressant effects are obtained after weeks of chronic treatment, indicating that long-term adaptive changes are important for therapeutic efficacy. The effects of SSRIs have been extensively investigated in a variety of behavioral pharmacological tests, such as the forced swim (Kelliher et al., 2003), the tail suspension (Teste et al., 1993) and the learned helplessness test (Takamori et al., 2001). Analysis of the relationship between the pharmacokinetics

(PK) and pharmacodynamics (PD) in these animal models could provide novel insights in the mechanisms of the time dependencies in the PD of psychotropic drugs. So far however, very few studies on the PK/PD correlations of psychotropic drugs in behavioral animal models have been reported (Della Paschoa et al., 1998; Jonker et al., 2003;Vis et al., 2001). A potential complicating factor in this respect is the interference of blood sampling with the measured PD effect. Even when chronically implanted cannulas are used, possible undesirable effects of blood sampling cannot be ignored. The limitations of blood sampling in behavioral investigations could be overcome by application of a so-called 'population approach'. Population PK/PD modeling is based on nonlinear mixed effects analysis in which observations on the PK and/or PD in several subjects are simultaneously analyzed, while explicitly taking into account both the inter-individual variability in the model parameters as well as the intra-individual residual error variability (Schoemaker and Cohen, 1996). The population PK approach offers the advantage over conventional data analysis that detailed information on the PK in individual rats can be obtained on the basis of unbalanced (sparse) PK data, PK data obtained on a different occasion in the same animals or even PK data obtained in separate group of animals. The concepts and application of population PK/PD modeling have been described in various reviews (Hashimoto and Sheiner, 1991;Sheiner and Ludden, 1992;Vozeh et al., 1996;Yuh et al., 1994). So far however limited information is available on the PK/PD correlations of psychotropic drugs in behavioral paradigms.

The aim of the current research was to develop and evaluate a population PK model for fluvoxamine in the rat. Information on the PK of fluvoxamine obtained in six different studies with a different experimental setup and/or study site was integrated for the analysis. Sampling designs were not identical for all studies and included dense as well as sparse sampling. The second aim was to evaluate the developed PK model for its utility in animal behavioral PK/PD studies. This was illustrated by simulation of a study on the PK/PD correlation of fluvoxamine for the effect on rapid eye movement (REM) sleep using a sparse PK sampling design. It is well established that SSRIs, including fluvoxamine, have significant effects on various sleep-wake stages in humans as well as in animals. Among these effects, a decrease in REM sleep duration is commonly observed after SSRI administration (Wilson et al., 2000b;Wilson et al., 2000a). In preliminary investigations it has been demonstrated that repeated blood sampling significantly influences sleep architecture in rats (Geldof et al., unpublished observations), thus complicating the PK/PD analysis of this effect.

Methods

Overall Study Design

The dataset used for the population PK analysis of fluvoxamine, contained data obtained from 6 separate studies, which were performed over a period of 4 years. In these studies, the relationship between the PK and various PD endpoints was analyzed in male Wistar rats after a 30-min intravenous (IV) infusion of fluvoxamine. The studies varied in surgery for the instrumentation of the rats, the number of rats, body weight, administered fluvoxamine dose, number of collected blood samples, time interval between collected blood samples, time of the investigation and study site (University of Leiden, Leiden, The Netherlands or Johnson and Johnson Pharmaceutical Research and Development, Beerse, Belgium), as depicted in Table 1.

Table 1. *Brief description of the characteristics of the six studies that are used in the population pharmacokinetic analysis of fluvoxamine in Wistar rats*

Study	Type of study	Instrumentation	n	Mean body weight ± S.D. (gram)	Dose (mg/kg)	Blood samples per rat	Time interval (min)	Study site
1	Microdialysis	2 cannulas + microdialysis probe	20	251 ± 22	1, 3.7, 7.3	10 - 12	10 - 315	Leiden
2	SERT occupancy	2 cannulas	36	248 ± 21	1	2 - 15	5 - 480	Leiden
3	SERT occupancy	2 cannulas	23	247 ± 13	7.3	2 - 6	2.5 - 600	Leiden
4	Microdialysis	2 cannulas + microdialysis probe	10	245 ± 11	3.7, 7.3	5 - 6	5 - 530	Leiden
5	PCA	2 cannulas	67	252 ± 16	1, 3.7, 7.3	2 - 18	5 - 720	Beerse
6	Sleep-wake	2 cannulas + EEG/EMG/EOG electrodes	17	282 ± 19	1, 3.7, 7.3	10 - 12	10 - 300	Beerse

S.E. = Standard Error
SERT = 5-HT transporter
PCA = para-chloroamphetamine
EEG = electroencephalogram
EMG = electromyogram
EOG = electro-oculogram

Sampling designs were different for the studies and consequently various rats did not contribute the same amount of information. All data were combined to one dataset, preserving the individual data structure, to examine the pharmacokinetic behavior of fluvoxamine by nonlinear mixed effects modeling. In total, 1463 plasma samples collected from 173 animals served as input for the analysis of the population PK of fluvoxamine.

Chemicals

Fluvoxamine maleate and clovoxamine fumarate were kindly provided by Solvay Pharmaceuticals (Weesp, The Netherlands). Acetonitrile and methanol were obtained from Acros (Geel, Belgium) and dimethylsulfoxide (DMSO) from Merck (Darmstadt, Germany). Ammonium acetate was obtained from Baker Chemicals (Deventer, The Netherlands) and Millipore water was obtained from a Milli-Q system (Millipore SA, Molsheim, France).

Animals

Male Wistar rats (Charles River Wiga GMBH, Sulzfeld, Germany) weighing 226-250 gram at arrival were housed in groups for 1-2 weeks, under standard environmental conditions (ambient temperature 21°C, humidity 60%, 12-h light/dark cycle) with free access to food (Laboratory Chow, Hope Farms, Woerden, The Netherlands for studies performed in Leiden and Onderhoudsvoeder A04C, Safe, France for studies performed in Beerse) and acidified water. After surgery, the animals were housed individually for 3-7 days after cannulation surgery, 1 week after implantation of a microdialysis probe and 3 weeks after implantation of electroencephalogram (EEG)/electromyogram (EMG)/electro-oculogram (EOG) electrodes (Table 1). All studies were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and approved by the Ethical Committee on Animal Experimentation of Leiden University or by the Animal Care and Use Committee of Johnson & Johnson Pharmaceutical Research and Development.

Surgical Procedures

Anesthesia was induced by Ketanest-S® ((S)-ketaminebase, Parke-Davis, Hoofddorp, The Netherlands) and Domitor® (medetomidine hydrochloride, Pfizer, Capelle a/d IJssel, The Netherlands) in studies 1-5 and by isoflurane (Abbott Laboratories, Queenborough, UK) in combination with N_2O and O_2 (3:2) using an Isotec evaporator (Ohmeda, Madison, USA) in study 6. Animals in all studies were

implanted with a permanent cannula in the right jugular vein for fluvoxamine administration and a permanent cannula in the left femoral artery for collection of blood samples. The animals in study 1 and 4 were instrumented with a microdialysis guide cannula (CMA/12, Aurora Borealis Control BV, Schoonebeek, The Netherlands) after implantation of the cannulas within the same operation. The animals in study 6 were instrumented with EEG/EMG/EOG electrodes, approximately 3 weeks before implantation of the cannulas. The skull of the animals was exposed and three small holes were drilled to receive three fixing stainless steel screws (diameter 1 mm) for polygraphic recording of frontal and parietal EEG. Two electrodes were placed stereotaxically on each side of the sagittal suture (AP: 2; L: -2.0 and AP: -6.0; L: 3.0 mm from bregma, in accordance with the atlas of Paxinos and Watson (Paxinos and Watson, 1982) from bregma and the third electrode (reference electrode) was screwed over the cerebellum. For recording of EOG and EMG, stainless steel wires were placed in peri-orbital and inserted into the nuckal muscle, respectively. Electrodes (stainless steel wires, Bilaney Consultants, Germany) were connected to a pin (Future Electronics, Lokeren, Belgium) with a small insert (track pins, Dataflex, Surrey, United Kingdom) and fitted into a 10 holes connector.

Dosage Regimen

Solutions of fluvoxamine in physiological saline (0.9%) were prepared on the day of administration. In the various studies, 1 and/or 3.7 and/or 7.3 mg/kg fluvoxamine was administered via a 30-min IV infusion (BAS BeeHive, Bioanalytical Systems Inc. USA) in the jugular vein cannula at a flow rate of 20 µl/min (Table 1). Fluvoxamine dosages were based on a pilot study (unpublished observations). The dosages and observed concentrations of fluvoxamine are expressed as the free base.

Blood Sampling

The number of arterial blood samples (100 µl) varied between 2 and 18 and were collected from each animal over a time period between 2.5 and 720 min at fixed time intervals after IV fluvoxamine administration (Table 1). After collection of each blood sample, an equal volume of heparinized (20 IU/ml, Pharmacy, Leiden University Medical Center, Leiden, The Netherlands) 0.9% NaCl (B. Braun Melsungen AG, Melsungen, Germany) was administered to the animal. Blood samples were collected in heparinized Eppendorf tubes and kept on ice during the

experiment. After centrifugation (10 min, 5000xg), 50 µl plasma was transferred into a glass tube and stored at -20°C until sample analysis.

Drug Analysis

All plasma samples were analyzed for fluvoxamine using liquid chromatography with tandem mass spectrometry (LC-MS/MS). Calibration standards and independent Quality Control (QC) samples were prepared by addition of fluvoxamine solutions in DMSO to control plasma. A volume of 50 µl of the calibration standards or independent Quality Control (QC) samples in DMSO was added to a volume of 50 µl blank plasma. For the plasma samples from the rats, a volume of 50 µl plasma was added to 50 µl DMSO. A volume of 50 µl of 500 ng/ml clovoxamine in DMSO was added to these samples as internal standard. Proteins were precipitated by adding 200 µl acetonitrile, the samples were centrifuged (10 min, 5000 x *g*) and a volume of 20 µl was injected into the system. Plasma samples were quantified on a reversed phase LC column (BDS Hypersil C18, 3 µm particle size, 100x4.6 mm I.D.; Thermo Hypersil-Keystone, Brussels, Belgium). The mobile phase consisted of 10mM ammonium acetate (solvent A), acetonitrile (solvent B) and methanol (solvent C). Chromatographic retention was obtained by isocratic elution (20% solvent A, 50% solvent B; 30% solvent C) at a flow rate of 1.2 ml/min. LC-MS/MS analysis was performed on an API-4000 MS/MS (Applied Biosystems, Toronto, Canada), coupled to an HPLC system (Agilent, Palo Alto, USA). The MS/MS operated in the positive ion mode using the TurboIonSpray-interface (electrospray ionization) optimized for the quantification of fluvoxamine. Detection by tandem mass spectrometry was based on precursor ion transitions to the strongest intensity, which was at m/z 71.16 (parent m/z 319.16) for fluvoxamine and at m/z 71.00 (parent m/z 285.10) for clovoxamine. The intra-batch accuracy from independent QC samples was between 80 and 120% over the entire range of the plasma samples and inter-assay variability ranged between 3% and 22%. The limit of quantification for fluvoxamine was 1 ng/ml.

Data Analysis

Population Pharmacokinetic Model Selection

All calculations were performed on a personal computer (Intel® Pentium® 4 processor) running under Windows NT using the Compaq Visual FORTRAN standard edition 6.1 (Compaq Computer Cooperation, Euston, Texas, USA) with the nonlinear mixed effects modeling software NONMEM (Version V, Level 1.1., NONMEM project group, University of California, San Francisco, USA). Plasma

fluvoxamine concentrations below the limit of quantification were excluded from analysis. Population PK values of fluvoxamine were estimated using the first order conditional estimation with interaction method. All steps in the development of the PK model were executed on the basis of the likelihood ratio test, goodness-of-fit plots, parameter correlations and precision in parameter estimates. An additional parameter was included in the structural model if the resulting change in MVOF was ≥ 10.8 ($p \leq 0.001$, one degree of freedom).

A linear three-compartment PK model described the concentration time profiles of fluvoxamine. This model was implemented in the PREDPP subroutine ADVAN 11 TRANS 4 in NONMEM. Using this routine the parameters systemic clearance (*CL* in ml/min), central volume of distribution (*V1* in ml), two peripheral volumes of distribution (*V2*, *V3* in ml) and inter-compartmental clearances (*Q2*, *Q3* in ml/min), were estimated (Figure 1).

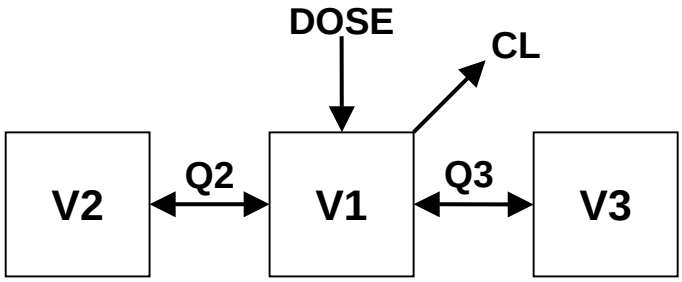

Figure 1. *Schematic representation of the population pharmacokinetic model for fluvoxamine. The model consists of three compartments describing the pharmacokinetics in plasma (V = volume of distribution; CL = clearance; Q = inter-compartmental clearance).*

The inter-individual variability on these parameters was modeled according to:

$$P_i = \theta \cdot \exp(\eta_i) \quad (1)$$

in which P_i is the estimate of parameter P for the i^{th} individual, θ is the population estimate for parameter P and $\exp(\eta_i)$ is the inter-individual random deviation of P_i from P. The values of η_i are assumed to be normally distributed with mean zero and variance ω^2.

Inter-individual variabilities (IIV) were analyzed on each with the requirement of a resulting change in MVOF ≥ 10.8 ($p \leq 0.001$) for significance. Residual variability (e.g. caused by measurement and experimental errors) was assumed to be proportional to the fluvoxamine concentration in plasma and an additive error was included to account for the increased uncertainty of the fluvoxamine plasma concentrations close to the quantification limit.

Accordingly, the residual error was characterized by a combination of a proportional and additive error model:

$$Cm_{ij} = Cp_{ij} \cdot (1 + \varepsilon_{1ij}) + \varepsilon_{2ij} \quad (2)$$

in which Cm_{ij} is the measured plasma concentration, Cp_{ij} is the j^{th} plasma concentration for the i^{th} individual predicted by the model and ε_{1ij} and ε_{2ij} account for the residual deviance of the predicted concentration from the observed concentration. The values for ε were assumed to be independently normally distributed with mean zero and variance σ^2.

Covariate Analysis

Body weight (BW) was modeled as a linear function of the parameters and standardized for the median value of 253 gram:

$$P_i = \theta_i + \theta_j \cdot (BW - 253) \quad (3)$$

in which P_i is the estimate for parameter P for the i^{th} individual, θ_i and θ_j are the intercept and slope of the relationship between model parameter P and body weight.

Body weight was selected to be a significant covariate on the basis of a significant reduction in MVOF of 20, which was ≥ 10.8 ($p \leq 0.001$) and reduction of the additive error component of the residual error. To refine the PK model, the covariance between the random effects in the model was explored using the OMEGA BLOCK option. High-scaled covariances were observed between the parameters *CL*, *V1*, *V2* and *Q2*. The covariance structure was simplified by reducing the covariance matrix to *CL* and *V1*, and scaling the IIV of *V1* to *V2* and *Q2* by estimated scaling factors.

Model Evaluation

The model was evaluated on the basis of a predictive check and a bootstrap analysis. A jackknife analysis was performed to evaluate the sensitivity of the model to each study from the dataset.

To determine whether the fitted model provides an adequate description of the data, a predictive check was performed (Cox et al., 1999;Yano et al., 2001). In this predictive check, 1000 individual curves were simulated from the final PK parameter estimates. Observations were normalized for fluvoxamine dose by applying a reference dose of 4 mg/kg. The median, lower (2.5%) and upper (97.5%) quantiles of the simulated concentrations were calculated for a time period between 0-300 min

after fluvoxamine administration. This time frame included 94% of the observations from the original dataset.

Bootstrapping is a computer-based data resampling method used for estimating parameter precision, confidence intervals and stability of the identified model. In the bootstrap analysis, a new replication of the original dataset was obtained by different random draws of individual data from the original dataset with replacement (Iwi et al., 1999). Each new dataset was then fitted using the final PK model. For this analysis, 1000 bootstrap runs were performed. Bootstrap runs with unsuccessful minimization were excluded from further analysis. The estimated model parameters were compared with each other to evaluate the stability of the final PK model.

Indications for an effective sampling design could be obtained, because the dataset included plasma concentrations of six studies with different sampling designs. By the jackknife method, the influence and sensitivity of each study on the parameters of the PK model were explored. Data from study 1-6 were excluded from the dataset one by one (Bonate et al., 2004), resulting in 6 new datasets that each contained fluvoxamine plasma concentrations of 5 studies. The final PK model was then fitted back to each of these datasets. Parameter estimates were compared to the estimates that were obtained by fitting the final model to the complete dataset of the PK data.

Utility of the PK Model in Animal Behavioral Studies

To illustrate the use of the developed population PK model in animal behavioral studies, a data set was simulated in which the effects of fluvoxamine on REM sleep duration were explored using a sparse PK sampling design. The profile of activity of fluvoxamine on sleep-wake architecture can be determined in rats that are chronically implanted with electrodes for recording the cortical electroencephalogram (EEG), electrical neck muscle activity (EMG), and ocular movements (EOG). Sleep-wake staging was executed per 2-second epoch for periods of 30 min, based on 5 EEG frequency domain values (δ: 0.4-4 Hz, θ: 4.2-8 Hz, α: 8.2-12 Hz, σ: 12.2-14 Hz, β: 14.2-30 Hz), integrated EMG, EOG and body activity level. The discriminative analysis uses classification rules for the final sleep stage assignment to each specific EEG epoch. Six sleep stages were classified of which one is indicative of REM sleep and can be characterized by low-voltage fast cortical waves with a regular theta rhythm, presence of rapid eye movements and absence of muscular and body movements. The detailed experimental conditions as well as the development of the PK/PD model for fluvoxamine on REM sleep will be described in more detail in a future publication (Geldof et al., **Chapter 10**).

In the simulation the relationship between fluvoxamine plasma concentration and the difference in REM sleep duration between saline-treated and fluvoxamine-treated rats as a percentage of the recording period of 30 min (ΔREM) was described by an E_{max} model:

$$E = \frac{E_{\max} \cdot Cp}{Cp + EC_{50}} \quad (4)$$

in which E is observed difference in REM sleep duration between saline-treated and fluvoxamine-treated rats, E_{max} is the maximum observed difference in REM sleep duration (20 %, which corresponds to 6 min), Cp is the plasma fluvoxamine concentration and EC_{50} is the plasma fluvoxamine concentration at which 50% of the maximum difference in REM sleep duration is observed (100 ng/ml).

The developed population PK model and the above described PK/PD relation for the effects of fluvoxamine on REM sleep duration was used to simulate a new data set using the SIMULATION option in NONMEM. In this virtual study 20 rats received 3.7 or 7.3 mg/kg of fluvoxamine (n=10 per dose). In the simulation only two blood samples were obtained at 220 and 355 min after dose administration, which was after the period in which the effects on REM sleep duration were observed. Together with the simulation of the (sparse) fluvoxamine plasma concentrations, a dense PD dataset was simulated (Eq.4) for the effects on REM sleep duration for each period of 30 min up to 360 min after dose administration.

The utility of the developed population PK model for PK/PD analysis based on sparse PK data was evaluated as follows. As the first step the population PK described in this article was used to predict the complete individual PK profiles in the animals by estimation of post hoc parameters based on the sparse data. The next step was the evaluation of the concentration-effect relationship for the effect on REM sleep duration using the predicted plasma concentration from the first step.

Results

Population Pharmacokinetic Model Selection

A three-compartment PK model provided an adequate description of the observed fluvoxamine concentration-time courses. In Figure 2, the observed and individual predicted fluvoxamine plasma concentration-time profiles are depicted, separated by study number and dose (Table 1).

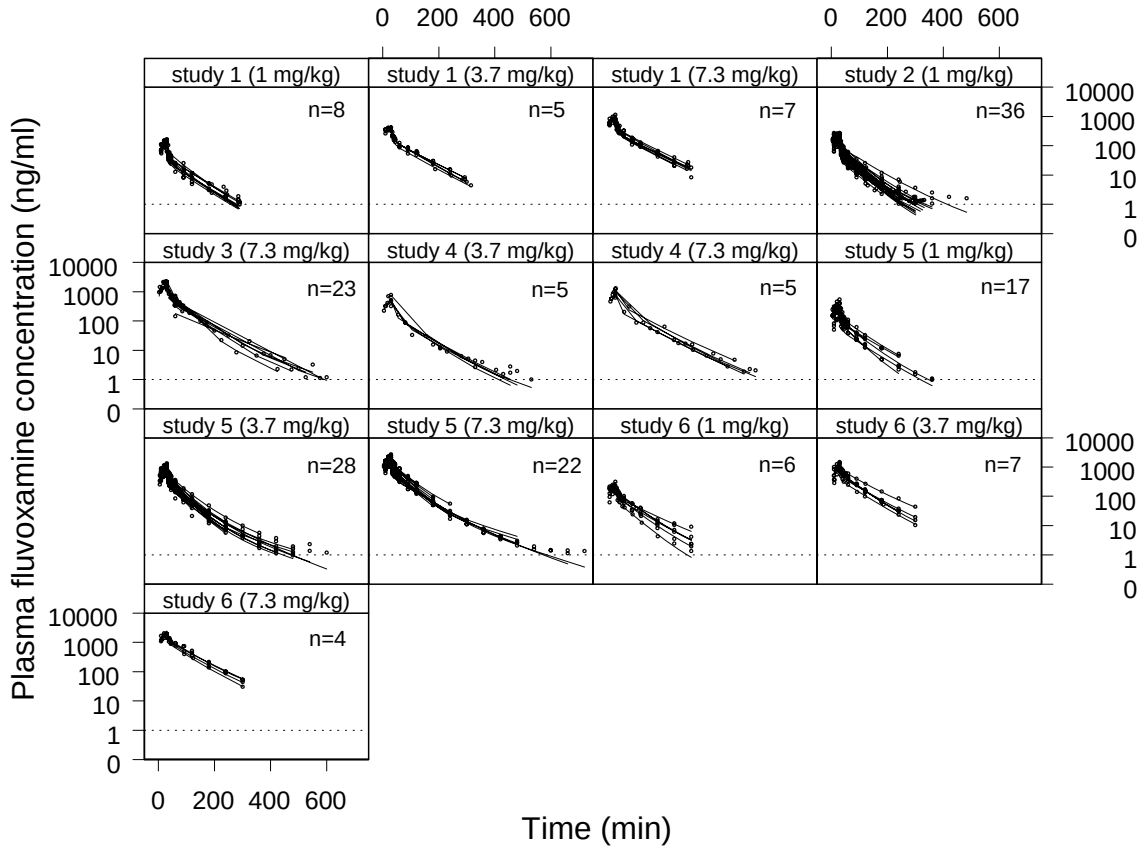

Figure 2. *Fluvoxamine concentration-time profiles in plasma, obtained after a 30-min IV infusion in Wistar rats. Depicted are the observed concentrations (dots), individual predictions (solid lines), separated by study number and fluvoxamine dose. The limit of quantification (1 ng/ml) is added for clarity (dashed horizontal line).*

The population three-compartment PK model was able to adequately describe observed fluvoxamine plasma concentrations for all studies and for each dose. Maximal observed fluvoxamine concentrations (C_{max}) were also adequately described by the PK model. All structural parameters of the population PK model for fluvoxamine could be adequately estimated (Table 2).

Table 2. *Population pharmacokinetic parameter estimates of fluvoxamine in plasma after IV administration in 30 min (1, 3.7 and 7.3 mg/kg) obtained by the three-compartment pharmacokinetic model. Depicted are the population mean estimates for CL, V1, V2, Q2, V3, Q3, σ^2 with corresponding coefficient of variation (C.V.) and inter-individual variability (IIV).*

Parameter	Unit	Value	C.V. (%)	IIV (%)[2]
CL	ml/min	25.1	3.1	39.5
V1	ml	256	6.0	43.5
V2	ml	721	4.8	50.1
Q2[1]	ml/min	30.0	5.1	25.7
V3	ml	136	32.7	-
Q3	ml/min	1.0	41.2	-
$\sigma^2\ \varepsilon_{1ij}$	-	0.026	10.2	-
$\sigma^2\ \varepsilon_{2ij}$	-	0.64	40.4	-

*C.V. = Coefficient of Variation (standard error / value * 100%)*

IIV = inter-individual variability ($100 \times \sqrt{\omega^2}$ *)*

1 Typical value for a rat of 253 gram

2 Scaled covariances: CL and V1: $\omega^2_{CL-V1} / \omega_{CL}\omega_{V1} = 0.83$, *V1 and V2 (assumption)* $\omega^2_{V1-V2} / \omega_{V2}\omega_{V1} = 1$ *and V1 and Q2 (assumption):* $\omega^2_{V1-Q2} / \omega_{Q2}\omega_{V1} = 1$

The mean population values for *CL*, *V1*, *V2*, *Q2* (typical body weight of 253 g), *V3* and *Q3* were estimated to be 25.1 ml/min, 256 ml, 721 ml, 30.0 ml/min, 136 ml and 1.0 ml/min, respectively. The coefficients of variation (C.V.) ranged between only 3 and 6% for the parameters of the first two PK compartments and were 33 and 41% for *V3* and *Q3*, respectively. Inter-individual variability could be characterized for *CL*, *V1*, *V2* and *Q2*, which were estimated to be 39.5, 43.5, 50.1 and 25.7%, respectively. Inter-individual variability in *V3* and *Q3* could not be identified. The covariate analysis showed that *CL* between the central compartment and the second peripheral compartment (*Q2*) was affected by body weight of the animals. No relevant correlation was present between fluvoxamine dose and the estimated population PK parameters. The goodness-of-fit plots for the population three-compartment model are depicted in Figure 3.

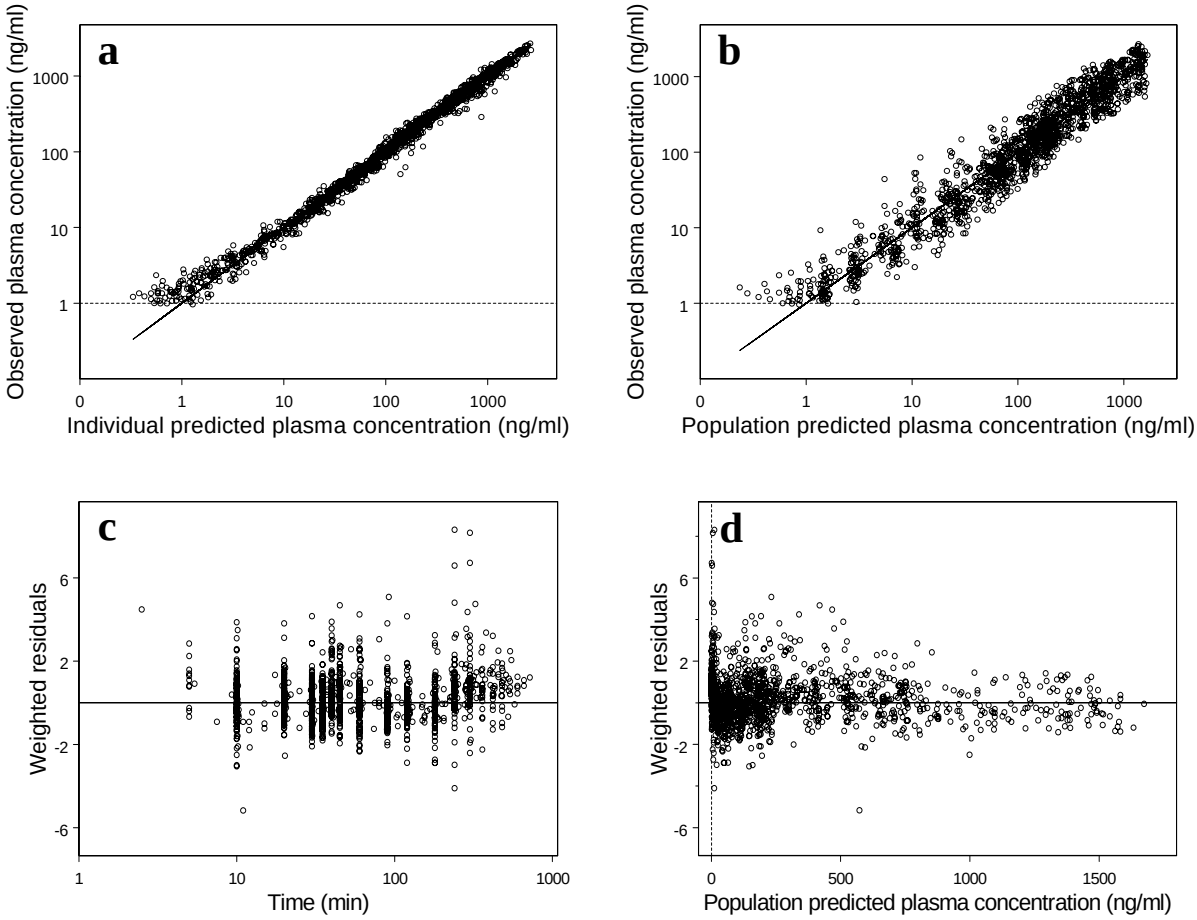

Figure 3. *Goodness-of-fit plots obtained for the final three-compartment pharmacokinetic model. Depicted are scatter plots of the observed fluvoxamine plasma concentrations versus the individual model predictions (a) and population model predictions (b) and scatter plots of the population weighted residuals versus time (c) and population model predictions (d). The limit of quantification (1 ng/ml) is added for clarity (dashed line).*

Fluvoxamine plasma concentrations could adequately be described, because no substantial deviation from the identity line was observed in the residual diagnostics. However, some bias was observed for the plasma concentrations that were close to the quantification limit in Figure 3a and 3b, as was already indicated in Figure 2.

Model Evaluation

The results of the predictive check, normalized for dose, between 0-300 min, in which 1000 datasets were simulated from the final PK parameter estimates, are depicted in Figure 4.

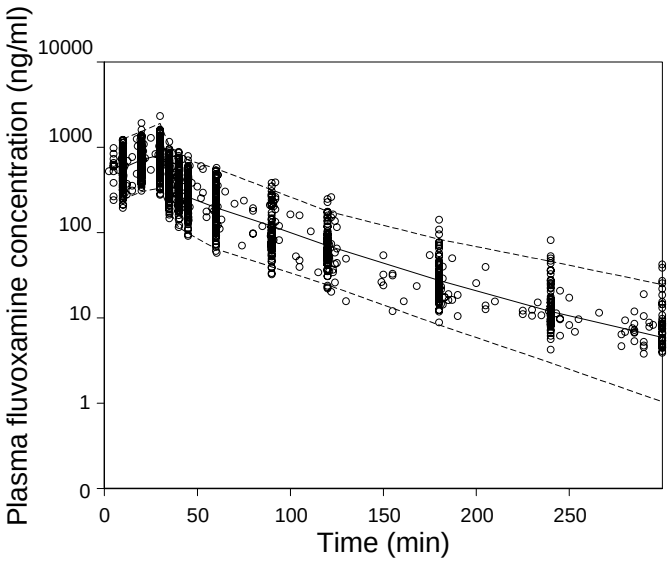

Figure 4. *Predictive check of the final three-compartment pharmacokinetic model between 0-300 min after fluvoxamine administration, normalized for dose by applying a reference dose of 4 mg/kg (includes 94% of observed data). A number of 1000 data sets were simulated from the final pharmacokinetic parameter estimates. Depicted are the observed fluvoxamine plasma concentrations (dots), upper (97.5%, upper dashed line) and lower (2.5%, lower dashed line) quantile of the simulated concentrations and median concentration (solid line) versus time.*

The majority of the observed fluvoxamine plasma concentrations are within the range of the simulated upper (97.5%) and lower (2.5%) quantiles of the simulated concentrations. In the bootstrap analysis, 974 out of 1000 bootstrap replicates succeeded to minimize successfully, indicating low sensitivity to variation in the individuals included in the dataset. The population estimates as well as the proportional and additive residual error of the final model are in very close agreement with the mean values of the 974 successful bootstrap replicates, as depicted in Table 3.

Table 3. *Population mean pharmacokinetic parameter estimates from the original data set and resulting 974 bootstrap replicates for CL, V1, V2, V3, Q2, Q3, σ^2 with corresponding coefficient of variation (C.V.).*

Parameter (unit)	Original dataset Value (C.V. %)	974 bootstrap replicates Value (C.V. %)
CL (ml/min)	25.1 (3.1)	25.0 (3.0)
V1 (ml)	256 (6.0)	254 (5.9)
V2 (ml)	721 (4.8)	717 (4.6)
Q2[1] (ml/min)	30.0 (5.1)	30.1 (5.0)
V3 (ml)	136 (32.7)	151 (27.7)
Q3 (ml/min)	1.0 (41.2)	1.12 (35.0)
$\sigma^2\ \varepsilon_{1ij}$	0.026 (10.2)	0.026 (10.1)
$\sigma^2\ \varepsilon_{2ij}$	0.64 (40.4)	0.63 (48.2)

*C.V = Coefficient of Variation (standard error / value * 100%)*
[1] *typical value for a rat of 253 gram*

Difference in the estimated parameters by the final model between the original observed plasma data and the combined bootstrap replicates was <1% for all parameter estimates, except for those of the third compartment (*V3*: 9.9% and *Q3*: 10.7%).

The estimated population PK parameters of the model fitted to the six datasets that were subjected to the jackknife method are depicted in Table 4.

Table 4. *Population pharmacokinetic parameter estimates of the final pharmacokinetic model fitted to the original dataset and datasets obtained by the jackknife method. Depicted are the population mean estimates for CL, V1, V2, V3, Q2, Q3, σ^2.*

Parameter (unit)	Values obtained after exclusion of study number						
	-	1	2	3	4	5	6
CL (ml/min)	25.1	23.3	23.7	25.7	24.5	27.6	26.2
V1 (ml)	256	239	266	265	246	249	254
V2 (ml)	721	656	737	732	695	766	721
Q2[1] (ml/min)	30.0	27.3	34.0	30.1	29.8	33.7	29.3
V3 (ml)	136	130	132	126	126	326	135
Q3 (ml/min)	1.0	0.93	0.89	0.99	1.01	2.77	0.95
$\sigma^2\ \varepsilon 1ij$	0.026	0.026	0.023	0.026	0.025	0.031	0.025
$\sigma^2\ \varepsilon 2ij$	0.64	0.55	0.73	0.70	0.68	0.24	0.61

[1] *typical value for a rat of 253 gram*

The estimated population parameters are in general in close agreement with those obtained by fitting the model to the original dataset. The only exceptions in this respect are the estimates of parameters *V3* and *Q3* when eliminating the data from study 5. Therefore, the developed PK model was stable and not very sensitive to the exclusion of a study from the dataset. However the inclusion of the results of study 5 appears to be important for the accurate identification of the parameters *V3* and *Q3*.

Utility of the PK Model in Animal Behavioral Studies

In Figure 5, the observed (simulated), individual and population predicted plasma concentration-time profiles of the study, in which the effects of fluvoxamine on REM sleep duration were simulated, are depicted.

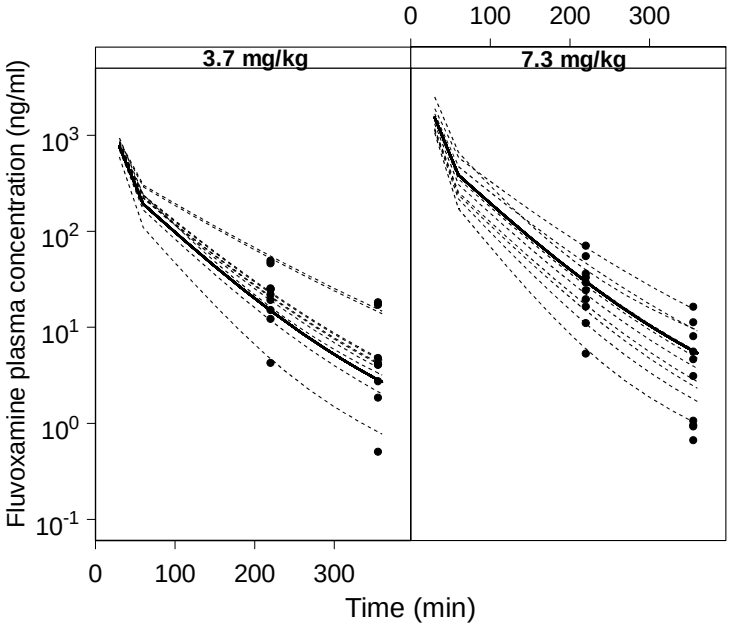

Figure 5. *Fluvoxamine concentration-time profiles in plasma, obtained in the simulated study in which a 30-min IV infusion of 3.7 and 7.3 mg/kg fluvoxamine was administered. Depicted are the observed concentrations (dots), individual predictions (dashed lines) and population predictions (solid lines), separated by fluvoxamine dose.*

In the simulation of the data set, a sparse sampling design was used in which only two blood samples per animal were collected at 220 and 355 min after fluvoxamine administration. By utilization of the developed population PK model, the complete fluvoxamine concentration *versus* time profiles could be predicted in individual animals from these sparse concentration data. The mean relative difference between the predicted parameter estimates, based on the blood samples collected at 220 and 355 min, and the actual parameter estimates was 12 and 18% for *CL* and *V1*,

respectively, indicating the accuracy of the obtained pharmacokinetic parameter estimates.

In Figure 6, the relationship between the individual predicted fluvoxamine plasma concentrations, as derived from Figure 5, and the difference in REM sleep duration between saline-treated and fluvoxamine-treated rats (ΔREM) are depicted.

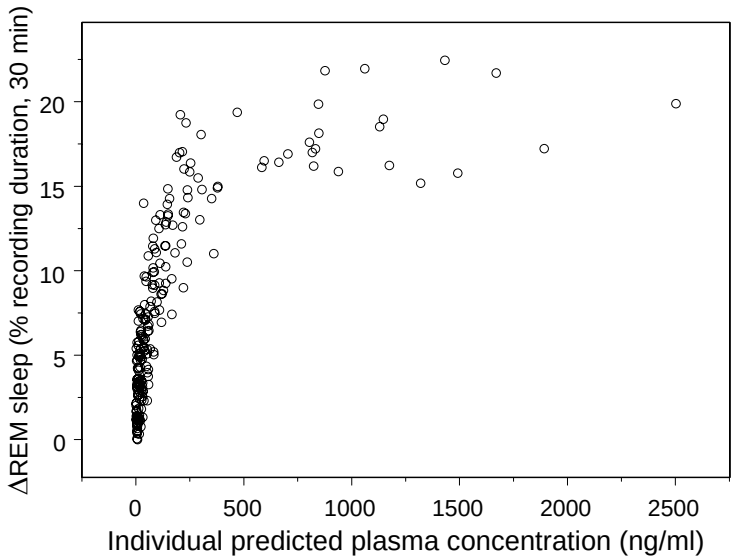

Figure 6. *The relationship between fluvoxamine plasma concentrations and the effect on ΔREM sleep (difference in REM sleep duration as a percentage of the recording period of 30 min between saline-treated animals and fluvoxamine-treated animals). This PK/PD relation is obtained in the simulated study after a 30-min IV infusion of 3.7 and 7.3 mg/kg fluvoxamine. At each time period of 30 min, the observed effects on REM sleep duration could be related to a predicted fluvoxamine plasma concentration.*

Figure 6 shows that the PK/PD relationship is fully recognizable when applying the population PK model to derive the complete concentration data set from the sparse data.

Discussion

Population Pharmacokinetic Model Selection

Compared to a fit of a two-compartment model, the three-compartment model showed a lower MVOF (-240) and a decreased bias in diagnostic plots. By the use of population modeling, the third compartment could thus be interpreted as a significant improvement of the model. This result was mainly due to the inclusion of study 5, which provides dense information clearly displaying three exponential phases. In some studies (e.g. study 5), deviation between the observed and individual predicted profiles is observed at concentrations that were close to the quantification limit. Attempts to improve the characterization of the time course of these plasma

concentrations included inclusion of an extra 4th compartment or a nonlinear term for *CL* (Michaelis-Menten elimination and/or combined Michaelis-Menten and linear elimination). However, none of these models showed significantly improved fits and/or generated adequate parameter estimates. Therefore, the linear three-compartment model was selected as optimal population PK model for description of the observed fluvoxamine plasma concentrations.

All structural parameters of the population PK model could be adequately estimated and inter-individual variability could be characterized for *CL*, *V1*, *V2* and *Q2* (Table 2). Incorporation of an additive error term in combination with a proportional error term for the residual error, significantly improved the model. The residual variability was estimated 0.026 for the proportional error and 0.64 for the additive error. Possible sources of variability were the type of surgery for the instrumentation (i.e. 2 cannulas, 2 cannulas + a microdialysis probe or 2 cannulas + EEG/EMG/EOG electrodes, of which this latter was performed in two separate surgeries), the time the animals were allowed to recover (varied between 3-7 days, 1 and 3 weeks) and the number of collected blood samples per animal (varied between a number of 2-18). In addition, the study site where the studies were performed (Leiden or Beerse) could also be a source of variation. However in the covariate analysis none of these factors was identified as a significant covariate for any of the pharmacokinetic parameters. Differences in the plasma concentration *versus* time profiles between the animals are presumably caused in part by variability in the metabolism of fluvoxamine. In humans, cytochrome P450 (CYP) isoenzymes play a major role in the biotransformation of fluvoxamine, which is extensive and associated high inter-individual variability (DeVane and Gill, 1997;Preskorn, 1997).

To our knowledge, the PK of fluvoxamine in rats has not been described before by compartmental analysis. The proposed population PK model will be of use in future studies on the PK/PD correlations fluvoxamine in behavioral paradigms.

Model Evaluation

The results of the predictive check demonstrated that the majority of the observed fluvoxamine plasma concentrations were within the range of the simulated upper (97.5%) and lower (2.5%) quantiles of the simulated concentrations. The solid line in Figure 4 that represents the predicted median plasma concentrations is positioned centrally in the observations. The high robustness of the identified PK model was confirmed in the bootstrap analysis, since only minimal differences in the estimated parameters were observed between the original observed plasma data and the

combined 974 successful bootstrap replicates. In addition, precision of the estimated population parameters was very high, since estimated values of C.V. were comparable. The jackknife analysis illustrated that the developed PK model was stable and not very sensitive to the exclusion of a study from the dataset, with the exception of study 5. By exclusion of study 5, estimated parameters of the third compartment significantly differed from those obtained with the original dataset, with values of *V3* and *Q3* which were 2.4 and 2.8 fold higher, respectively. This is not surprising, since fluvoxamine concentrations were measured over the longest time period in this study (up to 720 min), providing most information for characterization of the third compartment of the model. Clearly study 5 provided the most comprehensive structural information of the fluvoxamine PK in rats.

As part of the so-called 'separate study analysis' the developed model was also fitted to the data of each study separately to explore the influence of the various sampling designs in the studies (results not shown). This analysis revealed that the estimated parameters were significantly different from the parameter estimates obtained with the information of all studies. Estimated population PK parameters obtained after fitting the model to the dataset of study 5 were in closest agreement with those obtained with the datasets of all studies, which confirmed the results of the jackknife analysis. From the separate study analysis, only an indication of the influence of various sampling designs on the outcome of the study is obtained. For optimization of the sampling design, trial simulation should be preformed, as is now routinely done for clinical trials (Gieschke and Steimer, 2000). Using this approach, pertinent information on the optimal sampling (i.e. minimum number of blood samples, optimal sampling times) to adequately describe the PK can be obtained. The developed population PK model for fluvoxamine constitutes a unique basis for such trial simulations.

Utility of the PK Model in Animal Behavioral Studies

SSRIs have specific effects on sleep architecture (i.e. a reduction in REM sleep duration) both in laboratory animals and in man (Wilson et al., 2000b;Wilson et al., 2000a). Recent studies have shown that PK/PD analysis of the acute and chronic effects of fluvoxamine on REM sleep complicated by the effect of repeated blood sampling (Geldof et al., **Chapter 10**). This problem can be overcome by application of population PK modeling. In principle there are several options.

One of the options is to reduce the number of blood samples in the individual animals and then to use the population PK model for prediction of the individual

concentration *versus* time profiles by post hoc analysis. The advantage of this approach is that pertinent information on the PK is obtained at the same occasion as the behavioral study. It should be taken into consideration however that even a limited number of blood samples taken during the behavioral observations might still affect the sleep architecture. A second option is to determine the pharmacokinetics on a separate occasion in the same animals. This approach is particularly attractive in the situation where the inter-occasion variability is small relative to the interindividual variability. As a final alternative, the pharmacokinetics in the individual animals of the behavioral study might be predicted on the basis of a population pharmacokinetic model developed in separate group of animals. The latter approach requires that most of the inter-individual variability can be explained on the basis of relevant covariates.

In the present investigation we have explored the use of the population PK model for prediction of individual concentration time curves on the basis of sparse blood samples taken in the behavioral study. The second option (estimation of the pharmacokinetics on separate occasion) was not explored since no information on the inter-occasion variability in PK was available in our dataset. Furthermore, since no covariates could be identified which explain most of the inter-individual variability the third option (estimation of the pharmacokinetics in a separate group of animals) was not considered relevant.

In order to avoid possible interference of the blood sampling with the behavioral readout, the blood samples were collected after dissipation of the behavioral effect. Two blood samples at an interval of 135 minutes were collected as this enables the distinction between interindividual and residual variability. Although the simulated dataset contained only two PK samples, the complete individual fluvoxamine concentration *versus* time profiles could be accurately predicted on the basis of the post hoc estimates from the population PK model. This is illustrated in the high accuracy of the obtained pharmacokinetic parameter estimates.

This information was then successfully applied in the PK/PD analysis of the effect of fluvoxamine on the reduction in REM sleep.

In conclusion, a linear population three-compartment PK model, with body weight included as a covariate, could adequately describe observed fluvoxamine concentration time profiles in rat plasma following intravenous administration. Inter-individual variability could be characterized for *CL*, *V1*, *V2* and *Q2*. The results of the predictive check and the bootstrap analysis confirmed the predictive ability, model stability and precision of the parameter estimates of the PK model. The utility of the developed PK model in animal behavioral studies was shown for the effects of

fluvoxamine on REM sleep duration. By using the developed PK model, individual PK profiles were successfully predicted and in turn used for estimation of PK/PD profiles for the effect on REM sleep duration, with just a sparse PK sampling design.

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Chapter 6

Physiological Pharmacokinetic Modeling of Non-linear Brain Distribution of Fluvoxamine in the Rat

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Abstract

A physiological pharmacokinetic (PK) model is proposed for estimation of the brain distribution of fluvoxamine in the rat *in vivo*. Rats with permanent arterial and venous cannulas and a microdialysis probe in the frontal cortex, received a 30-min intravenous infusion of 1, 3.7 or 7.3. mg/kg of fluvoxamine. With increasing dose a disproportional increase in brain extracellular fluid (ECF) and tissue concentrations relative to plasma concentrations was observed.

The kinetics of the distribution between plasma and brain were estimated by simultaneous analysis of the plasma, ECF and total brain concentrations on the basis of a compartmental model. The proposed PK model consists of three compartments for description of the plasma fluvoxamine concentration in combination with two compartments for the ECF and brain concentrations. Within the brain, the mass exchange between a shallow perfusion-limited and a deep brain compartment is described by a passive diffusion term and a saturable active efflux term. The model resulted in precise estimates of the parameters describing the passive influx in the brain (k_{in}) of 0.16 1/min, the efflux rate from the shallow brain compartment (k_{out}) of 0.019 1/min and the fluvoxamine concentration in the deep brain compartment (ECF) at which 50% of the active efflux is reached (C_{50}) of 710 ng/ml. The proposed physiological brain distribution model constitutes a basis for precise characterization of the PK/PD correlation of fluvoxamine by taking into account the non-linearity in brain distribution.

Introduction

The Selective Serotonin Reuptake Inhibitors (SSRIs) are the first-line treatment for depression (Ables and Baughman, 2003;Isaac, 1999). SSRIs selectively and powerfully block the serotonin transporter (SERT) and thereby the reuptake of serotonin (5-HT) in the presynaptic nerve terminal resulting in increased extracellular 5-HT levels and enhancement of serotonergic neurotransmission (Bel and Artigas, 1992;Fuller, 1994). Despite their widespread use and numerous pre-clinical and clinical investigations, very few studies have addressed the pharmacokinetic/pharmacodynamic (PK/PD) correlations of SSRIs. This is remarkable since the PK/PD correlations of SSRIs appear to be rather complex. Upon chronic treatment important time dependencies in the pharmacodynamics of SSRIs have been observed (Bel and Artigas, 1996;Bosker et al., 1995;Benmansour et al., 1999).

In recent years, progress has been made in the field of mechanism-based PK/PD modeling. The objective of mechanism-based PK/PD modeling is to understand, in a strictly quantitative manner, the mechanisms that determine the time-course of the intensity of the drug effect *in vivo*. A pertinent feature of mechanism-based PK/PD models is that they contain specific expressions to describe processes on the causal path between drug administration and response (Danhof et al., 2005). These include target site distribution, target site binding, target site activation and homeostatic feedback. The development of mechanism-based PK/PD models relies on biomarkers, which characterize quantitatively the processes on the causal path (Rolan, 1997;Colburn and Lee, 2003).

In PK/PD modeling, target site distribution can be an important factor, which affects the intensity, the onset and the duration of the effect. Particularly for relatively large hydrophilic compounds and for compounds that are substrates for specific transporters, target site distribution may be restricted and non-linear. This has important implications for the PK/PD correlations for drugs, which act in tissues that are protected by specific barriers (i.e. the central nervous system).

In recent years important progress has been made in elucidating the functionality of the blood-brain barrier and the mechanisms of drug transport to the brain. It has been demonstrated that the distribution of drugs into the brain is restricted for hydrophilic drugs and for compounds, which are substrates for efflux transporters at the blood-brain barrier (Hammarlund-Udenaes, 2000). The present investigation focuses on the brain distribution of fluvoxamine, which is a member of the SSRIs. Recently, evidence has been obtained that fluvoxamine may be a substrate for efflux transporters at the blood-brain barrier. Specifically, it has been shown that fluvoxamine inhibits Pgp in MDR1 cells (model for human Pgp) as well as in primary porcine brain capillary endothelial cells (model for the blood-brain barrier) (Weiss et al., 2003). On the other hand, no significant Pgp mediated fluvoxamine efflux in MDR-1 cells was observed in another investigation (Doan et al., 2002). However, in the latter study the *in vitro* passive permeability of fluvoxamine was rather high, thereby possibly obscuring the contribution of the active efflux.

The objective of the present investigation was to characterize in a strictly quantitative manner the kinetics of the brain distribution of fluvoxamine in rats *in vivo*. To this end, the time-course of the fluvoxamine concentration in plasma, brain microdialysate and brain tissue was determined following intravenous administration of 1, 3.7 or 7.3 mg/kg. A physiological PK model is proposed which enables separate characterization of the passive influx, the passive efflux and the active efflux of

fluvoxamine to and from the brain. The model can be used in analyzing the PK/PD correlations of fluvoxamine, by taking into account the non-linearity in brain distribution.

Physiological Model for Fluvoxamine Brain Distribution

The proposed model for characterization of the brain distribution kinetics of fluvoxamine is illustrated in Figure 1.

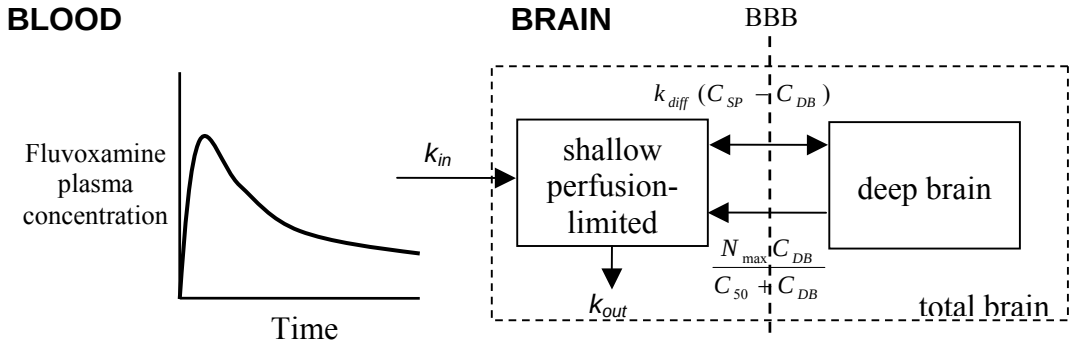

Figure 1. *Schematic representation of the proposed PK model for fluvoxamine in brain. Plasma fluvoxamine concentration versus time-courses were predicted by the previously developed population PK model and used as input function in the brain. The brain model consists of a shallow perfusion-limited brain compartment and a deep brain compartment. Between the two brain compartments, the BBB is present (dotted vertical line). The mass exchange of fluvoxamine between these compartments is composed of a passive diffusion term and an saturable active removal flux (k_{10} = rate constant from central plasma compartment; k_{in} = rate constant in the brain; k_{out} = rate constant from the brain; k_{diff} = diffusion rate constant between the shallow perfusion-limited and deep brain compartment; C_{SP} = concentration in shallow perfusion-limited compartment; C_{DB} = concentration in deep brain compartment; N_{max} = maximal active removal flux; C_{50} = fluvoxamine concentration in deep brain compartment at 50% saturation of the active removal flux).*

This mechanistic PK model contains specific expressions for various mechanisms of the distribution of fluvoxamine across the BBB, in which saturation of an active transport was assumed. Plasma fluvoxamine concentration *versus* time profiles were estimated by analysis of the plasma concentration data in combination with data from other investigations on the basis of a previously proposed population PK model. Individual predicted concentration *versus* time profiles served as fluvoxamine input function in the brain. The brain distribution model consisted of three compartments to describe the concentration *versus* time profile in plasma in combination with two compartments to describe the time-course of fluvoxamine concentrations in the brain. The first brain compartment is a shallow compartment that is in direct contact with the blood flow with an influx and efflux, which is determined by perfusion (shallow perfusion-limited compartment). The second compartment is a deep brain

compartment in which the concentration is equal to the measured fluvoxamine ECF concentrations (deep brain compartment). Fluvoxamine is not able to enter this compartment directly by perfusion, but only indirectly from the shallow perfusion-limited compartment by diffusion or active transport that could be mediated by P-glycoprotein (Pgp) and/or other transporters. Therefore, the mass balance in the brain is determined by perfusion of the outer shallow brain tissues (subscript SP) and exchange with the deep brain compartment (subscript DB):

$$\frac{dA_{SP}}{dt} = Q_B C_{in} - Q_B C_{out} + N_{SP-DB} \quad (1)$$

$$\frac{dA_{DB}}{dt} = -N_{SP-DB} \quad (2)$$

in which A_{SP} is the amount of fluvoxamine in the shallow perfusion-limited compartment, Q_B is the effective plasma perfusion rate, C_{in} is the concentration entering the shallow perfusion-limited compartment, C_{out} is the concentration leaving the shallow perfusion-limited compartment, $N_{SP\text{-}DB}$ is the net mass exchange between the shallow perfusion-limited compartment and deep brain compartment and A_{DB} is the amount of fluvoxamine in the deep brain compartment.

The mass exchange of fluvoxamine between the outer perfusion-limited brain compartment and the deep brain compartment contains two components, a passive diffusion term and an active removal flux. By assuming that this active removal flux is saturable the following relation for $N_{SP\text{-}DB}$ is obtained:

$$N_{SP-DB} = -k_{diff}(C_{SP} - C_{DB}) + N_{\max} C_{DB} / (C_{50} + C_{DB}) \quad (3)$$

in which k_{diff} is the diffusion rate constant between the shallow perfusion-limited and deep brain compartment, C_{SP} is the concentration in the shallow perfusion-limited compartment, C_{DB} is the concentration in the deep brain compartment, N_{max} is the maximal active removal flux and C_{50} is the fluvoxamine concentration in the deep brain compartment at which 50% of saturation of the active removal flux is reached.

The transport processes between the two brain compartments are difficult to identify, but by assuming that the exchange rate constants for the deep brain compartment from the shallow perfusion-limited compartment are large, the model is reduced to a model in which these transport processes can be identified. The assumption of large values of the exchange rate constants is reasonable since observed concentrations in ECF and total brain showed the same kinetic profile without any delay or different elimination kinetics (Figure 2). Therefore, rapid equilibrium between fluvoxamine concentrations in the shallow perfusion-limited and the deep brain compartment was

assumed resulting in the following relationship between the concentration in the shallow perfusion-limited compartment and deep brain compartment:

$$C_{SP} = C_{DB} + \frac{N_{\max}}{k_{diff}} C_{DB} /(C_{50} + C_{DB}) \quad (4)$$

The total amount of fluvoxamine in the brain can be described by the following equation:

$$\frac{dA_T}{dt} = \frac{dA_{SP}}{dt} + \frac{dA_{DB}}{dt} = Q_B C_{in} - Q_B C_{out} \quad (5)$$

in which A_T is the total amount of fluvoxamine in the brain.
The concentration entering the brain is assumed to be equal to the plasma concentration:

$$C_{in} = C_p \quad (6)$$

in which C_p is the plasma concentration.
The concentration leaving the brain is determined by the partition coefficient (P) between drug in plasma and the concentration in the shallow perfusion-limited brain area:

$$C_{out} = C_{SP} / P \quad (7)$$

In addition, the following constants can be defined:

$$k_{in} = Q_B / V_T \quad (8)$$
$$k_{out} = Q_B /(V_T P) \quad (9)$$

in which k_{in} is the influx rate constant in the brain, V_T is the volume of the brain and k_{out} is the efflux rate constant from the brain.
The differential equation for total fluvoxamine concentration in the brain can be described by:

$$\frac{dC_T}{dt} = k_{in} C_p - k_{out} C_{SP} \quad (10)$$

in which C_T is the total fluvoxamine concentration in the brain.
The relationships between the concentration in the deep brain compartment and total brain and the concentration in the shallow perfusion-limited brain compartment and total brain are defined by:

$$C_{DB} = f_{DB} C_T \quad (11)$$

$$C_{SP} = f_{SP} C_T \tag{12}$$

in which f_{DB} and f_{SP} are partition coefficients for the deep brain compartment and shallow perfusion-limited compartment, respectively.
The partition coefficient for the deep brain compartment can be calculated by the following formula:

$$f_{DB} = \frac{-N_{***\max} + C_T - C_{50} + \sqrt{(N_{***\max} - C_T + C_{50})^2 + 4C_T C_{50}}}{2C_T} \tag{13}$$

in which $N_{***\max}$ is a lumped parameter that can be defined by:

$$N_{***\max} = \frac{N_{\max} V_{SP}}{k_{diff} V_{DB} \alpha} > 0 \tag{14}$$

with α defined by:

$$\alpha = \left(\frac{V_{SP}}{V_{DB}} + 1 \right) > 1 \tag{15}$$

Furthermore, the partition coefficient for the shallow perfusion-limited compartment can be calculated by the following equation:

$$f_{SP} = 1 + V_{DB} / V_{SP} (1 - f_{DB}) \tag{16}$$

The complete mathematical derivations of the model are presented in appendix 1.

The schematic representation of the proposed PK model in Figure 1 shows that at low fluvoxamine levels relatively low fluvoxamine concentrations will be present in the deep brain compartment because of the action of the active efflux transport. At higher fluvoxamine concentrations, this active efflux transport becomes saturated and therefore relatively higher concentrations of fluvoxamine will be observed in the deep brain compartment. As a result, relatively slow elimination of fluvoxamine from the brain will occur at these higher concentrations since the rate of elimination from the brain is assumed to be dependent on the fluvoxamine concentration in the shallow perfusion-limited compartment (equation 7).

Materials and Methods

Chemicals

Fluvoxamine maleate and clovoxamine fumarate were kindly provided by Solvay Pharmaceuticals (Weesp, The Netherlands). Dimethylsulfoxide (DMSO), sodium

hydroxide (NaOH) and isoamylalcohol were purchased from Merck (Darmstadt, Germany). Isopentane, ethanol, acetonitrile and methanol were obtained from Acros (Geel, Belgium). Heptane was purchased from Sigma-Aldrich Laborchemikalien (Seelze, Germany) and ammonium acetate was obtained from Baker Chemicals (Deventer, The Netherlands). Millipore water was obtained from a Milli-Q system (Millipore SA, Molsheim, France).

Animals

Male Wistar rats (Charles River Wiga GMBH, Sulzfeld, Germany) weighing 226-250 gram were housed in groups for 6-10 days, under standard environmental conditions (ambient temperature 21°C, humidity 60%, 12-h light/dark cycle). The animals had free access to food (laboratory chow, Hope Farms, Woerden, The Netherlands) and acidified water. For the microdialysis studies 26 animals were used and for the brain sampling studies 35 animals were used. After surgery, the animals in the microdialysis studies were housed individually for 1 week and the animals in the brain sampling studies for 2 days. The study protocol was approved by the Ethical Committee on Animal Experimentation of Leiden University.

Experimental Procedures

Microdialysis Studies

Surgical Procedures

Animals were anaesthetized by a subcutaneous injection of 0.1 ml/100 g Ketanest-S® ((S)-ketaminebase, Parke-Davis, Hoofddorp, The Netherlands) and an intramuscular injection of 0.01 ml/100 g Domitor® (medetomidine hydrochloride, Pfizer, Capelle a/d IJssel, The Netherlands). A cannula was implanted in the right jugular vein (10 cm polyethylene tubing, Portex Limited, Hythe, United Kingdom; I.D.=0.58, O.D.=0.96 mm) for fluvoxamine administration and a cannula in the left femoral artery (4 cm polyethylene tubing, Portex Limited; I.D.=0.28, O.D.=0.61 mm heat-sealed to 18 cm polyethylene tubing, I.D.=0.58, O.D.=0.96 mm) for collection of blood samples. The cannulas were subcutaneously tunneled and externalized at the back of the neck. The venous cannula was filled with saline (0.9%, B. Braun Melsungen AG, Melsungen, Germany) containing heparin (20 IU/ml, Pharmacy, Leiden University Medical Center, Leiden, The Netherlands) and the arterial cannula was filled with 25% (w/v) polyvinylpyrrolidone (PVP, Brocacef, Maarssen, The Netherlands) solution in saline (0.9%) containing heparin (20 IU/ml) to prevent blockade by blood clotting. After implantation of the cannulas, the animals were

instrumented with a microdialysis guide cannula (CMA/12, Aurora Borealis Control BV, Schoonebeek, The Netherlands) within the same surgery. A microdialysis guide cannula with a dummy probe was implanted in the frontal cortex (AP: 3.2; L: -3.0; V: -1.5 mm from bregma, in accordance with the atlas of Paxinos and Watson (Paxinos and Watson, 1982)). Two support screws were placed and the guide was secured in place using dental cement (Simplex Rapid liquid and powder, Kemdent Associated Dental Products, United Kingdom).

Microdialysis

One day before implantation of the microdialysis probe, the probes (CMA/12, membrane length 3 mm, Aurora Borealis Control BV) were perfused *ex vivo* overnight with artificial cerebrospinal fluid (containing: 145 mM NaCl, 0.6 mM KCl, 1.0 mM $MgCl_2$, 1.2 mM $CaCl_2$, 0.2 mM ascorbic acid in 2 mM phosphate buffer pH 7.4) (Moghaddam and Bunney, 1989). The dummy probe was replaced by the microdialysis probe one day before start of the study. Because fluvoxamine appeared to be adhesive to the microdialysate tubing and microdialysis probe, the artificial cerebrospinal fluid additionally contained 0.5% (w/v) Bovine Serum Albumin (BSA, Sigma-Aldrich, Zwijndrecht, The Netherlands), which completely dissolved the sticking problems (data not shown). Flow rate during the whole experiment was constant at 2 µl/min and the outlet tubing was connected to a microdialysate fraction collector (Univentor 820, Antec Leyden BV, Zoeterwoude, The Netherlands). After intravenous administration of fluvoxamine, dialysate concentrations were measured over a period of 5 hrs. Sampling intervals were 20 or 30 min yielding a volume of 40 or 60 µl fluvoxamine dialysate sample in both microdialysis studies. At the end of the experiments, brains of the animals were removed and the location of the microdialysis probe was verified by visual inspection. Microdialysate samples were collected at a temperature of 4°C in the fraction collector, and subsequently stored at -20°C until analysis. Dosages and observed fluvoxamine concentrations are expressed as free base.

In Vivo Recovery

For 20 of the 26 animals used in the microdialysis studies, individual values for the *in vivo* recovery were determined by the method reverse dialysis or retrodialysis by drug after one hour of stabilization (Bouw and Hammarlund-Udenaes, 1998;de Lange et al., 1997). For the remaining 6 animals, the mean *in vivo* recovery value was used to be able to determine true unbound fluvoxamine concentrations in ECF from dialysate

concentrations. During the retrodialysis period, fluvoxamine concentrations (6, 12, 30 and 60 ng/ml) in artificial cerebrospinal fluid containing 0.5% BSA were locally infused in the frontal cortex. Six dialysate fractions of 20 min were collected, of which the median four fractions were used for determination of the *in vivo* recovery value. This *in vivo* recovery was calculated per probe by the loss of fluvoxamine from the fluvoxamine retrodialysis solution to the surrounding tissue according to the following equation:

$$\text{Recovery}_{invivo} = \frac{\left(C_{in-rec} - C_{out-rec}\right)}{C_{in-rec}} \qquad (17)$$

in which $C_{in\text{-}rec}$ is the fluvoxamine concentration entering the probe and $C_{out\text{-}rec}$ is the fluvoxamine concentration leaving the probe. At the end of the retrodialysis phase, the probes were perfused for 2 hrs with artificial cerebrospinal fluid containing 0.5% BSA after which no fluvoxamine was detected in dialysate samples and the administration of fluvoxamine in the jugular vein could be started.

Dosage Regimen

From the 26 animals used in the microdialysis studies, 8 animals received 1 mg/kg, 8 animals received 3.7 mg/kg and 10 animals received 7.3 mg/kg fluvoxamine via a 30-min intravenous infusion in the jugular vein cannula at a flow rate of 20 µl/min using a BAS BeeHive pump (Bioanalytical Systems Inc., West Lafayette, USA).

Blood Sampling

A number of 13 arterial blood samples (100 µl) were collected at variable fixed time intervals from the cannula in the femoral artery over a time period between 0 and 5 hrs after fluvoxamine administration. After collection of each blood sample, an equal volume of heparinized 0.9% NaCl (20 IU/ml) was administered to the animal. Blood samples were collected in heparinized Eppendorf tubes and kept on ice during the experiment. After centrifugation (10 min, 5000xg), 50 µl plasma was transferred into a glass tube and stored at -20°C until sample analysis.

Brain Sampling Studies

Surgical Procedures

Animals in the brain sampling studies were implanted with a cannula in the right jugular vein for fluvoxamine administration and a cannula in the left femoral artery

for collection of blood samples as described for the animals in the microdialysis studies.

Dosage Regimen

From the 35 animals used in the brain sampling studies, 19 animals received 1 mg/kg and 16 animals received 7.3 mg/kg fluvoxamine via the 30-min intravenous infusion in the jugular vein cannula, described for the microdialysis studies.

Blood Sampling

A number between 2 and 15 arterial blood samples (100 µl) were collected from the animals between 2.5 and 600 min from the cannula in the femoral artery after fluvoxamine administration, dependent on the time of brain sampling. Blood samples were collected, handled and stored as described for the microdialysis studies.

Brain Collection

From the 19 animals that received 1 mg/kg fluvoxamine, the brains were collected at 10, 20, 30, 45, 75, 90, 105, 120, 135, 150, 180, 210, 240, 270, 300, 330, 360, 390 and 420 min after fluvoxamine administration by destructive sampling. From the 16 animals that received 7.3 mg/kg fluvoxamine, the brains were collected at 15, 30, 45, 60, 75, 90, 105, 120, 135, 200, 250, 550, 600, 650, 700 and 750 min after fluvoxamine administration by destructive sampling. The animals were sacrificed by decapitation after which the brains were quickly isolated. The brains were frozen in dry ice-cooled isopentane that was surrounded with cold ethanol and stored at -20°C until analysis of fluvoxamine brain levels.

Drug Analysis

Fluvoxamine samples in the microdialysis studies and brain sampling studies were analyzed for fluvoxamine using liquid chromatography with tandem mass spectrometry (LC-MS/MS) as has been described earlier for fluvoxamine plasma (Geldof et al., 2007) and for fluvoxamine in ECF and brain (Geldof et al., in publication). Calibration standards and independent quality control samples were prepared by addition of fluvoxamine solutions in DMSO to control plasma, artificial cerebrospinal fluid or brain. Briefly, in plasma samples, proteins were precipitated by addition of acetonitrile and a volume of 20 µl of supernatant was injected into the system. For the ECF samples, a volume of 50 µl of the calibration standards or QC samples in DMSO were added to 50 µl artificial cerebrospinal fluid. For the ECF

samples from the rats, a volume of 50 µl DMSO, 500 µl H_2O and 50 µl of 500 ng/ml clovoxamine in DMSO were added. Addition of 100 µl NaOH increased the pH value to approximately 12. A volume of 4 ml of heptane-isoamylalcohol (95:5, v/v) was added, the solution was centrifuged (10 min, 3000xg) and the organic layer was evaporated to dryness under nitrogen at 65°C. The residues were dissolved in a mixture of 10mM ammonium acetate and acetonitrile (50:50, v/v) and a volume of 30 µl was injected into the system. Control brains and brains from fluvoxamine treated rats were weighed and 9 volumes of H_2O were added before homogenization. A volume of 100 µl of the calibration standards or QC samples in DMSO was added to 1 ml control brain homogenates. For the brain samples of the rats, a volume of 100 µl DMSO and 100 µl of 500 ng/ml clovoxamine in DMSO were added to 1 ml of brain homogenates. A volume of 2 ml of methanol was added for extraction and the samples were rotated for 15 min and centrifuged (10 min, 5000xg). The solutions were transferred in Eppendorf tubes, centrifuged for another time (10 min, 9727xg) and a volume of 20 µl was injected into the system. All fluvoxamine samples were quantified on a reversed phase LC column (BDS Hypersil C18, 3 µm particle size, 100x4.6 mm I.D.; Thermo Hypersil-Keystone, Brussels, Belgium). LC-MS/MS analysis was performed on an API-4000 MS/MS (Applied Biosystems, Toronto, Canada), coupled to an HPLC system (Agilent, Palo Alto, USA). The MS/MS operated in the positive ion mode using the TurboIonSpray-interface (electrospray ionization) was optimized for the quantification of fluvoxamine. For analysis of brain tissue samples, an additional guard cartridge (Hypersil ODS 5µm 10x4.0mm drop-in cartridge, Thermo Electron Corporation, Brussels, Belgium) with holder (Uniguard holder, Thermo Electron Corporation) was used. The intra batch accuracy from independent QC samples was between 80 and 120% over the entire range of the samples. The limit of quantification for fluvoxamine was 1 ng/ml in plasma, brain ECF and brain tissue.

Data Analysis

A nonlinear mixed effects modeling approach was used to describe the concentration-time profiles of fluvoxamine in plasma, brain ECF and brain tissue from all individual animals simultaneously. All fitting procedures were performed on a personal computer (Intel® Pentium® 4 processor) running under Windows XP using the Compaq Visual FORTRAN standard edition 6.1 (Compaq Computer Cooperation, Euston, Texas, USA) with the nonlinear mixed effects modeling

software NONMEM (Version V, Level 1.1., NONMEM project group, University of California, San Francisco, USA).

PK Analysis in Plasma

As described in the development of the physiological fluvoxamine brain distribution model, the PK information in plasma obtained in the present study was analyzed in combination with plasma concentration time data of other studies on the basis of a previously proposed population three-compartment PK model for fluvoxamine in plasma (Geldof et al., 2007). The individual plasma concentration *versus* time profiles in the animals included in the present study were estimated on the basis of the obtained post hoc estimates of the parameters and used as fluvoxamine input function in the brain. In Table 1, the mean post hoc estimates for the PK parameters of fluvoxamine in plasma obtained by the three-compartment PK model are depicted. Included are the post hoc estimates for systemic clearance (*CL*), central volume of distribution (*V1*), two peripheral volumes of distribution (*V2*, *V3*) and inter-compartmental clearances (*Q2*, *Q3*). On the basis of a covariate analysis, no differences in the pharmacokinetics of the three different dose groups nor between the rats in the microdialysis study and the brain sampling study could be detected.

PK Analysis in Brain ECF and Brain Tissue

All fluvoxamine data in plasma, brain ECF and brain tissue were implemented in the PREDD subroutine ADVAN 6 TOL5, which is a general nonlinear model that uses the numerical solution of the differential equations.

Inter-individual variability on the parameters was modeled by an exponential equation:

$$P_i = \theta \cdot \exp(\eta_i) \tag{18}$$

in which P_i is the individual estimate for parameter P for the i^{th} individual, θ is the population estimate for parameter P and η_i is the inter-individual random deviation of P_i from P. The values of η_i are assumed to be normally distributed with mean zero and variance ω^2 that distinguished the pharmacokinetic parameters for the i^{th} individual from the population typical value θ. Inter-individual variabilities were analyzed on each parameter and the inter-individual effects that did not significantly improve the model or could not be estimated were fixed to zero. Correlations between the inter-individual of the various parameters were explored using the OMEGA BLOCK option. Unexplained variability (e.g. caused by measurement and

experimental errors) in fluvoxamine concentrations in ECF and total brain were best described by a proportional error:

$$Cm_{ij} = C_{ij} \cdot (1 + \varepsilon_{ij}) \quad (19)$$

in which Cm_{ij} is the measured ECF or brain concentration, C_{ij} is the j^{th} ECF or brain concentration for the i^{th} individual predicted by the model and ε_{ij} account for the residual deviance of the predicted from the observed concentration. The values for ε were assumed to be independently normally distributed with mean zero and variance σ^2. Population pharmacokinetic values of θ, ω^2 and σ^2 were estimated using the first order conditional estimation with interaction method in NONMEM. Model fits were compared on the basis of the likelihood ratio test (Jolling et al., 2004;Kuipers et al., 2001), diagnostic plots, parameter correlations and precision in parameter estimates. An additional parameter was included in the structural model if the resulting change in minimum value of the objective function (MVOF) was ≥ 6.6 ($p \leq 0.01$).

Results

In Vivo Recovery

The average values of the recoveries were 0.20 ± 0.09, 0.36 ± 0.10, 0.27 ± 0.15, 0.25 ± 0.07 for retrodialysis fluvoxamine solutions of 6, 12, 30 and 60 ng/ml, respectively. Therefore, a mean recovery value of 0.27 ± 0.10 was used for the six animals in which no individual values of the *in vivo* recovery were obtained.

PK Analysis in Plasma

By application of the previously developed PK model, individual post hoc estimates for fluvoxamine in plasma could be obtained for *CL*, *V1*, *V2*, and *Q2* (Table 1).

Table 1. *Mean post hoc estimates for the pharmacokinetic parameters of fluvoxamine in plasma after IV administration in 30 min (1, 3.7 and 7.3 mg/kg) obtained by the three-compartment pharmacokinetic model. Depicted are the population mean estimates for CL, V1, V2, Q2, V3, and Q3.*

Study	PK parameter (unit)					
	CL (ml/min)	*V1* (ml)	*V2* (ml)	*Q2* (ml/min)	*V3* (ml)	*Q3* (ml/min)
Microdialysis + Brain sampling	31.6	321	949	33.7	136	1.0
Microdialysis	36.5	412	1255	39.4	136	1.0
Brain sampling	28.0	253	721	29.4	136	1.0

Inter-individual variability could not be identified on the parameters *V3* and *Q3*, and therefore the estimates for the population of 187 animals were used. The individual post hoc estimates were used in the current studies for each animal to estimate the fluvoxamine concentrations in plasma as input function in the brain

Physiological Model for Fluvoxamine Brain Distribution

In Figure 2, observed fluvoxamine concentrations in ECF (A) and total brain (B) are depicted as well as the upper and lower limit of interquantile range and the median concentration *versus* time for a number of 2000 datasets that were simulated from the obtained PK parameter estimates.

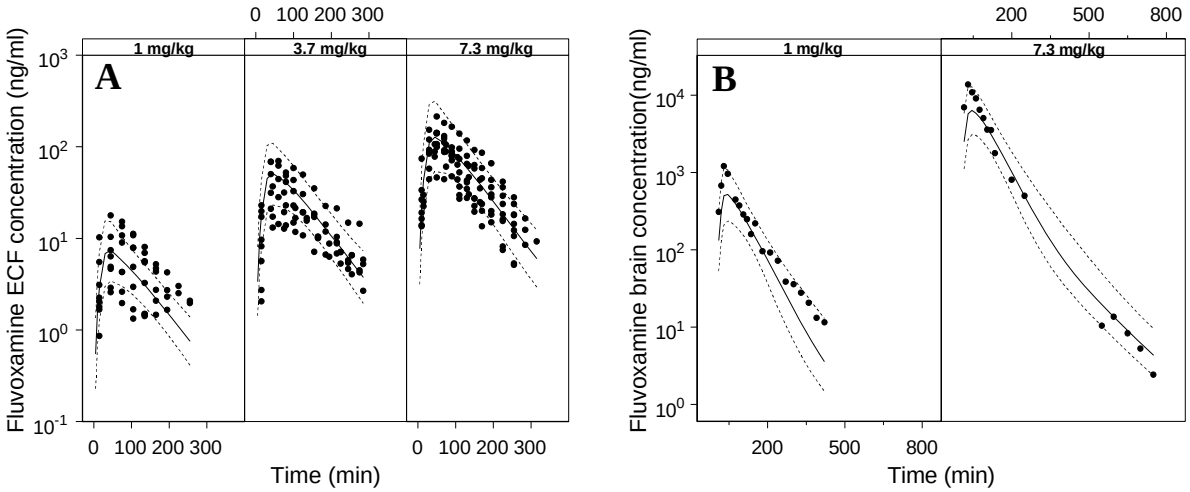

Figure 2. *Fluvoxamine concentration-time profiles in ECF (A) and total brain (B), obtained after a 30-min intravenous infusion in Wistar rats. Depicted are the observed fluvoxamine concentrations (dots), simulated upper limit of interquantile range (90%, upper dashed line), lower limit of interquantile range (10%, lower dashed line), median concentration (solid line) versus time. A number of 2000 datasets were simulated from the final PK parameter estimates.*

Fluvoxamine was transported rapidly into the brain and maximal ECF and brain concentrations were observed only about 20 min later than maximal plasma fluvoxamine concentration. Since maximum concentrations in ECF and brain were observed at the same time after administration, distribution between fluvoxamine levels in ECF and total brain was very rapid. Furthermore, fluvoxamine concentration-time curves in ECF and brain showed the same kinetic profiles without any delay of different elimination kinetics (Figure 2).

When observed fluvoxamine concentrations were normalized for dose (normalization dose 4 mg/kg), non-linearity could be observed in ECF and brain, which was not observed in plasma (Figure 3).

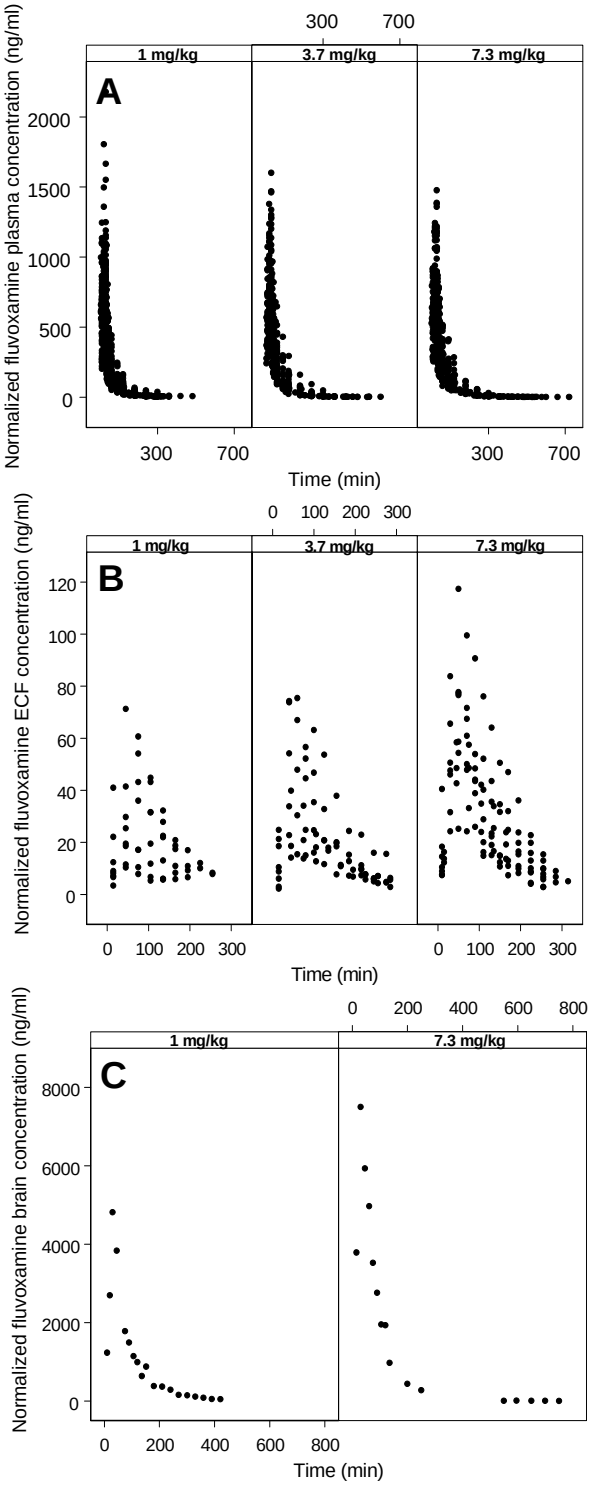

Figure 3. *Observed fluvoxamine concentration-time profiles in plasma (A), ECF (B) and total brain (C), after normalization for dose (normalization dose 4 mg/kg) separated by fluvoxamine dose.*

Specifically, with increasing fluvoxamine dose a disproportional increase in brain ECF and brain tissue concentrations relative to the plasma concentrations was observed. Therefore, the proposed physiological PK model with incorporated saturable efflux transport from the deep brain compartment to the shallow perfusion-limited brain compartment was compared with a model without such saturable efflux transport. The proposed physiological PK model with non-linearity in brain

distribution resulted in a significantly better description of fluvoxamine concentrations in brain ECF and brain tissue compared with the linear brain model on the basis of a decrease in MVOF of 37 points. Fluvoxamine concentrations in ECF and brain could be adequately described with this physiological model, as observed in Figure 2. These Figures also showed that the concentration-time curves of fluvoxamine in ECF and total brain followed the same kinetic profiles. Therefore, the assumption of the high rate constants between the compartments in the model was valid and the concentration-time profiles in ECF and brain were separated only by a scaling factor.

The structural parameters of the model could be simultaneously estimated with good precision (Table 2).

Table 2. *Population pharmacokinetic parameter estimates of fluvoxamine in ECF and brain after IV administration in 30 min (1, 3.7 and 7.3 mg/kg) obtained by the mechanistic conceptual PK model. Depicted are the population mean estimates for k_{in}, k_{out}, N_{***max}, C_{50}, ω^2, σ^2 with corresponding coefficient of variation (C.V.)*

Parameter	Unit	Value	C.V. (%)
k_{in}	1/min	0.16	13.6
k_{out}	1/min	0.019	8.1
N_{***max}	ng/hr	30700	92.5
C_{50}	ng/ml	710	96.8
$\omega^2\ \eta_{kin}$	-	0.50	25.7
$\omega^2\ \eta_{kout}$	-	0.17	28.5
$\omega\ \eta_{kin}/\omega\ \eta_{kout}$	-	0.24	32.9
$\sigma^2\ \varepsilon_{1ij}$	-	0.042	17.3

*C.V. = coefficient of variation (standard error / value * 100%)*

The influx rate constant in the brain (k_{in}) was estimated to be 0.16 1/min and the efflux rate from the brain by the shallow perfusion-limited brain compartment (k_{out}) was estimated to be 0.019 1/min.

Fluvoxamine concentration at which 50% of saturation of the active removal flux is reached (C_{50}) was equal to 710 ng/ml. Since measured observed fluvoxamine ECF concentrations ranged between only 1 and 214 ng/ml, full saturation of the active removal flux was not reached in the current studies. The residual error for fluvoxamine concentrations in ECF and total brain (ε_{1ij}) was estimated to be equal to 0.042 and significantly lower than the estimated value by the model without incorporated saturation kinetics. Therefore, more variation could be explained by the

model in which saturation of an active efflux transport was assumed, thus supporting the validity of the complex model. The coefficients of variation (C.V.) for the ECF and brain parameters were lower than 33% for all parameters, except for C_{50} and N_{***max} which were estimated as 96.8% and 92.5%, respectively. Since full saturation of the active removal flux was not reached in the current study, parameters correlated to the maximum ECF concentration for saturation of this process could not be estimated adequately. Inter-individual variability (IIV) for k_{in} was equal to 0.50 and was estimated for k_{out} to be 0.17. Inter-individual variability in the other parameters could not be adequately estimated and was fixed to zero. Correlation between the variability in the estimated PK parameters was observed for k_{in} and k_{out} and was implemented in the model. In Figure 4, goodness-of-fit plots for fluvoxamine concentrations in ECF of the frontal cortex and total brain are depicted.

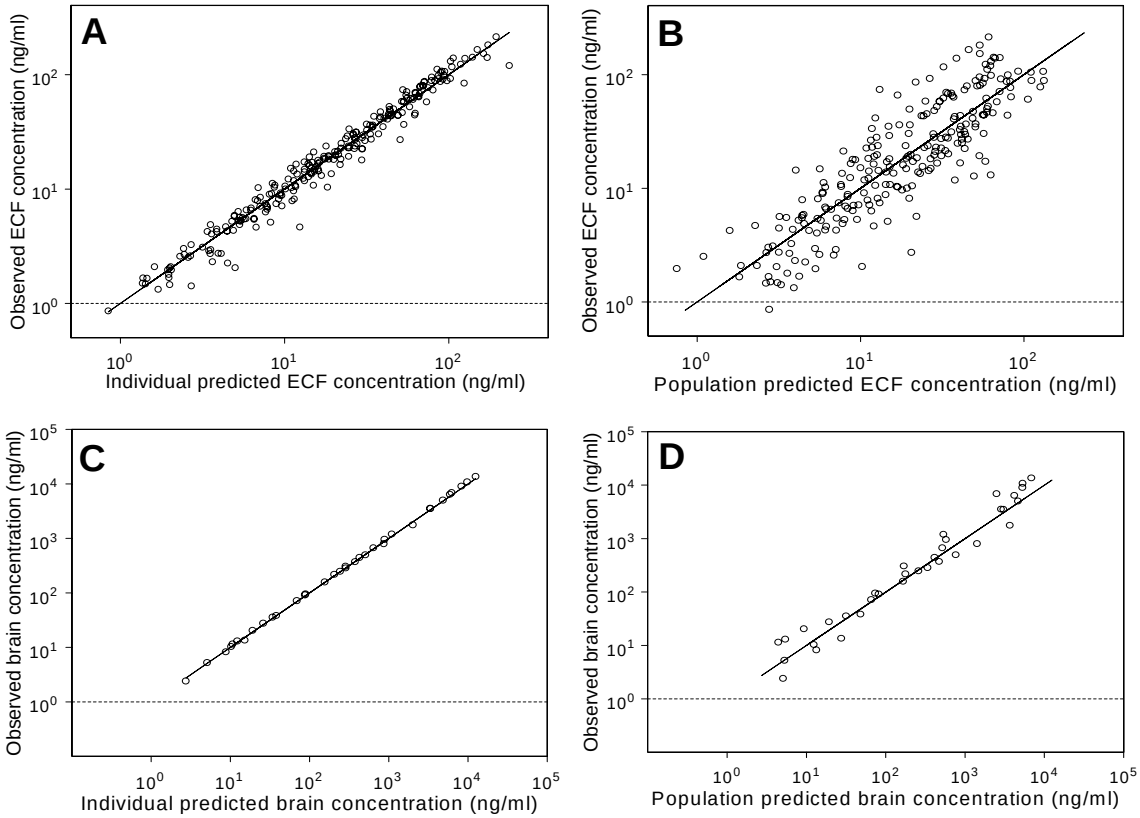

Figure 4. *Goodness-of-fit plots of fluvoxamine concentrations in ECF and total brain obtained for the physiological PK model. Depicted are scatter plots of the observed fluvoxamine ECF concentrations versus the individual model predictions (A) and population model predictions (B) and observed fluvoxamine brain concentrations versus the individual model predictions (C) and population model predictions (D). The limit of quantification (1 ng/ml) is added for clarity (dashed line).*

Observed ECF concentrations were in close agreement with individual predicted (A) and population predicted (B) ECF concentrations. No substantial or systemic

deviation from the identity line was observed indicating adequate description of observed fluvoxamine ECF concentrations. Furthermore, no substantial or systemic deviation from the identity line was present for the observed fluvoxamine brain concentrations vs. individual (C) and population (D) predicted brain concentrations. Although each observation from total brain was collected from a different animal by destructive sampling, fluvoxamine brain concentrations could be adequately described.

Discussion

In PK/PD modeling, target site distribution can be an important factor, which affects the intensity, the onset and the duration of the effect. Particularly for relatively large hydrophilic compounds and for compounds that are substrates for specific transporters, target site distribution may be restricted and non-linear, of which this latter was demonstrated for fluvoxamine brain distribution in the present study.

In the current study, brain ECF concentrations were measured (microdialysis studies) as well as the brain tissue (brain sampling studies). By this unique approach, the PK in brain could be determined up to 750 min as a result of higher fluvoxamine concentrations in brain tissue compared to fluvoxamine concentrations in plasma and brain ECF (Figure 2 and 3).

In the current modeling approach it was assumed that there was no uncertainty in determination of the *in vivo* recovery in each microdialysis probe. Furthermore, the median time point of the microdialysis sampling interval was used as being equal to the observation in microdialysate which actually was collected over a time period of 20 or 30 min. Therefore, linear PK was assumed within these intervals as well as small changes in the concentrations over time in each collection time interval (Patsalos et al., 1995;Stahle, 1992).

To overcome the sticking problem of fluvoxamine, artificial cerebrospinal fluid was required to be prepared in the presence of 0.5% BSA. Since this does not reflect the physiological situation in the brain, possible influence of the addition of this protein should be taken into account. Since BSA cannot be transported over the membrane of the microdialysis probe, it could be possible that more fluvoxamine in ECF was drawn from the brain over the membrane to the outlet of the probe and therefore measured fluvoxamine concentrations in ECF could be higher than under physiological conditions without BSA. However, it is likely that such an effect would be constant over time and therefore is a constant factor, which should not have

influence on the estimates predicted by the model. In addition, the presence of such an effect would probably be small because used BSA concentration is relatively low.
When fluvoxamine concentrations were normalized for dose, non-linearity was observed in ECF and in brain concentration *versus* time profiles, which was not the case for fluvoxamine plasma levels (Figure 3). This disproportional increase in ECF and brain concentrations at equal plasma concentrations was not caused by non-linear protein binding. In a separate study with the same experimental set-up and dosages, protein binding was determined at 30 and 60 min after fluvoxamine infusion, which was equal to 97.6 ± 0.4% (n=8) and 97.7 ± 0.6% (n=7).
The developed physiological PK model for description of non-linear brain distribution of fluvoxamine constitutes an extension of an earlier described model that has been proposed to describe the brain distribution of thiopental (Upton et al., 2000;Igari et al., 1982). In this membrane-limited brain model, a shallow flow-limited compartment and a deeper membrane-limited compartment were described, in which the entrance of drug into this deeper compartment was defined by a permeability term. The developed physiological model for description of fluvoxamine brain distribution is an extension of this previously described model by the addition of a term for an active saturable efflux between these brain compartments. In this manner, it was possible to adequately describe observed ECF and brain fluvoxamine concentrations (Figures 2 and 4). Since concentration-time curves in ECF and brain showed the same kinetic profiles without any delay or difference in elimination (Figure 2), rapid equilibrium between fluvoxamine in the shallow perfusion-limited compartment, deep brain compartment and total brain was assumed. Because the influx rate constant in the brain (k_{in}) was estimated as 0.16 1/min and the efflux rate from the brain by the shallow perfusion-limited compartment (k_{out}) was estimated around 0.019 1/min, relatively more fluvoxamine remains in the brain over time, indicating fluvoxamine brain accumulation.
Values for the C.V. for C_{50} and N_{***max} were estimated as 96.8% and 92.5% respectively. It was expected that the developed model could not precisely estimate the parameters for the maximal active removal flux since full saturation was not reached in the current study. This is also reflected in the relatively high value of the estimate for C_{50} (710 ng/ml) relative to the observed ECF concentrations in the current study. Nevertheless, the saturable function was required in the model for description of observed non-linearity in ECF and brain concentration-time curves at concentrations observed in the current study (Figure 3). Incorporation of the saturable active efflux term in the model significantly improved the adequacy of description of

observed data based on a decrease of the MVOF by 37 points. Furthermore, estimates of k_{in} and k_{out} were not influenced by the value of parameters values for C_{50} and therefore, were not dependent on an adequate description of the maximal concentrations by the model at which complete saturation of the active removal flux was reached.

The mechanism of the transport across the BBB barrier and blood-cerebrospinal fluid (CSF) barrier can involve passive diffusion through endothelial cells or via tight junctions, active transport into the brain by specific transport mechanisms and transport out of the brain by active efflux pumps. Furthermore, drugs can also be eliminated from the brain ECF by bulk flow through the perivascular space into the CSF or by metabolism. In particular, active efflux pumps appear to be very important at the level of the BBB and several transporter molecules have been identified as efflux pumps on the luminal side of the BBB, like P-glycoprotein (Pgp) and multidrug resistance protein (MRP) (Liang and Aszalos, 2006). Because Pgp is localized at the BBB in the apical membrane of brain capillary endothelial cells and therefore transports substrates toward the blood compartment, Pgp can effectively limit the penetration into and retention within the brain (Weiss et al., 2003). There are indications for such efflux transport of fluvoxamine across the BBB since it was shown *in vitro* that fluvoxamine induces intermediate Pgp inhibition in MDR1 cells (model for human Pgp) as well as in primary porcine brain capillary endothelial cells (model for the BBB) (Weiss et al., 2003), although this could not be determined in another study in MDR-1 cells (Doan et al., 2002). However, in this latter study the *in vitro* passive permeability of fluvoxamine was rather high (317 nm/s), thereby possibly obscuring the contribution of the active efflux. Furthermore, the current study demonstrated that the distribution of fluvoxamine to the brain was non-linear in the rat *in vivo*. Because it is not completely clear at this point which transporter at the BBB is important for fluvoxamine brain distribution, this could be investigated by analysis of fluvoxamine brain distribution in the presence of specific inhibitors of these transporters at the BBB.

Next to the model described in this article, an alternative model in which non-linear brain distribution was defined on the basis of non-linear tissue binding, rather than non-linear transport. In this respect, the concept of target-mediated disposition, assuming binding to the SERT as the mechanism of the non-linear brain distribution (Levy, 1994;Mager et al., 1991;Mager and Jusko, 2002), was considered. In this model one brain compartment was defined for fluvoxamine in the free phase and another compartment for fluvoxamine in the bound phase. Observed fluvoxamine

brain concentrations were equal to the sum of fluvoxamine in the free and bound phase. The concentration of fluvoxamine in the bound phase was composed of a saturable and linear component and related to the free fraction. This alternative model could also describe observed fluvoxamine concentrations in ECF and total brain as adequate as the model with incorporated saturable efflux transport mechanism. However, the values of the parameters describing the non-linear binding in the brain were completely different from the parameters describing the binding of fluvoxamine to SERT in a study in which the binding to SERT was estimated on the basis of imaging (Geldof et al., unpublished observations). Furthermore, the physiological model described in the current article appears to be more plausible constituting a mechanistic approach about how the transport of fluvoxamine across the BBB might occur.

In conclusion, a physiological PK model was developed that could adequately describe observed fluvoxamine concentrations in ECF of the frontal cortex and total brain after administration of 1, 3.7 and 7.3 mg/kg via a 30-min intravenous infusion in the rat. In this mechanistic model, saturation of an active removal flux was assumed between an outer shallow perfusion-limited compartment and a deep brain compartment next to passive diffusion. The developed physiological PK model will be used to explore the relationships between the plasma and brain PK and various behavioral PD endpoints for fluvoxamine in the rat (Geldof et al., in publication).

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Appendix 1

Derivations of the proposed physiological model for fluvoxamine brain distribution

Consider the brain to exist of two areas. The first area is a brain compartment that is in direct contact with the blood flow in which the mass exchange is perfusion limited. This compartment area is defined as shallow perfusion-limited compartment (subscript *SP*). The second brain area is a deep brain compartment and diffusion limited (subscript *DB*). This area is separated by the brain-blood barrier from the shallow area. The concentrations representing the measured fluvoxamine concentrations in ECF. Fluvoxamine cannot enter this area by perfusion, but only by diffusion or active transport that could be mediated by Pgp or other transporters. The shallow perfusion-limited compartment and deep brain compartment build the total brain. The total brain concentrations were also measured in the current study.

The differential equation for the mass balance in the brain is determined by perfusion of the outer shallow perfusion-limited tissues and exchange with the deep brain compartment:

$$\frac{dA_{SP}}{dt} = Q_B C_{in} - Q_B C_{out} + N_{SP-DB} \quad (1)$$

$$\frac{dA_{DB}}{dt} = -N_{SP-DB} \quad (2)$$

in which A_{SP} is the amount of fluvoxamine in shallow perfusion-limited compartment, Q_B is the effective plasma perfusion rate, C_{in} is the fluvoxamine concentration entering the shallow perfusion-limited compartment, C_{out} is the fluvoxamine concentration leaving the shallow perfusion-limited compartment, $N_{SP\text{-}DB}$ is the net mass exchange between the shallow perfusion-limited compartment and deep brain compartment and A_{DB} is the amount of fluvoxamine in the deep brain compartment.

The process of mass exchange between the shallow perfusion-limited compartment and the deep brain compartment is supposed to have two components, a passive diffusive term and an active removal flux. By assuming that the latter process is saturable, the following relation can be obtained:

$$N_{SP-DB} = -k_{diff}(C_{SP} - C_{DB}) + N_{\max} C_{DB} / (C_{50} + C_{DB}) \quad (3)$$

in which k_{diff} is the diffusion rate constant, C_{SP} is the fluvoxamine concentration in the shallow perfusion-limited compartment, C_{DB} is the fluvoxamine concentration in the deep brain compartment, N_{max} is the maximal active removal flux and C_{50} is the fluvoxamine concentration in deep brain compartment at which 50% of saturation of the active removal flux is reached

Consequently the following relations are obtained:

$$\frac{dA_{SP}}{dt} = Q_B C_{in} - Q_B C_{out} - k_{diff}(C_{SP} - C_{DB}) + N_{\max} C_{DB} / (C_{50} + C_{DB}) \quad (4)$$

$$\frac{dA_{DB}}{dt} = k_{diff}(C_{SP} - C_{DB}) - N_{\max} C_{DB} / (C_{50} + C_{DB}) \quad (5)$$

By assuming that the rate constants are high, which is the case if the blood-brain barrier is rather permeable for fluvoxamine, the following equations can be obtained:

$$\frac{1}{k_{diff}} \frac{dA_{DB}}{dt} = (C_{SP} - C_{DB}) - \frac{N_{\max}}{k_{diff}} C_{DB} / (C_{50} + C_{DB}) \quad (6)$$

$$0 = (C_{SP} - C_{DB}) - \frac{N_{\max}}{k_{diff}} C_{DB} / (C_{50} + C_{DB}) \quad (7)$$

$$C_{SP} = C_{DB} + \frac{N_{\max}}{k_{diff}} C_{DB} / (C_{50} + C_{DB}) \quad (8)$$

or

$$A_{SP} = A_{DB} \frac{V_{SP}}{V_{DB}} + \frac{N_{\max} V_{SP}}{k_{diff}} A_{DB} / (A_{50} + A_{DB}) \quad (9)$$

in which V_{SP} is the volume in shallow perfusion-limited compartment, V_{DB} is the volume in the deep brain compartment and A_{50} is the fluvoxamine amount in the deep brain compartment at which 50% of saturation of the active removal flux is reached.

The mass balance in the brain can be expressed in terms of one differential equation:

$$\frac{dA_T}{dt} = \frac{dA_{SP}}{dt} + \frac{dA_{DB}}{dt} = Q_B C_{in} - Q_B C_{out} \quad (10)$$

in which A_T is the total amount of fluvoxamine in the brain.

Assume that the entering concentration is equal to the plasma concentration:

$$C_{in} = C_p \quad (11)$$

in which C_p is the fluvoxamine concentration in plasma.

The concentration leaving the brain is determined by the partition coefficient between drug in plasma and the concentration in the shallow perfusion-limited compartment:

$$C_{out} = C_{SP} / P \quad (12)$$

in which P is the partition coefficient.

Accordingly:

$$\frac{dA_T}{dt} = Q_B C_p - Q_B C_{SP} / P \quad (13)$$

Dividing by brain volume results in the following equation:

$$\frac{dC_T}{dt} = Q_B C_p / V_T - Q_B C_{SP} / (V_T P) \quad (14)$$

in which C_T is the total fluvoxamine concentration in the brain and V_T is the total brain volume.

Now, define the following constants:

$$k_{in} = Q_B / V_T \tag{15}$$

$$k_{out} = Q_B /(V_T P) \tag{16}$$

in which k_{in} is the influx rate constant in the brain and k_{out} is the efflux rate constant from the brain.

As a result the following relationship is obtained:

$$\frac{dC_T}{dt} = k_{in} C_p - k_{out} C_{SP} \tag{17}$$

As a consequence we need to know the relationship between:

(1) The concentration in the deep brain compartment and total brain concentration:

$$C_{DB} = f_{DB} C_T \tag{18}$$

(2) The concentration in the shallow perfusion-limited compartment and total brain concentration:

$$C_{SP} = f_{SP} C_T \tag{19}$$

in which f_{DB} is the partition coefficient for the deep brain compartment and f_{SP} is the partition coefficient for the shallow perfusion-limited compartment

Equation 18 is needed to calculate the concentration in the deep brain compartment as a function of the total concentration. Equation 19 is needed for calculating the concentration in the shallow perfusion-limited compartment and for calculating the mass balance of the total brain in equation 17. Substitution of equation 19 in equation 17 yields:

$$\frac{dC_T}{dt} = k_{in} C_p - k_{out} f_{SP} C_T \tag{20}$$

The relationships in equation 18 and 19 can be found by solving equation 18:

$$A_{SP} = A_{DB} \frac{V_{SP}}{V_{DB}} + \frac{N_{\max} V_{SP}}{k_{diff}} A_{DB} /(A_{50} + A_{DB}) \tag{21}$$

Further the relation of the total amount of fluvoxamine in the brain is known:

$$A_T = A_{SP} + A_{DB} \tag{22}$$

and therefore:

$$A_T - A_{DB} = A_{DB} \frac{V_{SP}}{V_{DB}} + \frac{N_{\max} V_{SP}}{k_{diff}} A_{DB} /(A_{50} + A_{DB}) \tag{23}$$

$$A_T = A_{DB} \left(\frac{V_{SP}}{V_{DB}} + 1 \right) + \frac{N_{\max} V_{SP}}{k_{diff}} A_{DB} /(A_{50} + A_{DB}) \tag{24}$$

By defining:

$$\alpha = \left(\frac{V_{SP}}{V_{DB}} + 1 \right) > 1 \tag{25}$$

$$N_{*\max} = \frac{N_{\max} V_{SP}}{k_{diff}} > 0 \tag{26}$$

the following relationship for the total fluvoxamine amount in the brain is obtained:

$$A_T = A_{DB}\alpha + \frac{N_{*\max} A_{DB}}{A_{50} + A_{DB}} \tag{27}$$

Rearrangement results in:

$$\alpha A_{DB}^2 + (N_{*\max} - A_T + \alpha A_{50}) A_{DB} - A_T A_{50} = 0 \tag{28}$$

For which the solution is equal to:

$$A_{DECF} = \frac{-b + \sqrt{b^2 - 4ac}}{2a} \tag{29}$$

with:

$$c = -A_T A_{50} \tag{30}$$

$$b = N_{*\max} - A_T + \alpha A_{50} \tag{31}$$

$$a = \alpha \tag{32}$$

The concentration in the deep brain compartment can be calculated from the total brain concentration by:

$$\frac{C_{DB}}{C_T} = \frac{A_{DB}(V_{DB} + V_{SP})}{A_T V_{DB}} \tag{33}$$

$$C_{DB} = \frac{A_{DB}(V_{DB} + V_{SP})}{A_T V_{DB}} C_T \tag{34}$$

$$C_{DB} = f_{DB} C_T \tag{35}$$

with the fraction:

$$f_{DB} = \frac{(V_{DB} + V_{SP})}{V_{DB} A_T} \times \frac{-b + \sqrt{b^2 - 4ac}}{2a} \tag{36}$$

$$N_{***\max} = \frac{N_{\max} V_{SP}}{k_{diff}(1 + V_{DB})} > 0 \tag{37}$$

$$f_{DB} = \frac{-N_{***\max} + C_T - C_{50} + \sqrt{(N_{***\max} - C_T + C_{50})^2 + 4C_T C_{50}}}{2C_T} \tag{38}$$

To calculate the concentration in the shallow perfusion-limited compartment from the total brain concentration use:

$$C_{SP} = f_{SP} C_T \tag{19}$$

Recall that:

$$C_{DB} = f_{DB} C_T \tag{18}$$

$$A_{DB} / V_{DB} = f_{DB} A_T / (V_{DB} + V_{SP}) \tag{39}$$

And therefore the partition coefficient for the shallow perfusion-limited compartment can be calculated by:

$$f_{SP} = 1 + V_{DB} / V_{SP} (1 - f_{DB}) \tag{40}$$

Chapter 7

Pharmacokinetic/Pharmacodynamic Modeling of Fluvoxamine Serotonin Transporter Occupancy in Rat Frontal Cortex: Role of Non-linear Brain Distribution

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Abstract

The pharmacokinetic/pharmacodynamic correlation of fluvoxamine serotonin transporter (SERT) occupancy was determined in rat frontal cortex *in vivo*. Rats (n=47) with permanent arterial and venous cannulas received a 30-min intravenous infusion of 1 or 7.3. mg/kg of fluvoxamine. At various time points after administration, brain was collected for determination of fluvoxamine concentration and SERT occupancy. In addition, the time-course of fluvoxamine concentration in plasma was determined up to the time of brain collection. In a separate study (n=26), the time-course of fluvoxamine concentration in brain extracellular fluid (ECF) and plasma was determined. The results of the various investigations were interpreted by nonlinear mixed effects modeling (NONMEM).

Maximal SERT occupancy was reached instantly after administration and maintained for 1.5 and 7 hrs after 1 and 7.3 mg/kg, respectively. Thereafter, SERT occupancy decreased linearly at a rate of 8 %/hr.

SERT occupancy could be directly related to plasma, brain ECF and brain concentrations by a hyperbolic function (B_{max} model). Maximal SERT occupancy (B_{max}) was 95%. Estimated concentrations at half-maximal SERT occupancy (EC_{50}) in plasma, ECF and brain tissue were 0.48, 0.22 and 14.8 ng/ml respectively. Inter-individual variability in these values was 0.34, 0.25 and 0.25, respectively. The minimum value of the objective function (MVOF) decreased 12 points for ECF and brain tissue concentrations relative to plasma ($p< 0.01$), presumably as result of non-linear brain distribution.

The proposed PK/PD model constitutes a useful basis for prediction of the time-course of *in vivo* SERT occupancy in behavioral studies with SSRIs.

Introduction

Reduced serotonergic transmission is a well-known characteristic in the pathogenesis of depression (Coppen, 1967;Owens and Nemeroff, 1994). Not surprisingly, Selective Serotonin Reuptake Inhibitors (SSRIs) constitute the first line of treatment in depressive disorders (Ables and Baughman, III, 2003;Isaac, 1999). By blockade of the serotonin transporter (SERT) extracellular serotonin (5-hydroxytryptamine, 5-HT) concentrations are increased, resulting in enhancement of serotonergic transmission (Bel and Artigas, 1992;Fuller, 1994). The pharmacodynamics of SSRIs in depressive disorders are complex. Although SSRIs rapidly inhibit the reuptake of 5-HT, maximal antidepressant effects are only observed after weeks of chronic treatment, indicating that long-term adaptive changes are important for therapeutic efficacy. To

date, most long-term investigations of the effects of antidepressants have focused on the functionality of serotonergic autoreceptors and postsynaptic receptors, which appear to be sensitive to adaptive changes (Auerbach and Hjorth, 1995;Blier and Bouchard, 1994;Bosker et al., 1995). However, the functionality of SERT itself appears to be subjected to regulatory influences as well (Benmansour et al., 1999;Ginovart et al., 2003;Horschitz et al., 2001;Pineyro et al., 1994;Ramamoorthy and Blakely, 1999). Specifically, a decreased SERT gene expression (Lesch et al., 1993) and a decreased SERT density (Benmansour et al., 1999) have been observed after chronic treatment in rats.

Despite the widespread clinical use of SSRIs and numerous preclinical and clinical investigations, very few studies have addressed the pharmacokinetic/pharmacodynamic (PK/PD) correlations of SSRIs. In recent years, the concept of mechanism-based PK/PD modeling has gained considerable interest. The objective of mechanism-based PK/PD modeling is to understand, in a strictly quantitative manner, the mechanisms that determine the time-course of the intensity of the drug effect *in vivo*. A pertinent feature of mechanism-based PK/PD models is that they contain specific expressions to describe pertinent processes on the causal path between drug administration and response (Danhof et al., 2005). This includes among other factors the distribution of the drug to the target site, the binding to the target site, the activation of the target site and homeostatic feedback. The development of mechanism-based PK/PD models relies on biomarkers, which characterize quantitatively the processes on the causal path between drug administration and response (Rolan, 1997;Colburn and Lee, 2003).

Recently, we have initiated a series of investigations on the PK/PD correlations of fluvoxamine as a prototype for SSRIs. As the first step in this development, a population PK model of fluvoxamine in plasma has been proposed which enables full characterization of the plasma concentration time profile on the basis of sparse blood concentrations. This is important since in behavioral pharmacology investigations, blood sampling may interfere with assessment of the effect (Geldof et al., 2007). The next step has been the development of a physiological PK model describing the non-linear brain distribution of fluvoxamine, which enables prediction of fluvoxamine concentration in brain extracellular fluid (ECF) of the frontal cortex and brain tissue on the basis of plasma concentrations (Geldof et al., in publication). In the present investigation we propose a PK/PD model for fluvoxamine SERT occupancy in rat frontal cortex. This is important since SERT occupancy is an important intermediary step in the PD of SSRIs. Despite wide interest in the assessment of SERT occupancy

in clinical studies (Meyer et al., 2001;Suhara et al., 2003), very few investigations have addressed *in vivo* SERT occupancy in relation to behavioral responses in animal studies (Ginovart et al., 2003;Hirano et al., 2005), which can presumably be explained by the relative difficulty of quantifying *in vivo* SERT occupancy.
In this contribution, we present a PK/PD model, which enables characterization and prediction of the time-course of *in vivo* SERT occupancy in behavioral studies.

Overall Study Design
Two studies were performed to describe the PK/PD correlation of fluvoxamine SERT occupancy in rat frontal cortex. In the first study, brain was collected from 47 rats by destructive sampling at various time points after fluvoxamine administration for determination of the fluvoxamine concentration in brain tissue and SERT occupancy. In addition, the time-course of fluvoxamine concentration in plasma was determined in these animals until time of brain collection. In the second microdialysis study, the time-course of fluvoxamine concentration in brain ECF of the frontal cortex and plasma was determined in 26 animals by serial sampling. In addition, fluvoxamine concentration in brain tissue was determined in 16 of these animals at the end of the experiment as extra information for the model. The results of the latter study have also been reported separately (Geldof et al., in publication). In the present investigation, plasma fluvoxamine concentration *versus* time profiles were predicted in individual rats, by analysis of the plasma concentrations on the basis of a previously proposed PK model. The time-course of the fluvoxamine concentrations in brain ECF and brain tissue was predicted on the basis of a previously proposed physiological PK model describing non-linear brain distribution.
In the present investigation, information obtained in the two studies was integrated for development of a PK/PD model for fluvoxamine SERT occupancy in the rat, enabling prediction of the time-course of *in vivo* SERT occupancy in behavioral pharmacology studies.

Materials and Methods

Chemicals
Fluvoxamine maleate and clovoxamine fumarate were kindly provided by Solvay Pharmaceuticals (Weesp, The Netherlands). Acetonitrile, isopentane, ethanol and methanol were obtained from Acros (Geel, Belgium). Heptane was purchased from Sigma-Aldrich Laborchemikalien (Seelze, Germany) and isoamylalcohol, dimethylsulfoxide (DMSO) and sodium hydroxide (NaOH) from Merck (Darmstadt,

Germany). Ammonium acetate was obtained from Baker Chemicals (Deventer, The Netherlands) and Millipore water was obtained from a Milli-Q system (Millipore SA, Molsheim, France). The radioligand [^{3}H]-Citalopram was obtained from Amersham Biosciences Benelux (Roosendaal, The Netherlands).

Animals

Male Wistar rats (Charles River Wiga GMBH, Sulzfeld, Germany) weighing 226-250 gram were housed in groups for 1 week, under standard environmental conditions (ambient temperature 21°C, humidity 60%, 12-h light/dark cycle). The animals had free access to food (laboratory chow, Hope Farms, Woerden, The Netherlands) and acidified water. For the brain sampling studies 47 animals were used and for the microdialysis study 26 animals were used. After surgery, the animals in the brain sampling studies were housed individually for 2 days and the animals in the microdialysis studies for 7 days. All studies were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and approved by the Ethical Committee on Animal Experimentation of Leiden University.

Experimental Procedures

Brain Sampling Studies

Surgical Procedures

The animals were anaesthetized by a subcutaneous injection of 0.1 ml/100 g Ketanest-S® ((S)-ketaminebase, Parke-Davis, Hoofddorp, The Netherlands) and an intramuscular injection of 0.01 ml/100 g Domitor® (medetomidine hydrochloride, Pfizer, Capelle a/d IJssel, The Netherlands). Animals were implanted with a permanent cannula in the right jugular vein for administration of fluvoxamine and a permanent cannula in the left femoral artery for collection of blood samples. The cannulas were subcutaneously tunneled and externalized at the back of the neck. The venous cannula was filled with NaCl (0.9%, B. Braun Melsungen AG, Melsungen, Germany) containing heparin (20 IU/ml, Pharmacy, Leiden University Medical Center, Leiden, The Netherlands) and the arterial cannula was filled with 25% (w/v) polyvinylpyrrolidone (PVP, Brocacef, Maarssen, The Netherlands) solution in NaCl (0.9%) containing heparin (20 IU/ml) to prevent blockade by blood clotting. After surgery, the animals were housed individually for 2 days.

Dosage Regimen

From the 47 animals used in the brain sampling studies, 24 animals received 1 mg/kg and 23 animals received 7.3 mg/kg fluvoxamine via a 30-min intravenous infusion (BAS BeeHive, Bioanalytical Systems Inc. USA) in the jugular vein cannula at a flow rate of 20 µl/min (1 or 7.3 mg/kg). Solutions of fluvoxamine in physiological saline (0.9%) were prepared on the day of administration. Dosages and observed concentrations of fluvoxamine are expressed as free base.

Blood Sampling

A number between 2 and 15 arterial blood samples (100 µl) were collected from the animals between 2.5 and 600 min following fluvoxamine administration from the cannula in the femoral artery, dependent on the time of brain sampling. After collection of each blood sample, an equal volume of heparinized 0.9% NaCl (20 IU/ml) was administered to the animal. Blood samples were collected in heparinized Eppendorf tubes and kept on ice during the experiment. After centrifugation (10 min, 5000xg), 50 µl plasma was transferred into a glass tube and stored at -20°C until sample analysis.

Brain Collection

From the 24 animals that received 1 mg/kg fluvoxamine, the brains were collected at 10, 20, 30, 45, 60, 75, 90, 105, 120, 135, 150, 180, 210, 240, 270, 300, 330, 360, 390, 420, 450, 500, 550 and 600 min after fluvoxamine administration by destructive sampling. From the 23 animals that received 7.3 mg/kg fluvoxamine, the brains were collected at 15, 30, 45, 60, 80, 90, 110, 120, 140, 200, 250, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1100 and 1300 min after fluvoxamine administration by destructive sampling. The animals were sacrificed by decapitation after which the brains were quickly isolated. The brains were frozen in dry ice-cooled isopentane that was surrounded with cold ethanol after which they were stored at -20°C until sectioning and subsequent analysis.

Microdialysis Studies

Surgical Procedures

Animals in the microdialysis studies were implanted with a cannula in the right jugular vein for fluvoxamine administration and a cannula in the left femoral artery for collection of blood samples as described for the animals in brain sampling studies. The animals were instrumented with a microdialysis guide cannula (CMA/12, Aurora

Borealis Control BV, Schoonebeek, The Netherlands) within the same surgery. A microdialysis guide cannula with a dummy probe was implanted in the frontal cortex (AP: 3.2; L: -3.0; V: -1.5 mm from bregma, in accordance with the atlas of Paxinos and Watson (Paxinos and Watson, 1982)). Two support screws were placed and the guide was secured in place using dental cement (Simplex Rapid liquid and powder, Kemdent Associated Dental Products, United Kingdom). After surgery, the animals were housed individually for 1 week.

Dosage Regimen

From the 26 animals used in the microdialysis studies, 8 animals received 1 mg/kg, 8 animals received 3.7 mg/kg and 10 animals received 7.3 mg/kg fluvoxamine via the 30-min intravenous infusion, described for the brain sampling studies.

Microdialysis

The microdialysis study was performed as described before (Geldof et al., in publication). Briefly, 0.5% (w/v) Bovine Serum Albumin (BSA, Sigma-Aldrich, Zwijndrecht, The Netherlands) was added to artificial cerebrospinal fluid (containing: 145 mM NaCl, 0.6 mM KCl, 1.0 mM $MgCl_2$, 1.2 mM $CaCl_2$, 0.2 mM ascorbic acid in 2 mM phosphate buffer pH 7.4) (Moghaddam and Bunney, 1989) to prevent fluvoxamine from sticking to the microdialysate tubing and microdialysis probe. After fluvoxamine administration, dialysate concentrations were measured over a period of 5 hrs at a flow rate of 2 µl/min. Microdialysate samples were collected at a temperature of 4°C in the microdialysate fraction collector (Univentor 820, Antec Leyden BV, Zoeterwoude, The Netherlands) and subsequently stored at -20°C until analysis. Individual values for the *in vivo* recovery were determined by the method reverse dialysis or retrodialysis by drug (Bouw and Hammarlund-Udenaes, 1998;de Lange et al., 1997).

Blood Sampling

A number of 13 arterial blood samples (100 µl) were collected at variable fixed time intervals from the cannula in the femoral artery over a time period between 0 and 5 hrs after fluvoxamine administration. Blood samples were collected, handled and stored as described for the brain sampling studies.

Brain Collection

From the 8 animals that received 1 mg/kg fluvoxamine, the brains were collected at the end of the experiment at 290 (n=2), 300 (n=4), 305 and 310 min after fluvoxamine administration by destructive sampling. From the 8 animals that received 7.3 mg/kg fluvoxamine, the brains were collected at 300 (n=3), 310 (n=3), 365 and 380 min after fluvoxamine administration by destructive sampling. Brain samples were collected, handled and stored as described for the brain sampling studies.

Drug Analysis

Fluvoxamine samples were analyzed using liquid chromatography with tandem mass spectrometry (LC-MS/MS) as described earlier for plasma (Geldof et al., 2007) and for brain ECF and brain tissue (Geldof et al., in publication). A volume of 50 µl of the calibration standards or independent Quality Control (QC) samples in DMSO was added to a volume of 50 µl blank plasma. For the plasma samples from the rats, a volume of 50 µl plasma was added to 50 µl DMSO. A volume of 50 µl of 500 ng/ml clovoxamine in DMSO were added to these samples as internal standard. Proteins were precipitated by adding 200 µl acetonitrile, the samples were centrifuged (10 min, 5000xg) and a volume of 20 µl was injected into the system. For the ECF samples, a volume of 50 µl of the calibration standards or QC samples in DMSO were added to 50 µl artificial cerebrospinal fluid. For the ECF samples from the rats, a volume of 50 µl DMSO, 500 µl H_2O and 50 µl of 500 ng/ml clovoxamine in DMSO were added. Addition of 100 µl NaOH increased the pH value to approximately 12. A volume of 4 ml of heptane-isoamylalcohol (95:5, v/v) was added, the solution was centrifuged (10 min, 3000xg) and the organic layer was evaporated to dryness under nitrogen at 65°C. The residues were dissolved in a mixture of 10mM ammonium acetate and acetonitrile (50:50, v/v) and a volume of 30 µl was injected into the system. Control brain and brain from fluvoxamine treated rats were weighed and 9 volumes of H_2O were added before homogenization. A volume of 100 µl of the calibration standards or QC samples in DMSO was added to 1 ml control brain homogenates. For the brain samples of the rats, a volume of 100 µl DMSO and 100 µl of 500 ng/ml clovoxamine in DMSO were added to 1 ml of brain homogenates. A volume of 2 ml of methanol was added for extraction and the samples were rotated for 15 min and centrifuged (10 min, 5000xg). The solutions were transferred in Eppendorf tubes, centrifuged for another time (10 min, 9727xg) and a volume of 20 µl was injected into the system. All fluvoxamine samples were quantified on a

reversed phase LC column (BDS Hypersil C18, 3 μm particle size, 100x4.6 mm I.D.; Thermo Hypersil-Keystone, Brussels, Belgium). LC-MS/MS analysis was performed on an API-4000 MS/MS (Applied Biosystems, Toronto, Canada), coupled to an HPLC system (Agilent, Palo Alto, USA). The MS/MS operated in the positive ion mode using the TurboIonSpray-interface (electrospray ionization) was optimized for the quantification of fluvoxamine. For analysis of brain tissue samples, an additional guard cartridge (Hypersil ODS 5μm 10x4.0mm drop-in cartridge, Thermo Electron Corporation, Brussels, Belgium) with holder (Uniguard holder, Thermo Electron Corporation) was used. The intra batch accuracy from independent QC samples was between 80 and 120% over the entire range of the samples. The limit of quantification for fluvoxamine was 1 ng/ml in plasma, brain ECF and brain tissue.

Ex Vivo SERT Occupancy Analysis

The animals were sacrificed by decapitation at fixed times after fluvoxamine administration, which was the same time of determination of fluvoxamine PK in brain (section brain collection). Sections of 20 μm were cut at the level of the frontal cortex using a Leica CM 3050 cryostat-microtome (van Hopplynus, Belgium) and thaw-mounted on microscope slides (SuperFrost Plus Slides, LaboNord, France). Three adjacent brain slices from the same animal were collected per slide. The sections were stored at -20°C for approximately 48 hrs until use.

After thawing, the sections were dried under a stream of cold air. Two brain slices were used to analyze total binding and the third brain slice was used to determine nonspecific binding. The slices were not washed before incubation, to prevent dissociation of the complex of SERT with fluvoxamine. Total binding was analyzed by incubation of the sections for 10 min with 1 nM [^{3}H]-citalopram (81 Ci/mmol) in Tris-HCl buffer (50 mM, pH 7.4) containing 120 mM NaCl. Nonspecific binding was analyzed in the adjacent slice by addition of 10 μM fluoxetine (total 150 μl) to the incubation medium. Incubation was terminated by washing the slices in 50 mM Tris-HCl buffer, pH 7.4 at 4°C (1 x 1 min and 2 x 10 min) followed by a rapid dip in cold distilled H_2O and drying under a stream of cold air.

Slides were made conductive by disposing a copper foil tape (3M, Diegem, Belgium) on the free side. The radioligand binding signal on the slides was evaluated using a β-imager (BioSpace, Paris, France) (Langlois et al., 2001). The levels of bound radioactivity in the brain areas were directly determined by counting the number of β-particles emerging from the delineated area by using the Beta vision program (BioSpace). *Ex vivo* labeling by [^{3}H]citalopram in rat frontal cortex was expressed as

the percentage of SERT labeling in corresponding brain areas of control animals. Because only unoccupied transporters are available for the radioligand, *ex vivo* [^{3}H]citalopram labeling of SERT is inversely related to SERT occupancy by fluvoxamine.

Data Analysis

In the population analysis, the fluvoxamine concentrations in plasma, brain ECF or brain tissue and SERT occupancy from all individual animals were simultaneously analyzed. All fitting procedures were performed on a personal computer (Intel® Pentium® 4 processor) running under Windows XP using the Compaq Visual FORTRAN standard edition 6.1 (Compaq Computer Cooperation, Euston, Texas, USA) with the nonlinear mixed effects modeling software NONMEM (Version V, Level 1.1., NONMEM project group, University of California, San Francisco, USA).

PK Analysis in Plasma

As described in the overall study design, the PK information in plasma obtained in the brain sampling study and the microdialysis study were analyzed on the basis of a previously proposed population PK model for prediction of the plasma concentration *versus* time profiles in individual rats (Geldof et al., 2007). Plasma fluvoxamine concentrations in individual animals at the exact time point when fluvoxamine SERT occupancy were predicted on the basis of this model by application of the obtained post hoc estimates of the parameters.

PK Analysis in Brain ECF and Brain Tissue

As described in the overall study design, the PK information in brain ECF and brain tissue obtained in the brain sampling study and the microdialysis study were analyzed on the basis of a physiological PK model describing the non-linear brain distribution of fluvoxamine (Geldof et al., in publication). Fluvoxamine concentrations in brain ECF in individual animals were predicted at the exact time point when fluvoxamine SERT occupancy was determined on the basis of this model by application of the obtained post hoc estimates of the parameters. Fluvoxamine concentrations in brain tissue were calculated from the predicted fluvoxamine concentrations in brain ECF.

Pharmacokinetic/Pharmacodynamic Model

The predicted fluvoxamine concentrations in plasma, brain ECF and brain tissue at the exact time point when SERT occupancy was determined were related to

fluvoxamine SERT occupancy in three PK/PD models. Population PK and PD values of fluvoxamine were estimated using the first order conditional estimation with interaction method in NONMEM. All steps in the development of the PK/PD models were executed on the basis of the likelihood ratio test (Jolling et al., 2004;Kuipers et al., 2001), diagnostic plots (observed concentrations vs. individual and population predicted concentrations, weighted residuals vs. predicted time and concentrations), parameter correlations and precision in parameter estimates. An extra parameter was included in the structural model if the resulting change in the minimum value of the objective function (MVOF) was larger than 6.6 points ($p \leq 0.01$).

No hysteresis was observed between fluvoxamine concentrations in plasma, brain ECF, brain tissue and fluvoxamine SERT occupancy. Therefore, fluvoxamine SERT occupancy could be directly related to plasma, brain ECF and brain tissue concentrations. These relations could be analyzed by the B_{max} model, which is the simplest model that adequately describes non-linear relationships between drug effect and concentrations over the whole range of concentrations and is based on the hyperbolic relationship. In this model rapid equilibrium is assumed between plasma and biophase concentrations and was originally derived from the classical theory of drug-receptor interaction (Holford and Sheiner, 1981;Mager et al., 2003;Meibohm and Derendorf, 1997).

Therefore the relation between concentration and SERT occupancy was described by:

$$B = \frac{B_{\max} \cdot C}{C + EC_{50}} \tag{1}$$

in which B is SERT occupancy, B_{max} is maximum SERT occupancy, C is the simulated fluvoxamine concentration in plasma, brain ECF or brain tissue and EC_{50} is the fluvoxamine concentration in plasma, ECF or brain at which 50% of the SERT was occupied.

Although only one observation per animal for SERT occupancy could be determined and consequently a distinction between intra-individual and inter-individual variability could not be made, two random effects were explored in the model. Inter-individual variability on the parameters was modeled according to an exponential equation:

$$P_i = \theta \cdot \exp(\eta_i) \tag{2}$$

in which P_i is the estimate for parameter P for the i^{th} individual, θ is the population estimate for parameter P and $\exp(\eta_i)$ is the inter-individual random deviation of P_i

from *P*. The values of η_i are assumed to be normally distributed with mean zero and variance ω^2 that distinguished the PK parameters for the i^{th} individual from the population typical value θ. Inter-individual variabilities were analyzed on each parameter and the inter-individual effects that did not significantly improve the model or could not be estimated were fixed to zero. Correlations between the inter-individual variability of the various parameters were explored. Residual variability of SERT occupancy (e.g. caused by measurement and experimental errors) was described by a second level of random effects by an additive error:

$$Bo_{ij} = B_{ij} + \varepsilon_{1ij} \quad (3)$$

in which Bo_{ij} is observed SERT occupancy, B_{ij} is the j^{th} SERT occupancy for the i^{th} individual predicted by the model and ε_{1ij} accounts for the residual deviance of the predicted concentration from the observed concentration. The values for ε were assumed to be independently normally distributed with mean zero and variance σ^2.

Results

SERT Occupancy by Fluvoxamine

Figure 1 shows characteristic images of total and non-specific [^{3}H]citalopram binding on brain sections of the rat frontal cortex at various time points after a fluvoxamine dose of 1 mg/kg.

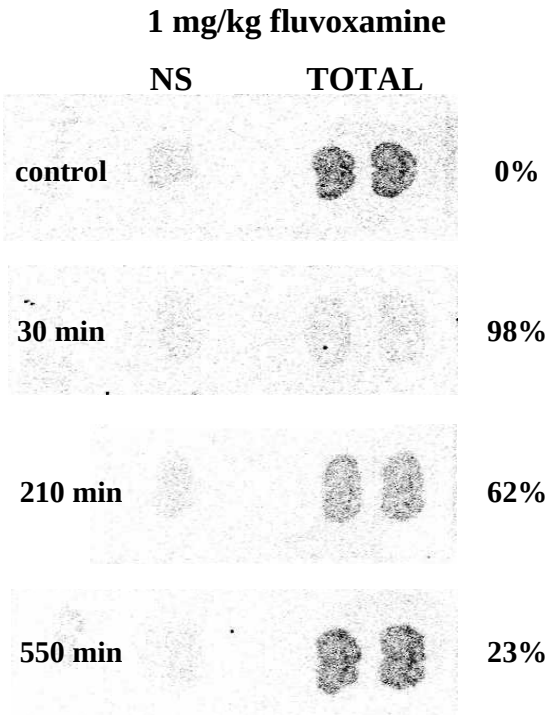

Figure 1. *Digital images of fluvoxamine SERT occupancy in rat frontal cortex after a 30-min infusion of 1 mg/kg. Depicted are images of control, 30, 210 and 550 min after fluvoxamine administration with resulting SERT occupancy obtained after 1.5 hr acquisition with the β-imager (ns: non-specific binding, total: total binding).*

Because only unoccupied transporters are available for the radioligand, *ex vivo* [^{3}H]citalopram labeling of SERT is inversely related to SERT occupancy by fluvoxamine. Most contrast was obtained in control brains in which no fluvoxamine was administered. Fluvoxamine was potent in occupying SERT in rat frontal cortex, since the contrast of the image decreased indicating high levels of fluvoxamine SERT occupancy, as shown by the maximal SERT occupancy for the digital image at 30 min after dose administration. In time, a decrease in fluvoxamine SERT occupancy was observed as shown by the increased contrast of the image at 210 and 550 min.

PK Analysis in Plasma

In Table 1, the mean post hoc estimates for the PK parameters of fluvoxamine in plasma obtained by the three-compartment PK model are depicted.

Table 1. *Mean post hoc estimates for the pharmacokinetic parameters of fluvoxamine in plasma after IV administration in 30 min (1, 3.7 and 7.3 mg/kg) obtained by the three-compartment pharmacokinetic model. Depicted are the population mean estimates for CL, V1, V2, Q2, V3, and Q3.*

Study	PK parameter (unit)					
	CL (ml/min)	*V1* (ml)	*V2* (ml)	*Q2* (ml/min)	*V3* (ml)	*Q3* (ml/min)
Brain sampling +Microdialysis	29.6	294.4	858.1	31.8	136.3	1.0
Brain sampling	27.1	253.8	722.4	29.2	136.3	1.0
Microdialysis	34.2	370.6	1113.3	36.7	136.3	1.0

Included are the post hoc estimates for systemic clearance (*CL*), central volume of distribution (*V1*), two peripheral volumes of distribution (*V2*, *V3*) and inter-compartmental clearances (*Q2*, *Q3*). On the basis of a covariate analysis, no differences in the pharmacokinetics of the different dose groups nor between the rats in the brain sampling studies and the microdialysis studies could be detected. Inter-individual variability could not be identified on the parameters *V3* and *Q3*, and therefore the estimates for the total population of 187 animals were used. The individual post hoc estimates were used in the current studies for each individual animal to estimate the fluvoxamine concentration in plasma at the time when fluvoxamine SERT occupancy was determined.

PK Analysis in Brain ECF and Brain Tissue

In Table 2, the mean post hoc estimates for the PK parameters of fluvoxamine in brain ECF obtained by the physiological PK model are depicted.

Table 2. *Mean post hoc estimates for the pharmacokinetic parameters of fluvoxamine in ECF of the frontal cortex after IV administration in 30 min (1, 3.7 and 7.3 mg/kg) obtained by the physiological brain distribution pharmacokinetic model. Depicted are the population mean estimates for k_{in}, k_{out} and C_{50}.*

Study	PK parameter (unit)		
	k_{in} (1/min)	k_{out} (1/min)	C_{50} (ng/ml)
Brain sampling + Microdialysis	0.2031	0.0183	710
Brain sampling	0.2034	0.0186	710
Microdialysis	0.2026	0.0178	710

Included are the post hoc estimates for the influx rate constant in the brain (k_{in}), the efflux rate from the brain (k_{out}). Inter-individual variability could not be identified on the parameter C_{50}, the fluvoxamine concentration at which 50% of saturation of the active removal flux is reached, and therefore the population estimate of 710 ng/ml was used for each animal. The individual post hoc estimates were used in the current studies for each individual animal to estimate the fluvoxamine concentration in brain ECF and brain tissue at the time when fluvoxamine SERT occupancy was determined.

Pharmacokinetic/Pharmacodynamic Model

In Figure 2, the time-courses of fluvoxamine SERT occupancy obtained after a 30-min intravenous infusion of 1 mg/kg (circles) or 7.3 mg/kg (triangles) are shown.

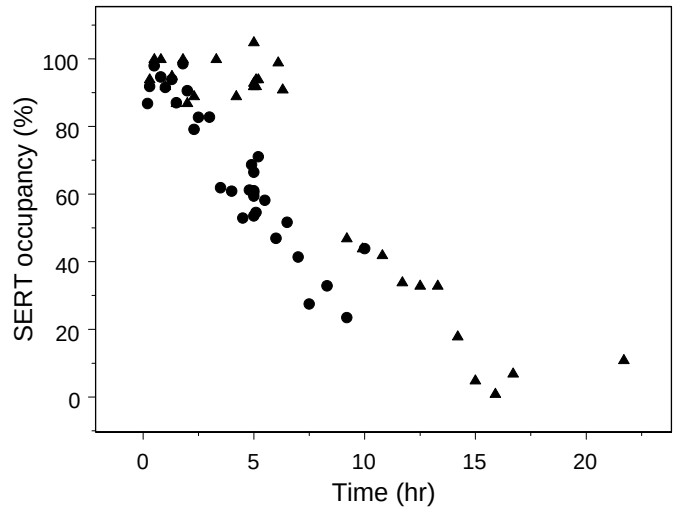

Figure 2. *Time-course of fluvoxamine SERT occupancy in rat frontal cortex obtained after a 30-min intravenous infusion of 1 mg/kg (circles) or 7.3 mg/kg (triangles).*

Maximal SERT occupancy was reached instantly after a dose of both 1 and 7.3 mg/kg. Maximal occupancy was observed for about 1.5 hrs after 1 mg/kg and for about 7 hrs after 7.3 mg/kg. Thereafter, fluvoxamine SERT occupancy linearly decreased in time at a rate of 8 %/hr, which was the same after both fluvoxamine dosages, indicated by the same slope of both curves. Fluvoxamine SERT occupancy reached 0% after about 15 hrs after a dose of 7.3 mg/kg. The relationships between the fluvoxamine concentrations in plasma, brain ECF and brain tissue and the degree of SERT occupancy are shown in Figure 3.

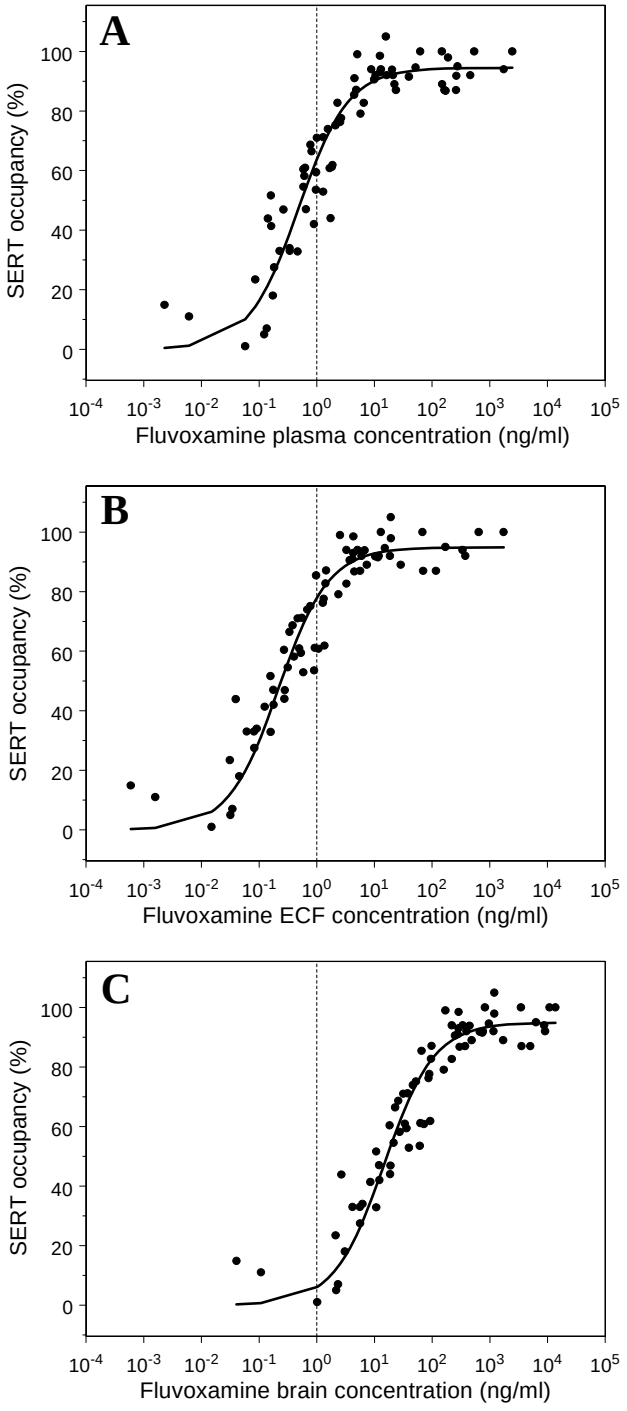

Figure 3. *The relationships between concentrations of fluvoxamine in plasma (A), ECF of the frontal cortex (B) and brain tissue (C) and the degree of fluvoxamine SERT occupancy. Dots are the observed SERT occupancies and the solid line represents the predicted SERT occupancies by fluvoxamine. The limit of quantification (1 ng/ml) is added for clarity (dashed line).*

The concentration-effect relations could be adequately described by relation of SERT occupancy to PK in plasma (A), brain ECF (B) and brain tissue (C). Figure 3 shows that the fluvoxamine concentrations in plasma and brain ECF were below the limit of quantification (1 ng/ml) for a significant part of the concentration-effect curves. However, by analysis of these profiles in plasma and brain ECF in relation with the much higher fluvoxamine concentrations in brain tissue in the previously described physiological model for fluvoxamine brain distribution (Geldof et al., in publication), it was possible to predict accurate fluvoxamine concentrations in plasma and brain ECF.

The structural PD parameters for fluvoxamine of the three PK/PD models could be estimated simultaneously with good accuracy and precision (Table 3).

Table 3. *Population PD parameter estimates of fluvoxamine SERT occupancy in rat frontal cortex after IV administration in 30 min (1 and 7.3 mg/kg) obtained by the developed PK/PD models. Fluvoxamine SERT occupancy was related to the simulated PK in plasma, ECF of frontal cortex or brain tissue. Depicted are the population mean estimates for* B_{max}, EC_{50}, ω^2, σ^2 *with corresponding coefficient of variation (C.V.) and values for MVOF.*

		Plasma		ECF		Brain	
Parameter	Unit	Value	C.V. (%)	Value	C.V. (%)	Value	C.V. (%)
B_{max}	%	94.5	1.1	94.9	1.1	94.9	1.1
EC_{50}	ng/ml	0.48	11.6	0.22	10.6	14.8	10.6
$\omega^2\ \eta_{EC50}$	-	0.34	36.2	0.25	38.0	0.25	38.0
$\sigma^2\ \varepsilon_{1ij}$	-	33.5	27.2	30.9	27.1	30.8	27.1
MVOF	-	382.9		370.7		370.7	

*C.V. = Coefficient of Variation (standard error / value * 100%)*

No significant correlation was observed between fluvoxamine dose and the pharmacodynamic parameter estimates. Although only one observation per animal for SERT occupancy could be determined, inter-individual variability could be identified for EC_{50}, which significantly improved description of the concentration-effect relationships by the PK/PD models. Improved description was reflected in a reduction in MVOF of 11.1, 3.8 and 3.8 points for plasma, brain ECF and brain tissue in addition to a reduction of the residual error by 52, 31 and 31, respectively.

When analyzing fluvoxamine SERT occupancy in relation to brain ECF and brain tissue concentrations, MVOF was decreased by 12.2 points compared to the model in which SERT occupancy was related to fluvoxamine plasma concentrations. In addition, both the residual error and inter-individual variability in EC_{50} were

significantly lower. Maximal SERT occupancy (B_{max}) was not significantly different when related to PK in plasma, brain ECF or brain tissue and was estimated as 95% by all models. Fluvoxamine concentration at half of the maximal SERT occupancy (EC_{50}) was 0.48 ng/ml in plasma, 0.22 ng/ml in brain ECF and 14.8 ng/ml in brain tissue.

In Figure 4, the goodness-of-fit plots obtained by the PK/PD model in which SERT occupancy was related to fluvoxamine brain concentrations are depicted.

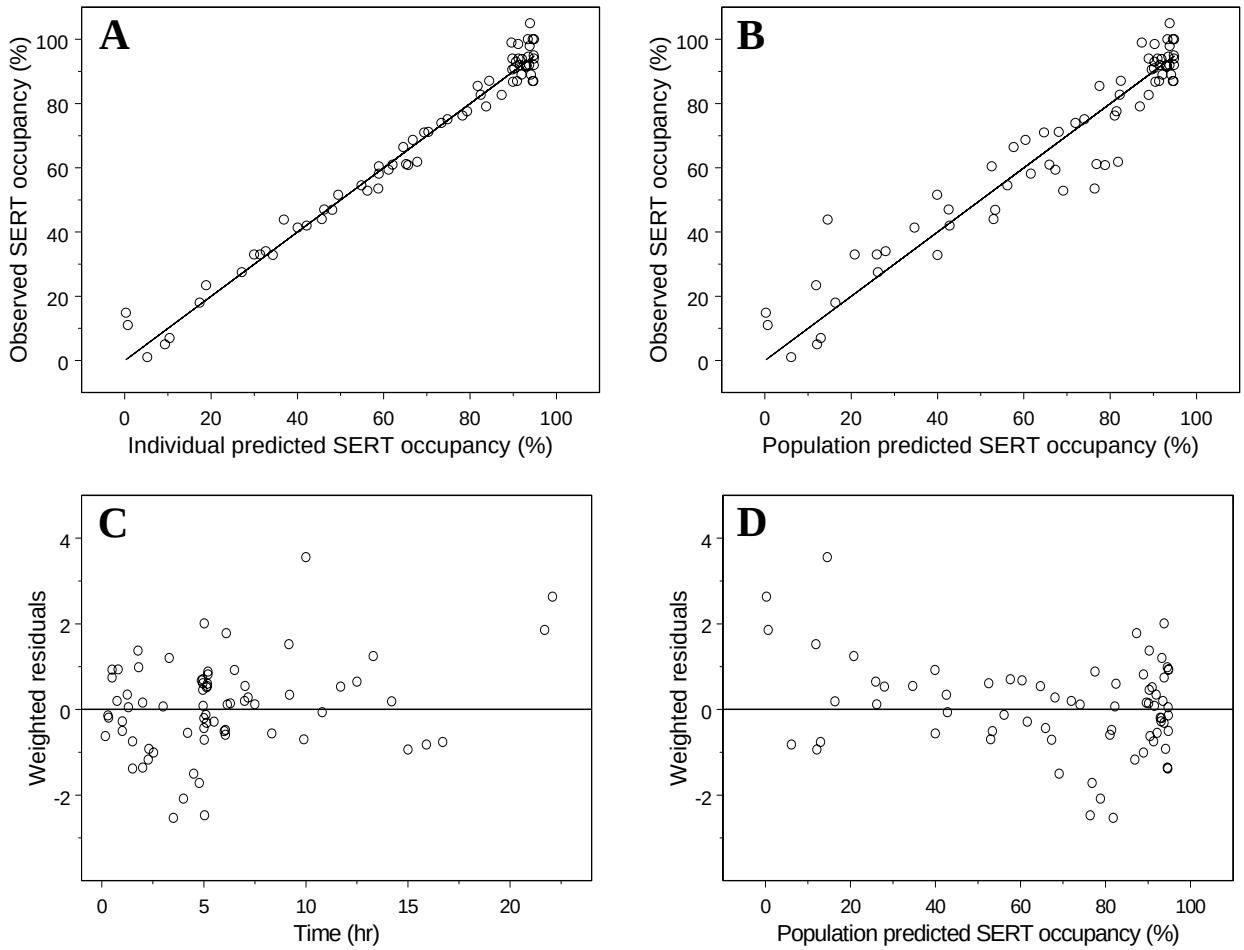

Figure 4. *Goodness-of-fit plots obtained by the developed PK/PD model in which SERT occupancy was related to fluvoxamine concentrations in brain tissue. Depicted are scatter plots of observed fluvoxamine SERT occupancy versus the individual model predictions (A) and versus population model predictions (B) and scatter plots of the population weighted residuals versus time (C) and versus population model predictions (D).*

Observed SERT occupancies were in close agreement with the predicted individual and population predicted SERT occupancies. Fluvoxamine SERT occupancy could be adequately described, since no substantial or systemic deviation from the identity line was observed in the residual diagnostics. The goodness-of-fit plots for the PK/PD

models in which SERT occupancy was related to fluvoxamine concentrations in plasma and brain ECF showed the same satisfactory results (plots not shown).

Discussion

The objective of this investigation was to develop a PK/PD model for SERT occupancy in rat frontal cortex following intravenous administration of fluvoxamine, which can be used for prediction of the time-course of SERT occupancy in behavioral pharmacology studies *in vivo*. This is important since SERT occupancy is an important intermediate step in the pharmacodynamics of SSRIs. The ultimate goal is to apply this model in behavioral studies with SSRIs to explore the relationships between drug concentration, SERT expression, SERT occupancy and the behavioral effect. An *ex vivo* technique was used to characterize *in vivo* fluvoxamine SERT occupancy. A limitation of this approach is that only a single observation of SERT occupancy is obtained. Furthermore, due to the high affinity of fluvoxamine for SERT, drug concentrations in biological fluids are typically below measurable values. In previous investigations we have proposed population PK models to describe the plasma concentration *versus* time profile of fluvoxamine on the basis of sparse sampling (Geldof et al., 2007) as well as a physiological PK model to describe non-linear brain distribution (Geldof et al., in publication). The latter model showed that fluvoxamine brain distribution is non-linear, presumably as result of the effect of an active efflux transporter at the blood-brain barrier. An important feature of these models is that by simultaneous analysis of fluvoxamine concentrations in plasma, brain ECF and brain tissue, fluvoxamine concentrations in plasma and brain ECF can be predicted over a much wider concentration range. In the present investigation, the previously proposed population PK in plasma and physiological brain distribution model were successfully applied to predict fluvoxamine concentrations in plasma and brain ECF in a relevant concentration range with regard to fluvoxamine SERT occupancy. No hysteresis between the fluvoxamine concentration in plasma, brain ECF, brain tissue and SERT occupancy was observed, and therefore these two could be directly related to each other. The relation between fluvoxamine concentrations and SERT occupancy were successfully described by a hyperbolic function (simple B_{max} model) allowing estimations of the relevant pharmacodynamic parameters.

Maximal SERT occupancy was reached instantly after administration and maintained for 1.5 and 7 hrs after 1 and 7.3 mg/kg, respectively. Thereafter, SERT occupancy decreased linearly at a rate of 8 %/hr after both fluvoxamine dosages. By relation of fluvoxamine SERT occupancy to fluvoxamine levels in brain ECF and brain tissue,

the MVOF was decreased by 12 points compared to the model with relation to fluvoxamine plasma levels and both the residual error and inter-individual variability in EC_{50} were significantly decreased, presumably as result of non-linear brain distribution. Furthermore, fluvoxamine levels in brain ECF and brain tissue have a lag time of about 20 min compared to fluvoxamine plasma levels because of the required time for transport of fluvoxamine from plasma to the brain. Fluvoxamine concentration at half of maximal SERT occupancy (EC_{50}) was 0.48 ng/ml in plasma, 0.22 ng/ml in brain ECF and 14.8 ng/ml in brain tissue, of which only this latter value was above the quantification limit (1 ng/ml) of the bioanalytical assay. Estimated EC_{50} values were very low, particularly in plasma and brain ECF, hence only very small levels of fluvoxamine must be present in plasma and brain ECF to be able to occupy SERT in rat frontal cortex. Because fluvoxamine SERT occupancy was determined in frontal cortex of the rat, it would be rational to relate SERT occupancy to the measured fluvoxamine ECF levels, since these were also measured in this brain region.

Because only one SERT occupancy observation could be obtained per animal, no distinction could be made between the inter- and intra-individual variability. However, in the developed PK/PD models, residual variability was described by an additive error in addition to the inter-individual variability identified for EC_{50}. By incorporation of the inter-individual variability for EC_{50}, MVOF was decreased for plasma, brain ECF and brain tissue and additionally, a reduction of the residual error was observed. For these reasons, inter-individual variability in EC_{50} was included next to the residual error model, but obviously no distinction between these two random effects could be made.

Only over recent years, the results of studies on the occupancy of various SSRIs to SERT in healthy volunteers and depressed patients have been described by application of the neuroimaging techniques positron emission tomography (PET) or single photon emission computed tomography (SPECT) (Meyer et al., 2001;Meyer et al., 2004;Suhara et al., 2003;Kugaya et al., 2004;Kugaya et al., 2003;Malison et al., 1998). These studies concluded that during chronic treatment with clinical SSRI dosages, about 80% of SERT is occupied in various regions of the brain, implicating that such a high SERT occupancy might be required to increase 5-HT levels to the degree that most therapeutic effects occur. The current acute study showed a high level of SERT occupancy at relatively low fluvoxamine levels in plasma, brain and ECF. In the current study, SERT occupancy was analyzed after acute fluvoxamine administration. However, similar experimental conditions could be applied to

determine the effects on fluvoxamine SERT occupancy after chronic administration to explore the possible subjectivity of SERT to adaptive changes in rat frontal cortex.
In conclusion, three PK/PD models were developed in which SERT occupancy was related to the PK of fluvoxamine in plasma, brain ECF and brain tissue. These relationships were characterized by the B_{max} model, which could adequately describe observed fluvoxamine SERT occupancy in rat frontal cortex. Based on a comparison of the MVOF, fluvoxamine SERT occupancy could be best described by the PK of fluvoxamine in brain ECF and brain tissue, which could possibly be explained by description of the previously observed non-linearity in these PK profiles. The proposed PK/PD model constitutes a useful basis for prediction of the time-course of *in vivo* SERT occupancy in behavioral studies with SSRIs.

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Chapter 8

Physiological Model for the Effect of Fluvoxamine on 5-HT and 5-HIAA Concentrations in Rat Frontal Cortex

Marian Geldof, Jan Freijer, Bert Peletier, Ludy van Beijsterveldt, Meindert Danhof

Abstract

A physiological model is proposed to predict the time course of the concentrations of 5-HT and its metabolite 5-hydroxyindolacetic acid (5-HIAA) in rat frontal cortex following administration of SSRIs. In the model, SSRIs increase synaptic 5-HT concentrations by reversible blockade of the SERT in a direct concentration-dependent manner, while the 5-HT response is attenuated by negative feedback via 5-HT autoreceptors. In principle, the model allows for the description of oscillatory patterns in the time course of 5-HT and 5-HIAA concentrations in brain extracellular fluid, which are commonly observed.

The model was applied in a pharmacokinetic-pharmacodynamic (PK/PD) investigation on the time course of the microdialysate 5-HT and 5-HIAA response in rat frontal cortex following a 30-min intravenous infusion of 3.7 and 7.3 mg/kg fluvoxamine.

Directly after administration of fluvoxamine, concentrations of 5-HT were increased to approximately 450–600% of baseline values while 5-HIAA concentrations were decreased. Thereafter 5-HT and 5-HIAA concentrations gradually returned to baseline values in 6-10 hours respectively.

The PK/PD analysis revealed that inhibition of 5-HT reuptake was directly related to the fluvoxamine concentration in plasma, with 50% inhibition of 5-HT reuptake occurring at a plasma concentration of 1.1 ng/ml (EC_{50}). The levels of 5-HT at which 50% of the inhibition of the 5-HT response was reached (IC_{50}) amounted to 272% of baseline. The model was unable to capture the oscillatory patterns in the individual concentration time curves, which appeared to occur randomly.

The proposed physiological model is the first step in modeling of complex neurotransmission processes. The model constitutes a useful basis for prediction of the time course of median 5-HT and 5-HIAA concentrations in the frontal cortex in behavioral pharmacology studies *in vivo*.

Introduction

Reduced serotonergic transmission is a well-known characteristic in the pathogenesis of depression (Coppen, 1967;Owens and Nemeroff, 1994). Not surprisingly, Selective Serotonin Reuptake Inhibitors (SSRIs) constitute the first line of treatment in depressive disorders (Ables and Baughman, III, 2003;Isaac, 1999). SSRIs selectively and powerfully block the serotonin transporter (SERT) and thereby the reuptake of serotonin (5-hydroxytryptamine, 5-HT) in the presynaptic nerve terminal

resulting in increased extracellular 5-HT concentrations and enhancement of serotonergic neurotransmission (Bel and Artigas, 1992;Fuller, 1994).

In many articles, the effects of SSRIs on microdialysate concentrations of 5-HT and its metabolite, 5-hydroxyindolacetic acid (5-HIAA), have been reported (Bel and Artigas, 1992;Bosker et al., 1995;Denys et al., 2004;Gartside et al., 1995;Invernizzi et al., 1992;Malagie et al., 1995;Shachar et al., 1997). Typically, single dose administration of SSRIs results in a rapid increase in the mean extracellular levels of 5-HT as well as in a decrease in 5-HIAA levels in the various investigated brain regions.

A limitation of many studies reported to date is that only the mean profiles of the 5-HT and 5-HIAA response have been described. As a result, much information on the processes underlying the observed effects is lost. Moreover, in the studies in which results in individual animals are described, typically a wide intra- and inter-animal variation in the 5-HT response is reported. This variability is usually attributed to various stressors that can occur during execution of a study, and which influence the 5-HT release (Fujino et al., 2002;Kirby and Lucki, 1997;Adell et al., 1997;Kalen et al., 1989). In addition, the 5-HT system is implicated in the regulation of sleep and vigilance states (Hery et al., 1977;McGinty and Harper, 1976;Quay, 1968;Semba et al., 1984). Presumably this circadian rhythm contributes to the observed variability in the extracellular concentrations of 5-HT (Cespuglio, 1982;Cespuglio et al., 1983;Puizillout et al., 1979).

Although the complex mechanisms of the physiological control of 5-HT release are well established, these mechanisms have not been implicated in the observed fluctuating 5-HT response patterns following SSRI administration in a mechanistic manner. Briefly, following the release in the synaptic cleft, 5-HT is subject to active reuptake by the SERT. Moreover, increased synaptic 5-HT concentrations attenuate the synthesis and release of 5-HT via somatodendritic 5-HT_{1A} and terminal 5-H_{1B} autoreceptors of the serotonergic neurons. In theory, under distinct conditions, this type of feedback systems may display oscillatory behavior, thus contributing to the observed intra- and inter-individual variability in 5-HT response.

In the present investigation we propose a physiological model to describe the complex time course of the 5-HT and 5-HIAA response in rat frontal cortex following administration of SSRIs. In the model, SSRIs increase synaptic 5-HT concentrations by reversible blockade of the SERT in a direct concentration-dependent manner, while the 5-HT response is attenuated by negative feedback via 5-HT autoreceptors.

Physiological Model for SSRI-induced Increase in 5-HT and 5-HIAA Concentrations in Rat Frontal Cortex

In Figure 1, the various processes occurring at the serotonergic synapse are shown.

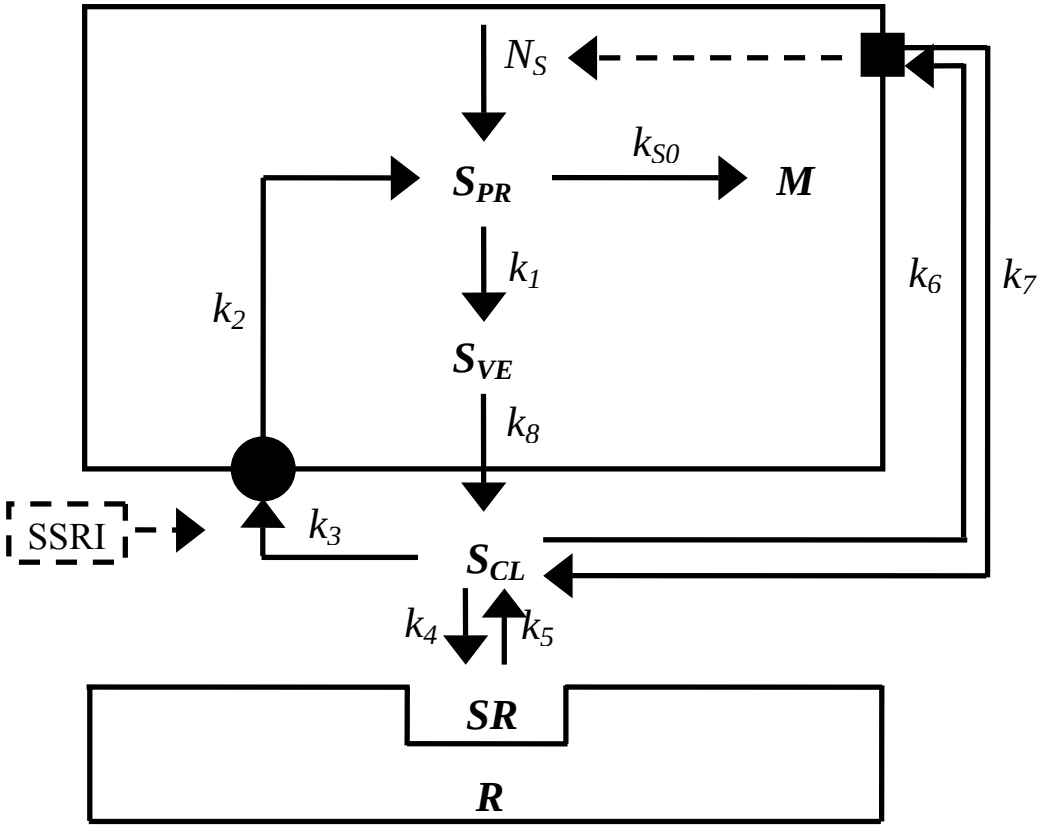


Figure 1. *Schematic presentation of serotonergic neurotransmission on which the developed PK/PD model is based. Depicted are the various processes important for the eventual serotonergic response. (SSRI: Selective Serotonin Reuptake Inhibitor, N_S: rate of formation of 5-HT and 5-HIAA in the presynaptic cell, S_{PR}: amount of 5-HT in the presynaptic cell, S_{VE} : amount of 5-HT stored in the vesicles of the presynaptic cell, S_{CL}: amount of 5-HT in the synaptic cleft, SR: Serotonin-Receptor complex, R: Response, M: Metabolite (5-HIAA), k_x: respective rate constants).*

The mechanisms which determine the serotonergic response include a) the synthesis of 5-HT in the presynaptic neuron, b) storage of 5-HT in the presynaptic vesicles, c) the release of 5-HT into the synaptic cleft, d) the binding of 5-HT to the postsynaptic receptors, the serotonin transporter (SERT) and the presynaptic autoreceptors, e) the reuptake of 5-HT via SERT and f) (intracellular) degradation of 5-HT into 5-HIAA. As the first step in the model development, a full mechanism-based model was constructed. To this end, differential equations were derived for the mass balance of 1) 5-HT in the presynaptic cell (free 5-HT which is important for 5-HIAA formation and 5-HT stored in vesicles which is important for the release of 5-HT in the synaptic

cleft), 2) 5-HT in the synaptic cleft, 3) 5-HT bound to SERT and free SERT, 4) 5-HT bound to autoreceptors and free autoreceptors and 5) 5-HT bound to receptors at the post synaptic cell and free receptors. An important feature of the model is the attenuation of 5-HT release from the presynaptic cell by a negative feedback mechanism via presynaptic autoreceptors. In the model it is assumed that inhibition of the synthesis of 5-HT in the presynaptic cell is maximal when the autoreceptor is fully occupied. Another important feature of the model is that it contains a differential equation for the production of 5-HIAA from free intracellular 5-HT. An important feature is that under distinct conditions this mechanistic model displays oscillatory behavior, which could possibly have been occurred in several individual rats (see results section).

Ultimately, the model describing the functioning of the serotonergic system was reduced by considering the values of the various rate constants and the mass balance terms. Specifically, the model was reduced to contain only expressions of the major rate limiting steps in serotonergic neurotransmission. This resulted in two differential equations describing the kinetics of 5-HT in the synaptic cleft and the kinetics of 5-HIAA in the presynaptic cell, which reflects 5-HIAA kinetics in the synaptic cleft. The effect of fluvoxamine was incorporated on the reuptake term ($k_3^* S_{CL}$). The following assumptions were made in the development of the reduced model: a) the binding of 5-HT to the autoreceptors is fast, b) the contribution of binding of 5-HT to postsynaptic receptors and presynaptic autoreceptors to the mass balance of 5-HT in the synaptic cleft is negligible, c) under normal conditions, the amount of transporter is not a limiting factor for 5-HT reuptake (i.e. SERT is not saturated with 5-HT), d) the amount of presynaptic 5-HT stored in the vesicles is large, e) equilibrium conditions are present at the transporter and f) the formation of 5-HIAA and elimination were rapid compared to the change of 5-HIAA concentration.

The kinetics of 5-HT in the synaptic cleft can be described by:

$$\frac{dS_{CL}}{dt} = N_{CL} - k_3^* I(C_{FLV}) S_{CL} \tag{1}$$

in which S_{CL} is the amount of 5-HT in the synaptic cleft, N_{CL} is the rate of release of 5-HT from the presynaptic cell into the synaptic cleft and $I(C_{FLV})$ is a function describing the inhibiting effect of fluvoxamine on the process of serotonergic neurotransmission.

In this equation, k_3^* is a rate constant and $I(C_{FLV})$ can be defined by:

$$I(C_{FLV}) = 1 - \frac{C_{FLV} E_{\max}}{EC_{50} + C_{FLV}} \quad (2)$$

in which C_{FLV} is the fluvoxamine plasma concentration, EC_{50} is the fluvoxamine plasma concentration at which 50% of the inhibition of the reuptake of 5-HT is reached and $E_{\max}$ is the maximal inhibition (*e.g.* 1).

The kinetics of the metabolite 5-HIAA (M) in the presynaptic cell can be described by:

$$\frac{dM_{PR}}{dt} = N_S^* I(S_{CL}) + k_3^{**} I(C_{FLV}) S_{CL} - k_0 M_{PR} \quad (3)$$

in which M_{PR} is the amount of M in the presynaptic cell, N_S^* is an aggregated parameter describing the rate of formation of 5-HIAA in the presynaptic cell, $I(S_{CL})$ describes the inhibition of the production of 5-HT in the presynaptic cell and k_3^{**} and k_0 are the respective rate constants. In equation (3), $I(S_{CL})$ is defined as:

$$I(S_{CL}) = 1 - \frac{S_{CL} I_{\max}}{IC_{50} + S_{CL}} \quad (4)$$

in which IC_{50} is the concentration of 5-HT (as a percentage of basal levels) at which 50% of the inhibition of the 5-HT response occurs and $I_{\max}$ is the maximal inhibition. The full details of the differential equations of the model describing serotonergic neurotransmission are presented in the appendix.

Materials and Methods

Chemicals

Fluvoxamine maleate and clovoxamine fumarate were kindly provided by Solvay Pharmaceuticals (Weesp, The Netherlands). Serotonin hydrochloride (5-hydroxytryptamine.HCl, 5-HT.HCl), 5-hydroxyindole-3-acetic acid (5-HIAA) and octanesulfonic acid were obtained from Sigma-Aldrich (Zwijndrecht, The Netherlands). Acetonitrile and methanol were obtained from Acros (Geel, Belgium). Dimethylsulfoxide (DMSO) and sodium hydroxide (NaOH) were purchased from Merck (Darmstadt, Germany). Acetic acid, potassium chloride (KCl), and ethylenediamine tetraacetic acid (EDTA) were obtained from Baker (Deventer, The Netherlands). Sodium dihydrogen phosphate ($NaH_2PO_4.H_2O$) was obtained from Fluka (Zwijndrecht, The Netherlands).

Animals

Male Wistar rats (Charles River Wiga GMBH, Sulzfeld, Germany, n=17) weighing 226-250 gram were housed in groups for 6-10 days under standard environmental conditions (ambient temperature 21°C, humidity 60%, 12-h light/dark cycle). The animals had free access to food (laboratory chow, Hope Farms, Woerden, The Netherlands) and acidified water. After surgery, the animals were housed individually for 1 week. The study was conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and approved by the Ethical Committee on Animal Experimentation of Leiden University.

Experimental Procedures

Surgical Procedures

Animals were anaesthetized by a subcutaneous injection of 0.1 ml/100 g Ketanest-S® ((S)-ketaminebase, Parke-Davis, Hoofddorp, The Netherlands) and an intramuscular injection of 0.01 ml/100 g Domitor® (medetomidine hydrochloride, Pfizer, Capelle a/d IJssel, The Netherlands). A cannula, filled with saline (0.9%, B. Braun Melsungen AG, Melsungen, Germany) containing heparin (20 IU/ml, Pharmacy, Leiden University Medical Center, Leiden, The Netherlands), was implanted in the right jugular vein (10 cm polyethylene tubing, Portex Limited, Hythe, United Kingdom; I.D.=0.58, O.D.=0.96 mm) for fluvoxamine administration and a cannula, filled with 25% (w/v) polyvinylpyrrolidone (PVP, Brocacef, Maarssen, The Netherlands) solution in saline (0.9%) containing heparin (20 IU/ml) to prevent blockade by blood clotting was implanted in the left femoral artery (4 cm polyethylene tubing, Portex Limited; I.D.=0.28, O.D.=0.61 mm heat-sealed to 18 cm polyethylene tubing, I.D.=0.58, O.D.=0.96 mm) for collection of blood samples. The cannulas were subcutaneously tunneled and externalized at the back of the neck. Subsequently, a microdialysis guide cannula (CMA/12, Aurora Borealis Control BV, Schoonebeek, The Netherlands) with a dummy probe was implanted in the frontal cortex (AP: 3.2; L: -3.0; V: -1.5 mm from bregma, in accordance with the atlas of Paxinos and Watson (Paxinos and Watson, 1982). Two support screws were placed and the guide was secured in place using dental cement (Simplex Rapid liquid and powder, Kemdent Associated Dental Products, United Kingdom).

Microdialysis Procedures

One day before implantation of the microdialysis probe (CMA/12, membrane length 3 mm, Aurora Borealis Control BV), they were perfused *ex vivo* overnight with

artificial cerebrospinal fluid (containing: 145 mM NaCl, 0.6 mM KCl, 1.0 mM $MgCl_2$, 1.2 mM $CaCl_2$, 0.2 mM ascorbic acid in 2 mM phosphate buffer pH 7.4) (Moghaddam and Bunney, 1989). The dummy probe was replaced by the microdialysis probe one day before start of the study. Flow rate during the whole experiment was constant at 2 µl/min and the outlet tubing was connected to a microdialysate fraction collector (Univentor 820, Antec Leyden BV, Zoeterwoude, The Netherlands). Four fractions were collected to determine basal concentrations of 5-HT and 5-HIAA, after a stabilization period of 1 hour following connection of the animals. After administration of fluvoxamine, microdialysate concentrations were measured for up to a (maximal) period of 10 hr. Sampling intervals were 30 min over the complete period yielding a volume of 60 µl microdialysate sample. At the end of the experiments, the brains of the animals were removed and the location of the microdialysis probe was verified by visual inspection. Microdialysate samples were collected in vials containing 20 µl 25 mM acetic acid at a temperature of 4°C in the fraction collector to prevent degradation of 5-HT and 5-HIAA, and immediately stored at -80°C until sample analysis.

Dosage Regimen

From the 17 animals used in the study, 9 animals received fluvoxamine at 3.7 mg/kg and 8 animals at 7.3 mg/kg via a 30-min intravenous infusion in the jugular vein at a flow rate of 20 µl/min using a BAS BeeHive pump (Bioanalytical Systems Inc., West Lafayette, USA). Solutions of fluvoxamine in physiological saline (0.9%) were prepared on the day of administration. Dosages and observed concentrations of fluvoxamine are expressed as free base.

Blood Sampling

A number of 6 arterial blood samples (100 µl) were collected at variable fixed time intervals from the cannula in the femoral artery over a time period between 5 and 530 min after fluvoxamine administration. After collection of each blood sample, an equal volume of heparinized 0.9% NaCl (20 IU/ml) was administered to the animal. Blood samples were collected in heparinized Eppendorf tubes and kept on ice during the experiment. After centrifugation (10 min, 5000 x *g*), 50 µl of plasma was transferred into a glass tube and stored at -20°C until sample analysis.

Drug Analysis

Fluvoxamine

Plasma samples were analyzed for fluvoxamine using liquid chromatography with tandem mass spectrometry (LC-MS/MS) as described earlier (Geldof et al., 2007). A volume of 50 µl of the calibration standards or independent Quality Control (QC) samples in DMSO was added to a volume of 50 µl blank plasma. For the plasma samples from the rats, a volume of 50 µl plasma was added to 50 µl DMSO. A volume of 50 µl of 500 ng/ml clovoxamine in DMSO were added to these samples as internal standard. Proteins were precipitated by adding 200 µl acetonitrile, the samples were centrifuged (10 min, 5000xg) and a volume of 20 µl was injected into the system. Plasma samples were quantified on a reversed phase LC column (BDS Hypersil C18, 3 µm particle size, 100x4.6 mm I.D.; Thermo Hypersil-Keystone, Brussels, Belgium). LC-MS/MS analysis was performed on an API-4000 MS/MS (Applied Biosystems, Toronto, Canada), coupled to an HPLC system (Agilent, Palo Alto, USA). The MS/MS operated in the positive ion mode using the TurboIonSpray-interface (electrospray ionization) was optimized for the quantification of fluvoxamine. The intra batch accuracy from independent QC samples was between 80 and 120% over the entire range of the samples. The limit of quantification for fluvoxamine was 1 ng/ml.

5-HT and 5-HIAA

Microdialysate samples of the frontal cortex were analyzed for 5-HT and 5-HIAA using HPLC with electrochemical detection. The system consisted of a LC-10AD HPLC pump (Shimadzu, ‘s Hertogenbosch, The Netherlands), a Waters 717 plus autosampler (Waters, Etten-Leur, The Netherlands), a pulse damper (Antec Leyden, Zoeterwoude, The Netherlands) and a DECADE electrochemical detector (Antec Leyden, The Netherlands) with a glassy carbon electrode. The electrochemical detector consisted of a VT-03 electrochemical flow cell with a 50 µm spacer and a standard Ag/AgCl reference electrode, filled with saturated KCl solution. The electrochemical detector was set at an optimal potential setting of 550 mV against the Ag/AgCl reference electrode, as determined by a voltammogram and sensitivity plot. Microdialysate samples were injected into the system without sample pre-treatment and quantified on a reversed phase LC column (Inertsil ODS-3, 3 µm particle size, 100x2.1 mm I.D.; Aurora Borealis Control BV, Schoonebeek, The Netherlands). A column oven (LKB, Woerden, The Netherlands) set at 30°C was used for both column and electrochemical detector. The mobile phase consisted of 75 mM

NaH_2PO_4, 0.1 mM EDTA, 0.2 mM octane sulphonic acid, adjusted to pH 4.8 with 1 M NaOH and 18% methanol. Mobile phase solvents were filtered through a 0.2 µm nylon filter (Alltech, Breda, The Netherlands), mixed and degassed continuously during analysis with helium. Flow rate was 0.2 ml/min. Fresh calibration standards and QC samples in artificial cerebrospinal fluid were added in each run. The detection limit for 5-HT was 0.5 fmol/fraction and for 5-HIAA the lowest point of the calibration curve was 1.6 pmol/fraction. Data were acquired and analyzed by the Millennium32 software (Waters, Etten-Leur, The Netherlands).

Data Analysis

Plasma fluvoxamine concentration *versus* time profiles were characterized by analysis of the actual plasma concentrations from the present study in combination with data from other investigations using a previously proposed population PK model (Geldof et al., 2007). Plasma fluvoxamine concentrations at the midpoints of the 5-HT and 5-HIAA collection intervals were predicted on the basis of the population PK parameter estimates.

The differential equations of the PK and PD model were implemented in the nonlinear mixed effects modeling software NONMEM (Version V, Level 1.1., NONMEM project group, University of California, San Francisco, USA) and using the subroutine ADVAN 6, which is a general nonlinear model that uses the numerical solution of the differential equations. Next, the model parameters were estimated by fitting the model to the data. All fitting procedures were performed on a personal computer (Intel® Pentium® 4 processor) running under Windows XP using the Compaq Visual FORTRAN standard edition 6.1 (Compaq Computer Cooperation, Euston, Texas, USA).

The model fit was evaluated on the basis of goodness of fit plots, parameter correlations and precision in parameter estimates. The residual variability in 5-HT and 5-HIAA levels in rat frontal cortex was assumed to be proportional to the 5-HT and 5-HIAA levels and was characterized by:

$$C_m = C_{p,5-HT/5-HIAA} \cdot (1 + \varepsilon_{5-HT/5-HIAA}) \quad (5)$$

in which C_m are the measured levels of 5-HT and 5-HIAA (as a percentage of basal levels), $C_{p,5-HT}$ and $C_{p,5-HIAA}$ are the population levels of 5-HT and 5-HIAA predicted by the model and values of ε_{5-HT} and ε_{5-HIAA} account for the residual deviance of the predicted increase in 5-HT or 5-HIAA levels from the observed increase in these

levels. Values for ε were assumed to be independently normally distributed with mean zero and variance σ^2.

Results

Pharmacokinetics of Fluvoxamine in Plasma

The PK parameter estimates for systemic clearance (*CL*), central volume of distribution (*V1*), two peripheral volumes of distribution (*V2*, *V3*) and inter-compartmental clearances (*Q2*, *Q3*) are depicted in Table 1. The population estimates were used in the present investigation to predict the fluvoxamine plasma concentration at the middle time point of the 5-HT and 5-HIAA collection interval.

Table 1. *Population estimates for the pharmacokinetic parameters of fluvoxamine in plasma after intravenous administration in 30 min (1, 3.7 and 7.3 mg/kg) obtained in the previously developed three-compartment pharmacokinetic model (Geldof et al., 2007).*

Parameter	Unit	Value
CL	ml/min	25.1
V1	ml	256
V2	ml	721
Q2[1]	ml/min	30.0
V3	ml	136
Q3	ml/min	1.0

[1] *typical value for a rat of 253 gram*

Effects of Fluvoxamine on Individual 5-HT and 5-HIAA levels in Rat Frontal Cortex

No time-related differences were observed in 5-HT and 5-HIAA levels in control rats after 30-min intravenous administration of saline (results not shown). Before fluvoxamine administration (from -120 until 0 min), averaged 5-HT and 5-HIAA levels were 11.5 ± 9.6 pg/ml (n=16) and 30.3 ± 11.9 ng/ml (n=14), respectively (values not corrected for recovery).

In Figure 2, the individual time courses of the 5-HT and 5-HIAA response in microdialysate of the frontal cortex following the administration of 3.7 or 7.3 mg/kg fluvoxamine are depicted.

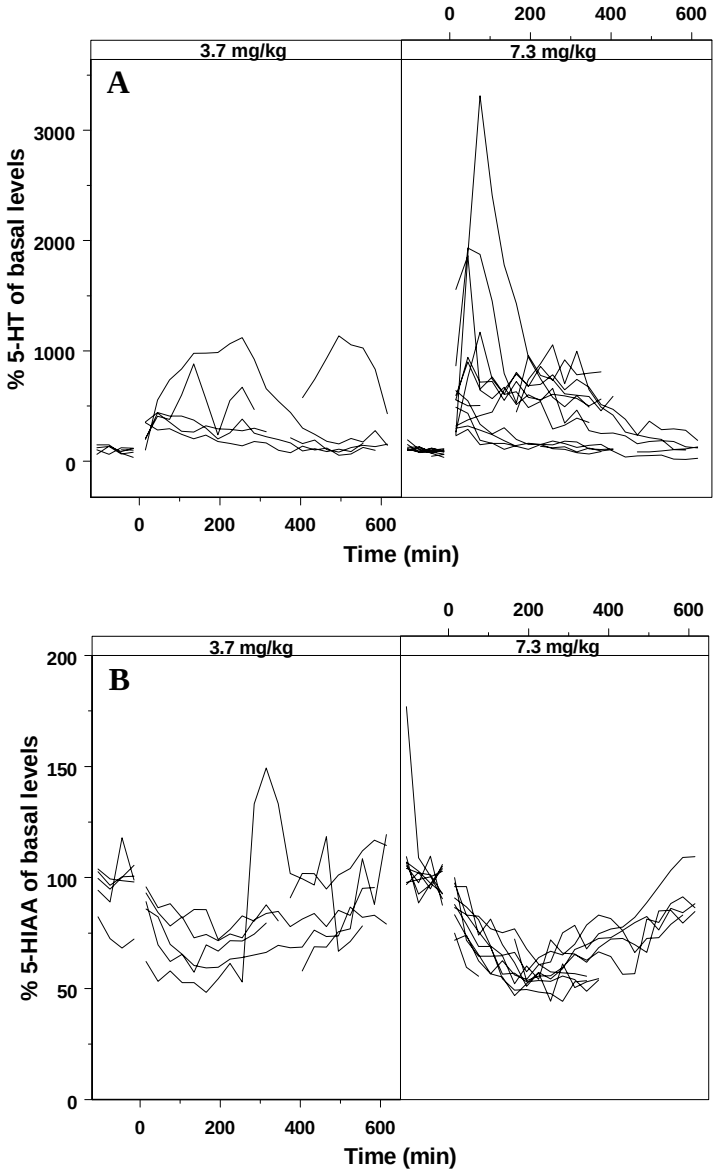


Figure 2. *Time course of 5-HT (a) and 5-HIAA (b) concentrations in microdialysate of the frontal cortex in individual rats. Levels of 5-HT and 5-HIAA are represented as a percentage of basal levels following a 30-min intravenous infusion of 3.7 and 7.3 mg/kg fluvoxamine.*

The 5-HT and 5-HIAA responses are represented as the percentage of the mean value of the 4 baseline samples, obtained after 1 hour of stabilization. Infusion of fluvoxamine resulted in an instant effect on both 5-HT (a) and 5-HIAA (b) microdialysate levels in rat frontal cortex.

Concentrations of 5-HT were immediately increased after administration of fluvoxamine while 5-HIAA levels were decreased after fluvoxamine administration. These concentrations gradually returned to baseline values within 6–10 hours respectively. Within and between individual animals significant variation in 5-HT concentrations was observed. No consistent pattern could be detected in the concentration *versus* time profiles of 5-HT as various distinctly different profiles

were observed. In some animals, an oscillating pattern in 5-HT levels was observed, which could be indicative of complex homeostatic feedback mechanisms at the level of the release of 5-HT. However, since the cause of most of the observed intra-individual variability in 5-HT concentrations could not be specified, ultimately, the observed variability was regarded to be random variation (see discussion). The variation between the individual profiles of 5-HIAA levels was much less compared to the variation in the 5-HT levels. The complete mechanistic model (appendix) was too complex for an adequate fit to the available data of the 5-HT and 5-HIAA response in individual animals. Therefore, the model was ultimately reduced as described in the introduction.

Effect of Fluvoxamine on Median 5-HT and 5-HIAA levels in Rat Frontal Cortex

The results of the effect of fluvoxamine on median 5-HT and 5-HIAA levels in the frontal cortex of the rat are depicted in Figure 3.

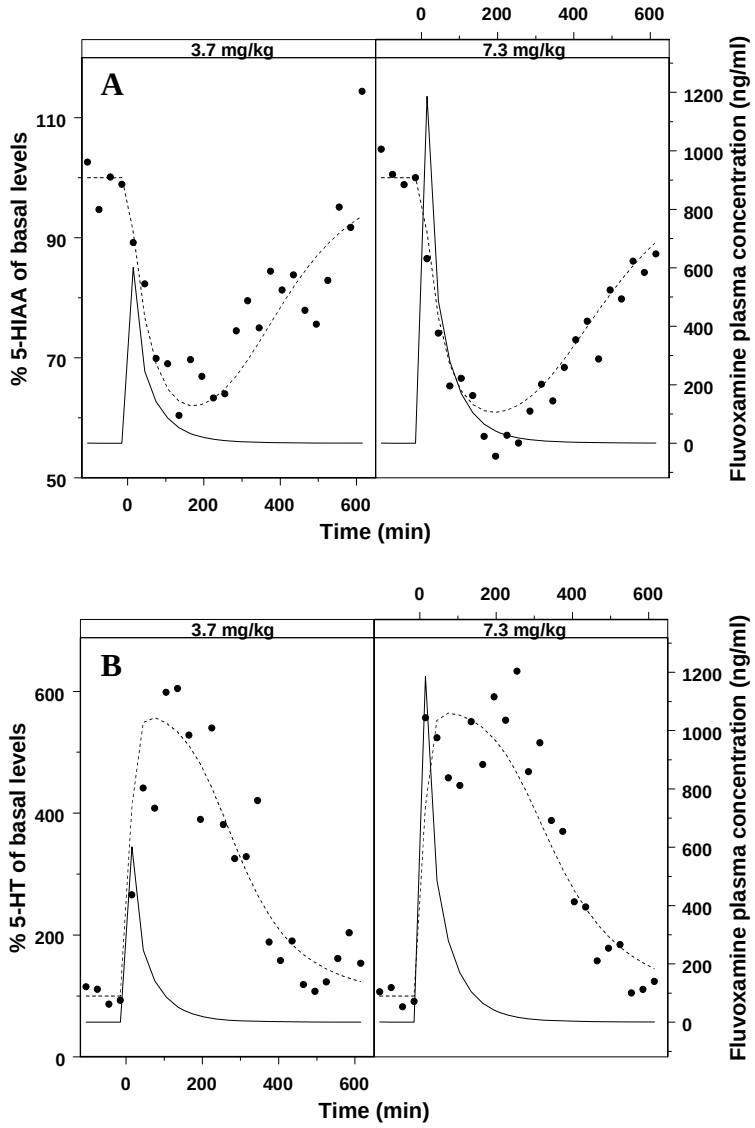
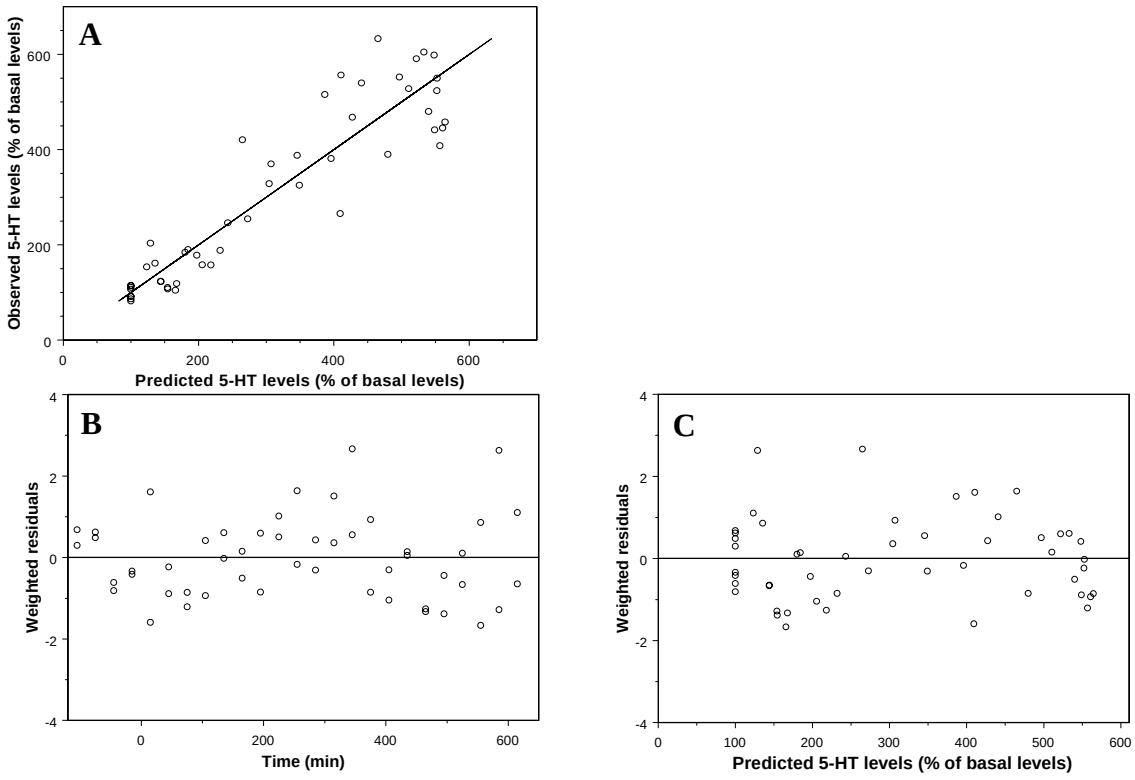


Figure 3. *Time course of the plasma concentration (solid line) and the resulting effects on median 5-HT (a) and 5-HIAA (b) concentrations (dashed line depicts the model fit, dots depict the observations) in microdialysate of rat frontal cortex (as a percentage of basal levels) following a 30-min intravenous infusion of 3.7 and 7.3 mg/kg fluvoxamine in rats.*

After an immediate increase in the first microdialysate fraction (15 min post-dose), the median 5-HT response remained fairly constant between 450-600% over a period of about 15-225 min (Figure 3a) after fluvoxamine administration. Thereafter, median 5-HT concentrations decreased to values slightly above basal levels at approximately 375 min until the end of the collection period at 615 min. After a fluvoxamine dose of 7.3 mg/kg, a similar profile was observed albeit that the increase in 5-HT response lasted longer compared to the lower dose. The maximum 5-HT response was similar with values between 450-600% over a period for approximately 15-315 min. Thereafter, median 5-HT response decreased in time to values slightly above basal levels at approximately 465 min until the end of the collection period at 615 min.
Median values of 5-HIAA response decreased after 3.7 mg/kg fluvoxamine (Figure 3b). Maximal decrease was observed at 75 min and remained fairly constant at values between 60-70% of basal levels until about 255 min after dose administration. Thereafter, median 5-HIAA response returned to baseline values at the end of the collection period at 615 min. After 7.3 mg/kg fluvoxamine, median 5-HIAA response reached a maximal decrease of 54% at 195 min. Thereafter, median 5-HIAA levels increased and reached slightly lower than median basal levels at 615 min.

PD Analysis of the Effects of Fluvoxamine on median 5-HT and 5-HIAA levels
The proposed mechanism-based PK/PD model could adequately describe the time course of the median 5-HT and 5-HIAA response in rat frontal cortex, as is illustrated in Figure 3. In Figure 4, the goodness-of-fit plots are depicted.



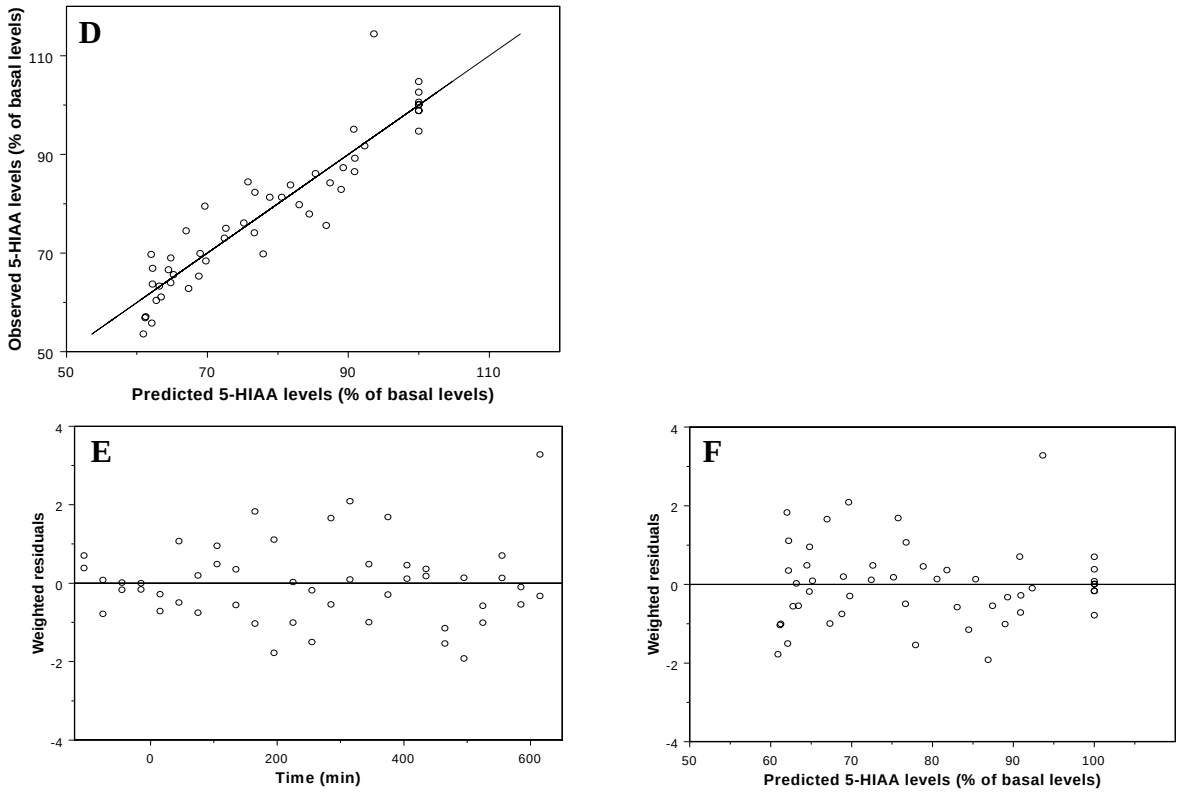


Figure 4. *Goodness-of-fit plots obtained by the developed PK/PD model in which the effects on median 5-HT and 5-HIAA levels were related to fluvoxamine concentrations in plasma. Depicted are scatter plots of observed effects on median 5-HT levels (as a percentage of basal levels) versus the model predictions (a), scatter plots of the weighted residuals versus time (b) and versus population model predictions (c) for 5-HT and for 5-HIAA (d-f).*

Observed 5-HT and 5-HIAA responses were in close agreement with the predicted median 5-HT and 5-HIAA concentrations. Specifically, no substantial or systemic deviation from the identity line was observed in the residual diagnostics.

The values of the structural PD parameters are presented in Table 2.

Table 2. *Parameter estimates in the PK/PD model describing the median 5-HT and 5-HIAA microdialysate levels in rat frontal cortex after intravenous administration in 30 min (3.7 and 7.3 mg/kg). Median 5-HT and 5-HIAA levels were related to the simulated fluvoxamine plasma concentrations, predicted using the previously developed PK model (Geldof et al, 2007); Table 1). Depicted are the estimates for* k_3^*, k_3^{**}, k_0, IC_{50}, $I_{\max}$, EC_{50}, $E_{\max}$ *and* σ^2 *with corresponding coefficient of variation (C.V.).*

Parameter	Unit	Value	C.V. (%)
k_3^*	1/min	0.41	53
k_3^{**}	1/min	0.0050	58
k_0	1/min	0.019	21
IC_{50}	%	272	24
$I_{\max}$	-	1.0	29
EC_{50}	ng/ml	1.1	5.4
$E_{\max}$	-	0.83	0.8
$\sigma^2 \varepsilon_{5-HT}$	-	0.049	17
$\sigma^2 \varepsilon_{5-HIAA}$	-	0.0046	29

*C.V. = Coefficient of Variation (standard error / value * 100%)*

The values of the parameters k_3^*, k_3^{**} and k_0 were estimated to be 0.41, 0.0050 and 0.019 1/min, respectively. The levels of 5-HT (as a percentage of basal levels) at which 50% of the inhibition of the 5-HT response was reached (IC_{50}) was estimated as 272% and the maximal inhibition ($I_{\max}$) as 1.0. The fluvoxamine plasma concentration at which 50% of the inhibition of the reuptake of 5-HT was reached (EC_{50}) was 1.1 ng/ml and the maximal inhibition ($E_{\max}$) 0.83. The coefficients of variation (C.V.) for the parameter estimates ranged between 0.8 and 58%.

Discussion

Since the aim of the present investigation was to describe the effects of fluvoxamine on the 5-HT and 5-HIAA response rather than a description of the absolute extracellular concentrations, no individual values for the *in vivo* recoveries for 5-HT and 5-HIAA were determined.

In the present investigation, a new mechanistic model for the effects of SSRIs on serotonergic transmission has been proposed. The physiological model can be used to predict the time course of the 5-HT and 5-HIAA response in rat frontal cortex following administration of SSRIs. In the model, SSRIs increase synaptic 5-HT concentrations by reversible blockade of the SERT in a direct concentration-dependent manner. A unique feature of the model is that it contains a function to

describe negative feedback via presynaptic 5-HT autoreceptors. In various studies it has been shown that the release of 5-HT from serotonergic nerve terminals is under the control of inhibitory 5-HT autoreceptors (for reviews see Briley et al., 1997 and Göthert and Schlicker, 1997) and (Blier and de Montigny, 1987;Briley and Moret, 1993;Moret and Briley, 1997;Sotelo et al., 1990;Sprouse and Aghajanian, 1987). Activation of somatodendritic 5-HT_{1A} autoreceptors and terminal 5-HT_{1B} autoreceptors decreases 5-HT neuronal firing and, in turn, the synthesis, metabolism and release of 5-HT. It should be remembered that a mathematical model is always a simplification of the actual situation. Similar, the model described in the present investigation is based on the main principles of serotonergic neurotransmission. Additional features like the suggestion that 5-HT cells are auto-controlled via long feed-back loops activated by postsynaptic 5-HT_{1A} receptors as well (Hajos et al., 1999) are not included in the model at this point.

Another important feature of the present investigation is that the 5-HIAA response was analyzed simultaneously with the 5-HT response, yielding additional information for the developed model.

The proposed model was applied in a PK/PD study in which detailed information was obtained on the time course of the microdialysate 5-HT and 5-HIAA response in the rat frontal cortex following administration of two different dosages of fluvoxamine. Observed effects on median levels of 5-HT and 5-HIAA were in accordance with previously reported studies on the acute effects of fluvoxamine on the mean levels of these neurotransmitters (Bel and Artigas, 1992;Bosker et al., 1995;Denys et al., 2004;Gartside et al., 1995;Invernizzi et al., 1992;Malagie et al., 1995;Shachar et al., 1997). Observed 5-HT and 5-HIAA response could be adequately described by the proposed model.

The observed decrease in 5-HIAA levels after fluvoxamine administration is in agreement with earlier observations (Bel and Artigas, 1992;Kalen et al., 1988). It has been shown that changes in extracellular 5-HIAA are poor indicators of changes in neuronal activity, and that variations in 5-HIAA primarily reflect intraneuronal 5-HT metabolism. The proposal that 5-HIAA primarily reflects intraneuronal metabolism of 5-HT and only to a minor degree the metabolism of 5-HT released from the nerve terminals and not release of 5-HT was made by (Grahame-Smith, 1971), and an increasing body of evidence now supports this conclusion (Wolf et al., 1985;Commissiong, 1985). If it is assumed that the rate of formation and elimination of 5-HIAA is equal to the rate of formation of 5-HT, then the rate of elimination of 5-

HIAA under steady-state conditions will be a direct measure of 5-HT synthesis (Neff and Tozer, 1968;Neckers and Meek, 1976).

In some animals, an oscillating pattern in 5-HT levels was observed, which is indicative of complex homeostatic feedback mechanisms at the level of the release of 5-HT. The original mechanistic model (before reduction) was able to show this oscillatory behavior in the 5-HT response, depending on the values of the specific system parameters. However, it is not known whether these oscillations are actually present because of the observed additional random variation of the 5-HT response within and between the animals. The cause of the observed large intra-individual variability in 5-HT concentrations could not be precisely described by the model and presumably reflects also the random variation caused by environmental factors such as stress. As a result, the oscillating patterns were no longer observed in the mean and median 5-HT concentration *versus* time profiles at both dosages. For these reasons, the observed variability was treated as random variation in this investigation. The reduced mechanistic model was able to adequately describe the observed median 5-HT and 5-HIAA concentrations.

In the development and reduction of the model, a number of assumptions were made. These assumptions were made on a rational basis, focusing on the main capacity limiting steps in the process of serotonergic neurotransmission. The assumption that binding of 5-HT to the autoreceptors is relatively fast (and therefore not a rate-limiting step) is reasonable, given the fast response and the observation in other studies that the increase in 5-HT levels by SSRIs is even further enhanced in combination with autoreceptor antagonists (Artigas, 1993;Hjorth, 1993;Hjorth and Auerbach, 1994). Furthermore, it seems reasonable that under normal conditions the amount of SERT is not a (capacity) limiting factor for the reuptake of 5-HT (i.e. SERT is not saturated with 5-HT). Under basal conditions, the 5-HT concentrations are low, which is indicative of the high efficiency of the 5-HT reuptake process. This makes it unlikely that the reuptake process is saturable under physiological conditions. Furthermore, in the present and other studies, a clear effect of fluvoxamine (via binding to SERT) was observed. The observed variation in the 5-HT response in individual animals was probably not caused by a difference in the amount of 5-HT present in the synaptic vesicles (S_{VE}), which presumably is available in large excess. This is supported by the observation that various behavioral effects could be observed in the rat, instantly after administration of PCA (*para*-chloroamphetamine), which acts via depletion of 5-HT from the synaptic vesicles into the synaptic cleft (Geldof et al., *in preparation*). These behavioral effects could not

be observed after administration of fluvoxamine alone. Apparently, the behavioral effects after PCA administration result indeed from the very large increases in extracellular 5-HT caused by the releasing drugs; the smaller increases caused by SSRIs are insufficient to elicit the effects. The observation that the behavioral effects could not be observed after administration of fluvoxamine alone indicates that the amount of 5-HT in the presynaptic vesicles is very large and constant and that there is a constant supply of 5-HT in the synaptic cleft. In contrast, the amount of free 5-HT is not constant but changes over time, which is important for the concentrations of the metabolite 5-HIAA.

The problem of variability in the response variable, complicating the identifiability of the model is clearly not specific for 5-HT levels. Also, in other investigations, manipulations of the data and/or the model have been described to simplify the system and model. An example of simplification of a PK/PD model is the proposed model for a selective irreversible antagonist (Hutmacher et al., 2005). In this investigation, the techniques used for model reduction were comparable to the investigation described in the present article. The problems of uncertainties inherent to kinetic data in relation to model complexity have been reviewed by Bernillon and Bois with regard to a Bayesian perspective (Bernillon and Bois, 2000).

In a recent investigation, the acute effects of escitalopram (S-citalopram), which is also a SSRI, on the brain extracellular 5-HT levels in the hippocampus were explored (Bundgaard et al., 2006). Generally, comparable profiles of the mean 5-HT concentrations could be observed as in the present investigation. It was observed that increasing the dose from 5 to 10 mg/kg did not result in further enhancement of the acute 5-HT response. This is in the line with the results of the present investigation in which a similar increase in 5-HT levels was observed at both fluvoxamine dosages. In contrast to the results for escitalopram, 5-HT concentrations seemed to decrease at a later time point after administration of the higher fluvoxamine dose. Bundgaard and coworkers developed a feedback turnover model that mimicked the drug-induced effects of acute escitalopram administration. The turnover model included an inhibitory feedback moderator component, which adequately described observed 5-HT concentrations. The authors state that the inhibitory feedback moderator compartment, which was included in the feedback turnover model acting on kin, reflected the inhibitory segment of the pharmacological response. However, no experimental data were in the model for this moderator compartment making the model somewhat hypothetical. The authors considered the model as having a mechanistic background in terms of the acute action of SSRIs on the 5-HT turnover

in the rat. In the present investigation for the serotonergic response to fluvoxamine, a more mechanistic approach was used for description of the various processes occurring at serotonergic neurotransmission. Differential equations were derived for description of the most important processes at serotonergic neurotransmission. The model was reduced on a rational basis for description of the median 5-HT and 5-HIAA response. Another important advantage of the present investigation is that the response of both 5-HIAA and 5-HT were included in the model in contrast to the study of Bundgaard and coworkers. By simultaneous analysis of the metabolite and 5-HT concentrations in individual animals, additional information on the serotonergic system was obtained.

In a previous investigation, the PK/PD correlation for the occupancy of fluvoxamine to the SERT in rat frontal cortex was explored (Geldof et al., *in preparation*). This investigation showed that maximal SERT occupancy was reached instantly after a 30-min intravenous fluvoxamine infusion of 1 and 7.3 mg/kg, which maintained for 1.5 and 7 h after dosing, respectively. Thereafter, SERT occupancy decreased linearly at a rate of 8 %/hr. In the present investigation, median 5-HT concentrations decreased at approximately 6 h after 7.3 mg/kg fluvoxamine, which could be related to the occupancy of fluvoxamine to SERT. The relationship between SERT occupancy and 5-HT and 5-HIAA concentrations in rat frontal cortex will be the subject of future investigations in our laboratory.

In future investigations, the placebo response should be characterized and analyzed in more detail as well. Such a solid dataset on the 5-HT and 5-HIAA response in control rats would make it possible to develop a PK/PD model for the baseline responses. The effect of fluvoxamine could then be modeled on top of these control values instead of relating the observed response to the basal values, as described in the present investigation.

In conclusion, a physiological model was proposed to predict the time course of the concentrations of 5-HT and 5-HIAA in rat frontal cortex following administration of SSRIs. The PK/PD investigation showed that considerable random variation in the 5-HT concentration *versus* time profiles could be observed in individual animals. Since the observed large intra-individual variability in 5-HT concentrations could not be precisely described by the model it was ultimately regarded to be random variation. Various parameters important for the serotonergic system could not be identified using the developed model. Therefore, the model was reduced, enabling an adequate description of the median responses of 5-HT and 5-HIAA. The proposed physiological model is the first step in modeling of complex neurotransmission

processes and the model constitutes a useful basis for prediction of the time course of median 5-HT and 5-HIAA concentrations in the frontal cortex in behavioral pharmacology studies *in vivo*, which is an important intermediate step in the pharmacodynamics of SSRIs. Ultimately, additional studies need to be performed for a detailed characterization of serotonergic neurotransmission.

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Appendix 1

Derivation of the proposed PK/PD model for the description of serotonergic neurotransmission and the effect of fluvoxamine on this process

The PK/PD model for the description of the effects of fluvoxamine on serotonergic neurotransmission was obtained in three steps: 1) differential equations were developed for the description of the various processes occurring at a serotonergic synapse, 2) reduction of the comprehensive model and 3) implementation of the effects of fluvoxamine in the reduced model.

1. Mass balance

The mass balance of 5-HT (serotonin, S) at a synapse (Figure 2) can be described by the following set of differential equations.

The mass balance in the intracellular space of the presynaptic cell can be described by:

$$\frac{dS_{PR}}{dt} = N_S I(SA) - k_1 S_{PR} + k_2 ST - k_{S0} S_{PR} \quad (1)$$

in which S_{PR} is the amount of free 5-HT in the presynaptic cell, N_S is the production of 5-HT in the presynaptic cell, I is the inhibition term (between 0 and 1) on the production of 5-HT in the presynaptic cell, SA is the Serotonin-Autoreceptor complex, ST is the Serotonin-(reuptake)Transporter complex and k_1, k_2 and k_{S0} are the respective rate constants.

The mass balance in the serotonergic vesicles in the presynaptic neuron can be described by:

$$\frac{dS_{VE}}{dt} = k_1 S_{PR} - k_8 S_{VE} \quad (2)$$

in which S_{VE} is the amount of 5-HT stored in the vesicles of the presynaptic cell and k_8 is the respective rate constant.

The amount of 5-HT in the synaptic cleft (S_{CL}) can be described by:

$$\frac{dS_{CL}}{dt} = k_8 S_{VE} - k_3 S_{CL} T - k_4 S_{CL} R + k_5 SR - k_6 S_{CL} A + k_7 SA \quad (3)$$

in which T is the reuptake Transporter, R is postsynaptic Receptor, SR is the Serotonin-postsynaptic Receptor complex, A is the Autoreceptor and k_3, k_4, k_5, k_6 and k_7 are the respective rate constants.

The mass balance of 5-HT bound to the reuptake transporter and the mass balance of free reuptake transporter can be described by:

$$\frac{dST}{dt} = k_3 S_{CL} T - k_2 ST \quad (4)$$

$$\frac{dT}{dt} = -k_3 S_{CL} T + k_2 ST \quad (5)$$

The mass balance of 5-HT bound to the Autoreceptor (A) and mass balance of free autoreceptor can be described by:

$$\frac{dSA}{dt} = k_6 S_{CL} A - k_7 SA \tag{6}$$

$$\frac{dA}{dt} = -k_6 S_{CL} A + k_7 SA \tag{7}$$

The mass balance of 5-HT bound to the receptors at the postsynaptic cell can be expressed by:

$$\frac{dSR}{dt} = k_4 S_{CL} R - k_5 SR \tag{8}$$

$$\frac{dR}{dt} = -k_4 S_{CL} R + k_5 SR \tag{9}$$

When exploring the release of 5-HT from the presynaptic cell into the synaptic cleft, a negative feedback mechanism is of interest. This mechanism assumes that inhibition of the production of 5-HT in the presynaptic cell is maximum when the occupancy of 5-HT to the Autoreceptor is saturated, i.e. $I(SA)$ becomes zero.

$$I(SA) = 1 - \frac{SA}{SA + I_{50}} \tag{10}$$

in which I_{50} corresponds to 50% inhibition on the production of 5-HT in the presynaptic cell.

The production of the metabolite 5-HIAA (M) in the presynaptic cell is described by the following differential equation:

$$\frac{dM_{PR}}{dt} = k_{S0} S_{PR} - k_{M0} M_{PR} \tag{11}$$

in which M_{PR} is the amount of M in the presynaptic cell and k_{S0} and k_{M0} are the respective rate constants.

2. Model reduction

The model system described in section 1 can now be reduced to a more simple system of two differential equations when considering the orders of magnitude of the rate constants and the mass balance terms.

First, it is assumed that binding of 5-HT to the autoreceptor occurs relatively fast. Therefore, the differential equation for the binding to this receptor can be expressed by the following equilibrium equation:

$$\lim_{k \to \infty} \frac{1}{k_6} \frac{dSA}{dt} = S_{CL} A - \frac{k_7}{k_6} SA = 0 \tag{12}$$

Because the free amount of autoreceptor relates to the total amount of autoreceptor (A_0) by:

$$A = A_0 - SA \tag{13}$$

and the distribution coefficient k_{DA} is defined as:

$$k_{DA} = \frac{k_7}{k_6} \tag{14}$$

equation 12 results in the following relationship:

$$SA = \frac{S_{CL} A_0}{k_{DA} + S_{CL}} \tag{15}$$

Substitution of equation 15 in equation 10 results in:

$$I(SA) = 1 - \frac{\dfrac{S_{CL} A_0}{k_{DA} + S_{CL}}}{\dfrac{S_{CL} A_0}{k_{DA} + S_{CL}} + I_{50}} \tag{16}$$

$$I(S_{CL}) = 1 - \frac{S_{CL} A_0}{S_{CL} A_0 + I_{50}(k_{DA} + S_{CL})} \tag{17}$$

$$I(S_{CL}) = 1 - \frac{S_{CL} A_0 /(I_{50} + A_0)}{I_{50} k_{DA} /(I_{50} + A_0) + S_{CL}} \tag{18}$$

$$I(S_{CL}) = 1 - \frac{S_{CL} I_{\max}}{IC_{50} + S_{CL}} \tag{19}$$

in which the maximal inhibition ($I_{\max}$) is described by:

$$I_{\max} = A_0 /(I_{50} + A_0) < 1 \tag{20}$$

and

$$IC_{50} = I_{50} k_{DA} /(I_{50} + A_0) \tag{21}$$

in which IC_{50} corresponds to the % 5-HT at which 50% of the maximal inhibition of the 5-HT response is reached.

Second, it is assumed that the mass balance of 5-HT in the synaptic cleft is hardly influenced by binding to the various receptor sites at the postsynaptic cell as well as to the autoreceptor, since these amounts of 5-HT are small compared to the amount of 5-HT present in the synaptic cleft and the amount involved in the reuptake.

Further, it is assumed that under natural conditions the amount of transporter is not a limiting factor for reuptake, such that T in equation 3 can be considered as a constant.

Another consideration relates to the amount of presynaptic 5-HT present in serotonergic vesicles. Since the storage capacity of 5-HT in the vesicles is high, the production term in the synaptic cleft can be considered as a constant ($k_8 << k_1$).

Therefore, equation 3 becomes:

$$\frac{dS_{CL}}{dt} = N_{CL} - k_3^* S_{CL} \tag{22}$$

with

$$k_3^* = k_3 T \tag{23}$$

$$N_{CL} = k_8 S_{VE} \tag{24}$$

It is further assumed that binding of 5-HT to the transporter occurs relatively fast. Therefore, the differential equation for the binding to this transporter can be expressed by the following equilibrium equation:

$$\lim_{k\to\infty}\frac{dST}{dt} = k_3^* S_{CL} - k_2 ST \tag{25}$$

$$ST = \frac{k_3^*}{k_2} S_{CL} \tag{26}$$

resulting in the following equation:

$$\frac{dS_{PR}}{dt} = N_S I(S_{CL}) - k_1 S_{PR} + k_3^* S_{CL} - k_{S0} S_{PR} \tag{27}$$

Third, it is assumed that the formation and elimination of the metabolite (M) occur rapid compared to the change of metabolite concentration:

$$\frac{dM_{PR}}{dt} << (k_{S0} S_{PR} - k_{M0} M_{PR}) \tag{28}$$

Then:

$$S_{PR} = \frac{k_{M0}}{k_{S0}} M_{PR} \tag{29}$$

and:

$$\frac{dS_{PR}}{dM_{PR}} = \frac{k_{M0}}{k_{S0}} \tag{30}$$

Accordingly:

$$\frac{dM_{PR}}{dt} = \frac{k_{S0}}{k_{M0}} \left\{ N_S I(S_{CL}) - k_1 \frac{k_{M0}}{k_{S0}} M_{PR} + k_3^* S_{CL} - k_{S0} \frac{k_{M0}}{k_{S0}} M_{PR} \right\} \tag{31}$$

$$\frac{dM_{PR}}{dt} = N_S^* I(S_{CL}) + k_3^{**} S_{CL} - k_0 M_{PR} \tag{32}$$

with:

$$N_S^* = \frac{k_{S0} N_S}{k_{M0}} \tag{33}$$

$$k_3^{**} = \frac{k_{S0} k_3^*}{k_{M0}} \tag{34}$$

$$k_0 = k_{S0} + k_1 \tag{35}$$

Summarizing, the following two differential equations can describe the kinetics of 5-HT in the synaptic cleft and kinetics of its metabolite in the presynaptic cell:

$$\frac{dS_{CL}}{dt} = N_{CL} - k_3^* S_{CL} \tag{36}$$

$$\frac{dM_{PR}}{dt} = N_S^* I(S_{CL}) + k_3^{**} S_{CL} - k_0 M_{PR} \tag{37}$$

with

$$I(S_{CL}) = 1 - \frac{S_{CL} I_{\max}}{IC_{50} + S_{CL}} \tag{38}$$

3. Reduced model in the presence of fluvoxamine (FLV)

In the presence of fluvoxamine, the serotonin reuptake is inhibited, which can be expressed by adding a drug effect on the reuptake term, $k_3^* S_{CL}$:

$$\frac{dS_{CL}}{dt} = N_{CL} - k_3^* I(C_{FLV}) S_{CL} \tag{39}$$

$$\frac{dM_{PR}}{dt} = N_S^* I(S_{CL}) + k_3^{**} I(C_{FLV}) S_{CL} - k_0 M_{PR} \tag{40}$$

$$I(S_{CL}) = 1 - \frac{S_{CL} I_{\max}}{IC_{50} + S_{CL}} \tag{41}$$

$$I(C_{FLV}) = 1 - \frac{C_{FLV} E_{\max}}{EC_{50} + C_{FLV}} \tag{42}$$

Chapter 9

Pharmacokinetic/Pharmacodynamic Modeling of the Effect of Fluvoxamine on *p*-Chloroamphetamine-induced behavior

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Abstract

The pharmacokinetic/pharmacodynamic (PK/PD) correlation of the effect of fluvoxamine on *para*-chloroamphetamine (PCA)-induced behavior was determined in the rat.

Rats (n=72) with permanent arterial and venous cannulas received a 30-min intravenous infusion of 1.0, 3.7 or 7.3 mg/kg fluvoxamine. At various time points after the start of fluvoxamine administration, a single dose of PCA (2.5 mg/kg) was injected in the tail vein and resulting behavioral effects, excitation (EXC), flat body posture (FBP) and forepaw trampling (FT), were immediately scored (scores: 0, 1, 2 or 3) over a period of 5 min. In each individual animal the time course of the fluvoxamine plasma concentration was determined up to the time of PCA administration. Observed behavioral effects were related to fluvoxamine plasma concentrations.

Fluvoxamine pharmacokinetics was successfully described by a population 3-compartment pharmacokinetic model. The effects of fluvoxamine on PCA-induced behavior (probability of EXC, FBP and FT) were directly related to fluvoxamine plasma concentration on the basis of the proportional odds model. For EXC, EC_{50} values for the cumulative probabilities (P(Y<1), P(Y<2), P(Y<3) were 237 ± 39 ng/ml, 174 ± 28 ng/ml and 100 ± 20 ng/ml, respectively. Slightly higher EC_{50} values were obtained for the corresponding effects on FBP and FT.

This investigation demonstrates the feasibility of PK/PD modeling of categorical drug effects in animal behavioral pharmacology. This constitutes a basis for the future development of a mechanism-based PK/PD model for fluvoxamine in this paradigm.

Introduction

Reduced serotonergic neurotransmission is a well-known characteristic in the pathogenesis of depression (Coppen, 1967;Owens and Nemeroff, 1994). Selective Serotonin Reuptake Inhibitors (SSRIs) constitute the first line of treatment in depressive disorders (Ables and Baughman, III, 2003;Isaac, 1999). By blockade of the serotonin transporter (SERT), extracellular serotonin (5-hydroxytryptamine, 5-HT) concentrations are increased, resulting in enhancement of serotonergic neurotransmission (Bel and Artigas, 1992;Fuller, 1994). Although SSRIs rapidly inhibit the reuptake of 5-HT, maximal antidepressant effects are only observed after weeks of chronic treatment, indicating that complex regulatory control mechanisms are an important determinant of the clinical effect.

The effects of SSRIs have been extensively investigated in a wide array of behavioral paradigms, such as the forced swim (Kelliher et al., 2003), the tail suspension (Teste et al., 1993) and the learned helplessness test (Takamori et al., 2001). Analysis of the relationship between the pharmacokinetics (PK) and pharmacodynamics (PD) in these animal behavioral models could provide novel insights in the mechanisms of the time dependencies in the PD of SSRIs. Furthermore, PK/PD modeling is of potential interest for the prediction of the effective drug concentrations in humans on the basis of information from preclinical investigations (Danhof et al., 2006).

In recent years, important progress has been made in the field of mechanism-based PK/PD modeling. The objective of mechanism-based PK/PD modeling is to characterize, in a mechanistic and strictly quantitative manner, the time-course of the intensity of the drug effect *in vivo*. A pertinent feature of mechanism-based PK/PD models is that they contain specific expressions to describe processes on the causal path between drug administration and effect (Danhof et al., 2005). Recently, novel mechanism-based PK/PD models have been proposed with improved properties for extrapolation and prediction, specifically also with regard to the extrapolation from animals to humans (Rosario et al., 2005). So far however, very few studies have been reported on the PK/PD modeling of behavioral paradigms with psychotropic drugs (Della Paschoa et al., 1998;Jonker et al., 2003;Vis et al., 2001).

Potential complicating factors of PK/PD modeling in behavioral pharmacology include the interference of blood sampling with the measured effect, limitations with regard to the collection of PK and/or PD data and the fact that often non-continuous pharmacodynamic endpoints are obtained. These complexities could in part be overcome by application of the population approach. In the population approach, (sparse) observations on the PK and/or PD in several subjects are simultaneously analyzed, while explicitly taking into account both the inter-individual variability in the model parameters as well as the intra-individual residual error variability (Schoemaker and Cohen, 1996). The concepts and applications of population PK/PD modeling have been described in various reviews (Hashimoto and Sheiner, 1991;Sheiner and Ludden, 1992;Vozeh et al., 1996;Yuh et al., 1994). In the meantime population PK/PD models have been successfully applied to continuous and non-continuous measures of drug effect, for both direct (Karlsson et al., 1995;Minto et al., 1997;Schnider et al., 1996) and indirect (Bouillon et al., 1996;Minto et al., 1997a) PD models.

In the present article, we describe the population PK/PD modeling of the effect of fluvoxamine on behavioral effects induced by administration of *para*-

chloroamphetamine (PCA). PCA is taken up into serotonergic nerve terminals by the SERT, after which it accumulates in the synaptic vesicles. In the synaptic vesicles, PCA displaces 5-HT, which is subsequently released, ultimately resulting in depletion of tissue 5-HT stores (Berger et al., 1989;Fuller et al., 1975;Fuller, 1992). By increasing the extracellular concentrations of 5-HT in the synaptic cleft, synaptic 5-HT receptors are activated yielding typical behavioral effects, such as excitation (EXC), flat body posture (FBP) and forepaw trampling (FT) (Fuller, 1992;Hutson and Curzon, 1989;Trulson and Jacobs, 1976). Since PCA produces its biochemical and behavioral effects only after uptake into serotonergic neurons via SERT, its effects are inhibited by SSRIs (Berger et al., 1989;Lassen, 1978;Fuller, 1980).

A specific complexity for PK/PD modeling PCA-induced behavior is that, within the time frame of PK/PD investigations only a singe behavioral observation is feasible in each animal because PCA administration results in a depletion of 5-HT stores (Fuller, 1992). Furthermore, observed behavioral effects are not continuous but ordered categorical data, which requires specific data analysis techniques to be applied. Ordered categorical data are frequently collected in clinical trials, for example when using pain scores and sedation scales as pharmacodynamic endpoints (Sheiner, 1994;Mandema and Stanski, 1996;Knibbe et al., 2002;Olofsen et al., 2005). In the present investigation, the behavioral observations were measured on an ordered categorical scale with four levels (score 0, 1, 2 or 3). A commonly used model for ordered categorical data is a generalization of the logistic regression model (Harrell, Jr. et al., 1988), which estimates the probability of the data to be in a certain category, on a scale with multiple categories (Agresti, 1999). The model uses the logit link function to link the observations to the cumulative probabilities of the model parameters. Therefore, rather than modeling the categories themselves, a transformation of the probability of being in a particular set of categories is modeled. The model assumes that the treatment parameters have the same effect on all categories or, in other words, have an identical effect on the predictors for each cumulative probability (parallel slopes assumption). The model is therefore often referred to as the proportional odds model. The proportional odds model was introduced in the field of PK/PD modeling by Sheiner in 1994 (Sheiner, 1994).

In previous investigations, a population PK model of fluvoxamine in plasma has been proposed which enables full characterization of the plasma concentration *versus* time profile in individual animals on the basis of sparse blood concentrations (Geldof et al., 2007). In the present study, this model is applied to derive relevant plasma concentrations of fluvoxamine at the time of the behavioral observations. Observed

PD effects, EXC, FBP and FT were implemented in the nonlinear mixed effects modeling program NONMEM using the proportional odds model and related to fluvoxamine plasma concentrations in a PK/PD model.

Methods

Chemicals

Fluvoxamine maleate and clovoxamine fumarate were kindly provided by Solvay Pharmaceuticals (Weesp, The Netherlands). Dimethylsulfoxide (DMSO) was obtained from Merck (Darmstadt, Germany) and acetonitrile from Acros (Geel, Belgium). *Para*-chloroamphetamine (PCA) was obtained from Sigma-Aldrich (Bornem, Belgium) and millipore water from a Milli-Q system (Millipore SA, Molsheim, France).

Animals

Male Wistar rats (Charles River Wiga GMBH, Sulzfeld, Germany) were housed in groups for 1 week, under standard environmental conditions (ambient temperature 21±2°C, relative humidity 65±15%, 12-h light/dark cycle). Animals had free access to food (A04C fine ground diet, SAFE, Augy, France) and tap water. After surgery, the animals were housed individually for 3 days and weighed 212-287 gram at the start of dose administration. The animal care and use committee of Johnson & Johnson Pharmaceutical Research and Development approved all studies.

Surgical Procedures

The animals were anaesthetized by a subcutaneous injection of Ketanest-S® ((S)-ketaminebase (0.1 ml/100 g), Parke-Davis, Hoofddorp, The Netherlands) and an intramuscular injection of Domitor® (medetomidine hydrochloride (0.01 ml/100 g), Pfizer, Capelle a/d IJssel, The Netherlands). Animals were implanted with a permanent cannula in the right jugular vein for fluvoxamine administration and a permanent cannula in the left femoral artery for collection of blood samples. The cannulas were subcutaneously tunneled and externalized at the back of the neck. The venous cannula was filled with NaCl (0.9%, B. Braun Melsungen AG, Melsungen, Germany) containing heparin (20 IU/ml, Pharmacy, Leiden University Medical Center, Leiden, The Netherlands) and the arterial cannula was filled with 25% (w/v) polyvinylpyrrolidone (PVP, Brocacef, Maarssen, The Netherlands) solution in NaCl (0.9%) containing heparin (20 IU/ml) to prevent blockade by blood clotting.

Dosage Regimen

Fluvoxamine (1.0, 3.7 or 7.3 mg/kg) was administered via a 30-min intravenous infusion (BAS BeeHive, Bioanalytical Systems Inc. USA) in the jugular vein cannula at a flow rate of 20 µl/min. Solutions of fluvoxamine in physiological saline (0.9%) were prepared on the day of administration. Dosages and observed concentrations of fluvoxamine are expressed as free base.

Blood Sampling

A number between 2 and 18 arterial blood samples (100 µl) were collected from the animals between 5 and 720 min after fluvoxamine administration from the cannula in the femoral artery, dependent on the time of PCA administration (see section '*PCA assay*'). After collection of each blood sample, an equal volume of heparinized 0.9% NaCl (20 IU/ml) was administered to the animal. Blood samples were collected in heparinized Eppendorf tubes and kept on ice during the experiment. After centrifugation (10 min, 5000xg), 50 µl plasma was transferred into a glass tube and stored at -20°C until sample analysis.

Drug Analysis

Fluvoxamine samples were analyzed using the liquid chromatography with tandem mass spectrometry (LC-MS/MS) as described earlier (Geldof et al., 2007). Briefly, volumes of 50 µl of the calibration standards or the independent Quality Control (QC) samples in DMSO were added to a volume of 50 µl of blank plasma. For the samples from the animals, a volume of 50 µl plasma and 50 µl of a 500 ng/ml clovoxamine solution in DMSO, the internal standard, were added to 50 µl DMSO. Proteins were precipitated by adding 200 µl acetonitrile, the samples were centrifuged (10 min, 5000xg) and a volume of 20 µl was injected into the system. Fluvoxamine samples were quantified on a reversed phase LC column (BDS Hypersil C18, 3 µm particle size, 100x4.6 mm I.D.; Thermo Hypersil-Keystone, Brussels, Belgium). LC-MS/MS analysis was performed on an API-4000 MS/MS (Applied Biosystems, Toronto, Canada), coupled to an HPLC system (Agilent, Palo Alto, USA). The MS/MS operated in the positive ion mode using the TurboIonSpray-interface (electrospray ionization), optimized for the quantification of fluvoxamine. The intra batch accuracy from independent QC samples was between 80 and 120% over the entire range of the samples. The limit of quantification for fluvoxamine in plasma was 1 ng/ml.

PCA Test

At fixed time points after the start of the fluvoxamine infusion (0.25, 0.5, 1, 2, 4, 8, 12 and 16 hr), a single dose of PCA (2.5 mg/kg) was injected in the tail vein. For each time point and for each of the 3 fluvoxamine dose groups, 3 animals were included, resulting in a total of 72 animals in the present study. The behavioral effects, EXC, FBP and FT, were observed over a time period of 5 min, starting immediately after the administration of PCA. The behavioral effects were scored on an ordinal categorical scale by 0 (no effects), 1 (mild effects), 2 (moderate effects) or 3 (severe effects). Trained technicians performed the scorings. Control injections of saline were included in each experimental session. Animals were tested in separate daily experimental sessions in order to account for day-to-day variability and to minimize systematic errors.

Data Analysis

The relationships between fluvoxamine concentration in plasma and the PCA-induced behavioral effects from all individual animals were simultaneously analyzed. All fitting procedures were performed on a personal computer (Intel® Pentium® 4 processor) running under Windows XP using the Compaq Visual FORTRAN standard edition 6.1 (Compaq Computer Cooperation, Euston, Texas, USA) with the nonlinear mixed effects modeling software NONMEM (Version V, Level 1.1., NONMEM project group, University of California, San Francisco, USA). The proportional odds model was used for analysis of the 4 categories of behavioral scores.

PK Analysis in Plasma

Plasma concentration *versus* time profiles were estimated in individual animals by analysis of the plasma concentration data obtained in the present study in combination with data from other investigations on the basis of a previously proposed population PK model (Geldof et al., 2007). Briefly, body weight was identified as significant covariate in the developed linear three-compartment PK model. Inter-individual variability could be characterized for the parameters of the first and second compartments. Predictive ability, model stability and precision of the parameter estimates of the PK model were confirmed by a predictive check and bootstrap analysis. Plasma concentrations in individual animals at the time of the behavioral observation were predicted on the basis of this PK model by application of the obtained post hoc estimates of the parameters. In this respect, it is important that the

effects of fluvoxamine on PCA-induced behavior could be measured for a longer time than measurable fluvoxamine concentrations could be detected in plasma. Specifically, fluvoxamine concentrations decreased in time below the limit of quantification (1 ng/ml) within the time frame of the experiments. Thus the population PK model enabled the prediction of fluvoxamine concentrations in plasma below the limitation of quantification.

Pharmacokinetic/Pharmacodynamic Model

The predicted fluvoxamine plasma concentrations at the time of behavioral observations were related to PCA-induced behavior using the proportional odds model for each of the three behavioral effects, EXC, FBP and FT.

The proportional odds model can be described by the following equation (Nestorov et al., 2001):

$$\text{logit}\left(\Pr(Y_i < k)\right) = \log\left(\frac{\Pr(Y_i < k)}{1 - \Pr(Y_i < k)}\right) \quad (1)$$

$$= \sum_{h=1}^{k} \theta_h + \beta * C_p \quad , k = 1,2,3 \quad (2)$$

in which Pr(.) is the probability, Y is the categorical observation, logit(Pr(Y))=log{Pr(Y)/(1-Pr(Y))} is the logit transformation, k is the category, $\sum_{h=1}^{k} \theta_h$ is the cut point (probability of being in the first k categories for $C_p = 0$) up to category k, β is the vector of model parameters (drug effect) and C_p is the fluvoxamine plasma concentration. For a categorical response variable with 4 categories, there are 3 (4-1) parallel lines on the log odds scale that correspond to the different sets of cumulative probabilities. In the model, cut points are calculated, that can vary between -∞ and +∞, after which they are scaled between a value of 0 and 1 by the logit transformation.

Results

PK Analysis in Plasma

In Figure 1, the observed and individual predicted fluvoxamine plasma concentration-time profiles are depicted, separately for each dose.

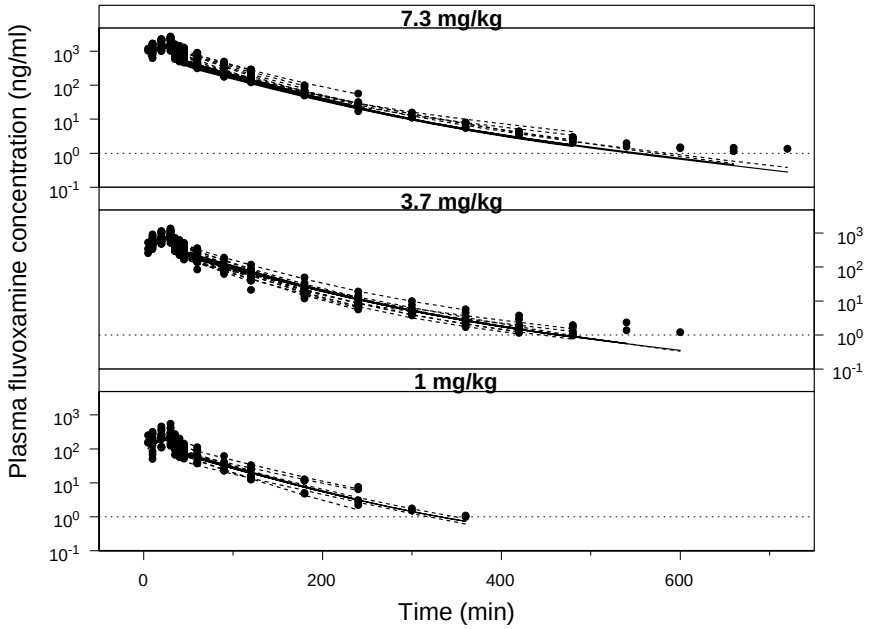

Figure 1. *Fluvoxamine concentration-time profiles in plasma, obtained after start of a 30-min IV infusion in rats. Depicted are the observed concentrations (dots), individual predictions (dotted lines) and population predictions (solid lines), separately for each fluvoxamine dose. The limit of quantification (1 ng/ml) is added for clarity.*

The three-compartment PK model was able to adequately describe observed fluvoxamine plasma concentrations. In Table 1, the mean post hoc estimates for the PK parameters of fluvoxamine in plasma obtained by the three-compartment PK model are depicted.

Table 1. *Mean post hoc estimates for the pharmacokinetic parameters of fluvoxamine in plasma after a 30-min intravenous administration (1, 3.7 and 7.3 mg/kg) obtained by the three-compartment model. Depicted are the mean parameter estimates for CL, V1, V2, Q2, V3, and Q3.*

CL (ml/min)	*V1* (ml)	*V2* (ml)	*Q2* (ml/min)	*V3* (ml)	*Q3* (ml/min)
22.3	224	625	27.7	136	1.0

Included are the post hoc estimates for systemic clearance (*CL*), central volume of distribution (*V1*), two peripheral volumes of distribution (*V2*, *V3*) and inter-compartmental clearances (*Q2*, *Q3*). On the basis of a covariate analysis, no differences in the pharmacokinetics of the different dose groups were observed. Inter-individual variability could not be identified on the parameters *V3* and *Q3*, and therefore the estimates for the total population of 187 animals were used (Geldof et al., 2007). The individual post hoc estimates were used in the current studies for each

individual animal to estimate the fluvoxamine plasma concentration at the time of the behavioral observations.

Inhibition of PCA-Induced Behavior by Fluvoxamine

No single animal showed any of the behavioral effects after just administration of fluvoxamine (score 0). In Figure 2, the time course of the PCA-induced behavioral response following the administration of the different doses of fluvoxamine is depicted.

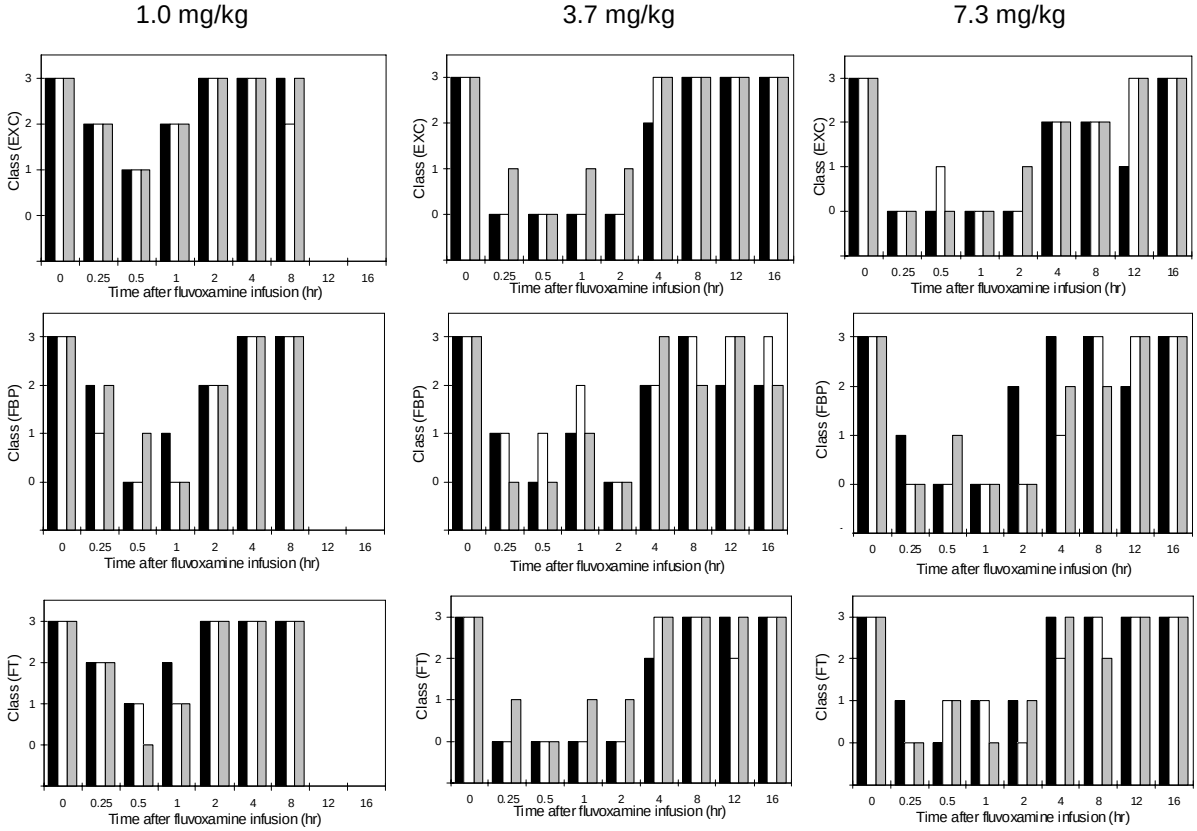

Figure 2. *Time-courses of the effect on PCA-induced behavioral effects (excitation (EXC), flat body posture (FBP) and forepaw trampling (FT)) after start of a 30-min intravenous infusion of 1.0, 3.7 or 7.3 mg/kg fluvoxamine. The time after fluvoxamine administration depicted on the x-axis corresponds to the time of PCA administration and each bar represents a different animals (n=3).*

Control animals that received a saline infusion followed by PCA administration at various time points, all showed maximum scores (3) for EXC, FBP and FT. The patterns for the time course of the effect of fluvoxamine on the three behavioral paradigms were similar. The time course of the effect was clearly dose-dependent. Following the 1.0 mg/kg dose a maximum reduction for EXC was observed at 0.5 hr following the start of the infusion (corresponding to the end of the infusion) and the

effect returned to baseline at 2 hrs. Following the highest dose (7.3 mg/kg) the return to baseline did not occur until 12 hours after administration.

Pharmacokinetic/Pharmacodynamic Model

Figure 3, shows the distribution of the observed behavioral scores as a function of the plasma concentration of fluvoxamine.

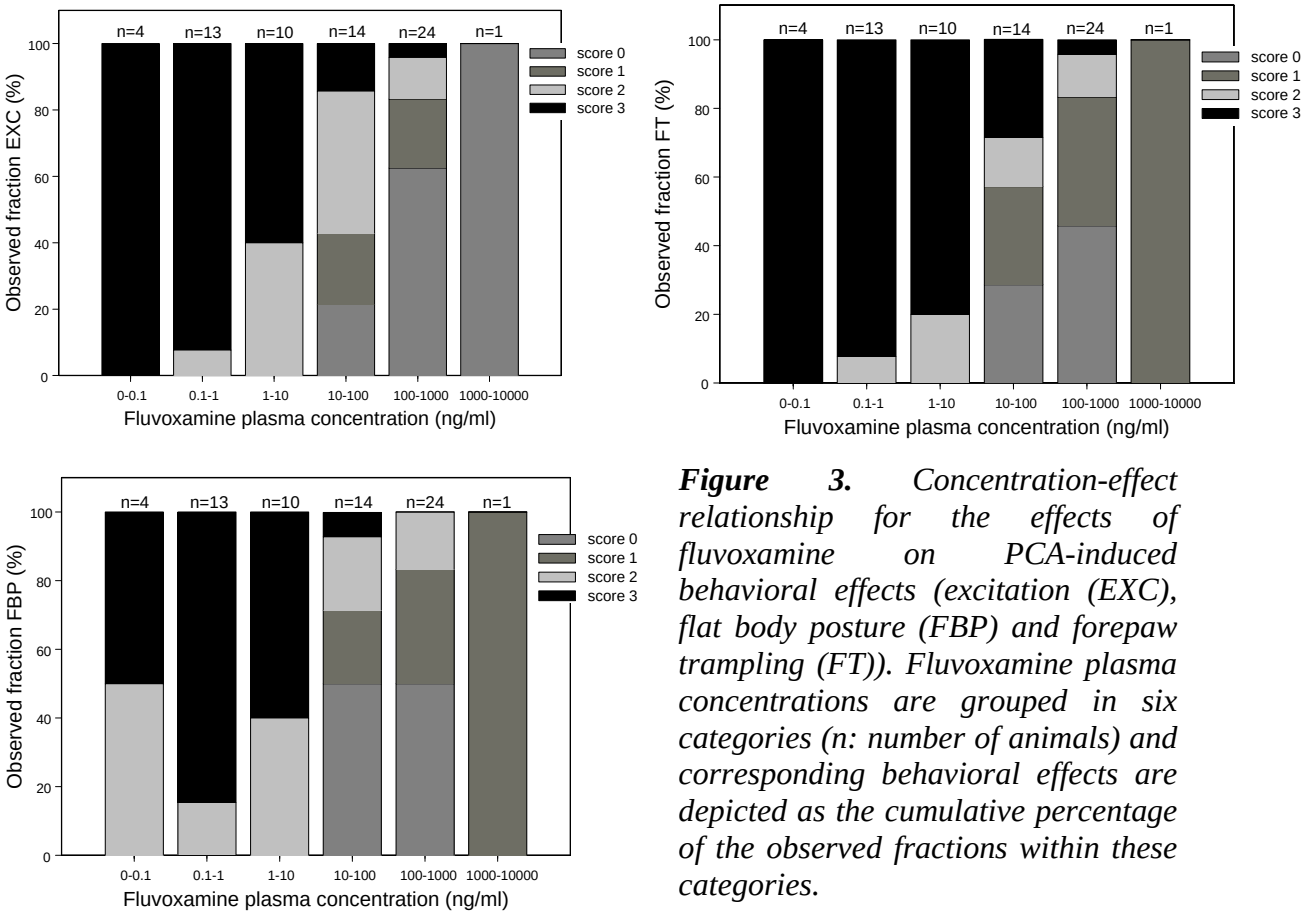

Figure 3. *Concentration-effect relationship for the effects of fluvoxamine on PCA-induced behavioral effects (excitation (EXC), flat body posture (FBP) and forepaw trampling (FT)). Fluvoxamine plasma concentrations are grouped in six categories (n: number of animals) and corresponding behavioral effects are depicted as the cumulative percentage of the observed fractions within these categories.*

In this analysis the fluvoxamine plasma concentrations at the time of the behavioral observations were grouped in six categories (0.0-0.1, 01.-1, 1-10, 10-100, 100-1000 and 1000-10000 ng/ml) and the corresponding observed behavioral effects were depicted as the cumulative percentage of the observed fractions within these categories. Generally, an increase in the fluvoxamine plasma concentrations was associated with in a progressive decrease of the behavioral effect score from the value of 3 to the value of 0. The resulting concentration-effect relationships from the PK/PD analysis on the basis of the proportional odds model are depicted in Figure 4.

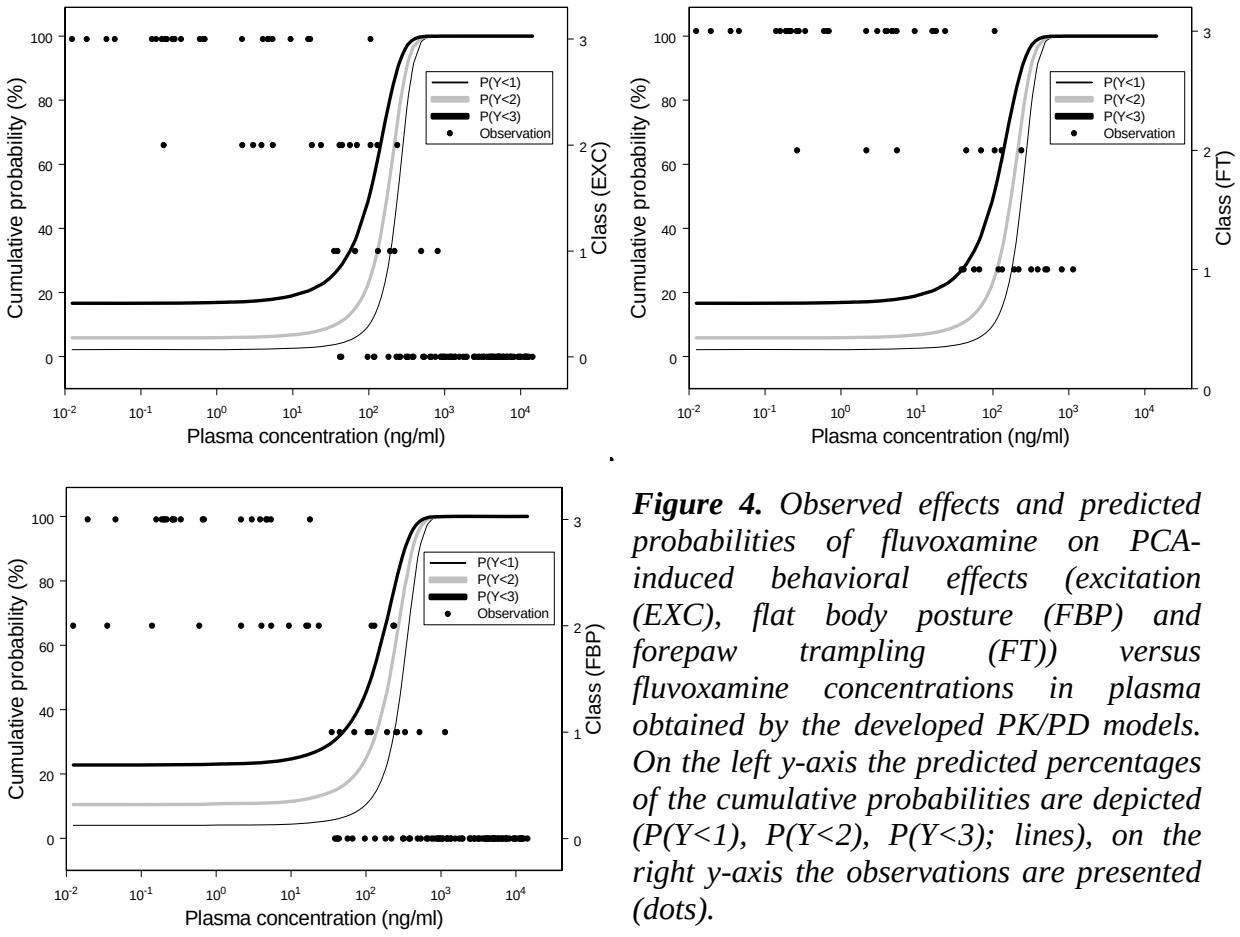

Figure 4. *Observed effects and predicted probabilities of fluvoxamine on PCA-induced behavioral effects (excitation (EXC), flat body posture (FBP) and forepaw trampling (FT)) versus fluvoxamine concentrations in plasma obtained by the developed PK/PD models. On the left y-axis the predicted percentages of the cumulative probabilities are depicted (P(Y<1), P(Y<2), P(Y<3); lines), on the right y-axis the observations are presented (dots).*

Specifically this figure shows the observed (right y-axis) and predicted effects (left y-axis; the predicted percentages of the cumulative probabilities) of the PCA-induced behavioral effects *versus* the fluvoxamine plasma concentrations (x-axis). The plots show that the model adequately describes the observed behavioral effects. In general, the observations and predictions were about similar for each of the three behavioral effects.

The PD parameter estimates for the inhibitory effect of fluvoxamine on the PCA-induced behavioral effects EXC, FBP and FT, with the corresponding standard errors are depicted in Table 2.

Table 2. *Pharmacodynamic parameter estimates for the inhibitory effects of fluvoxamine on PCA-induced behavioral effects (excitation (EXC), flat body posture (FBP) and forepaw trampling (FT)). Depicted are the parameter estimates for θ_1, θ_2, θ_3, β, with corresponding standard errors (S.E.) and ΔMVOF for each behavioral effect. For explanation of the symbols, see equation (2)*

	EXC		*FBP*		*FT*	
Parameter	*Value*	*S.E.*	*Value*	*S.E.*	*Value*	*S.E.*
θ_1	-3.8	0.51	-3.2	0.41	-3.4	0.44
θ_2	1.02	0.35	1.03	0.29	1.27	0.33
θ_3	1.18	0.31	0.92	0.23	0.55	0.19
β	0.016	0.0028	0.010	0.0020	0.0081	0.0016
ΔMVOF	76		56		49	

The parameters could be estimated simultaneously with good accuracy and precision for each of the three behavioral effects.

Based on a comparison of the difference in the minimum value of the objective function after fluvoxamine administration compared to saline administration (ΔMVOF), the inhibitory effects of fluvoxamine on PCA-induced behavioral effects could be best described for EXC, followed by FBP and FT, respectively.

With the developed categorical PK/PD models, parameters of pharmacological interest like the predicted fluvoxamine plasma concentration at half of the maximal inhibitory effects of PCA-induced behavior (EC_{50}, corresponding to the plasma fluvoxamine concentration at a cumulative probability of 50% in Figure 4), for the various cumulative probabilities (P(Y<1), P(Y<2), P(Y<3)) could be calculated (Table 3).

Table 3. *Predicted values for EC_{50} and standard error (S.E., calculated by stochastic simulation with n=1000) for the cumulative probabilities (P(Y<1), P(Y<2), P(Y<3)) of the inhibitory effects of fluvoxamine on PCA-induced behavioral effects (excitation (EXC), flat body posture (FBP) and forepaw trampling (FT)).*

P	*EXC (ng/ml)*	*FBP (ng/ml)*	*FT (ng/ml)*
P(Y<1)	237 ± 39	305 ± 27	421 ± 48
P(Y<2)	174 ± 28	206 ± 22	264 ± 34
P(Y<3)	100 ± 20	117 ± 15	196 ± 28

In addition, the standard errors of the EC_{50} values could be estimated by stochastic simulation taking into account the covariance matrix between the parameter estimates. In this simulation (n=1000), extreme EC_{50} values were excluded by

allowing only values between the 5 and 95% quantiles. From Table 3 it can be concluded that the behavioral effect EXC was the most sensitive for the effects of fluvoxamine, followed by FBP and FT, respectively.

Discussion

The PK/PD correlation of the effect of fluvoxamine on PCA-induced behavior was determined in the rat. The PCA assay described in the current article is widely applied to screen for activity of SSRIs and antidepressants (Pellegrino and Bayer, 2000;Andres et al., 2005;Andres et al., 2003;Bartolome et al., 2005). However to our knowledge, PK/PD modeling of the effects of these compounds in the PCA assay has not been achieved. This could be of potential interest in prediction of the effective concentrations in humans.

The assessment of the PK/PD correlation of fluvoxamine in the PCA assay is complicated by the availability of only sparse data for the behavioral effects, since only one observation is feasible in each animal for each behavioral effect. Furthermore, the readout of the PCA assay is non-continuous (score 0, 1, 2 or 3), requiring an alternative approach to the modeling of the data.

In the present article, we propose population PK modeling to describe the plasma concentrations of fluvoxamine and the proportional odds model to account for the non-continuous categorical behavioral observations.

Fluvoxamine plasma concentrations at the times of the behavioral observations were determined by analysis of the sparse PK information from the present investigation on the basis of a previously proposed population PK model (Geldof et al., 2007). Specifically, by using the individual post hoc estimates of the various PK parameters (*CL*, *V1*, *V2*, *Q2*, *V3* and *Q3*) for each animal, the fluvoxamine plasma concentration at the exact time of the behavioral effects could be predicted. This enabled analysis of the PK/PD correlation.

Fluvoxamine by itself did not induce EXC, FT and FBP or any other overt behavioral effects. When PCA was injected after saline administration in the control animals, the behavioral effects could be clearly observed in all animals at the maximum score of 3. When PCA was administered at different times following fluvoxamine administration, the behavioral effects were either partially inhibited or even absent, resulting in a decrease in the categorical scores. The magnitude of this effect was dependent on the administered dose and the time after fluvoxamine administration (Figure 2). Specifically, inhibition of PCA-induced behavioral effects by fluvoxamine could already be observed at 15 min after start of the infusion for each fluvoxamine

dose. The inhibitory effects of fluvoxamine on PCA-induced behavior were comparable for EXC, FBP and FT. At the end of the fluvoxamine infusion after 30 min, fluvoxamine completely inhibited behavior induced by PCA at each dose (score 0). At later time points after fluvoxamine administration, the inhibition of PCA-induced behavior was reduced and the behavioral effects returned. The time course of this effect was dependent on the administered dose of fluvoxamine, with the highest dose resulting in the longest duration of the effect (Figure 2).

In an exploratory analysis the relation between plasma concentration of fluvoxamine and the behavioral effect was evaluated. By grouping of the fluvoxamine plasma concentrations at the time of PCA administration in six categories, the effect of the fluvoxamine plasma concentration on PCA-induced behavioral effects could be clearly observed (Figure 3). In general, an increase in the inhibition of the PCA-induced behavioral effects could be observed, hence a reduction in the behavioral scores, with increasing fluvoxamine concentrations in plasma.

The proportional odds model was used to account for the non-continuous categorical behavioral observations EXC, FT and FBP. A direct relationship could be observed between the fluvoxamine plasma concentration and the measured PD effect. The proportional odds model could adequately describe observed behavioral effects for EXC, FBP and FT (Figure 4) and the PD parameter estimates for the inhibitory effects of fluvoxamine on PCA-induced behavior could be adequately described (Table 2). The predicted cumulative probabilities (P(Y<1), P(Y<2) and P(Y<3)) for these effects could adequately predict observed behavioral scores at the corresponding fluvoxamine concentrations in plasma. The predicted cumulative probabilities and probabilities were approximately the same for EXC, FT and FBP. By incorporation of the effect of fluvoxamine on the PCA-induced behavior in the models, the description of the observed data was significantly improved compared to models in which this drug effect was not incorporated. By excluding the effect of fluvoxamine (β=0), the MVOF was increased by 76, 56 and 49 for EXC, FBP and FT, respectively (=ΔMVOF, Table 2). Therefore, fluvoxamine showed a significant effect on PCA-induced behavior and the behavioral observations could be best described for EXC, FBP and FT, respectively.

With the modeling approach described in the present article, unique PK and PD parameters have been obtained which are independent of the administered fluvoxamine dose. The effect of fluvoxamine in the PCA assay occurs at very high plasma concentrations. For EXC the EC_{50} values for the cumulative probabilities (P(Y<1), P(Y<2), P(Y<3) were 237 ± 38.9 ng/ml, 174 ± 28.3 ng/ml and 100 ± 20.1

ng/ml, respectively (Table 3). Slightly higher EC_{50} values were obtained for the corresponding effects on FBP and FT, and therefore it could be concluded that EXC was the most sensitive behavioral effect for the inhibitory effects of fluvoxamine after PCA administration. Hence, at equal fluvoxamine plasma concentration, the decrease in behavioral scores could be more rapidly observed for EXC than for FBP, followed by FT. An important question is how the observed EC_{50} concentrations in the present investigations relate to comparable values in other pharmacological test systems and in particular also the degree of SERT occupancy.

Recently, we have developed a physiological PK model, which enables prediction of the fluvoxamine concentration in extracellular fluid (ECF) of the frontal cortex and in brain tissue on the basis of plasma concentrations (Geldof et al., in publication). This analysis showed that the brain distribution of fluvoxamine is non-linear. Moreover, this analysis also showed that, at steady state, the free drug concentrations in the frontal cortex amount to on average 5% of the total plasma concentrations. Correction of the observed EC_{50} values for this brain distribution factor yields values in the range of 5 - 21 ng/ml, exceeding reported values for the affinity of fluvoxamine to SERT in *in vitro* bioassays (Goodnick and Goldstein, 1998), indicating that the PCA effect occurs at near maximal SERT occupancy. This is confirmed in the results of a recent study on the SERT occupancy *in vivo*. Specifically, a PK/PD model has been developed describing the relationship between fluvoxamine concentration in plasma and the occupancy of SERT in the frontal cortex of the rat (Geldof et al., in publication). In this investigation SERT occupancy could be directly related to fluvoxamine plasma concentrations by a hyperbolic function (B_{max} model). The estimated fluvoxamine plasma concentration at half-maximal SERT occupancy was 0.48 ng/ml, which is much lower than obtained EC_{50} values in the present investigation for the cumulative probabilities of PCA-induced behavior confirming that very high fluvoxamine SERT occupancy is required for the inhibition of PCA-induced behavior. This can be explained by the nature of the PCA test. Specifically the PCA test is probably based on the competitive interaction between on the one hand the SSRI (i.e. fluvoxamine) and on the other hand PCA for SERT. It is well established that the magnitude of competitive drug-drug interaction depends on the relative concentrations of the two interacting agents (Jonker et al., 2005). Specifically, at relatively high PCA doses also relatively high concentrations of SSRIs are required to counteract the PCA-induced behavior and *vice versa*. As a result, the pharmacodynamic parameter estimates of SSRIs in the PCA test depend on the administered PCA dose. This has important implications for the prediction of

effective drug concentrations of SSRIs on the basis of PK/PD modeling of effects in the PCA test. Specifically, modeling of the competitive pharmacodynamic interaction between PCA and the SSRI of interest is required to derive the relevant *in vivo* concentration range. In recent years the successful modeling of competitive drug-drug interactions *in vivo* has been reported for a number of CNS-active drugs including benzodiazepines (Mandema et al., 1992a;Mandema et al., 1992b) and 5-HT_{1A} receptor agonists (Zuideveld et al., 2002). This constitutes a useful basis for the modeling of the interaction between PCA and SSRIs and will be the subject of future investigations in our laboratory.

In conclusion, PK/PD models, based on the proportional odds model could adequately describe the relationships between fluvoxamine plasma concentration and its effects on the probability for PCA-induced behavioral effects EXC, FBP and FT in the rat. Administration of fluvoxamine before PCA injection resulted in immediate inhibition of the behavioral effects, dependent on the fluvoxamine plasma concentration and was comparable for the three effects. As fluvoxamine plasma concentrations decreased in time, the inhibition of PCA-induced behavior was reduced and behavioral effects could be observed again. The developed model could adequately describe observed behavioral effects and the values of PD parameter estimates could be adequately estimated. The effects of fluvoxamine on PCA-induced behavior could be best described for EXC, FBP and FT, respectively. At equal plasma concentration of fluvoxamine, the decrease in behavioral scores could be more rapidly observed for EXC, FBP and FT, respectively, based on EC_{50} values for the cumulative probabilities (P(Y<1), P(Y<2), P(Y<3)). This investigation demonstrates the feasibility of PK/PD modeling of categorical drug effects in animal behavioral pharmacology, which constitutes a basis for the future development of a mechanism-based PK/PD model for fluvoxamine in this paradigm.

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Chapter 10

Preliminary Studies on the Pharmacokinetic/Pharmacodynamic correlation of the Effect of Fluvoxamine on Rapid Eye Movement (REM) Sleep in Rats

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Abstract

A pharmacodynamic (PD) model for the effects of fluvoxamine on Rapid Eye Movement (REM) sleep is presented. Rats chronically implanted with electrodes for recording of the cortical electroencephalogram (EEG), electrical neck muscle (EMG) activity and ocular movements (EOG) received an intraperitoneal (IP) injection of 1, 3.7 or 7.3 mg/kg of fluvoxamine. The effects on the various sleep-wake states were determined over a period of 14 hrs. In parallel, the time course of the fluvoxamine concentration in plasma was determined in a satellite group of rats equipped with electrodes under identical conditions. The results of the investigations were analyzed by nonlinear mixed effects modeling.

A two-compartment PK model with dose dependency on the inter-individual variability (IIV) in *V2* described the fluvoxamine plasma concentrations following intraperitoneal administration in individual animals. Population PK parameter estimates for *ka*, *CL*, *Q*, *V2* and *V3* were 0.024 1/min, 143 ml/min, 37.1 ml/min, 338 ml and 3260 ml, respectively. Inter-individual variability was identified on *V2* (17.4 %) and *V3* (34.5 %).

Fluvoxamine showed a dose-dependent inhibition of the onset of the increase in REM sleep. Thereafter, REM sleep remained constant around a value of 10-15% (3-4.5 min of the total recording periods of 30 min) in all dose groups up to about 9 h, after which REM sleep decreased again, which was one hour before the dark period. In the PD model, the effects of fluvoxamine on REM sleep were characterized by an indirect response model with stimulation of k_{in}, which was controlled by a REM sleep generation pulse function accounting for the changes in REM sleep. The administered fluvoxamine dose was related to the onset of the increase in REM sleep, which is of clinical relevance and of high interest within a translational medicine paradigm in drug development. All structural parameters of the model could be adequately described and population values for k_{in}, k_{out}, the offset of the increase in REM sleep (t_2) and the fraction determining the value of k_{in} (REM_{max}) were estimated as 2.8 %/min, 1.2 1/min, 9.3 h and 4.5. The values for the onset of the increase in REM sleep (t_1) were 0.73, 1.2 and 1.7 h at a fluvoxamine dose of 1, 3.7 and 7.3 mg/kg, respectively and zero after administration of saline.

This investigation is a first step towards comprehensive PD modeling of the effect of Selective Serotonin Reuptake Inhibitors (SSRIs) on sleep-wake cycle following acute and chronic administration.

Introduction

A reduced activity of serotonergic neurotransmission has been implicated in the pathogenesis of depression (Coppen, 1967;Owens and Nemeroff, 1994). As a result Selective Serotonin Reuptake Inhibitors (SSRIs) constitute the first-line of treatment for depression. SSRIs selectively and powerfully block the serotonin transporter (SERT) and thereby the reuptake of serotonin (5-hydroxytryptamine, 5-HT) in the presynaptic nerve terminal. (Bel and Artigas, 1992;Fuller, 1994). Although SSRIs rapidly inhibit 5-HT uptake and induce an immediate series of electrophysiological changes, maximal antidepressant effects are observed only after weeks of chronic treatment, suggesting that long-term adaptive changes are an important determinant of the therapeutic efficacy.

Recently, we have initiated a series of investigations in the rat on the pharmacokinetic/pharmacodynamic (PK/PD) correlations of fluvoxamine, as a prototype for SSRIs. In these investigations the concept of population PK modeling for the prediction of individual plasma concentration *versus* time profiles has been proposed (Geldof et al., 2007). This is important since blood sampling may confound assessment of the effect of SSRIs in behavioral pharmacology paradigms.

Many studies have shown that antidepressants, including SSRIs, produce significant effects on various sleep-wake stages in patients, in healthy volunteers and in animals, both after acute and chronic administration. Suppression of REM sleep and increase of REM latency are the most consistent replicable effects on sleep (Hendrickse et al., 1994;Kerkhofs et al., 1990;Kupfer et al., 1991;Nicholson and Pascoe, 1988;Rush et al., 1998;Vasar et al., 1994;Wilson et al., 2000a;Wilson et al., 2000b). Specifically, in rats and cats, acute and chronic treatment with SSRIs has been shown to induce a sustained decrease in REM sleep (Hilakivi et al., 1987;Kleinlogel and Burki, 1987;Neckelmann et al., 1996;Pastel and Fernstrom, 1987;Sommerfelt and Ursin, 1991). In contrast the effects of SSRIs on slow-wave sleep and waking are less consistent, since both increases and decreases in slow-wave sleep have been reported. (Nicholson and Pascoe, 1986;Nicholson et al., 1986;Nicholson and Pascoe, 1988;Oswald and Adam, 1986;Kupfer et al., 1991).

To date, comprehensive PK/PD modeling of the effects of SSRIs on sleep-wake architecture has not been accomplished. In preliminary investigations it has been demonstrated that repeated blood sampling significantly influences sleep architecture in rats after acute and, although to a lesser extent, also after chronic fluvoxamine administration (Geldof et al., unpublished observations), thus complicating the PK/PD analysis of this effect. Furthermore, it has been shown to be impossible to

administer SSRIs via an intravenous infusion in such investigations, since this also significantly influences the sleep-wake architecture. This necessitates the intraperitoneal administration of SSRIs in PK/PD investigations on the effect on sleep. Moreover, advanced PK modeling approaches are needed for the description of the time course of the drug concentration.
The objective of the present investigation was to characterize the PK/PD correlation of the effect of fluvoxamine on REM sleep. We present a) a population PK model for fluvoxamine following intraperitoneal administration and b) a PD model for the effects on REM sleep. The investigations described in this article are a first step towards a comprehensive PK/PD analysis of the effect of SSRIs on sleep after acute and chronic administration.

Materials and Methods

Chemicals

Fluvoxamine maleate and clovoxamine fumarate were kindly provided by Solvay Pharmaceuticals (Weesp, The Netherlands). Dimethylsulfoxide (DMSO) was obtained from Merck (Darmstadt, Germany) and acetonitrile for LC-MS/MS analysis of the plasma samples was obtained from Acros (Geel, Belgium).

Animals

Male Sprague Dawley rats (IFFA Credo, Brussels, Belgium) weighing between 386-540 gram at the start of dosing were used. Animals were housed in full-view Plexiglas cages (25x33 cm, 18 cm high) that belong to IVC-racks (Individually Ventilated Cages) located in a sound-attenuated chamber. Animals were housed under controlled environmental conditions (ambient temperature 22 ± 2°C, relative humidity 60%, 12-h light/dark cycle with lights on from 12:00 to 24:00 hrs and intensity of ~ 100 lux). The animals had free access to food (Onderhoudsvoeder A04C, Safe, France) and tap water. All studies were approved by the animal care and use committee of Johnson & Johnson Pharmaceutical Research and Development.
Animals were randomly selected from a test population. Only rats that complied with pre-set criteria for inclusion were used in the experiments, i.e. weight of animals 300-700 g, a wash out period of at least 14 days and adequate signal quality.

Surgical Procedures

For optimal comparison, electrodes were implanted both in the animals in which the sleep-wake architecture was explored and in the animals in which the fluvoxamine

pharmacokinetics was determined. Anesthesia was induced with 4% (*v/v*) isoflurane (Abbott Laboratories, Queenborough, UK) in combination with N_2O and O_2 (3:2) at a total flow rate of 0.5 l/min, using an Isotec evaporator (Ohmeda, Madison, USA) and anesthesia was maintained with 2% (*v/v*) isoflurane. The skull of the animals was exposed and three small holes were drilled into the cranial bone without perforating the dura, to receive three fixing stainless steel screws (diameter 1 mm) for polygraphic recording of the frontal and parietal electroencephalogram (EEG). Two electrodes were placed stereotaxically on each side of the sagittal suture (AP: 2; L: -2.0 and AP: -6.0; L: 3.0 mm from bregma, in accordance with the atlas of Paxinos and Watson (Paxinos and Watson, 1982) and the third electrode (reference electrode) was screwed over the cerebellum. For recording of the electro-oculogram (EOG) and electromyogram (EMG), stainless steel wires were placed in periorbital and inserted into the nuckal muscle, respectively. Electrodes (stainless steel wires, Bilaney Consultants, Germany) were connected to a pin (Future Electronics, Lokeren, Belgium) with a small insert (track pins, Dataflex, Surrey, United Kingdom) and fitted into an 8 holes connector. The electrodes were fixed with dental cement to the cranium. The animals were allowed to recover after surgery for ten days and thereafter, the animals were handled and habituated to the recording procedure in their home cages for two weeks. The rats were connected at regular intervals by a cable to a rotating swivel, allowing free movement while EEG, EOG and EMG activities were monitored. The spontaneous online EEG/EMG recording procedure consisted of a 24-h pre-recording habituation in the recording chambers with the electrode cables connected so as to minimize environmental stress, and a 24-h recording session immediately after the habituation.

Dosage Regimen

The animals (total n=58; sleep-wake study: n=32; PK study: n=26) were randomly assigned to four treatment conditions (n=8 per treatment in each study, except control in PK study: n=2). Fluvoxamine was dissolved in physiological saline (0.9%) and administered via an intraperitoneal (IP) injection of 1, 3.7 or 7.3 mg/kg in a volume of 10 ml/kg body weight. An equivalent volume of saline was administered in control animals. Dosages and observed concentrations of fluvoxamine are expressed as free base.

Sleep-wake architecture

To study the effects of fluvoxamine on sleep-wake architecture, the present study was conducted according to a crossover design whereby two EEG recording sessions were performed. The first recording session started at 14:00 hr and lasted 14 hours after the administration of saline (n=32). The second recording session was performed on the following day at the same time of the circadian day and for the same duration following administration of saline (n=8) or a fluvoxamine dosage of 1, 3.7 or 7.3 mg/kg (n=8 per dose). The EEG, EOG and EMG activities and body movement signals were monitored for 14 hours. Digital recording was conducted on-line at a sample rate of 200 Hz. The EEG, EOG, EMG and body movement signals were passed via a bipolar recorder system (Embla) developed by Flaga Medical Devices (Iceland) to a computer and managed by software package Somnologica (Flaga Medical Devices, Iceland) that turns the computer into a polygraphic workstation for signal recording. Off-line, the automated rat sleep analysis system was applied to 14 continuous hours following the injection of fluvoxamine or saline. The sleep-wake staging was executed per 2-second epoch for periods of 30 min, based on 5 EEG frequency domain values (δ: 0.4-4 Hz, θ: 4.2-8 Hz, α: 8.2-12 Hz, σ: 12.2-14 Hz, β: 14.2-30 Hz), integrated EMG, EOG and body activity level. The discriminative analysis uses classification rules for the final sleep stage assignment to each specific EEG epoch. Six sleep stages were classified as being active wake (AW), passive wake (PW), light slow wave sleep (SWS1), deep slow wave sleep (SWS2), intermediate stage (IS) or rapid eye movement (REM) sleep. Briefly, the different vigilance states were characterized as follows: AW, low-voltage fast EEG activity, high EMG activity, numerous eye movements and high body activity; PW, low-voltage fast EEG activity, high EMG activity, numerous eye movements and absence of body activity; SWS1, high-voltage slow cortical waves interrupted by low-voltage fast waves and reduced EMG activity; SWS2, continuous high-amplitude slow-wave activity in EEG absence of EMG, EOG and body activities; IS: spindles activity with theta rhythm, absence of EOG and body movements; REM sleep, low-voltage fast cortical waves with a regular theta rhythm, presence of rapid eye movements and absence of muscular and body movements. Time spent in each vigilance state including REM sleep, which was the focus of the present investigation, was expressed as percentage of the recording period. Since the effect on REM sleep is the most prominent effect of SSRIs on sleep-wake architecture, a PD model was developed for the effects of fluvoxamine on REM sleep.

Blood Sampling

The fluvoxamine concentration in plasma was determined in a satellite group of animals by blood sampling from the orbital plexus at various time points after fluvoxamine administration. A number of 10 blood samples (100 µl) were collected at fixed time intervals between 4 and 375 min after fluvoxamine administration. Blood samples were collected in Multivette® 600 K3E tubes (Sarstedt, Nümbrecht, Germany) and kept on ice during the experiment. After centrifugation (10 min, 5000x*g*), 50 µl plasma was transferred into a glass tube and stored at -20°C until sample analysis.

Drug Analysis

Fluvoxamine plasma samples were analyzed using liquid chromatography with tandem mass spectrometry (LC-MS/MS) as described earlier (Geldof et al., 2007). A volume of 50 µl of the calibration standards or independent Quality Control (QC) samples in DMSO was added to a volume of 50 µl blank plasma. For the plasma samples from the rats, a volume of 50 µl plasma was added to 50 µl DMSO. A volume of 50 µl of 500 ng/ml clovoxamine in DMSO was added to these samples as internal standard. Proteins were precipitated by adding 200 µl acetonitrile, the samples were centrifuged (10 min, 5000xg) and a volume of 20 µl was injected into the system. Fluvoxamine samples were quantified on a reversed phase LC column (BDS Hypersil C18, 3 µm particle size, 100x4.6 mm I.D.; Thermo Hypersil-Keystone, Brussels, Belgium). LC-MS/MS analysis was performed on an API-4000 MS/MS (Applied Biosystems, Toronto, Canada), coupled to an HPLC system (Agilent, Palo Alto, USA). The MS/MS operated in the positive ion mode using the TurboIonSpray-interface (electrospray ionization) was optimized for the quantification of fluvoxamine. The intra batch accuracy from independent QC samples was between 80 and 120% over the entire range of the samples. The limit of quantification for fluvoxamine was 1 ng/ml.

Data Analysis

The fluvoxamine concentrations in plasma and mean effects on REM sleep were analyzed by using a population PK and a dose driven PD model. All fitting procedures were performed on a personal computer (Intel® Pentium® 4 processor) running under Windows XP using the Compaq Visual FORTRAN standard edition 6.1 (Compaq Computer Cooperation, Euston, Texas, USA) with the nonlinear mixed effects modeling software NONMEM (Version V, Level 1.1., NONMEM project

group, University of California, San Francisco, USA). Model fits were assessed based on diagnostic plots (observed concentrations vs. individual and population predicted concentrations, weighted residuals vs. predicted time and concentrations), parameter correlations and precision in parameter estimates. The likelihood ratio test is based on a comparison of the minimum value of the objective function (MVOF) of two models. An additional parameter was included in the structural model if the resulting change in MVOF was ≥ 6.6 ($p \leq 0.01$), assuming that the log likelihood ratio is χ^2 distributed. The dosages of fluvoxamine were evaluated as the actual amounts administered to the animals.

Pharmacokinetic Model

A linear two-compartment model PK model with first-order absorption and a dose-dependency included on the inter-individual variability of the volume of distribution (*V2*), described the concentration *versus* time profiles of fluvoxamine following intraperitoneal administration. This model was implemented in the PREDPP subroutine ADVAN6 TRANS5 in NONMEM. Using this routine, the parameters first-order absorption rate constant (*ka* in 1/min), systemic clearance (*CL* in ml/min), inter-compartmental clearance (*Q* in ml/min) and two peripheral volumes of distribution (*V2*, *V3* in ml), were estimated (Figure 1).

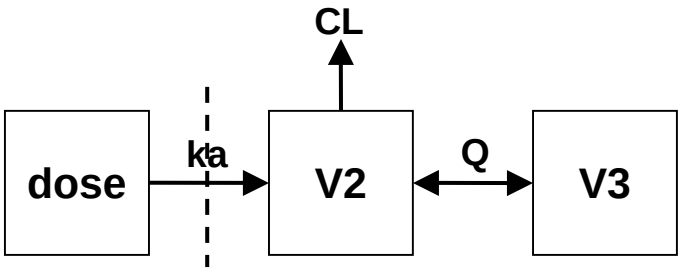

Figure 1. *Schematic representation of the population pharmacokinetic model for fluvoxamine. The model consists of two compartments describing the pharmacokinetics in plasma (ka = absorption rate constant, V = volume of distribution, CL = clearance and Q = inter-compartmental clearance).*

The inter-individual variability on these parameters was modeled by an exponential equation:

$$P_i = \theta \cdot \exp(\eta_i) \qquad (1)$$

in which P_i is the individual estimate for parameter P for the i^{th} individual, θ is the population estimate for parameter P and η_i is the inter-individual random deviation of P_i from P. The values of η_i are assumed to be normally distributed with mean zero

and variance ω^2 that distinguished the PK parameters for the i^{th} individual from the population typical value θ.

Inter-individual variabilities were analyzed on each parameter by the method of stepwise forward inclusion and backward elimination with the requirement of a resulting change in MVOF ≥ 6.6 ($p \leq 0.01$) for significance. Inter-individual effects that did not significantly improve the model by reduction of the MVOF by at least 6.6 points or which could not be estimated were fixed to zero. Correlations between the inter-individual variability of the various parameters were explored using the OMEGA BLOCK option. Unexplained residual variability (e.g. caused by measurement and experimental errors) in fluvoxamine plasma concentrations was described by a second level of random effects, which was assumed to be proportional to the fluvoxamine concentration in plasma and was described by a proportional error:

$$Cm_{ij} = Cp_{ij} \cdot (1 + \varepsilon_{ij}) \quad (2)$$

in which Cm_{ij} is the measured fluvoxamine plasma concentration, Cp_{ij} is the j^{th} plasma concentration for the i^{th} individual predicted by the model and ε_{ij} accounts for the residual deviance of the predicted concentration from the observed concentration. The values for ε were assumed to be normally distributed with mean zero and variance σ^2.

The plasma fluvoxamine concentrations were predicted at the exact time points when the effect on REM sleep was measured (periods of 30 min) on the basis of the developed PK model by application of the obtained population estimates of the parameters.

Pharmacodynamic Model for the Effect of Fluvoxamine on REM Sleep

The fluvoxamine dose, the predicted fluvoxamine plasma concentrations at the time points of the REM sleep measurements and mean PD effects on REM sleep were implemented in the subroutine ADVAN6, which is a general nonlinear model that uses the numerical solution of the differential equations. REM sleep was analyzed as a percentage of the subsequent recording periods of 30 min.

The PD model used to describe the effects of fluvoxamine on the amount of REM sleep time was a type III indirect response model with stimulation of k_{in} accounting for the observed delay in REM sleep due to administration of fluvoxamine (Dayneka et al., 1993;Sharma and Jusko, 1996):

$$\frac{dREM}{dt} = k_{in} * (1 + REMS) - k_{out} * REM \quad (3)$$

in which *REM* is the total duration of REM sleep (as a percentage of the recording time in a 30 min period), k_{in} is the zero-order rate constant for production of REM sleep, *REMS* is the REM sleep state describing the relationship between fluvoxamine dose and REM sleep, k_{out} is the first-order rate constant for the loss of REM sleep.
The indirect response model is controlled by a REM sleep generation function accounting for the change in REM sleep. This block pulse function is the forcing condition for REM sleep. The dosages of fluvoxamine were used as explanatory factor for the observed effects of fluvoxamine on REM sleep and the administered fluvoxamine dose was related to the onset of the increase in REM sleep (t_1).
The pulse function for the change in REM sleep was described by blocked pulse in REMS:

$$if: \quad t_2 \le t \le t_1, REMS = 0 \quad (4)$$
$$else: \quad REMS = REM_{\max} \quad (5)$$

in which t_1 and t_2 are the start times of the onset and the offset of the increase in REM sleep, respectively. REM_{max} is the fraction determining the value of k_{in} (REM sleep with respect to baseline REM sleep minus 1). The value of t_1 is dependent on the administered fluvoxamine dose. Its value is equal to zero after administration of saline and has a specific value after each dose of fluvoxamine.
Since the mean PD effect of fluvoxamine on REM sleep was analyzed (see results), inter-individual variability on the parameters could not be modeled and as a result, population parameter estimates are obtained. The residual variability in REM sleep, e.g. caused by measurement and experimental errors, was characterized by an additive error:

$$REMm = REMp + \varepsilon \quad (6)$$

in which *REMm* is the measured REM sleep (as a % of the recording period), *REMp* is the REM sleep predicted by the model and ε accounts for the residual variability of the predicted REM sleep from the observed REM sleep. The values for ε were assumed to be normally distributed with mean zero and variance σ^2.

Results

Pharmacokinetic Analysis of Fluvoxamine Plasma Concentrations following intraperitoneal administration

In Figure 2, the observed and individual predicted fluvoxamine plasma concentration-time profiles are depicted, grouped by fluvoxamine dose.

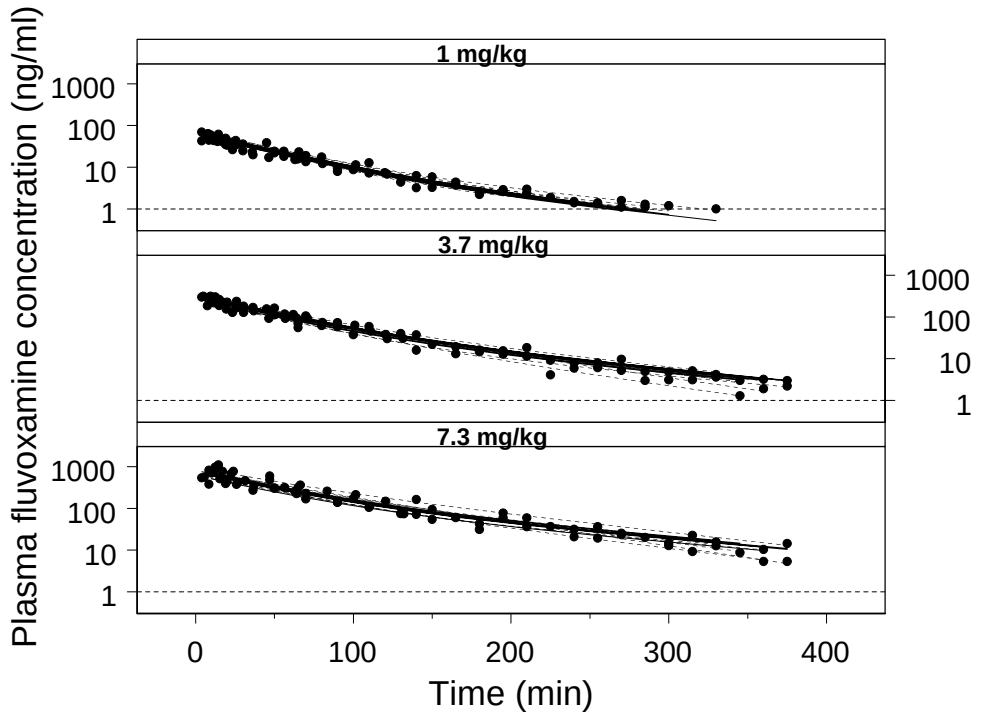

Figure 2. *Fluvoxamine concentration versus time profiles in plasma, obtained after an IP injection in Sprague Dawley rats. Depicted are the observed concentrations (dots), individual predictions (dashed lines) and population predictions (solid lines), separated by fluvoxamine dose. The limit of quantification (1 ng/ml) is added for clarity (dashed horizontal line).*

The two-compartment PK model accurately described observed fluvoxamine plasma concentrations after IP administration for each dose. Maximal fluvoxamine plasma concentration (C_{max}) was reached rapidly after dosing and observed already at the first measurement.

In Table 1, the estimates for the PK parameters in plasma obtained by the two-compartment PK model are depicted.

Table 1. *Pharmacokinetic parameter estimates of fluvoxamine in plasma after IP administration (1, 3.7 and 7.3 mg/kg) obtained by the two-compartment pharmacokinetic model. Depicted are the population estimates for ka, CL, Q, V2, V3, σ^2 with corresponding coefficient of variation (C.V.) and inter-individual variability (IIV).*

Parameter	Unit	Value	C.V. (%)	IIV (%)
ka	1/min	0.024	5.2	-
CL	ml/min	143	6.2	-
Q	ml/min	37.1	15.0	-
V2	ml	338	35.2	17.4
V3	ml	3260	11.6	34.5
$\sigma^2\ \varepsilon_{ij}$	-	0.026	14.7	-

*C.V. = Coefficient of Variation (standard error / value * 100%)*

IIV = inter-individual variability ($100 \times \sqrt{\omega^2}$ *)*

The mean population values for *ka*, *CL*, *Q*, *V2* and *V3* were estimated to be 0.024 1/min, 143 ml/min, 37.1 ml/min, 338 ml and 3260 ml, respectively. The coefficients of variation (C.V.) for all parameters ranged between 5.2 and 35.2%. Inter-individual variability could be characterized for *V2* and *V3*, which were estimated to be 17.4 and 34.5%, respectively. The covariate analysis showed no significant relationship between body weight of the animals and any of the parameters. The residual variability was estimated to be 0.026 (Table 1).

The goodness-of-fit plots for the population two-compartment model are depicted in Figure 3.

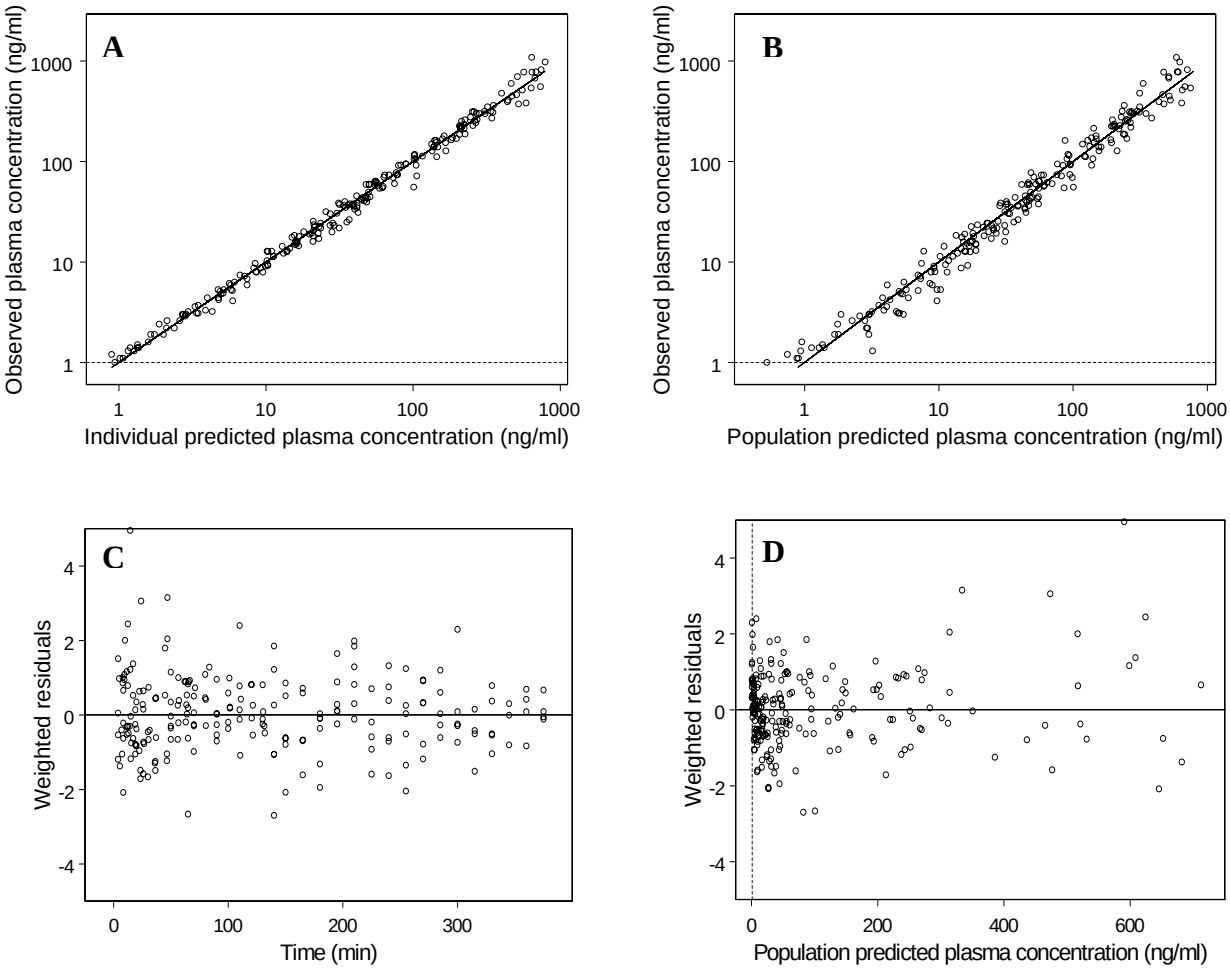

Figure 3. *Goodness-of-fit plots obtained for the final two-compartment pharmacokinetic model. Depicted are scatter plots of the observed fluvoxamine plasma concentrations versus the individual model predictions (a) and population model predictions (b) and scatter plots of the population weighted residuals versus time (c) and population model predictions (d). The limit of quantification (1 ng/ml) is added for clarity (dashed line).*

Fluvoxamine plasma concentrations could adequately be described, since no substantial deviation from the identity line was observed in the residual diagnostics. The observed plasma concentrations were in close agreement with the predicted individual and population plasma concentrations. However, some bias was observed for the plasma concentrations that were close to the quantification limit in Figure 3b. The observed unidirectional bias in the plots of the observed vs. the population predicted concentrations at these low concentrations might be explained by influence of the quantification limit.

Effects of Fluvoxamine on REM Sleep

The animals showed a normal circadian sleep-wake pattern as compared to patterns after saline injection (data not shown). A clear effect on the various sleep-wake states

of the lights going off could be observed, which started already some time before the lights were actually turned off. This is a well-known anticipatory circadian effect on the lights going off. Fluvoxamine showed clear effects on the various sleep-wake states; light sleep was increased whereas intermediate state, REM sleep and deep sleep were decreased (data not shown) compared to baseline. The mean (n=8) time courses of the effect on REM sleep after IP administration of saline or 1, 3.7 or 7.3 mg/kg fluvoxamine are depicted in Figure 4.

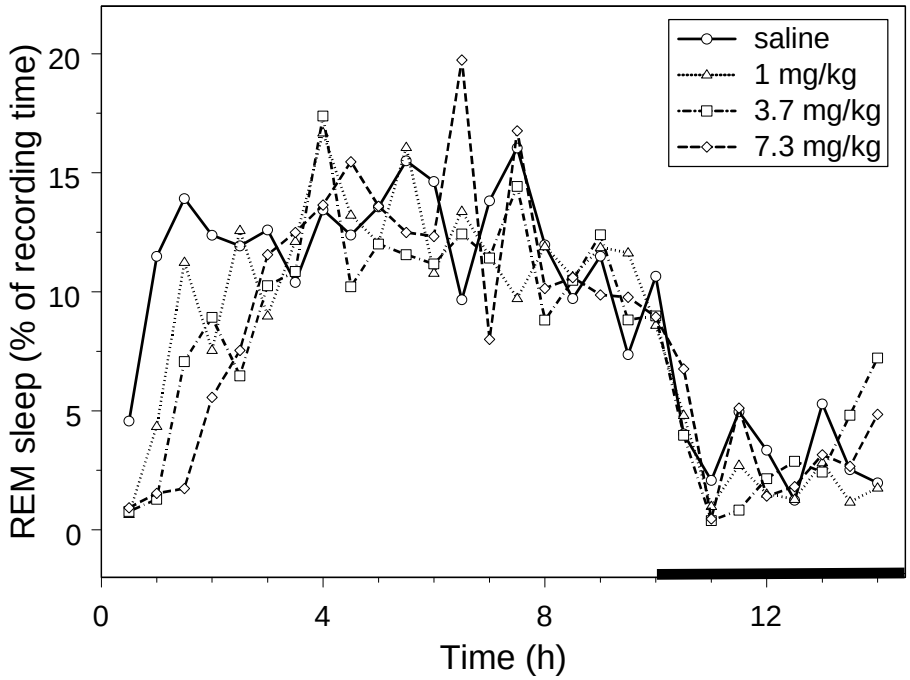

Figure 4. *Time course of REM sleep in Sprague Dawley rat, obtained after an IP injection of saline (circles) or fluvoxamine at 1 (triangles), 3.7 (boxes) or 7.3 mg/kg (diamonds). The effects on REM sleep are expressed as a percentage of a 30 min recording period for 14 hr after dose administration. The black bar indicates the dark period. Values are mean of 8 animals per experimental group.*

The effects on REM sleep are expressed as a percentage of the total recording time of subsequent 30 min periods for 14 hr after dose administration; so a value of 20% corresponds with a time of 6 min. Fluvoxamine showed a dose-dependent decrease effect on the onset of the increase in REM sleep compared to saline administration. After saline administration, the animals showed already significant REM sleep at the first time point of 30 min, in contrast to the animals that received fluvoxamine at the three dosages, where the onset of the increase in REM sleep was delayed in a dose dependent manner. Thereafter, REM sleep remained constant at a value of around 10-15%, corresponding to a REM sleep time of 3-4.5 min. REM sleep in untreated animals and in animals treated with fluvoxamine decreased after about 9 h. Thus the latency of the onset of the increase in REM sleep (time between drug administration

and occurrence of REM sleep for at least 30 sec) was increased compared to saline. Depending on the administered dose, fluvoxamine-treated animals reached these REM sleep percentages of 10-15% with a delay.

Pharmacodynamic Analysis of the Effects of Fluvoxamine on Mean REM Sleep

In Figure 5, the observed and predicted effects of fluvoxamine on mean values of REM sleep are depicted (as a percentage of the total recording period of 30 min), grouped by administered fluvoxamine dose.

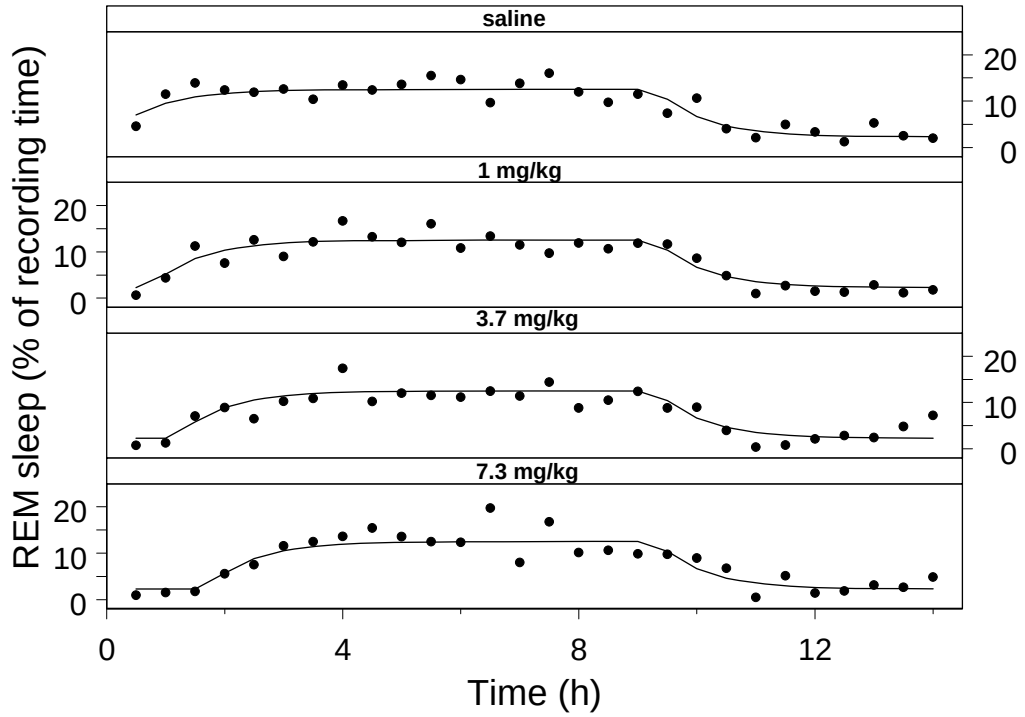

Figure 5. *Observed and predicted effects by the PD model for mean REM sleep values, as a percentage of the recording period, after an IP injection of saline or 1, 3.7 or 7.3 mg/kg fluvoxamine, which was used as explanatory factor in the model. Dots are the observed mean REM sleep times and the solid line represents the predicted mean REM sleep times by the developed PD model (n=8 per group).*

The developed PD model was able to adequately describe observed effects on REM sleep after IP administration of fluvoxamine at each dosage. All structural parameters of the model could be adequately estimated (Table 2).

Table 2. *Population PD parameter estimates for REM sleep (as a percentage of the recording period) after intraperitoneal administration (1, 3.7 and 7.3 mg/kg) obtained by the developed PD model. Mean effects on REM sleep were related to the fluvoxamine dose. Depicted are the population mean estimates for* t_2, k_{in}, k_{out}, REM_{MAX}, $t_{1\,(1\ mg/kg\ FLV)}$, $t_{1\,(3.7\ mg/kg\ FLV)}$, $t_{1\,(7.3\ mg/kg\ FLV)}$ *and* σ^2 *with corresponding coefficient of variation (C.V.).*

Parameter	Unit	Value	C.V. (%)
t_2	h	9.3	2.9
k_{in}	%/min	2.8	5.1
k_{out}	1/min	1.2	16
$REM_{\max}$	-	4.5	11
$t_{1\,(1\ mg/kg\ FLV)}$	h	0.73	5.4
$t_{1\,(3.7\ mg/kg\ FLV)}$	h	1.2	2.2
$t_{1\,(7.3\ mg/kg\ FLV)}$	h	1.7	3.3
$\sigma^2\ \varepsilon$	-	4.4	9.6

*C.V. = Coefficient of Variation (standard error / value * 100%)*

The population values for k_{in} and k_{out} were 2.8 %/min and 1.2 1/min. Estimated offset of the increase in REM sleep (t_2) was 9.3 h and the fraction determining k_{in} ($REM_{\max}$) was 4.5. Hence, during REM sleep, value of k_{in} was 5.5 times of the value of k_{in} during non-REM sleep. The onset time of the increase in REM sleep (t_1) was estimated as 0.73, 1.2 and 1.7 h at a fluvoxamine dose of 1, 3.7 and 7.3 mg/kg, respectively. Values of C.V. ranged between 2.2 and 16% for all parameters. The residual variability was estimated to be 4.4.

The goodness-of-fit plots for the developed PD model are depicted in Figure 6.

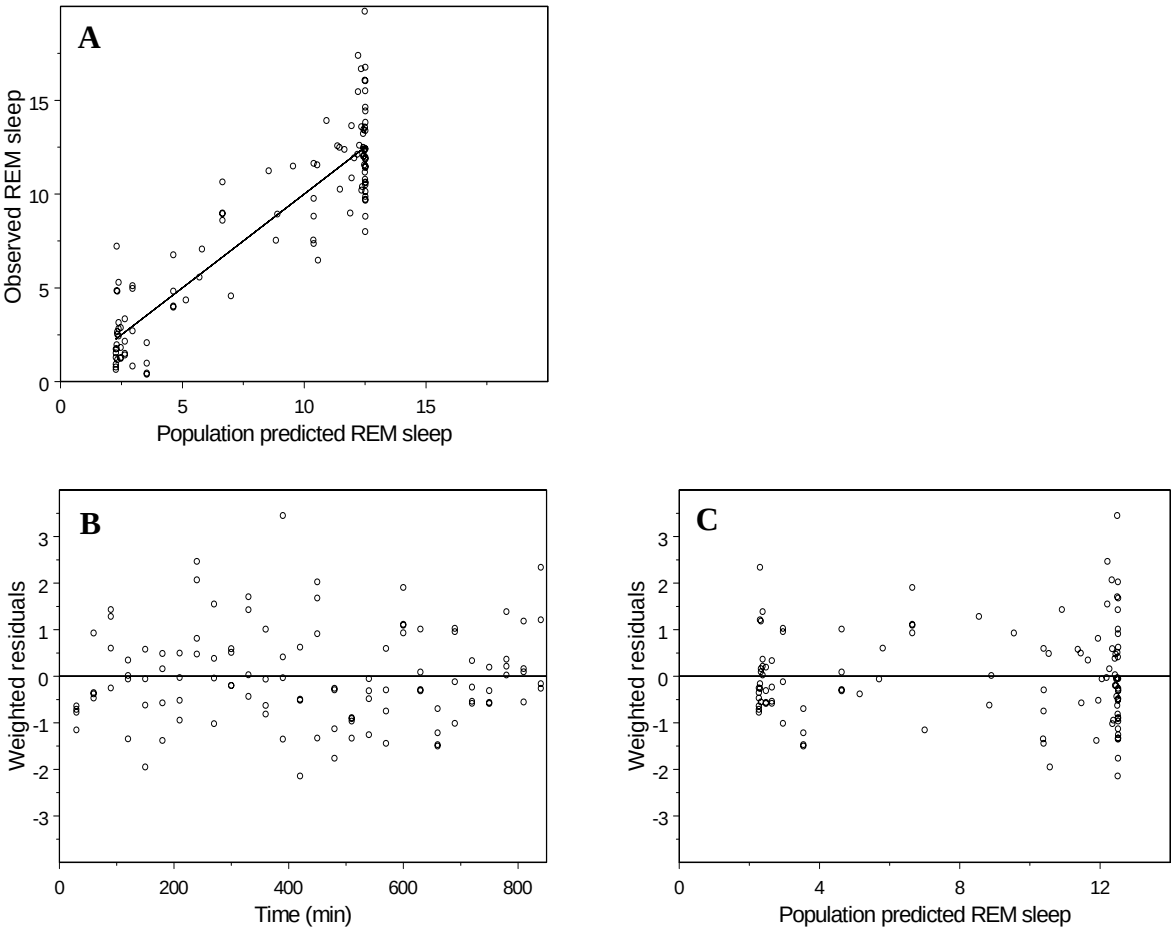

Figure 6. *Goodness-of-fit plots obtained by the developed PD model. Depicted are scatter plots of observed REM sleep times versus population model predictions (A) and scatter plots of the population weighted residuals versus time (B) and versus population model predictions (C).*

Observed effects on mean REM sleep could adequately be described, since no substantial deviation from the identity line was observed in the residual diagnostics. The observed REM sleep times were in close agreement with the predicted population REM sleep times.

Discussion

Disturbances of sleep are typical for most depressed patients and belong to the core symptoms of the disorder, in which disturbance of sleep is even used as a (differential) diagnostic. Many depressed patients suffer from sleep disturbances and mechanisms involved in the regulation of sleep are closely linked to the regulation of mood. It is well known that the 5-HT system is involved in the regulation of sleep and vigilance states (Hery et al., 1977;McGinty and Harper, 1976;Quay, 1968;Semba et al., 1984). Polysomnographic sleep research has demonstrated that depressed

patients typically show a REM sleep disinhibition with a shortened latency of REM sleep and a higher REM density (Berger and Riemann, 1993;Kupfer and Foster, 1972;Kupfer, 1976;Kupfer et al., 1994;Vogel et al., 1980;Vogel, 1983). Furthermore, it is known that selective REM sleep or total sleep deprivation result in a rapid improvement in mood, albeit often short-lived, in about 70% of patients with major depressive disorder (Vogel et al., 1980;Wu and Bunney, 1990;Wirz-Justice and Van den Hoofdakker, 1999;Berger et al., 1997;Riemann et al., 1999;Vollmann and Berger, 1993). Many studies have shown that antidepressants including SSRIs produce significant effects on various sleep-wake stages, both in patients, healthy volunteers and in animals. Suppression of REM sleep and increase of REM latency are the most consistent replicable effects (Hendrickse et al., 1994;Kerkhofs et al., 1990;Kupfer et al., 1991;Nicholson and Pascoe, 1988;Rush et al., 1998;Vasar et al., 1994;Wilson et al., 2000a;Wilson et al., 2000b). Similar changes have been observed in laboratory animals. In rats and cats, acute and chronic treatment with SSRIs induced a sustained decrease in REM sleep (Hilakivi et al., 1987;Kleinlogel and Burki, 1987;Neckelmann et al., 1996;Pastel and Fernstrom, 1987;Sommerfelt and Ursin, 1991).

Not many studies have examined the PK/PD relationship for the effects of SSRIs on sleep-wake architecture. A simple PK/PD investigation was performed for the effects of fluoxetine on sleep EEG in healthy subjects (Feige et al., 2002), in which the relationship between drug plasma concentrations and log spectral power values were described with an E_{max} model. It was found that REM latency and spectral power effects correlated best with total SSRI plasma concentration, but only in the time interval after discontinuation. Various statistical models have been developed to describe the natural sleep-wake cycle and state-to-state transitions in adult humans (Achermann et al., 1993;Borbely, 1982;Yang and Hursch, 1973) and in infants (Holditch-Davis et al., 1998). In another study, a Markov mixed-effects model has been proposed for the effects of temazepam (benzodiazepine) on sleep (Karlsson et al., 2000). Contrary to an earlier Markov model of sleep stage transitions (Kemp and Kamphuisen, 1986), this model integrates interactions between daytime measures, drug dosage, nighttime and the sleep process. In another study, a two-state stochastic model of REM sleep architecture in the rat was proposed (Gregory and Cabeza, 2002) which is a Markov-type model exploring the REM sleep architecture in particular.

The eventual aim of the research described in this article is comprehensive PK/PD modeling of the effect of SSRIs on sleep after acute and chronic administration. PK/PD modeling of the effect on sleep is of considerable interest given the fact that it is a reliable physiological read-out showing relevance to the clinic. Efficacious and

tolerable doses of 50-300 mg/day have been suggested for clinical use of fluvoxamine (Claghorn et al., 1996). It should be noted that the effects of fluvoxamine on sleep in the clinic seem to depend on the patient group involved. Next to its therapeutic efficacy, fluvoxamine has been reported to exert beneficial effects on sleep in for example patients suffering from major depression or from post-traumatic stress disorder (Neylan et al., 2001;Dalery and Honig, 2003), whereas in obsessive-compulsive disorders fluvoxamine treatment resulted in circadian rhythm sleep disorder (Hermesh et al., 2001).

Notwithstanding this clinical complexity, the change in latency to REM sleep onset has clear clinical value because ample evidence supports the role of changes in density and latency to onset of REM sleep as a biomarker for clinical response and anticipated efficacy in depression, provided that these changes are adequately monitored and PK/PD effects correctly modeled (Rijnbeek et al., 2003;Murck et al., 2003). A predictive PK/PD model is therefore of great value in understanding the close relationship between effects of SSRIs on REM sleep parameters. More specifically, because a close agreement on such effects exists between animals and humans, such a model renders unique opportunities in translational medicine and is therefore of invaluable importance in drug discovery and development.

Since the effects on sleep are similar between animals and humans, modeling of the effect on sleep does constitute a valid basis for a further, more advanced study of the inter-species scaling of the effects of SSRIs. In this respect it should be noted that next to many similarities there are also some distinct differences between human and rodent sleep. For example, rodents, being nocturnal animals, have a circadianly dispersed sleep rhythm whereas man has a clear diurnally entrained rhythm. Moreover, the dynamics of rat sleep are of a higher magnitude with shorter sleep cycles and a higher basal fragmentation in the sleep-wake architecture compared to man. Yet, the effects of psychoactive compounds on rodent sleep are often surprisingly similar and it has been convincingly demonstrated that the sleep-wake states in the rat and humans can indeed be defined along comparable lines (Ruigt et al., 1989;Drinkenburg and Ahnaou, 2004). A complicating, practical factor in the analysis of the PK/PD correlations of SSRIs on sleep architecture is the fact that no blood samples can be collected during the pharmacodynamic studies, as even automated blood sampling interferes with the behavioral observations regarding changes in vigilance states. Moreover, intravenous infusion of the study drugs is not feasible for the same reason. This underscores the need for the development of

alternative approaches to the assessment of the pharmacokinetics in investigations of the effects if SSRIs on sleep architecture.

In the present investigation, results are presented of the first studies on the PK and PD of fluvoxamine on sleep-wake organization. We have presented a population PK model for fluvoxamine in plasma after IP administration of different dosages. This model can be used as the basis for prediction of the concentration in future PK/PD studies by application of the population approach. In addition, a PD model has been developed for the effects of fluvoxamine on REM sleep. The dosage of fluvoxamine was related to the observed REM sleep profiles. The studies presented in this investigation are a first step towards a more extensive PK/PD modeling of the effect of SSRIs on sleep after acute and chronic administration.

Pharmacokinetic Model Selection

The two-compartment PK model significantly improved description of the observed fluvoxamine concentration-time courses compared to a one-compartment PK model as is reflected in a reduction of the MVOF by 142 points and a decreased bias in the diagnostic plots. The model was further refined because of the observed dose-dependency in the fluvoxamine concentrations. Specifically, following a dose of 7.3 mg/kg the observed concentrations were higher than the model predicted concentrations. Likewise, following a dose of 1 mg/kg, the observed concentrations were lower than predicted by the model. In the pharmacokinetic analysis strong correlation was observed between fluvoxamine dose and distribution volume (*V*), which is indicative of a dose-dependency in the bioavailability of fluvoxamine following IP administration. Specifically, this non-linearity in pharmacokinetics is not caused by saturation of the elimination, since no non-linearity is observed following intravenous administration in the same dose range (Geldof et al., 2007). In the PK model the observed non-linearity was accounted for by including dose dependency on the inter-individual variability of the value of the parameter *V2*, yielding a reduction in the MVOF by 32 points compared to the model without such a dose effect. Furthermore, description of the population predicted fluvoxamine concentration *versus* time curves was significantly improved, particularly for the dosages of 1 and 7.3 mg/kg. Finally, no correlation between the inter-individual variability of *V2* and dose was present anymore.

Inter-individual variability could be characterized for *V2* and *V3*, which were estimated to be 17.4 and 34.5%, respectively. IIV in the other parameters could not be adequately estimated and was fixed to zero. In our previously developed population

three-compartment PK model describing the plasma concentration *versus* time profiles of fluvoxamine following intravenous administration in Wistar rats, IIV could be characterized for four out of six PK parameters (Geldof et al., 2007). In this study, IIV could be characterized on *CL* (39.5%), *V1* (43.5%), *V2* (50.1%) and *Q2* (25.7%) and thus were in the same range as obtained IIV in the present investigation after IP administration of fluvoxamine.

No significant covariates could be identified in model development to explain possible inter-individual variability between the animals. This result is in contrast to the previously developed population PK model after intravenous fluvoxamine infusion in Wistar rats in which body weight could be identified as a significant covariate of the inter-compartmental clearance *Q2* (Geldof et al., 2007).

The residual variability was estimated to be 0.026 (Table 1). Differences in the plasma concentration *versus* time profiles between the animals are presumably caused in part by variability in the metabolism of fluvoxamine. In humans, cytochrome P450 (CYP) isoenzymes play a major role in the biotransformation of fluvoxamine, which is extensive and associated high inter-individual variability (DeVane and Gill, 1997;Preskorn, 1997).

Since blood sampling has been shown to interfere with the assessment of the effects on sleep, an important question is how estimates of the pharmacokinetics in individual animals can be obtained, using the population PK model. In theory, there are several options. One of the options is to reduce the number of blood samples in the individual animals and then to use the population PK model for prediction of the individual concentration *versus* time profiles by post hoc analysis. The advantage of this approach is that pertinent information on the PK is obtained at the same occasion as the behavioral study. It should be taken into consideration however that even a limited number of blood samples taken during the behavioral observations might still affect the sleep architecture. A solution might be to obtain blood samples after the behavioral observations. A second alternative option is to determine the pharmacokinetics on a separate occasion in the same animals. This approach is particularly attractive in the situation where the inter-occasion variability is small relative to the inter-individual variability. As a final alternative, the pharmacokinetics in the individual animals of the behavioral study might be predicted on the basis of a population pharmacokinetic model developed in separate group of animals. The latter approach requires that most of the inter-individual variability can be explained on the basis of relevant covariates. The exploration of these options will be the subject of future investigations in our laboratory.

Pharmacodynamic Model for the Effect of Fluvoxamine on REM Sleep

In the present investigation, an important effect of SSRIs on sleep was successfully characterized. The proposed indirect response PD model adequately described the effects of fluvoxamine on the onset of the increase in REM sleep. The controlling REM sleep generation function accounting for the change in REM sleep and the administered fluvoxamine dose of fluvoxamine was related to the onset of the increase in REM sleep (t_1). The onset of the increase in REM sleep was significantly dependent on the administered dosage of fluvoxamine and was estimated as 0.73, 1.2 and 1.7 h at 1, 3.7 and 7.3 mg/kg, respectively. The corresponding range of fluvoxamine plasma concentrations, predicted with the developed PK model, was 27-37, 64-93 and 91-204 ng/ml after 1, 3.7 and 7.3 mg/kg, respectively. Since the effect on the onset of the increase in REM sleep roughly occurs at different concentrations of fluvoxamine, further investigations on the precise PK/PD correlations of fluvoxamine on REM sleep are needed. In contrast, the start time of the offset of the increase in REM sleep was not influenced by the administered fluvoxamine dose and therefore presumably independent of the fluvoxamine plasma concentrations.

The dataset of the effects of fluvoxamine on REM sleep as well as on the other sleep-wake states was very rich and powerful. Therefore, next to the modeling approach that was used in the present study, the data could be modeled by other modeling techniques as well. Besides modeling the data in a continuous manner, they could also have been modeled using a categorical (binomial or multinomial) model or by using a Markov model. The developed population PK model is useful in these future investigations for prediction of the fluvoxamine plasma concentrations. An example of categorical modeling was described in our previous study in which the effects of fluvoxamine on the *para*-chloroamphetamine (PCA)-induced behavioral effects were explored. Sleep data have been described previously by Markov models (Karlsson et al., 2000;Kemp and Kamphuisen, 1986) or a type of Markov model (Gregory and Cabeza, 2002). In these studies, models were developed to describe the probability of moving from one sleep stage to another. The data obtained in the present study could also be modeled by this type of Markov modeling using Markov chains for the probability transitions between the six sleep states, which are only dependent on the state before. However, this analysis was beyond the scope of the article.

In conclusion, a linear two-compartment PK model with absorption and a dose-dependency on the inter-individual variability in *V2* described the fluvoxamine plasma concentration *versus* time profiles in individual rats. Mean population PK parameter estimates for *ka*, *CL*, *Q*, *V2* and *V3* were 0.024 1/min, 143 ml/min, 37.1

ml/min, 338 ml and 3260 ml, respectively. Inter-individual variability was identified on *V2* (17.4 %) and *V3* (34.5 %). In the PD model, the effects of fluvoxamine on REM sleep were characterized by an indirect response model with stimulation of k_{in}. All structural parameters of the model could adequately be described and mean population values for k_{in}, k_{out}, start time of the decrease in REM sleep (t_2) and the fraction determining k_{in} (REM_{max}) were estimated as 2.8 %/min, 1.2 1/min, 9.3 h and 4.5. The values for the start time of the increase in REM sleep (t_1) were estimated as 0.73, 1.2 and 1.7 h after a fluvoxamine dose of 1, 3.7 and 7.3 mg/kg, respectively and zero after administration of saline. This investigation is a first step towards comprehensive PK/PD modeling of the effect of SSRIs on sleep following acute and chronic administration.

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SECTION 3

Conclusions and General Discussion

Chapter 11

Mechanism-based PK/PD Modeling of SSRIs: Summary & Conclusions

11.1 Introduction

The main objective of the investigations described in this thesis was to explore the PK/PD correlations of fluvoxamine, as a prototype for the Selective Serotonin Reuptake Inhibitors (SSRIs). In the various investigations, a spectrum of different biomarkers was used, each reflecting a specific process on the causal path between drug administration and response. In PK/PD models, the information obtained on the different biomarkers was integrated for the various effects.

A reduced activity of serotonergic neurotransmission is an important feature in the pathogenesis of depression (Coppen, 1967;Owens and Nemeroff, 1994). SSRIs selectively and powerfully block the reuptake of serotonin (5-hydroxytryptamine, 5-HT) in the presynaptic nerve terminal via the serotonin transporter (SERT), resulting in increased extracellular 5-HT levels (Bel and Artigas, 1992;Fuller, 1994). SSRIs constitute the first line of treatment in depressive disorders (Ables and Baughman, III, 2003;Isaac, 1999). Although SSRIs rapidly inhibit the reuptake of 5-HT, maximal antidepressant effects are only observed after weeks of chronic treatment, indicating that long-term adaptive changes are important for therapeutic efficacy (Bel and Artigas, 1996;Bosker et al., 1995;Benmansour et al., 1999). The observed adaptation in the functioning of SSRIs is in support of a systematical approach for analysis of the serotonergic system. In this approach, various important intermediate steps from drug administration to the eventual response are analyzed, as has been performed in this thesis. The relation between the various biomarkers is important for understanding the complete biological system. This is particularly important for investigation of the effects after chronic administration of SSRIs. Although pilot studies have been performed on the chronic effects of fluvoxamine on sleep-wake architecture, the investigations presented in this thesis have focused on the effects after acute SSRI administration. This actually is the first step in understanding the processes underlying the various effects of SSRIs.

Over the years, several animal models, such as the forced swim and tail suspension test, have been developed that can detect specific behavioral changes that are sensitive to the effects of antidepressants (Kelliher et al., 2003;Teste et al., 1993;Takamori et al., 2001). By analysis of the relationship between the PK and PD in behavioral animal models, novel insights in the mechanisms of the time dependencies in the PD of SSRIs could be provided. However, very few studies have addressed the PK/PD correlations of SSRIs and other psychotropic drugs in these animal models (Della Paschoa et al., 1998;Jonker et al., 2003;Vis et al., 2001), probably caused by the potentially complicating factors including a) the interference

of blood sampling with the measured PD effect, b) the availability of only sparse PK and/or PD data and c) the fact that often the PD endpoints are non-continuous. These complexities can in part be overcome by application of a mixed effects modeling approach as was illustrated in **Chapter 5**. Population PK/PD modeling is based on nonlinear mixed effects analysis and characterizes the pharmacokinetics and concentration-effect relationships in populations rather than in individual subjects (Hashimoto and Sheiner, 1991;Sheiner and Ludden, 1992). PK/PD models have been successfully applied to continuous and non-continuous measures of drug effect, for both direct (Karlsson et al., 1995;Minto et al., 1997b;Schnider et al., 1996) and indirect (Bouillon et al., 1996;Minto et al., 1997a) PD models. In recent years, progress has been made in the field of mechanism-based PK/PD modeling, of which the objective is to understand, in a strictly quantitative manner, the mechanisms that determine the time-course of the intensity of the drug effect *in vivo*. A pertinent feature of mechanism-based PK/PD models is that they contain specific expressions to describe processes on the causal path between drug administration and response, such as the distribution of the drug to the target site, the binding to the target, the activation of the target and homeostatic feedback (Danhof et al., 2005;Danhof et al., 2006). The development of mechanism-based PK/PD models relies on biomarkers, which characterize quantitatively the processes on the causal path between drug administration and response (Colburn and Lee, 2003;Rolan, 1997). In our investigations on the PK/PD correlations for fluvoxamine, a cascade of biomarkers in the rat was used.

11.2 Summary and Discussion

The various biomarkers each reflected a specific process on the causal path between the administration of fluvoxamine and the observed effects. The investigations steps are illustrated in Figure 1.

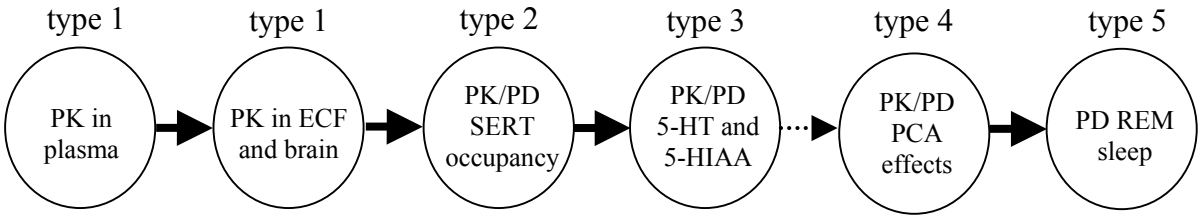

Figure 1. *Schematic illustration of the various biomarkers used in the present thesis, each reflecting a specific aspect of the mechanism of action of fluvoxamine in the rat as a prototype for SSRIs.*

The investigations in the rat included the development of a population PK model for fluvoxamine in plasma (type 1 biomarker according to (Danhof et al., 2005)), the development of a PK model for the transport of fluvoxamine across the blood-brain barrier in brain extracellular fluid (ECF) of the frontal cortex and brain tissue (type 1), the development of a PK/PD model for the effect of fluvoxamine on the occupancy to SERT in frontal cortex (type 2), the development of a PK/PD model for the effects of fluvoxamine on brain 5-HT and 5-hydroxyindoleacetic acid (5-HIAA, main metabolite) levels (type 3). Then, a categorical PK/PD model for the effects of fluvoxamine on PCA (*para*-chloroamphetamine)-induced behavioral effects was developed as a sort of intermediary biomarker (type 4). Ultimately, a PD model for the effect of fluvoxamine on rapid eye movement (REM) sleep was developed (type 5). The information on the different biomarkers was integrated in PK/PD models for the various effects.

11.2.1 Population PK Model of Fluvoxamine in Rats

In order to characterize the PK/PD relationships for fluvoxamine, a population PK model was developed, describing the plasma concentration *versus* time profiles obtained after a 30 min intravenous infusion of 1, 3.7 or 7.3 mg/kg fluvoxamine to Wistar rats (**Chapter 5**). The PK model was developed on the basis of PK information obtained in six separate studies with a different experimental setup and/or study site, in which fluvoxamine was administered via a 30 min intravenous infusion. Hence, all studies described in this thesis, except for the study on the effects of fluvoxamine on REM sleep (**Chapter 10**), since fluvoxamine was administered via an intraperitoneal injection in this study. Sampling designs were not identical for all studies and included dense as well as sparse sampling. The problem of the interference of blood sampling on the measured effect can be overcome by application of population PK modeling. In principle there are several options. One of the options is to reduce the number of blood samples in the individual animals and then to use the population PK model for prediction of the individual concentration *versus* time profiles by post hoc analysis. The advantage of this approach is that pertinent information on the PK is obtained at the same occasion as the behavioral study. It should be taken into consideration however that even a limited number of blood samples taken during the behavioral observations might still affect the sleep architecture. A second option is to determine the pharmacokinetics on a separate occasion in the same animals. This approach is particularly attractive in the situation where the inter-occasion variability is small relative to the inter-individual variability.

As a final alternative, the pharmacokinetics in the individual animals of the behavioral study might be predicted on the basis of a population pharmacokinetic model developed in separate group of animals. The latter approach requires that most of the inter-individual variability can be explained on the basis of relevant covariates. In the present investigation we have explored the use of the population PK model for prediction of individual concentration time curves on the basis of sparse blood samples taken in the behavioral study. The second option (estimation of the pharmacokinetics on separate occasion) was not explored since no information on the inter-occasion variability in PK was available in our dataset. Furthermore, since no covariates could be identified which explain most of the inter-individual variability the third option (estimation of the pharmacokinetics in a separate group of animals) was not considered relevant. It was shown that on the basis of this model the full concentration *versus* times profiles of fluvoxamine in individual rats could be described on the basis of information from sparse data, which is important, since blood sampling readily interferes with pharmacodynamic observations in behavioral models.

All the information on the PK of fluvoxamine was simultaneously analyzed by population PK modeling. The developed three-compartment PK model enabled full characterization of the plasma concentration *versus* time profile on the basis of sparse blood concentrations. All structural parameters of the population PK model could be adequately estimated and mean population values for *CL*, *V1*, *V2*, *Q2* (typical body weight of 253 g), *V3* and *Q3* were estimated to be 25.1 ml/min, 256 ml, 721 ml, 30.0 ml/min, 136 ml and 1.0 ml/min, respectively. Inter-individual variability could be characterized for *CL*, *V1*, *V2* and *Q2*, which were estimated to be 39.5, 43.5, 50.1 and 25.7%, respectively. Body weight was identified as a significant covariate of the inter-compartmental clearance *Q2*. The pharmacokinetics was independent of factors such as dose, surgery (for instrumentation) and study site. A predictive check and bootstrap analysis confirmed the predictive ability, model stability and precision of the parameter estimates.

To investigate the utility of the model to overcome the problem of the effect of blood sampling, simulation of a study was performed for the effects of fluvoxamine on REM sleep using a sparse PK sampling design with collection of only two blood samples per animal. In order to avoid possible interference of the blood sampling with the behavioral readout, the blood samples were collected after dissipation of the behavioral effect. Two blood samples at an interval of 135 minutes were collected as this enables the distinction between inter-individual and residual variability. It is well

established that SSRIs, including fluvoxamine, have significant effects on various sleep-wake stages in humans as well as in animals. Among these effects, a decrease in REM sleep duration is commonly observed after SSRI administration (Wilson et al., 2000b;Wilson et al., 2000a). In preliminary investigations it has been demonstrated that repeated blood sampling significantly influences sleep architecture in rats (Geldof et al., unpublished observations), thus complicating the PK/PD analysis of this effect. Even when chronically implanted cannulas are used, possible undesirable effects of blood sampling cannot be ignored. In a pilot study on the chronic effects of fluvoxamine on sleep-wake architecture in the rat using osmotic minipumps and a limited number of blood sampling via the tail vein, it was shown that although the influence on the sleep-wake cycle was less compared to these effects observed after acute fluvoxamine administration with more intensive blood sampling, normal sleeping pattern in the rats was not completely restored, even after 3 weeks of chronic dosing (Geldof et al., unpublished observations). The simulations with the PK/PD model showed that although the simulated dataset contained only two PK samples, the complete individual fluvoxamine concentration *versus* time profiles could be adequately predicted on the basis of the post hoc estimates from the developed population PK model (Figure 2).

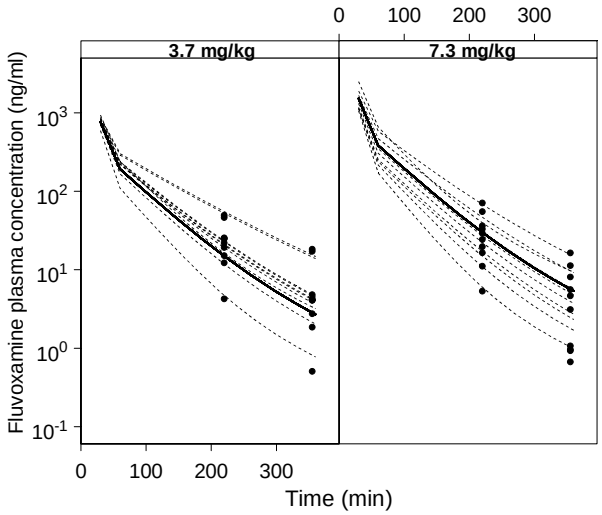

Figure 2. *Fluvoxamine concentration versus time profiles in plasma, obtained in the simulation study in which a 30 min IV infusion of 3.7 and 7.3 mg/kg fluvoxamine was administered. Depicted are the observed concentrations (dots), individual predictions (dashed lines) and population predictions (solid lines), separated by fluvoxamine dose.*

This was illustrated in the high accuracy of the obtained PK parameter estimates. By using the pertinent information from the population PK model, individual PK profiles and the PK/PD correlation could be adequately described.

To our knowledge, the PK of fluvoxamine in rats has not been described before by compartmental analysis. The proposed population PK model was used in the following studies described in this thesis on the PK/PD correlations fluvoxamine.

Another application of the model could be related to optimization of the sampling design. For optimization of the sampling design, trial simulation should be preformed, as is now routinely done for clinical trials (Gieschke and Steimer, 2000). Using this approach, pertinent information on the optimal sampling (i.e. minimum number of blood samples, optimal sampling times) to adequately describe the PK can be obtained. The developed population PK model for fluvoxamine constitutes a unique basis for such trial simulations.

11.2.2 Physiological PK Modeling of Non-Linear Brain Distribution of Fluvoxamine in the Rat

After developing a population PK model describing the plasma concentration *versus* time profiles of fluvoxamine in individual rats, the next step was to study the time course of fluvoxamine concentrations in the brain. In PK/PD modeling, target site distribution can be an important factor, which affects the intensity, the onset and the duration of the effect. Particularly for relatively large hydrophilic compounds and for compounds that are substrates for specific transporters, target site distribution may be restricted and non-linear, of which this latter was demonstrated for fluvoxamine brain distribution in the present study. This has important implications for the PK/PD correlations for drugs, which act in tissues that are protected by specific barriers (i.e. the central nervous system). The mechanism of the transport across the blood-brain barrier (BBB) and blood-cerebrospinal fluid (CSF) barrier can involve passive diffusion through endothelial cells or via tight junctions, active transport into the brain by specific transport mechanisms and transport out of the brain by active efflux pumps. Furthermore, drugs can also be eliminated from the brain ECF by bulk flow through the perivascular space into the CSF or by metabolism. In particular, active efflux pumps appear to be very important at the level of the BBB and several transporter molecules have been identified as efflux pumps on the luminal side of the BBB, like P-glycoprotein (Pgp) and multidrug resistance protein (MRP). Because Pgp is localized at the BBB in the apical membrane of brain capillary endothelial cells and therefore transports substrates toward the blood compartment, Pgp can effectively limit the penetration into and retention within the brain (Weiss et al., 2003). There are indications for such efflux transport of fluvoxamine across the BBB since it was shown *in vitro* that fluvoxamine induces intermediate Pgp inhibition in MDR1 cells (model for human Pgp) as well as in primary porcine brain capillary endothelial cells (model for the BBB) (Weiss et al., 2003), although this could not be determined in another study in MDR-1 cells (Doan et al., 2002). However, in this

latter study the *in vitro* passive permeability of fluvoxamine was rather high (317 nm/s), thereby possibly obscuring the contribution of the active efflux. Furthermore, the current study demonstrated that the distribution of fluvoxamine to the brain was non-linear in the rat *in vivo*. Because it is not completely clear at this point which transporter at the BBB is important for fluvoxamine brain distribution, this could be investigated by analysis of fluvoxamine brain distribution in the presence of specific inhibitors of these transporters at the BBB.

A physiological PK model was proposed for estimation of the brain distribution of fluvoxamine in the rat (**Chapter 6**). The individual post hoc estimates for *CL*, *V1*, *V2*, and *Q2* and the population post hoc estimates for *V3* and *Q3* obtained from the previously developed population PK model in plasma were used to estimate the fluvoxamine concentrations in plasma, which served as input function in the brain. Fluvoxamine was transported rapidly into the brain and maximal ECF and brain concentrations were observed only about 20 min later than maximal plasma fluvoxamine concentration. Since maximum concentrations in ECF and brain were observed at the same time after administration, distribution between fluvoxamine levels in ECF and total brain was very rapid. Furthermore, fluvoxamine concentration *versus* time curves in ECF and brain showed the same kinetic profiles without any delay of different elimination kinetics.

When observed fluvoxamine concentrations were normalized for dose, non-linearity could be observed in ECF and brain, which was not observed in plasma. Specifically, with increasing fluvoxamine dose a disproportional increase in brain ECF and brain tissue concentrations relative to the plasma concentrations was observed (Figure 3).

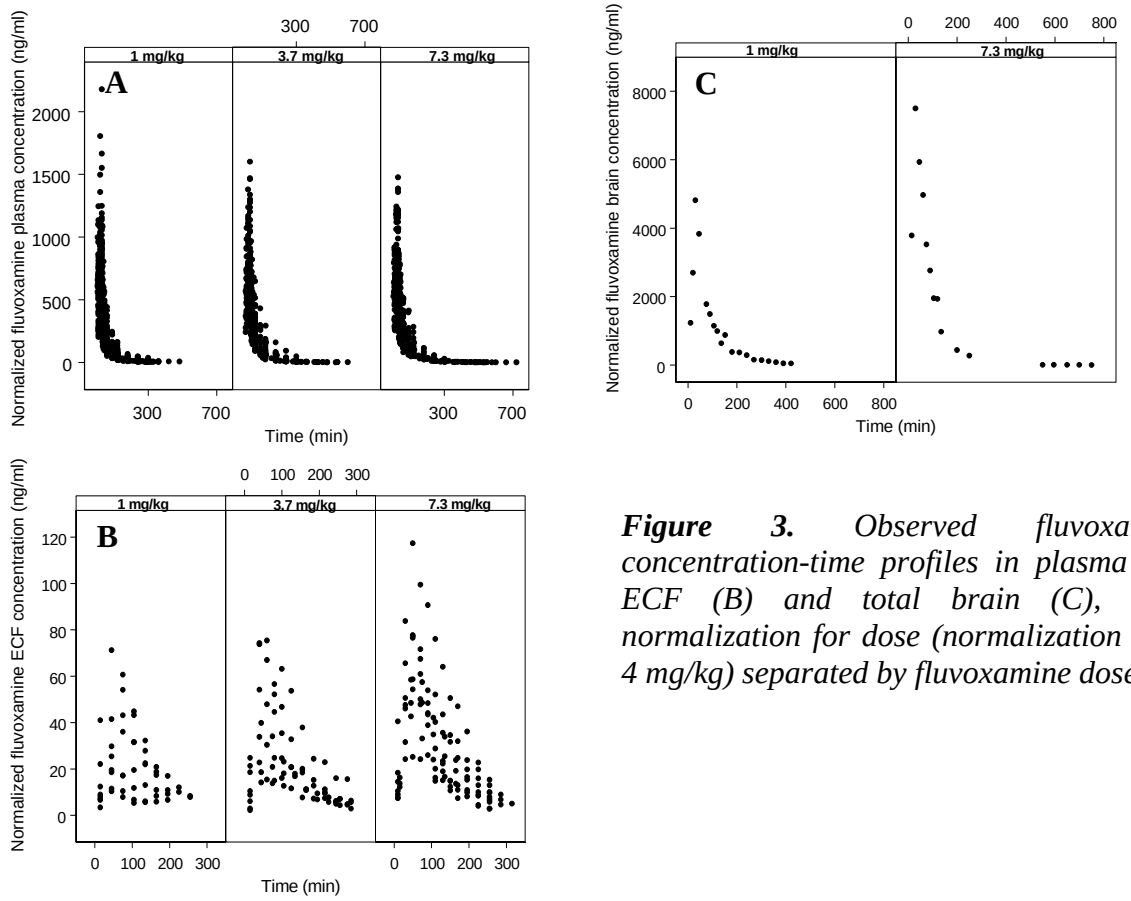

Figure 3. *Observed fluvoxamine concentration-time profiles in plasma (A), ECF (B) and total brain (C), after normalization for dose (normalization dose 4 mg/kg) separated by fluvoxamine dose.*

The observed disproportional increase in ECF and brain concentrations at equal plasma concentrations was not caused by non-linear protein binding. In a separate study with the same experimental set-up and dosages, protein binding was determined at 30 and 60 min after fluvoxamine infusion, which was equal to 97.6 ± 0.4% (n=8) and 97.7 ± 0.6% (n=7).

The kinetics of the distribution between plasma and brain were estimated by simultaneous analysis of the plasma, ECF and total brain concentrations on the basis of a compartmental model. The developed model was able to predict the time course of the fluvoxamine concentration in brain ECF of the frontal cortex and brain tissue on the basis of fluvoxamine plasma concentrations. The physiological PK model enabled the separate characterization of the passive influx, the passive efflux and the active efflux of fluvoxamine to and from the brain. The proposed PK model consisted of three compartments for description of the plasma fluvoxamine concentration in combination with two compartments for the ECF and brain concentrations. Within the brain, the mass exchange between a shallow perfusion-limited and a deep brain compartment was described by a passive diffusion term and a saturable active efflux term. The model resulted in precise estimates of the parameters describing the passive influx in the brain (k_{in}) of 0.16 1/min, the efflux rate from the shallow brain

compartment (k_{out}) of 0.019 1/min and the fluvoxamine concentration at which 50% of the active efflux was reached (C_{50}) of 710 ng/ml. Since measured observed fluvoxamine ECF concentrations ranged between only 1 and 214 ng/ml, full saturation of the active removal flux was not reached in the current studies. Nevertheless, the saturable function was required in the model for description of observed non-linearity in ECF and brain concentration *versus* time curves at concentrations observed in the current study. Incorporation of the saturable active efflux term in the model significantly improved the adequacy of description of observed data based on a decrease of the minimum value of the objective function (MVOF) by 37 points. Furthermore, estimates of k_{in} and k_{out} were not influenced by the value of parameters values for C_{50} and therefore, were not dependent on an adequate description of the maximal concentrations by the model at which complete saturation of the active removal flux was reached.

The developed physiological PK model for description of non-linear brain distribution of fluvoxamine constitutes an extension of an earlier described model that has been proposed to describe the brain distribution of thiopental (Upton et al., 2000;Igari et al., 1982). In this membrane-limited brain model, a shallow flow-limited compartment and a deeper membrane-limited compartment were described, in which the entrance of drug into this deeper compartment was defined by a permeability term. The developed physiological model for description of fluvoxamine brain distribution is an extension of this previously described model by the addition of a term for an active saturable efflux between these brain compartments. In this manner, it was possible to adequately describe observed ECF and brain fluvoxamine concentrations. Since concentration-time curves in ECF and brain showed the same kinetic profiles without any delay or difference in elimination, rapid equilibrium between fluvoxamine in the shallow perfusion-limited compartment, deep brain compartment and total brain was assumed.

Next to the model described in **Chapter 6**, an alternative model in which non-linear brain distribution was defined on the basis of non-linear tissue binding, rather than non-linear transport. In this respect, the concept of target-mediated disposition, assuming binding to the SERT as the mechanism of the non-linear brain distribution (Levy, 1994;Mager et al., 1991;Mager and Jusko, 2002), was considered. In this model one brain compartment was defined for fluvoxamine in the free phase and another compartment for fluvoxamine in the bound phase. Observed fluvoxamine brain concentrations were equal to the sum of fluvoxamine in the free and bound phase. The concentration of fluvoxamine in the bound phase was composed of a

saturable and linear component and related to the free fraction. This alternative model could also describe observed fluvoxamine concentrations in ECF and total brain as adequately as the model with incorporated saturable efflux transport mechanism. However, the values of the parameters describing the non-linear binding in the brain were completely different from the parameters describing the binding of fluvoxamine to SERT in a study in which the binding to SERT was estimated on the basis of imaging (**Chapter 7**). Furthermore, the physiological model described in the current article appears to be more plausible constituting a mechanistic approach about how the transport of fluvoxamine across the BBB might occur.

To overcome the adhesion of fluvoxamine to the microdialysis probe, artificial cerebrospinal fluid was required to be prepared in the presence of 0.5% BSA. Since this does not reflect the physiological situation in the brain, the possible influence of the addition of this protein should be taken into account. Since BSA cannot be transported over the membrane of the microdialysis probe, it could be possible that more fluvoxamine in ECF was drawn from the brain over the membrane to the outlet of the probe and therefore measured fluvoxamine concentrations in ECF could be higher than under physiological conditions without BSA. However, it is likely that such an effect would be constant over time and be reflected in the *in vivo* recovery estimate. Moreover, since this is a constant factor it should not have influence on the estimates predicted by the model. In addition, the presence of such an effect would probably be small because used BSA concentration is relatively low.

The proposed physiological brain distribution model constituted a basis for precise characterization of the PK/PD correlation of fluvoxamine by taking into account the non-linearity in brain distribution. The PK model was used in the following studies described in this thesis to explore the relationships between the plasma and brain PK and PD endpoints for fluvoxamine in the rat.

11.2.3 PK/PD Modeling of Fluvoxamine Serotonin Transporter Occupancy

Since the concentrations of fluvoxamine could be adequately described in plasma, brain ECF and brain tissue in individual rats, the next step in our investigations was to explore the PD effects of these concentrations. In the first study on the PK/PD correlations of fluvoxamine in the rat, the effects of fluvoxamine on the occupancy of the serotonin transporter (SERT) in rat frontal cortex were explored (**Chapter 7**).

To date, most investigations on the long term effects of antidepressants have focused on changes in the functionality of serotonergic autoreceptors and postsynaptic receptors, which appear to be sensitive to adaptive changes (Auerbach and Hjorth,

1995;Blier and Bouchard, 1994;Bosker et al., 1995). However, the functionality of SERT itself appears to be subject to regulatory influences as well (Benmansour et al., 1999;Ginovart et al., 2003;Horschitz et al., 2001;Pineyro et al., 1994;Ramamoorthy and Blakely, 1999). Specifically, a decreased SERT gene expression (Lesch et al., 1993) and a decreased SERT density (Benmansour et al., 1999) have been observed after chronic treatment in rats.

The PK/PD correlation for the occupancy of fluvoxamine to SERT in rat frontal cortex is an important intermediary step in the PK/PD analysis of SSRIs. Despite the wide interest in the assessment of SERT occupancy in clinical studies (Meyer et al., 2001;Suhara et al., 2003), very few investigations have addressed *in vivo* SERT occupancy in relation to behavioral responses in animal studies (Ginovart et al., 2003;Hirano et al., 2005), which can presumably be explained by the relative difficulty of quantifying *in vivo* SERT occupancy. In this contribution, we present a PK/PD model, which enables characterization and prediction of the time-course of *in vivo* SERT occupancy in behavioral studies. An *ex vivo* technique was used to characterize *in vivo* fluvoxamine SERT occupancy. A limitation of this approach is that only a single observation of SERT occupancy is obtained. Furthermore, due to the high affinity of fluvoxamine for SERT, drug concentrations in biological fluids are typically below measurable values. The results of the obtained information on the PK in plasma, brain ECF, brain tissue and SERT occupancy were therefore interpreted by nonlinear mixed effects modeling. Because only unoccupied transporters are available for the radioligand, *ex vivo* [^{3}H]citalopram labeling of SERT is inversely related to SERT occupancy by fluvoxamine.

The effect *versus* time curve showed that maximal SERT occupancy was reached instantly after administration and maintained for 1.5 and 7 hrs after 1 and 7.3 mg/kg, respectively. Thereafter, fluvoxamine SERT occupancy linearly decreased in time at a rate of 8 %/hr, which was the same after both fluvoxamine dosages. Fluvoxamine SERT occupancy reached 0% after about 15 hrs following a dose of 7.3 mg/kg. The individual post hoc estimates for the pharmacokinetic parameters *CL*, *V1*, *V2*, and *Q2* and the population post hoc estimates for *V3* and *Q3* obtained from the previously developed population PK model in plasma were used to estimate the fluvoxamine concentration in plasma at the time when fluvoxamine SERT occupancy was determined. The individual post hoc estimates for the influx rate constant in the brain (k_{in}), the efflux rate from the brain (k_{out}) and the population post hoc estimates for the fluvoxamine concentration at which 50% of saturation of the active removal flux was reached (C_{50}) obtained from the previously developed physiological PK brain

distribution model were used to estimate the fluvoxamine concentration in brain ECF and brain tissue at the time when fluvoxamine SERT occupancy was determined. The fluvoxamine concentrations in plasma and brain ECF were below the limit of quantification (1 ng/ml) for a significant part of the concentration-effect curves. However, by analysis of these profiles in plasma and brain ECF in relation with the much higher fluvoxamine concentrations in brain tissue in the previously described physiological model for fluvoxamine brain distribution, it was possible to predict accurately the fluvoxamine concentrations in plasma and brain ECF. Fluvoxamine concentrations in plasma and brain ECF could be predicted over a much wider concentration range. In the present investigation, the previously proposed population PK in plasma and physiological brain distribution model were successfully applied to predict fluvoxamine concentrations in plasma and brain ECF in a relevant concentration range with regard to fluvoxamine SERT occupancy. No hysteresis between the fluvoxamine concentration in plasma, brain ECF, brain tissue and SERT occupancy was observed, and therefore these two could be directly related to each other. The relation between fluvoxamine concentrations and SERT occupancy were successfully described by a hyperbolic function (B_{max} model) allowing estimations of the relevant pharmacodynamic parameters (Figure 4).

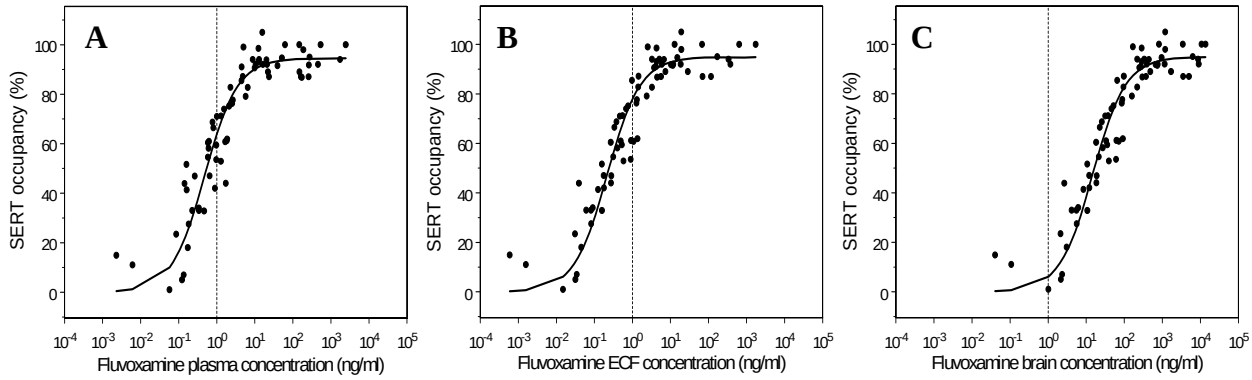

Figure 4. *The relationships between fluvoxamine concentrations in plasma (A), ECF of the frontal cortex (B) and brain tissue (C) and the degree of fluvoxamine SERT occupancy. Dots are the observed SERT occupancies and the solid line represents the predicted SERT occupancies by fluvoxamine. The limit of quantification (1 ng/ml) is added for clarity (dashed line).*

When analyzing fluvoxamine SERT occupancy in relation to brain ECF and brain tissue concentrations, MVOF was decreased by 12.2 points ($p < 0.01$) compared to the model in which SERT occupancy was related to fluvoxamine plasma concentrations, presumably as result of non-linear brain distribution. In addition, both the residual error and inter-individual variability in EC_{50} were significantly lower. Furthermore, fluvoxamine levels in brain ECF and brain tissue have indeed a small

lag time of about 30 min compared to fluvoxamine plasma levels because of the required time for transport of fluvoxamine from plasma to the brain. Maximal SERT occupancy (B_{max}) was not significantly different when related to PK in plasma, brain ECF or brain tissue and was estimated as 95% by all models. Fluvoxamine concentration at half of the maximal SERT occupancy (EC_{50}) was 0.48 ng/ml in plasma, 0.22 ng/ml in brain ECF and 14.8 ng/ml in brain tissue, of which only this latter value was above the quantification limit (1 ng/ml) of the bioanalytical assay. Estimated EC_{50} values were very low, particularly in plasma and brain ECF, hence only very small levels of fluvoxamine must be present in plasma and brain ECF to be able to occupy SERT in rat frontal cortex. Because fluvoxamine SERT occupancy was determined in frontal cortex of the rat, it would be rational to relate SERT occupancy to the measured fluvoxamine ECF levels, since these were also measured in this brain region.

The developed PK/PD model might also be used to explore the effects of fluvoxamine on SERT occupancy after chronic administration. This is important since it is suggested that long-term adaptive changes are important for therapeutic efficacy (Bel and Artigas, 1996;Bosker et al., 1995;Benmansour et al., 1999). With this method and model, the effects on the possible occurrence of up- or down-regulation of the SERT after chronic SSRI administration could be explored in future experiments.

Only over recent years, the results of studies on the occupancy of various SSRIs to SERT in healthy volunteers and depressed patients have been described by application of the neuroimaging techniques positron emission tomography (PET) or single photon emission computed tomography (SPECT) (Meyer et al., 2001;Meyer et al., 2004;Suhara et al., 2003;Kugaya et al., 2004;Kugaya et al., 2003;Malison et al., 1998). These studies concluded that during chronic treatment with clinical SSRI dosages, about 80% of SERT is occupied in various regions of the brain, implicating that such a high SERT occupancy might be required to increase 5-HT levels to the degree that most therapeutic effects occur. The current acute study showed that a high level of SERT occupancy was present at relatively low fluvoxamine plasma levels as well as fluvoxamine concentrations in brain ECF and brain tissue. In the current study, SERT occupancy was analyzed after acute fluvoxamine administration. However, similar experimental conditions could be applied to determine the effects on fluvoxamine SERT occupancy after chronic administration to explore the possible subjectivity of SERT to adaptive changes in rat frontal cortex.

The proposed PK/PD model constituted a useful basis for characterization and prediction of the time-course of *in vivo* SERT occupancy, which is an important intermediate step in the pharmacodynamics of SSRIs, in behavioral studies with SSRIs. This model can be applied in behavioral studies with SSRIs to explore the relationships between drug concentration, SERT occupancy and the behavioral effect.

11.2.4 Physiological Model for the Effect of Fluvoxamine on 5-HT and 5-HIAA Concentrations in Rat Frontal Cortex

As a next step in exploring the PK/PD relations of fluvoxamine, a physiological model was proposed for the effect of fluvoxamine on 5-HT and 5-HIAA (major metabolite) in the rat frontal cortex (**Chapter 8**). As described, SSRIs increase extracellular levels of 5-HT by blockade of the presynaptic SERT and thereby the reuptake of 5-HT.

A limitation of many studies reported to date is that only the mean profiles of the 5-HT and 5-HIAA response have been described. As a result, much information on the processes underlying the observed effects is lost. Moreover, in the studies in which results in individual animals are described, typically a wide intra- and inter-animal variation in the 5-HT response is reported. This variability is usually attributed to various stressors that can occur during execution of a study, and which influence the 5-HT release (Fujino et al., 2002;Kirby and Lucki, 1997;Adell et al., 1997;Kalen et al., 1989). In addition, the 5-HT system is implicated in the regulation of sleep and vigilance states (Hery et al., 1977;McGinty and Harper, 1976;Quay, 1968;Semba et al., 1984). Presumably this circadian rhythm contributes to the observed variability in the extracellular concentrations of 5-HT (Cespuglio, 1982;Cespuglio et al., 1983;Puizillout et al., 1979).

Although the complex mechanisms of the physiological control of 5-HT release are well established, these mechanisms have not been implicated in the observed fluctuating 5-HT response patterns following SSRI administration in a mechanistic manner. Briefly, following the release in the synaptic cleft, 5-HT is subject to active reuptake by the SERT. In various studies it has been shown that the release of 5-HT from serotonergic nerve terminals is under the control of inhibitory 5-HT autoreceptors (for reviews see Briley et al., 1997 and Göthert and Schlicker, 1997) and (Blier and de Montigny, 1987;Briley and Moret, 1993;Moret and Briley, 1997;Sotelo et al., 1990;Sprouse and Aghajanian, 1987). Activation of somatodendritic 5-HT_{1A} autoreceptors and terminal 5-HT_{1B} autoreceptors decreases 5-HT neuronal firing and, in turn, the synthesis, metabolism and release of 5-HT. In

theory, under distinct conditions, this type of feedback systems may display oscillatory behavior, thus contributing to the observed intra- and inter-individual variability in 5-HT response.

In **Chapter 8**, a physiological model was proposed to describe the complex time course of the 5-HT and 5-HIAA response in rat frontal cortex following administration of SSRIs (3.7 and 7.3 mg/kg via a 30-min intravenous infusion). In the model, SSRIs increase synaptic 5-HT concentrations by reversible blockade of the SERT in a direct concentration-dependent manner, while the 5-HT response is attenuated by negative feedback via 5-HT autoreceptors. The PK/PD model for the description of the effects of fluvoxamine on serotonergic neurotransmission was obtained in three steps: 1) differential equations were developed for the description of the various processes occurring at a serotonergic synapse, 2) reduction of the comprehensive model and 3) implementation of the effects of fluvoxamine in the reduced model. An important feature of the model is the attenuation of 5-HT release from the presynaptic cell by a negative feedback mechanism via presynaptic autoreceptors. Furthermore, the model contains a differential equation for the production of 5-HIAA from free intracellular 5-HT. Another important feature is that under distinct conditions this mechanistic model displays oscillatory behavior, which could possibly have occurred in several individual rats.

The proposed model was applied in a PK/PD study in which detailed information was obtained on the time course of the microdialysate 5-HT and 5-HIAA response in the rat frontal cortex following a 30-min intravenous infusion of 3.7 and 7.3 mg/kg fluvoxamine. Directly after administration of fluvoxamine, median concentrations of 5-HT were increased to approximately 450–600% of baseline values while median 5-HIAA concentrations were decreased. Thereafter, 5-HT and 5-HIAA concentrations gradually returned to baseline values in 6-10 hours respectively. The population PK estimates were used to predict the fluvoxamine plasma concentration at the midpoint of the 5-HT and 5-HIAA collection interval on the basis of the previously developed PK model (Geldof et al., 2007). Within and between individual animals significant variation in 5-HT concentrations was observed. In some animals, an oscillating pattern in 5-HT levels was observed, which could be indicative of complex homeostatic feedback mechanisms at the level of the release of 5-HT. The original mechanistic model (before reduction) was able to show this oscillatory behavior in the 5-HT response, depending on the values of the specific system parameters. However, it is not known whether these oscillations are actually present because of the observed additional random variation of the 5-HT response within and between

the animals. The cause of the observed large intra-individual variability in 5-HT concentrations could not be precisely described by the model and presumably reflects also the random variation. Moreover, the oscillating patterns were no longer observed in the mean and median 5-HT concentration *versus* time profiles at both dosages. For these reasons, the observed variability was treated as random variation in this investigation. The complete mechanistic model was not identifiable on the basis of the available data on the 5-HT and 5-HIAA response in individual animals. Therefore, the model was ultimately reduced to contain two differential equations describing the kinetics of 5-HT in the synaptic cleft and the kinetics of 5-HIAA in the presynaptic cell (reflecting 5-HIAA kinetics in the synaptic cleft). The reduced mechanistic model was able to adequately describe the observed median 5-HT and 5-HIAA concentrations (Figure 5). Observed 5-HT and 5-HIAA responses were in close agreement with the predicted median 5-HT and 5-HIAA concentrations.

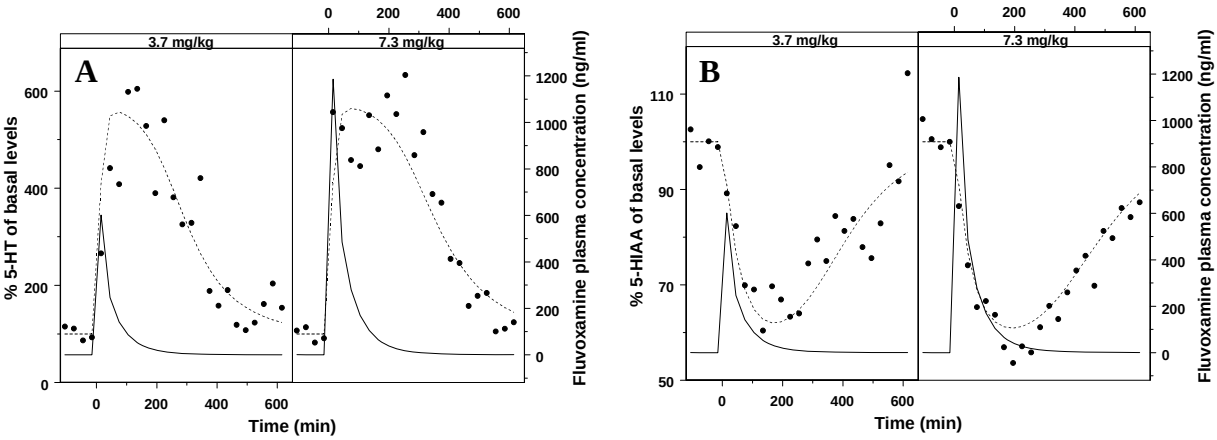

Figure 5. *Time course of the plasma concentration (solid line) and the resulting effects on median 5-HT (A) and 5-HIAA (B) concentrations (dashed line depicts the model fit, dots depict the observations) in microdialysate of rat frontal cortex (as a percentage of basal levels) following a 30-min intravenous infusion of 3.7 and 7.3 mg/kg fluvoxamine in rats.*

The PK/PD analysis revealed that inhibition of 5-HT reuptake was directly related to the fluvoxamine concentration in plasma, with 50% inhibition of 5-HT reuptake occurring at a plasma concentration of 1.1 ng/ml (EC_{50}). The levels of 5-HT at which 50% of the inhibition of the 5-HT response was reached (IC_{50}) amounted to 272% of baseline. The coefficients of variation (C.V.) for the parameter estimates ranged between 0.8 and 58%.

In a recent investigation, the acute effects of escitalopram (S-citalopram), which is also a SSRI, on the brain extracellular 5-HT levels in the hippocampus were explored (Bundgaard et al., 2006). Generally, comparable profiles of the mean 5-HT concentrations were observed as in the present investigation. It was observed that

increasing the dose from 5 to 10 mg/kg did not result in further enhancement of the acute 5-HT response. This is in the line with the results of the present investigation in which a similar increase in 5-HT levels was observed at both fluvoxamine dosages. In contrast to the results for escitalopram, 5-HT concentrations seemed to decrease at a later time point after administration of the higher fluvoxamine dose. Bundgaard and coworkers developed a feedback turnover model that mimicked the drug-induced effects of acute escitalopram administration. The turnover model included an inhibitory feedback moderator component, which adequately described observed 5-HT concentrations. The authors state that the inhibitory feedback moderator compartment which was included in the feedback turnover model acting on k_{in} reflected the inhibitory segment of the pharmacological response. However, no experimental data were in the model for this moderator compartment making the model somewhat hypothetical. The authors considered the model as having a mechanistic background in terms of the acute action of SSRIs on the 5-HT turnover in the rat. In the present investigation for the serotonergic response to fluvoxamine, a more mechanistic approach was used for description of the various processes occurring at serotonergic neurotransmission. Differential equations were derived for description of the most important processes at serotonergic neurotransmission. The model was reduced on a rational basis for description of the median 5-HT and 5-HIAA response. Another important advantage of the present investigation is that the response of both 5-HIAA and 5-HT were included in the model in contrast to the study of Bundgaard and coworkers. By simultaneous analysis of the metabolite and 5-HT concentrations in individual animals, additional information on the serotonergic system was obtained. However, it should be still remembered that a mathematical model is always a simplification of the actual situation and the developed model is based on the main principles of serotonergic neurotransmission. Additional features like the suggestion that 5-HT cells are auto-controlled via long feed-back loops activated by postsynaptic 5-HT_{1A} receptors as well (Hajos et al., 1999) are not included in the model at this point.

The problem of variability in the response variable, complicating the identifiability of the model is clearly not specific for 5-HT levels. Also, in other investigations, manipulations of the data and/or the model have been described to simplify the system and model. An example of simplification of a PK/PD model is the proposed model for a selective irreversible antagonist (Hutmacher et al., 2005). In this investigation, the techniques used for model reduction were comparable to the investigation described in the present article. The problems of uncertainties inherent

to kinetic data in relation to model complexity have been reviewed by Bernillon and Bois with regard to a Bayesian perspective (Bernillon and Bois, 2000).

In the previous investigation on the PK/PD correlation for the occupancy of fluvoxamine to the SERT in rat frontal cortex (**Chapter 7**), maximal SERT occupancy was reached instantly after a 30-min intravenous fluvoxamine infusion of 1 and 7.3 mg/kg, which maintained for 1.5 and 7 h after dosing, respectively. Thereafter, SERT occupancy decreased linearly at a rate of 8 %/hr. In the present investigation, median 5-HT concentrations decreased at approximately 6 h after 7.3 mg/kg fluvoxamine, which could be related to the occupancy of fluvoxamine to SERT. The relationship between SERT occupancy and 5-HT and 5-HIAA concentrations in rat frontal cortex will be the subject of future investigations in our laboratory.

In future investigations, the (circadian) placebo response should be characterized and analyzed in more detail as well. Such a solid dataset on the 5-HT and 5-HIAA response in control rats would make it possible to develop a PK/PD model for the baseline responses. The effect of fluvoxamine could then be modeled in an integrative manner with these control values instead of relating the observed response to the basal values, as described in the present investigation.

The proposed physiological model is the first step in modeling of complex neurotransmission processes. The model constitutes a useful basis for prediction of the time course of median 5-HT and 5-HIAA concentrations in the frontal cortex in behavioral pharmacology studies *in vivo*, which is an important intermediate step in the pharmacodynamics of SSRIs. Ultimately, additional studies need to be performed for a detailed characterization of serotonergic neurotransmission. For example, a possible next step in the investigations could be to explore the effects of fluvoxamine in the presence of a serotonergic autoreceptor agonist (such as buspirone or 8-OH-DPAT) or an autoreceptor antagonist (such as WAY 100635) and thereby to explore the stimulating or inhibiting effects on the serotonergic system. The additional information could be used in identifying a more complex physiological model for serotonergic neurotransmission.

11.2.5 PK/PD Modeling of the Effect of Fluvoxamine on PCA-induced Behavior

In the next step on the investigations on the relations of fluvoxamine, a PK/PD model was proposed for the effects of fluvoxamine on behavioral effects induced by administration of PCA (**Chapter 9**). PCA is taken up into serotonergic nerve terminals by the SERT, after which it accumulates in the synaptic vesicles. In the

synaptic vesicles, PCA displaces 5-HT, which is subsequently released, ultimately resulting in depletion of tissue 5-HT stores (Berger et al., 1989;Fuller et al., 1975;Fuller, 1992). By increasing the extracellular concentrations of 5-HT in the synaptic cleft, postsynaptic 5-HT receptors are activated yielding typical behavioral effects, such as excitation (EXC), flat body posture (FBP) and forepaw trampling (FT) (Fuller, 1992;Hutson and Curzon, 1989;Trulson and Jacobs, 1976). Since PCA produces its biochemical and behavioral effects only after uptake into serotonergic neurons via SERT, its effects are inhibited by SSRIs including fluvoxamine (Berger et al., 1989;Lassen, 1978;Fuller, 1980). The PCA assay described in (**Chapter 9**) is widely applied to screen for activity of SSRIs and antidepressants (Pellegrino and Bayer, 2000;Andres et al., 2005;Andres et al., 2003;Bartolome et al., 2005). However to our knowledge, PK/PD modeling of the effects of these compounds in the PCA assay has not been achieved. This could be of potential interest in prediction of the effective concentrations in humans. A specific complexity for PK/PD modeling PCA-induced behavior is that, within the time frame of PK/PD investigations only a singe behavioral observation is feasible in each animal for each behavioral effect because PCA administration results in a depletion of 5-HT stores (Fuller, 1992). Furthermore, observed behavioral effects are not continuous but ordered categorical data (score 0, 1, 2 or 3), which requires specific data analysis techniques to be applied. Ordered categorical data are frequently collected in clinical trials, for example when using pain scores and sedation scales as pharmacodynamic endpoints (Sheiner, 1994;Mandema and Stanski, 1996;Knibbe et al., 2002;Olofsen et al., 2005). A commonly used model for ordered categorical data is a generalization of the logistic regression model (Harrell et al., 1988), which estimates the probability of the data to be in a certain category, on a scale with multiple categories (Agresti, 1999). Therefore, rather than modeling the categories themselves, a transformation of the probability of being in a particular set of categories is modeled. The model is often referred to as the proportional odds model and has been introduced in the field of PK/PD modeling by Sheiner in 1994 (Sheiner, 1994).

The individual post hoc estimates for CL, V1, V2, and Q2 and the population post hoc estimates for V3 and Q3 obtained from the previously developed population PK model in plasma were used to estimate the fluvoxamine concentrations in plasma at the time of the behavioral observations. Fluvoxamine by itself did not induce EXC, FT and FBP or display any other overt behavioral effects. When PCA was injected after saline administration in the control animals, the behavioral effects could be clearly observed in all animals at the maximum score of 3. When PCA was

administered at different times following fluvoxamine administration, the behavioral effects were either partially inhibited or even absent, resulting in a decrease in the categorical scores. The magnitude of this effect was dependent on the administered dose and the time after fluvoxamine administration. The inhibitory effects of fluvoxamine on PCA-induced behavior were comparable for EXC, FBP and FT. Following the 1.0 mg/kg dose a maximum reduction for EXC was observed at 0.5 hr following the start of the infusion (corresponding to the end of the infusion) and the effect returned to baseline at 2 hrs. Following the highest dose (7.3 mg/kg) the return to baseline did not occur until 12 hours after administration. In general, an increase in the inhibition of the PCA-induced behavioral effects could be observed, hence a reduction in the behavioral scores, with increasing fluvoxamine concentrations in plasma. The effects of fluvoxamine on PCA-induced behavior (probability of EXC, FBP and FT) were directly related to the fluvoxamine plasma concentration on the basis of the proportional odds model (Figure 6).

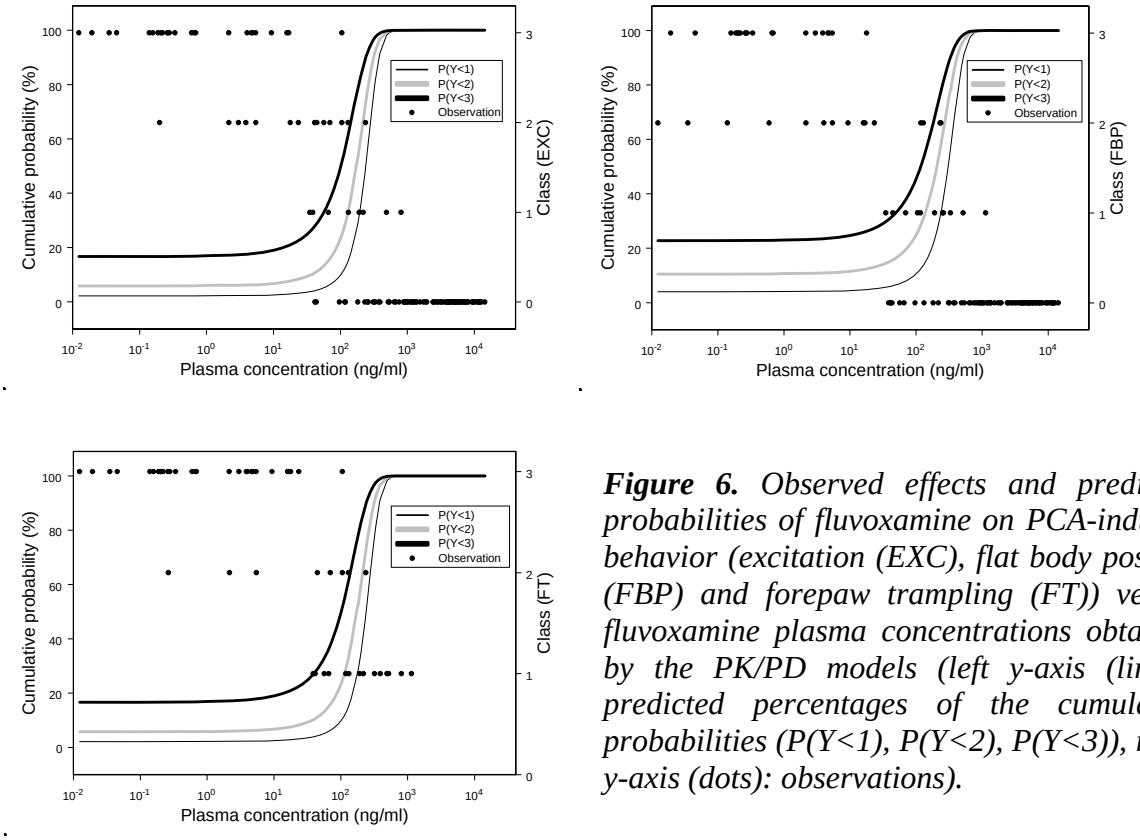

Figure 6. *Observed effects and predicted probabilities of fluvoxamine on PCA-induced behavior (excitation (EXC), flat body posture (FBP) and forepaw trampling (FT)) versus fluvoxamine plasma concentrations obtained by the PK/PD models (left y-axis (lines): predicted percentages of the cumulative probabilities (P(Y<1), P(Y<2), P(Y<3)), right y-axis (dots): observations).*

Although only one behavioral observation per animal could be obtained and the readout of the behavioral test was non-continuous, the relationship between fluvoxamine plasma concentration and the effects of fluvoxamine on PCA-induced behavioral effects for EXC, FBP and FT could be successfully described by the

developed PK/PD model. The predicted cumulative probabilities (P(Y<1), P(Y<2) and P(Y<3)) for these effects could adequately predict observed behavioral scores at the corresponding fluvoxamine concentrations in plasma. The predicted cumulative probabilities and probabilities were approximately the same for EXC, FT and FBP. Fluvoxamine showed a significant effect on PCA-induced behavior. Based on a comparison of the difference in the minimum value of the objective function after fluvoxamine administration compared to saline administration (ΔMVOF), the inhibitory effects of fluvoxamine on PCA-induced behavioral effects could be best described for EXC, followed by FBP and FT, respectively.

With this modeling approach for categorical observations, unique PK and PD parameters have been obtained which are independent of the administered fluvoxamine dose. Parameters of pharmacological interest like the predicted fluvoxamine plasma concentration at half of the maximal inhibitory effects of PCA-induced behavior (EC_{50}, corresponding to the plasma fluvoxamine concentration at a cumulative probability of 50%), for the various cumulative probabilities (P(Y<1), P(Y<2), P(Y<3)) could be calculated. In addition, the standard errors of the EC_{50} values could be estimated by stochastic simulation taking into account the covariance matrix between the parameter estimates. The effect of fluvoxamine in the PCA assay occurs at very high plasma concentrations. For EXC the EC_{50} values for the cumulative probabilities (P(Y<1), P(Y<2), P(Y<3) were 237 ± 38.9 ng/ml, 174 ± 28.3 ng/ml and 100 ± 20.1 ng/ml, respectively. Slightly higher EC_{50} values were obtained for the corresponding effects on FBP and FT, and therefore it could be concluded that EXC was the most sensitive behavioral effect for the inhibitory effects of fluvoxamine after PCA administration. Hence, at equal fluvoxamine plasma concentration, the decrease in behavioral scores could be more rapidly observed for EXC than for FBP, followed by FT. An important question is how the observed EC_{50} concentrations in the present investigations relate to comparable values in other pharmacological test systems and in particular also the degree of SERT occupancy. In the previously developed physiological PK model for description of the fluvoxamine concentrations in ECF of the frontal cortex and in brain tissue on the basis of plasma concentrations, it was shown that the brain distribution of fluvoxamine is non-linear (**Chapter 6**). Moreover, this analysis also showed that, in steady-state, the free drug concentrations in the frontal cortex amount to on average 5% of the total plasma concentrations. Correction of the observed EC_{50} values for this brain distribution factor yields values in the range of 5-21 ng/ml, exceeding reported values for the affinity of fluvoxamine to SERT in *in vitro* bioassays (Goodnick and Goldstein,

1998), indicating that the PCA effect occurs at near maximal SERT occupancy. This is confirmed in the results of the investigation in which the effects of fluvoxamine on the SERT occupancy in the rat frontal cortex were explored (**Chapter 5**). The estimated fluvoxamine plasma concentration at half-maximal SERT occupancy was 0.48 ng/ml, which is much lower than obtained EC_{50} values in the present investigation for the cumulative probabilities of PCA-induced behavior confirming that very high fluvoxamine SERT occupancy is required for the inhibition of PCA-induced behavior. This can be explained by the nature of the PCA test. Specifically the PCA test is probably based on the competitive interaction between on the one hand the SSRI (i.e. fluvoxamine) and on the other hand PCA for SERT. It is well established that the magnitude of competitive drug-drug interaction depends on the relative concentrations of the two interacting agents (Jonker et al., 2005). Specifically, at relatively high PCA doses also relatively high concentrations of SSRIs are required to counteract the PCA-induced behavior and *vice versa*. As a result, the pharmacodynamic parameter estimates of SSRIs in the PCA test depend on the administered PCA dose. This has important implications for the prediction of effective drug concentrations of SSRIs on the basis of PK/PD modeling of effects in the PCA test. Specifically, modeling of the competitive pharmacodynamic interaction between PCA and the SSRI of interest is required to derive the relevant *in vivo* concentration range. In recent years the successful modeling of competitive drug-drug interactions *in vivo* has been reported for a number of CNS-active drugs including benzodiazepines (Mandema et al., 1992a;Mandema et al., 1992b) and 5-HT_{1A} receptor agonists (Zuideveld et al., 2002). This constitutes a useful basis for the modeling of the interaction between PCA and SSRIs and will be the subject of future investigations in our laboratory.

11.2.6 Preliminary Studies on the PK/PD Correlation of the Effect of Fluvoxamine on REM Sleep in Rats

In the final investigation on the PK/PD relationships of fluvoxamine, preliminary studies were performed on the PK/PD correlation of the effect of fluvoxamine on Rapid Eye Movement (REM) sleep in the Sprague Dawley rat (**Chapter 10**).

Disturbances of sleep are typical for most depressed patients and belong to the core symptoms of the disorder, in which disturbance of sleep is even used as a (differential) diagnostic. Many studies have shown that antidepressants, including SSRIs, produce significant effects on various sleep-wake stages in patients, in healthy volunteers and in animals, both after acute and chronic administration. Suppression of

REM sleep and increase of REM latency are the most consistent replicable effects on sleep (Hendrickse et al., 1994;Kerkhofs et al., 1990;Kupfer et al., 1991;Nicholson and Pascoe, 1988;Rush et al., 1998;Vasar et al., 1994;Wilson et al., 2000a;Wilson et al., 2000b). Similar changes have been observed in laboratory animals. In rats and cats, acute and chronic treatment with SSRIs induced a sustained decrease in REM sleep (Hilakivi et al., 1987;Kleinlogel and Burki, 1987;Neckelmann et al., 1996;Pastel and Fernstrom, 1987;Sommerfelt and Ursin, 1991).

To date, comprehensive PK/PD modeling of the effects of SSRIs on sleep-wake architecture has not been accomplished. In preliminary investigations it has been demonstrated that repeated blood sampling significantly influences sleep architecture in rats after acute and, although to a lesser extent, also after chronic fluvoxamine administration (Geldof et al., unpublished observations), thus complicating the PK/PD analysis of this effect. Furthermore, it has been shown to be impossible to administer SSRIs via an intravenous infusion in such investigations, since this also significantly influences the sleep-wake architecture. This necessitates the intraperitoneal administration of SSRIs in PK/PD investigations on the effect on sleep. Moreover, advanced PK modeling approaches are needed for the description of the time course of the drug concentration.

The objective of the present investigation was to characterize the PK/PD correlation of the effect of fluvoxamine on REM sleep. Rats chronically implanted with electrodes for recording of the cortical electroencephalogram (EEG), electrical neck muscle (EMG) activity and ocular movements (EOG) received an intraperitoneal (IP) injection of 1, 3.7 or 7.3 mg/kg of fluvoxamine. The effects on the various sleep-wake states were determined over a period of 14 hrs. In parallel, the time course of the fluvoxamine concentration in plasma was determined in a satellite group of rats equipped with electrodes under identical conditions. In **Chapter 10**, we presented a) a population PK model for fluvoxamine following intraperitoneal administration and b) a PD model for the effects on REM sleep.

A two-compartment PK model with dose dependency on the inter-individual variability (IIV) in *V2* described the fluvoxamine plasma concentrations following intraperitoneal administration in individual animals. Maximal fluvoxamine plasma concentration (C_{max}) was reached rapidly after dosing and observed already at the first measurement. Fluvoxamine plasma concentrations could adequately be described, since no substantial deviation from the identity line was observed in the residual diagnostics. Mean population PK parameter estimates for *ka*, *CL*, *Q*, *V2* and *V3* were 0.024 1/min, 143 ml/min, 37.1 ml/min, 338 ml and 3260 ml, respectively. Inter-

individual variability was identified on *V2* (17.4 %) and *V3* (34.5 %). In our previously developed population three-compartment PK model describing the plasma concentration *versus* time profiles of fluvoxamine following intravenous administration in Wistar rats (**Chapter 5**), IIV could be characterized for four out of six PK parameters. In this study, IIV could be characterized on *CL* (39.5%), *V1* (43.5%), *V2* (50.1%) and *Q2* (25.7%) and thus were in the same range as obtained IIV in the present investigation after IP administration of fluvoxamine. No significant covariates could be identified in model development to explain possible inter-individual variability between the animals. This result is in contrast to the previously developed population PK model (**Chapter 5**) after intravenous fluvoxamine infusion in Wistar rats in which body weight could be identified as a significant covariate of the inter-compartmental clearance *Q2*.

Fluvoxamine showed a dose-dependent inhibition of the onset of the increase in REM sleep compared to saline administration. Thereafter, REM sleep remained constant around a value of 10-15% (3-4.5 min of the total recording periods of 30 min) in all dose groups up to about 9 h, after which REM sleep decreased again, which was one hour before the dark period. In the PD model, the effects of fluvoxamine on REM sleep were characterized by an indirect response model with stimulation of k_{in}, which was controlled by a REM sleep generation pulse function accounting for the changes in REM sleep. The administered fluvoxamine dose was related to the onset of the increase in REM sleep (t_1), which is of clinical relevance and of high interest within a translational medicine paradigm in drug development.

In Figure 7, the observed and predicted effects of fluvoxamine on mean values of REM sleep are depicted (as a percentage of the total recording period of 30 min), separated by administered fluvoxamine dose.

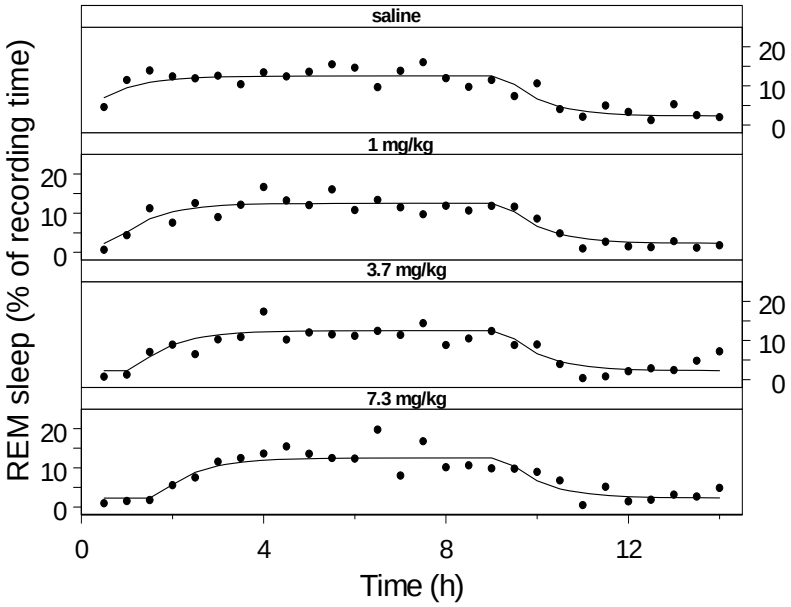

Figure 7. *Observed and predicted effects for mean REM sleep values (as % of the recording period, after an IP injection of saline or 1, 3.7 or 7.3 mg/kg fluvoxamine. Dots are the observed mean REM sleep times and the solid line represents the predicted mean REM sleep times by the developed PD model (n=8 per group).*

The developed PD model was able to adequately describe observed effects on REM sleep after IP administration of fluvoxamine at each dosage. All structural parameters of the model could be adequately described. Values of C.V. ranged between 2.2 and 16% for all parameters. Observed effects on mean REM sleep could adequately be described, since no substantial deviation from the identity line was observed in the residual diagnostics. The mean population values for k_{in} and k_{out} were 2.8 %/min and 1.2 1/min. Estimated offset of the increase in REM sleep (t_2) was 9.3 h and the fraction determining k_{in} ($REM_{\max}$) was 4.5. Hence, during REM sleep, value of k_{in} was 5.5 times of the value of k_{in} during non-REM sleep. The onset time of the increase in REM sleep (t_1) was estimated as 0.73, 1.2 and 1.7 h at a fluvoxamine dose of 1, 3.7 and 7.3 mg/kg, respectively, and zero after administration of saline. The corresponding range of fluvoxamine plasma concentrations, predicted with the developed PK model, was 27-37, 64-93 and 91-204 ng/ml after 1, 3.7 and 7.3 mg/kg, respectively. Since the effect on the onset of the increase in REM sleep roughly occurs at different concentrations of fluvoxamine, further investigations on the precise PK/PD correlations of fluvoxamine on REM sleep are needed. In contrast, the start time of the offset of the increase in REM sleep was not influenced by the administered fluvoxamine dose and therefore presumably independent of the fluvoxamine plasma concentrations.

The eventual aim of the research described in this article is comprehensive PK/PD modeling of the effect of SSRIs on sleep after acute and chronic administration. PK/PD modeling of the effect on sleep is of considerable interest given the fact that it is a reliable physiological read-out showing relevance to the clinic. Efficacious and tolerable doses of 50-300 mg/day have been suggested for clinical use of fluvoxamine (Claghorn et al., 1996). It should be noted that the effects of fluvoxamine on sleep in the clinic seem to depend on the patient group involved. Next to its therapeutic efficacy, fluvoxamine has been reported to exert beneficial effects on sleep in for example patients suffering from major depression or from post-traumatic stress disorder (Neylan et al., 2001;Dalery and Honig, 2003), whereas in obsessive-compulsive disorders fluvoxamine treatment resulted in circadian rhythm sleep disorder (Hermesh et al., 2001). Notwithstanding this clinical complexity, the change in latency to REM sleep onset has clear clinical value because ample evidence supports the role of changes in density and latency to onset of REM sleep as a biomarker for clinical response and anticipated efficacy in depression, provided that these changes are adequately monitored and PK/PD effects correctly modeled (Rijnbeek et al., 2003;Murck et al., 2003). A predictive PK/PD model is therefore of

great value in understanding the close relationship between effects of SSRIs on REM sleep parameters. More specifically, because a close agreement on such effects exists between animals and humans, such a model renders unique opportunities in translational medicine and is therefore of invaluable importance in drug discovery and development.

Since the effects on sleep are similar between animals and humans, modeling of the effect on sleep does constitute a valid basis for a further, more advanced study of the inter-species scaling of the effects of SSRIs. In this respect it should be noted that next to many similarities there are also some distinct differences between human and rodent sleep. For example, rodents, being nocturnal animals, have a circadianly dispersed sleep rhythm whereas man has a clear diurnally entrained rhythm. Moreover, the dynamics of rat sleep are of a higher magnitude with shorter sleep cycles and a higher basal fragmentation in the sleep-wake architecture compared to man. Yet, the effects of psychoactive compounds on rodent sleep are often surprisingly similar and it has been convincingly demonstrated that the sleep-wake states in the rat and humans can indeed be defined along comparable lines (Ruigt et al., 1989; Drinkenburg and Ahnaou, 2004). A complicating, practical factor in the analysis of the PK/PD correlations of SSRIs on sleep architecture is the fact that no blood samples can be collected during the pharmacodynamic studies, as even automated blood sampling interferes with the behavioral observations regarding changes in vigilance states. Moreover, intravenous infusion of the study drugs is not feasible for the same reason. This underscores the need for the development of alternative approaches to the assessment of the pharmacokinetics in investigations of the effects if SSRIs on sleep architecture.

The dataset of the effects of fluvoxamine on REM sleep as well as on the other sleep-wake states was very rich and powerful. Therefore, next to the modeling approach that was used in the present study, the data could be modeled by other modeling techniques as well. Besides modeling the data in a continuous manner, they could also have been modeled using a categorical (binomial or multinomial) model or by using a Markov model. The developed population PK model is useful in these future investigations for prediction of the fluvoxamine plasma concentrations. An example of categorical modeling was described in our previous study in which the effects of fluvoxamine on the *para*-chloroamphetamine (PCA)-induced behavioral effects were explored (**Chapter 9**). Not many studies have examined the PK/PD relationship for the effects of SSRIs on sleep-wake architecture. A simple PK/PD investigation was performed for the effects of fluoxetine on sleep EEG in healthy subjects (Feige et al.,

2002), in which the relationship between drug plasma concentrations and log spectral power values were described with an E_{max} model. It was found that REM latency and spectral power effects correlated best with total SSRI plasma concentration, but only in the time interval after discontinuation. Various statistical models have been developed to describe the natural sleep-wake cycle and state-to-state transitions in adult humans (Achermann et al., 1993;Borbely, 1982;Yang and Hursch, 1973) and in infants (Holditch-Davis et al., 1998). In another study, a Markov mixed-effects model has been proposed for the effects of temazepam (benzodiazepine) on sleep (Karlsson et al., 2000). Contrary to an earlier Markov model of sleep stage transitions (Kemp and Kamphuisen, 1986), this model integrates interactions between daytime measures, drug dosage, nighttime and the sleep process. In another study, a two-state stochastic model of REM sleep architecture in the rat was proposed (Gregory and Cabeza, 2002) which is a Markov-type model exploring the REM sleep architecture in particular. In these studies, models were developed to describe the probability of moving from one sleep stage to another. The data obtained in the present study could also be modeled by this type of Markov modeling using Markov chains for the probability transitions between the six sleep states, which are only dependent on the state before. However, this analysis was beyond the scope of the article.

In the present investigation, results are presented of the first studies on the PK and PD of fluvoxamine on sleep-wake organization. We have presented a population PK model for fluvoxamine in plasma after IP administration of different dosages. This model can be used as the basis for prediction of the concentration in future PK/PD studies by application of the population approach. In addition, a PD model has been developed for the effects of fluvoxamine on REM sleep. The dosage of fluvoxamine was related to the observed REM sleep profiles. This investigation is a first step towards comprehensive PK/PD modeling of the effect of Selective Serotonin Reuptake Inhibitors (SSRIs) on sleep-wake cycle following acute and chronic administration.

11.3 Conclusions and Perspectives

In the present thesis, a mechanism-based cascading PK/PD model for SSRIs has been developed. The model enables the prediction, in a quantitative manner of *in vivo* drug effects on the basis of biomarker information. To this end, a spectrum of different biomarkers has been validated in the various investigations described. These biomarkers each reflected a specific process on the causal path between drug administration and response. The effects of fluvoxamine have been explored in six

investigation steps; from the relatively simple description of the pharmacokinetics of fluvoxamine in plasma and brain (type 1 biomarker) to the more complex relationships with the effects on SERT occupancy (type 2), 5-HT and 5-HIAA levels (type 3) and REM sleep (type 5 biomarker). In the PCA study, a categorical PK/PD model was proposed for the effects of fluvoxamine on PCA-induced behavioral effects as a kind of intermediary biomarker (type 4).

It appears that there are important aspects in the PK/PD relationships of fluvoxamine, which was already indicated in the well-known delayed therapeutic effect of SSRIs. The cascading PK/PD model enables the prediction of the effects of functional adaptation upon long-term administration. For SSRIs, adaptation may occur at various levels in the biological system. The various biomarkers that have been studied provide a basis to determine at which level of the biological system functional adaptation occurs (i.e. target site distribution, target expression, turnover of neurotransmitters, transduction mechanisms).

An important point in the examination of drug effects is always the relationship between target occupancy and effect. For agonists this is often analyzed on the basis of concepts of receptor theory. On the basis of the operational model of agonism it has been demonstrated that many G-protein coupled receptors function with a high degree of receptor reserve. For A_1 adenosine receptors (Van der Graaf et al., 1999), μ opioid receptor agonists (Cox et al., 1998;Garrido et al., 2000), and 5-HT_{1A} receptor agonists (Zuideveld et al., 2004) it has been demonstrated that only partial target occupancy is required for maximal drug effect. Moreover, for benzodiazepines it has been demonstrated that they act as partial agonists at the $GABA_A$ receptor, which implies that the intensity of the drug effect is directly proportional to the degree of target occupancy (Visser et al., 2003). For SSRIs, the opposite is shown: for these antagonists a high degree of target occupancy is required for the effect. This is shown in the present thesis for the effects of fluvoxamine on 5-HT and 5-HIAA response (**Chapter 8**) and for the effects on REM sleep (**Chapter 10**).

The various models that have been proposed in the present thesis may also be applied to other neurotransmitter reuptake inhibitors. Next to serotonin, the monoamines norepinephrine (NE) and dopamine (DA) are also important neurotransmitters and play an important modulatory role in the CNS and are involved in numerous physiological functions and pathological conditions. Like presynaptic plasma membrane transporter for serotonin (SERT) investigated in the present thesis, the presynaptic plasma membrane transporters for NE (NET) and DA (DAT) control synaptic actions of these monoamines by rapidly clearing the released amine from the

synaptic cleft after which it is restored into synaptic vesicles (Jayanthi and Ramamoorthy, 2005;Iversen, 2000;Iversen, 2006). Because of the similar mechanism of action as the SSRIs, the PK/PD correlations for reuptake inhibitors of NE and DA could also be investigated by the mechanistic approach described in the present thesis, to obtain more information on the processes on the causal path between drug administration and response. Next to the monoamines 5-HT, NE and DA, already PK/PD models have been developed for compounds acting on other neurotransmitter systems like draflazine, a nucleoside (adenosine) transport inhibitor (Snoeck et al., 1998;Snoeck et al., 1997) and tiagabine, a gamma-aminobutyric acid (GABA) reuptake inhibitor (Cleton et al., 2000;Cleton et al., 1999).

An important aspect for SSRIs as well as other CNS compound is the possibility for interspecies extrapolation (i.e. the prediction of the effect in man on the basis of preclinical information) of the relationships between the PK and PD, for which biomarkers may be used. This is important since up to now, rational drug development for CNS disorders has been seriously hampered by limitations in the translation of results from animal experiments to human. Despite the introduction of a range of biomarkers in recent years, such as neuroimaging techniques and functional challenge tests, few attempts have been made establish quantitative relations between preclinical and clinical data that could assist in progressing drug development in this area to a more rational and efficient process. Setting up a mechanistic PK/PD framework for CNS drugs, should support in the translation of animal data to preclinical setting, thus allowing an earlier selection of optimal drug candidates to be progressed into clinical development. For example, establishing the relationship between preclinical SERT occupancy and clinical SERT occupancy, followed by scaling to the concentrations of the drug in plasma or brain in humans would yield important information for drug development of antidepressants.

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Chapter 12

Samenvatting in het Nederlands (Synopsis in Dutch)

Depressie en SSRIs

Depressie is een ziekte die wordt gekenmerkt door een verscheidenheid aan fysieke en psychiatrische symptomen die de basale activiteit van mensen ernstig dempt. De oorzaak van depressie is niet goed duidelijk. Wel is de kennis over het ontstaan van depressie sterk verbeterd op basis van verschillende risicofactoren zoals erfelijke aanleg, chemie in de hersenen, psychosociale factoren en omgevingsfactoren. Er zijn veel aanwijzingen dat verstoringen van de signaaloverdracht door de neurotransmitters serotonine (5-hydroxytryptamine, 5-HT), noradrenaline (NA) en dopamine (DA) belangrijk zijn bij het ontstaan van depressie. Een algemeen bekend kenmerk bij het ontstaan van depressie is een vermindering van de serotonerge neurotransmissie. Het is dan ook niet verwonderlijk dat stoffen die de heropname van serotonine remmen, de selectieve serotonine heropname remmers (SSRIs), geneesmiddelen van de eerste keuze zijn in de behandeling van depressie. De SSRIs blokkeren selectief en krachtig de serotonine transporter (SERT) en daardoor de heropname van 5-HT in het presynaptische zenuweinde. Dit resulteert in een verhoging van de extracellulaire 5-HT concentraties in de synaptische spleet en uiteindelijk in een verhoging van de serotonerge neurotransmissie. Desondanks wordt aanvankelijk de serotonerge neurotransmissie verlaagd wat tot uiting komt in een daling in de activiteit van de serotonerge neuronen. De activatie van verscheidene 5-HT (auto)receptoren en een vermindering van de 5-HT afgifte van de zenuwuiteindes speelt daarbij een belangrijke rol. Echter, vele studies hebben laten zien dat deze autoreceptoren ongevoelig worden na chronische SSRI behandeling van ongeveer twee weken. Als gevolg van deze desensibilisatie worden de auto-inhiberende processen gereduceerd, wat uiteindelijk resulteert in een verhoogde afgifte van 5-HT van de zenuwuiteindes, waardoor de totale serotonerge neurotransmissie wordt versterkt. Dit wordt verondersteld een verklaring te zijn voor de therapeutische effecten van SSRIs, die zich langzaam ontwikkelen na chronische behandeling van een aantal weken. Echter, de resultaten van de verschillende studies zijn niet allemaal in overeenstemming met elkaar en zelfs nu zijn er nog altijd veel vragen die beantwoord moeten worden.

Onderzoek naar gedrag in proefdiermodellen

Voor elke ziekte is het duidelijk dat het enige perfecte model de menselijke ziekte zelf is en ook dat het ideale diermodel niet bestaat. Diermodellen zijn echter wel nuttig voor het bestuderen van de werkingsmechanismen van geneesmiddelen. Bij het ontwikkelen van diermodellen van een ziekte trachten wetenschappers syndromen in

proefdieren te ontwikkelen die lijken op de waargenomen syndromen in mensen, zodat geselecteerde aspecten van de menselijke psychopathologie kunnen worden bestudeerd. Omdat depressie een heterogene ziekte is met symptomen op het psychologische, gedrag en fysiologische niveau, is het extra moeilijk om de ziekte in een proefdier na te bootsen. Omdat veel van de symptomen van depressie bij de mens niet bij een proefdier kunnen worden waargenomen, zal men nooit weten of een proefdier inderdaad depressief is. In tegenstelling tot andere ziektes waarvan de pathofysiologie beter gekarakteriseerd en beter meetbaar is (zoals diabetes of de ziekte van Parkinson) is de onderliggende pathofysiologie van depressie nog altijd niet volledig bekend. Er zijn geen symptomen of klinische kenmerken die typisch zijn voor depressie. In feite kunnen veel van de symptomen van depressie ook waargenomen worden bij andere ziekten. Ondanks de moeilijkheden bij het bestuderen van depressie in proefdieren zijn er talrijke pogingen gedaan om diermodellen van depressie te creëren, of tenminste modellen van de symptomen van depressie. Over de jaren zijn verscheidene diermodellen ontwikkeld waarin specifieke gedragsveranderingen kunnen worden waargenomen die gevoelig zijn voor de effecten van antidepressiva. De effecten van SSRIs zijn uitvoerig onderzocht in veel van deze gedragsfarmacologische testen, zoals de geforceerde zwemtest, de aangeleerde hulpeloosheidstest en de test waarbij een proefdier aan zijn staart hangt.

Farmacokinetische/farmacodynamische (PK/PD) modellering

Farmacokinetiek (PK) is een term die wordt gebruikt om de processen te beschrijven die, na toediening, het verloop van de concentratie van een geneesmiddel in de tijd bepalen. Het verloop van de (plasma) concentratie in de tijd van een geneesmiddel wordt bepaald door de processen van absorptie (wanneer de route van toediening niet systemisch is), distributie (inclusief de distributie naar de plaats van werking), metabolisme en excretie. Farmacodynamiek (PD) is een term die wordt gebruikt om de relatie te beschrijven tussen de (plasma) concentratie van het geneesmiddel (en/of metabolieten) en de grootte van het farmacologische effect (bv. bloeddruk, hartslag). Farmacodynamiek houdt rekening met de verscheidenheid van processen die het (verloop van het) effect van het geneesmiddel bepalen, met inbegrip van de binding aan de receptor, de activatie van de receptor en de signaal transductie processen die leiden tot het uiteindelijke effect. PK/PD modellering verbindt deze twee klassieke disciplines van de farmacologie. Het is een wiskundige beschrijving die de verandering van de concentratie in de tijd (PK) verbindt met de verandering van de intensiteit van het farmacologische effect in de tijd (PD). Het voornaamste doel van

PK/PD modellering is de identificatie van de belangrijkste eigenschappen van een geneesmiddel *in vivo*, waardoor het mogelijk is om het verloop van de effecten van een geneesmiddel te karakteriseren en te voorspellen, zowel onder fysiologische als onder pathologische condities.
PK/PD modellering heeft zich ontwikkeld van een empirische en beschrijvende aanpak naar een meer wetenschappelijke methode die gebaseerd is op de (patho)fysiologische mechanismen achter de PK/PD relaties en die kan worden toegepast in alle fasen van de ontwikkeling van een geneesmiddel. Het doel van op mechanisme gebaseerde PK/PD is om, op een nauwkeurige kwantitatieve manier, de mechanismen te begrijpen die het verloop van de intensiteit van het effect van het geneesmiddel in de tijd *in vivo* bepalen. In op mechanisme gebaseerde modellen wordt getracht om de mechanismen die een bijdrage leveren aan de werking van het geneesmiddel *in vivo* te begrijpen en mee te nemen in het model. In een strikte betekenis verwijst op mechanisme gebaseerde PK/PD modellering naar de beschrijving van het functioneren van het geïntegreerde biologische systeem *in vivo* en is daarom gelijk aan de analyse van het biologische systeem. Op mechanisme gebaseerde PK/PD modellen verschillen van empirische beschrijvende modellen in dat ze specifieke uitdrukkingen bevatten die, op een kwantitatieve manier, processen beschrijven op de causale weg tussen toediening van een geneesmiddel en het effect. Op mechanisme gebaseerde PK/PD modellen hebben dan ook sterk verbeterde eigenschappen voor extrapolatie en voorspelling. De ontwikkeling van op mechanisme gebaseerde PK/PD modellen steunt op biomarkers die op een kwantitatieve manier de processen op de causale route tussen de toediening van een geneesmiddel en de respons beschrijven. Deze processen omvatten: 1) distributie naar de plaats van werking (distributie tussen plasma en de plaats van werking in perifere weefsels), 2) binding en activatie van doeleiwitten op de plaats van werking, 3) farmacodynamische interacties, 4) transductie en 5) invloed van *in vivo* homeostatische terugkoppeling mechanismen. Uiteindelijk moeten op mechanisme gebaseerde PK/PD modellen ook de effecten op ziekte processen en het verloop van een ziekte beschrijven.

Biomarkers

In de context van op mechanisme gebaseerde PK/PD modellering wordt een biomarker gedefinieerd als een meting die, op een strikt kwantitatieve manier, een proces beschrijft dat op de causale weg ligt tussen de toediening van een geneesmiddel en het effect. Onlangs is een nieuwe classificatie van biomarkers

voorgesteld, waarin elke biomarker een specifiek aspect van het werkingsmechanisme van een geneesmiddel *in vivo* weergeeft. Het nieuwe classificatie systeem maakt onderscheid tussen zeven types van biomarkers: type 0, genotype/fenotype die de respons van het geneesmiddel bepaalt; type 1, de concentratie van het geneesmiddel en/of geneesmiddel metabolieten; type 2, bezetting aan het moleculaire doel; type 3, activatie van het moleculaire doel; type 4, fysiologische metingen; type 5, pathofysiologische metingen; en type 6, klinische classificaties. Deze onderverdeling is gebaseerd op de voornaamste intermediaire stappen in de farmacodynamiek, hoewel het niet noodzakelijk is om informatie te verkrijgen over elke intermediaire stap. Met deze biomarkers kan een cascade van modellen worden ontwikkeld waarmee de effecten van een geneesmiddel van het ene proces in de keten naar het volgende proces kan worden beschreven. Dit is belangrijk omdat de relaties tussen de verschillende processen bepaald worden door het functioneren van het biologische systeem, met andere woorden specifiek zijn voor het systeem, en daarom onafhankelijk zijn van het geneesmiddel. Op mechanisme gebaseerde modellering vormt een wetenschappelijke basis voor de voorspelling van de uiteindelijke klinische effecten van nieuwe geneesmiddelen gebaseerd op de respons van een biomarker. Bovendien kunnen studies over de respons van een biomarker de basis vormen voor het begrijpen van, in mechanistische zin, en de voorspelling van de variabiliteit in de respons van een geneesmiddel.

PK/PD gedragsstudies in proefdieren

Onderzoek naar de relatie tussen de farmacokinetiek en farmacodynamiek in proefdiermodellen kan nieuwe inzichten geven in de mechanismen van de tijdsafhankelijkheden in de farmacodynamiek van SSRIs. Er zijn maar zeer weinig studies beschreven over de PK/PD correlaties van SSRIs, ondanks hun frequente gebruik en de verscheidene preklinische en klinische experimenten. Dit is opvallend omdat de PK/PD correlaties van SSRIs complex zijn, in die zin dat SSRIs de heropname van 5-HT snel remmen maar de maximale antidepressieve effecten pas lijken op te treden na chronische behandeling van enkele weken. Dit wijst erop dat aanpassingen in het biologische systeem een bepalende factor kunnen zijn voor het therapeutische effect van SSRIs. Bestudering van de PK/PD correlaties van SSRIs is om verschillende redenen complex. Complicerende factoren in dergelijke studies kunnen zijn: a) de verstoring van het farmacodynamische effect door het nemen van bloedmonsters, b) de beschikbaarheid van slechts schaarse farmacokinetische en/of farmacodynamische gegevens en c) het feit dat de farmacodynamische eindpunten

vaak niet continu zijn. Deze complicerende factoren kunnen gedeeltelijk worden overwonnen door toepassing van de zogeheten "gemengde effecten" modelleringmethode. Populatie PK/PD analyse is gebaseerd op niet-lineaire gemengde effecten analyse om de PK en/of PD te beschrijven in populaties in plaats van in individuen. Dit heeft als voordeel dat het mogelijk is om geïntegreerde informatie te verkrijgen over de PK en/of de PD, op basis van schaarse en onevenwichtige data in een groep van subjecten. Een bijkomend voordeel is dat met deze benadering potentiële bronnen kunnen worden onderzocht die de respons significant beïnvloeden op basis van een covariaat analyse.

Doel en overzicht van de onderzoeken beschreven in dit proefschrift

Het doel van de studies die beschreven zijn in dit proefschrift was om de farmacokinetische/farmacodynamische correlaties van SSRIs te bestuderen. In de verschillende onderzoeken werd fluvoxamine gebruikt als prototype voor de SSRIs. Er werd een reeks van verschillende biomarkers gebruikt die elk een specifiek proces op het causale pad weergeven tussen de toediening van het geneesmiddel en de respons. De informatie van de verschillende biomarkers werd geïntegreerd in PK/PD modellen voor beschrijving van de verschillende effecten.

Populatie farmacokinetisch model voor fluvoxamine in de rat: bruikbaarheid voor toepassing in gedragsstudies in proefdieren

Hoofdstuk 5 laat zien dat de beperkingen van het nemen van bloedmonsters in PK/PD gedragsstudies in diermodellen gedeeltelijk kunnen worden overwonnen door gebruik te maken van een gemengde effecten modelleringsaanpak. Hier werd de ontwikkeling en evaluatie van een populatie PK model voor fluvoxamine in plasma van de rat beschreven, op basis van een niet-lineaire gemengde effecten modellering. Hiertoe werden de gegevens over de farmacokinetiek van fluvoxamine, die was verzameld in zes afzonderlijke studies met een verschillende experimentele opzet, plaats van de studie en/of bloedafname schema, gelijktijdig geanalyseerd. Een populatie model met drie compartimenten kon de fluvoxamine plasma concentraties goed beschrijven. Het lichaamsgewicht van de ratten werd meegenomen als een significante covariaat in het PK model. De analyse liet verder zien dat de farmacokinetiek onafhankelijk was van de factoren dosis, operatie, en plaats van de studie. Interindividuele variabiliteit kon worden vastgesteld voor vier van de zes geschatte PK parameters. Een zgn. voorspellende controle en een bootstrap analyse bevestigden het voorspellende vermogen en de stabiliteit van het model en de nauwkeurigheid van de parameter

schattingen. Er werd aangetoond dat op basis van dit model het volledige concentratie *versus* tijdsprofiel van fluvoxamine in individuele ratten kon worden beschreven op basis van informatie van schaarse data. Dit is belangrijk omdat het nemen van bloedmonsters gemakkelijk de farmacodynamische waarnemingen in gedragsstudies in proefdieren kan verstoren. De bruikbaarheid van het model in PK/PD gedragsstudies in proefdieren werd geïllustreerd door simulatie van de PK/PD correlatie van fluvoxamine voor de effecten op REM (rapid eye movement) slaap. Hierbij werd gebruik gemaakt van een schaarse opzet voor de afname van bloedmonsters voor beschrijving van de farmacokinetiek. Door gebruik te maken van de relevante informatie van het ontwikkelde populatie PK model konden de individuele PK profielen en de PK/PD correlatie nauwkeurig worden beschreven ondanks dat er maar een klein aantal bloedmonsters werd afgenomen in elk proefdier.

Fysiologische farmacokinetische modellering van de niet-lineaire distributie van fluvoxamine in de hersenen in de rat

In hoofdstuk 6 wordt een fysiologisch PK model voorgesteld voor schatting van de hersendistributie van fluvoxamine in de rat. Ratten met permanente arteriële (voor bloedafname) en veneuze (voor toediening van fluvoxamine) canules en een microdialyse probe geïmplanteerd in de frontale cortex van de hersenen ontvingen een intraveneus infuus (30 min) van fluvoxamine. De fluvoxamine concentraties werden gemeten in de extracellulaire vloeistof (ECF) van de frontale cortex en in het totale weefsel van de hersenen. Door deze unieke aanpak kon de farmacokinetiek in de hersenen worden bepaald tot 750 min na toediening van fluvoxamine als gevolg van de hogere fluvoxamine concentraties in de totale hersenen in vergelijking met de concentraties in plasma en ECF.

Bij een toenemende dosis werd een onevenredige verhoging waargenomen in de fluvoxamine ECF concentraties en in hersenweefsel in vergelijking met de fluvoxamine plasma concentraties. Deze disproportionele verhoging in ECF en totale hersen concentraties bij gelijke plasma concentraties werd niet veroorzaakt door een niet-lineaire plasma eiwitbinding. Er zijn aanwijzingen in de literatuur dat fluvoxamine actief wordt getransporteerd via een efflux transport uit de hersenen naar het bloed compartiment, waarschijnlijk via P-glycoproteine (Pgp). Pgp is dus eigenlijk een actieve efflux pomp waardoor de distributie naar en het verblijf in de hersenen wordt beperkt. Deze hypothese zou in de toekomst nog onderzocht moeten worden door de analyse van de hersendistributie van fluvoxamine in de aanwezigheid van specifieke inhibitoren van Pgp en andere transporters op de bloed-hersen barrière.

De kinetiek van de distributie van fluvoxamine tussen plasma en de hersenen werd geschat door gelijktijdige analyse van de plasma, ECF en totale hersen concentraties op basis van een compartimenteel model. Het voorgestelde fysiologische PK model was een uitbreiding van een eerder beschreven model en bestond uit drie compartimenten om de fluvoxamine plasma concentraties te beschrijven in combinatie met twee compartimenten voor beschrijving van de ECF en hersen concentraties. In de hersenen werd de uitwisseling van massa tussen een ondiep perfusie-gelimiteerd compartiment en een diep hersen compartiment beschreven door een passieve diffusie term en een verzadigbare actieve efflux term. Het model resulteerde in nauwkeurige schattingen van de parameters die de passieve influx in de hersenen beschreven, de efflux snelheid van het ondiepe hersen compartiment en de fluvoxamine concentratie waarbij 50% van de actieve efflux werd bereikt. Het voorgestelde fysiologische hersen distributie model maakt het mogelijk om, op basis van het verloop van de fluvoxamine plasma concentratie, het verloop van de concentratie ECF van de frontale cortex en hersenweefsel te voorspellen in de tijd. Het ontwikkelde fysiologische hersen distributie model vormt een basis voor een nauwkeurige karakterisatie van de PK/PD correlatie van fluvoxamine door rekening te houden met de waargenomen niet-lineariteit in de hersen distributie.

Farmacokinetische/farmacodynamische modellering van de bezetting van fluvoxamine aan de serotonine transporter in de frontale cortex in de rat: rol van niet-lineaire distributie in de hersenen

Hoofdstuk 7 beschrijft de PK/PD correlatie voor de bezetting van fluvoxamine aan de serotonine transporter (SERT) in de frontale cortex van de rat. Dit is een belangrijke tussenliggende stap in de farmacodynamiek van SSRIs. In dit experiment werden ratten geïnstrumenteerd met een permanente veneuze en arteriële canule voor toediening van fluvoxamine en de afname van bloedmonsters. Op verschillende tijdstippen na toediening van fluvoxamine werden de hersenen verzameld voor bepaling van de bezetting van fluvoxamine aan de SERT en bepaling van de fluvoxamine concentratie in de hersenen. Ook werd het verloop van de fluvoxamine plasma concentratie bepaald tot aan het tijdspunt van het verzamelen van hersenweefsel. In een andere studie, beschreven in hoofdstuk 6, werd het verloop van de fluvoxamine concentratie in ECF van de frontale cortex en hersenweefsel bepaald. De resultaten van de verschillende studies werden geïntegreerd door middel van niet-lineaire gemengde effecten modellering. Direct na toediening van fluvoxamine werd een maximale bezetting van fluvoxamine aan de serotonine transporter waargenomen,

die aanwezig bleef tot 1.5 en 7 uur na toediening van respectievelijk 1 en 7.3 mg/kg fluvoxamine. Hierna daalde de fluvoxamine bezetting aan de SERT lineair in de tijd met een snelheid van 8% per uur. In dit onderzoek werden de eerder ontwikkelde PK modellen voor de fluvoxamine concentraties in plasma (hoofdstuk 5) en de fluvoxamine concentraties in hersen ECF en hersenweefsel (hoofdstuk 6) met succes toegepast om de fluvoxamine concentraties in plasma, hersen ECF en hersenweefsel te voorspellen in een relevant concentratie gebied met betrekking tot de bezetting aan de SERT. Het was mogelijk om de bezetting van fluvoxamine aan de SERT direct te relateren aan de fluvoxamine concentraties in plasma, hersen ECF en hersenweefsel door middel van een hyperbolische functie (B_{max} model), die elk een goede beschrijving konden geven van de waargenomen fluvoxamine bezetting aan de SERT. De maximale bezetting aan de serotonine transporter (B_{max}) was gelijk aan 95% en de geschatte concentraties op de helft van de maximale bezetting aan de serotonine transporter (EC_{50}) waren gelijk aan 0.48, 0.22 en 14.8 ng/ml in respectievelijk plasma, hersen ECF en hersenweefsel. De geschatte waardes voor de EC_{50} waren erg laag, in het bijzonder in plasma en hersen ECF en daarom moeten er dus maar zeer lage niveaus van fluvoxamine aanwezig te zijn voor een maximale bezetting van de SERT in de frontale cortex. De minimale waarde van de objectieve functie (MVOF) daalde met 12 punten voor de analyse op basis van hersen ECF en hersenweefsel concentraties ten opzichte van plasma concentraties, dat waarschijnlijk veroorzaakt wordt door de niet-lineaire hersen distributie, zoals beschreven in hoofdstuk 6. Het voorgestelde PK/PD model vormt een unieke basis voor de karakterisatie en voorspelling van het verloop van de *in vivo* bezetting van fluvoxamine aan de serotonine transporter in de tijd in gedragsstudies met SSRIs.

Fysiologisch model voor de effecten van fluvoxamine op de microdialysaat concentraties van 5-HT en 5-HIAA in de frontale cortex in de rat

Hoofdstuk 8 beschrijft de ontwikkeling van een fysiologisch model voor het effect van fluvoxamine op de microdialysaat concentraties van 5-HT en zijn voornaamste metaboliet 5-HIAA (5-hydroxyindole azijnzuur) in de frontale cortex van de rat. SSRIs verhogen de extracellulaire niveaus van 5-HT door een blokkade van de presynaptische serotonine transporter (SERT). In dit onderzoek werd een fysiologisch model voorgesteld dat het verloop van de 5-HT en 5-HIAA concentraties in de frontale cortex van de rat kon voorspellen na toediening van SSRIs. Hiervoor werden differentiaal vergelijkingen afgeleid voor de beschrijving van de verschillende processen die plaatsvinden tijdens serotonerge neurotransmissie. In het model

verhoogden de SSRIs de synaptische 5-HT concentraties door een omkeerbare blokkade van de serotonine transporter op een directe concentratieafhankelijke manier, terwijl de 5-HT respons werd verminderd door een negatieve terugkoppeling via presynaptische 5-HT autoreceptoren. Door middel van simulaties werd aangetoond dat, afhankelijk van de waardes van de specifieke systeem parameters, de 5-HT respons oscillerend gedrag kon vertonen. Hierdoor kon (een deel van) de waargenomen aanzienlijke variabiliteit in de 5-HT concentraties tussen de verschillende ratten worden verklaard. Het model werd toegepast in een PK/PD experiment onderzoek waarin het verloop van de microdialysaat respons van 5-HT en 5-HIAA in de frontale cortex van de rat werd bekeken na toediening van fluvoxamine. De PK/PD analyse liet zien dat de inhibitie van de heropname van 5-HT direct gerelateerd was aan de fluvoxamine plasma concentratie, met een 50% inhibitie van de remming van de 5-HT heropname die plaatsvond bij een fluvoxamine plasma concentratie van 1.1 ng/ml (EC_{50}). De niveaus van 5-HT waarbij 50% van de auto-inhibitie van de 5-HT response werd bereikt (IC_{50}) was gelijk aan 272% ten opzichte van de basislijn waarde. Het voorgestelde fysiologische model is een eerste stap in het modelleren van complexe neurotransmissie processen. Het model vormt een nuttige basis voor de voorspelling van het verloop van de mediane 5-HT en 5-HIAA concentraties in de frontale cortex van de rat in farmacologische gedragsstudies *in vivo*.

Farmacokinetische/farmacodynamische modellering voor het effect van fluvoxamine op gedragseffecten geïnduceerd door *p*-chloroamphetamine (PCA)

In hoofdstuk 9 wordt een categorisch PK/PD model voorgesteld voor beschrijving van de effecten van fluvoxamine op de gedragseffecten in de rat die worden geïnduceerd door injectie van *para*-chloroamphetamine (PCA). In deze studie kregen ratten die permanent geïnstrumenteerd waren met een arteriële en veneuze canule, wederom een 30-min intraveneus infuus van fluvoxamine. Op verschillende tijdspunten na de start van het infuus werd PCA toegediend (2.5 mg/kg) via een injectie in de ader van de staart van de rat. Direct hierna werden de resulterende gedragseffecten, excitatie (EXC), platte lichaamshouding (FBP) en het trappelen van de voorpoten (FT), gescoord (scores: 0, 1, 2 of 3) over een periode van 5 min. De effecten van PCA worden geremd door SSRIs omdat PCA zijn biochemische en gedragseffecten slechts produceert na opname in serotonerge neuronen via de serotonine transporter. Het verloop van de fluvoxamine plasma concentratie werd bepaald tot aan het tijdspunt van PCA toediening en de waargenomen

gedragseffecten werden gerelateerd aan de fluvoxamine plasma concentraties. De kinetiek van fluvoxamine in plasma kon succesvol worden beschreven op basis van het eerder ontwikkelde populatie drie compartimenteel PK model. Toediening van fluvoxamine resulteerde in een directe inhibitie van de verschillende gedragseffecten, die afhankelijk was van de fluvoxamine plasma concentratie. Wanneer de fluvoxamine plasma concentraties lager werden in de tijd, werd de inhibitie van de door PCA geïnduceerde gedragseffecten gereduceerd en konden de gedragseffecten opnieuw worden waargenomen. De effecten van fluvoxamine op de door PCA geïnduceerde gedragseffecten (de kans op EXC, FBP of FT) werden direct gerelateerd aan de fluvoxamine plasma concentratie op basis van het proportionele kans model. Hoewel slechts een enkele gedragsobservatie per dier kon worden verkregen en de uitlezing van de gedragstest niet-continu was, was het mogelijk om de relatie tussen de fluvoxamine plasma concentratie en de effecten van fluvoxamine op de door PCA geïnduceerde gedragseffecten te beschrijven. Voor EXC werden de EC_{50} waardes voor de cumulatieve kansen (P(Y<1), P(Y<2), P(Y<3) geschat op 237 ± 39, 174 ± 28 and 100 ± 20 ng/ml, respectievelijk. Enigszins hogere EC_{50} waardes werden verkregen voor de corresponderende effecten op FBP en FT. Dit onderzoek laat zien dat het ook mogelijk is om PK/PD modelleren toe te passen voor categorische effecten van geneesmiddelen in farmacologische gedragsstudies in proefdieren. Dit vormt een basis voor de toekomstige ontwikkeling van een op mechanisme gebaseerde PK/PD model voor fluvoxamine in dit paradigma.

Farmacodynamisch model voor de effecten van fluvoxamine op de REM (Rapid Eye Movement) slaap in de rat

In het laatste experimentele hoofdstuk 10, wordt een farmacodynamisch model gepresenteerd voor de beschrijving van de effecten van fluvoxamine op de start van de verhoging van de REM (rapid eye movement) slaap in de rat. In dit experiment werden de ratten chronisch geïmplanteerd met elektrodes voor het opnemen van het corticale elektro-encefalogram (EEG), voor de elektrische activiteit van de nekspier (EMG) en voor bewegingen van de ogen (EOG). De ratten ontvingen een intraperitoneale injectie van fluvoxamine waarna de effecten op de verschillende slaap-waak stadia werden bepaald over een periode van 14 uur. In een aparte groep van ratten met dezelfde geïmplanteerde instrumentatie en toegediende fluvoxamine doseringen werd het verloop van de fluvoxamine concentratie in plasma in de tijd bepaald. De resultaten van deze onderzoeken werden geïnterpreteerd met behulp van niet-lineaire gemengde effecten modellering. Een twee compartimenteel PK model

met een dosis afhankelijkheid op de interindividuele variabiliteit in *V2* was in staat om de waargenomen fluvoxamine plasma concentraties in individuele ratten nauwkeurig te beschrijven. Fluvoxamine liet een dosisafhankelijk effect zien op de start van de REM slaap tussen 1 en 3 uur na toediening. Daarna bleef de REM slaap ongeveer constant rond een waarde van 10-15% (komt overeen met 3-4.5 min) in alle dosis groepen tot aan ongeveer 9 uur na toediening van fluvoxamine (gelijk aan een uur voordat de lichten uit gingen). Hierna nam de REM slaap weer verder af. In het farmacodynamische model werden de effecten van fluvoxamine op de REM slaap beschreven door een indirect respons model met een stimulatie van de parameter k_{in}. Alle structurele parameters van het model konden adequaat worden beschreven en de gemiddelde populatie waardes voor k_{in}, k_{out}, einde van de verhoging van de REM slaap (t_2) and de fractie die de waarde van k_{in} bepaald (REM_{MAX}) werden geschat als 2.8 1/min, 1.2 1/min, 9.3 h and 4.5. De waardes voor de start van de verhoging van de REM slaap (t_1) werden geschat als 0.73, 1.2 and 1.7 h bij een fluvoxamine dosering van respectievelijk 1, 3.7 and 7.3 mg/kg, en als 0 h na toediening van 0.9% NaCl. Dit onderzoek is een eerste stap richting een meer uitgebreide PK/PD modellering van de effecten van SSRIs op verschillende slaap-waak stadia na acute en chronische toediening.

Conclusies

In de verschillende onderzoeken beschreven in dit proefschrift werden de PK/PD relaties van fluvoxamine onderzocht op een systematische en mechanistische manier. Met deze mechanistische aanpak werden de processen op de causale route tussen de toediening van fluvoxamine en de respons kwantitatief gekarakteriseerd. Daarbij werd gebruik gemaakt van een reeks van verschillende biomarkers die elk een specifiek proces op deze route reflecteerde. De effecten van fluvoxamine werden bestudeerd in zes stappen; van de relatief eenvoudige beschrijving van de farmacokinetiek van fluvoxamine in plasma en de hersenen (type 1 biomarker) naar de meer complexe relaties met de effecten van fluvoxamine op de bezetting van de serotonine transporter (type 2), op de 5-HT en 5-HIAA niveaus (type 3) en op de REM slaap (type 5). In de PCA studie werd een categorisch PK/PD model voorgesteld als een soort van intermediaire biomarker voor de effecten van fluvoxamine op gedragseffecten, die geïnduceerd worden door toediening van PCA (type 4). Naast de inhibitoren van het serotonerge systeem zouden ook andere neurotransmissie systemen kunnen worden onderzocht met de in dit proefschrift beschreven aanpak om meer informatie te krijgen over de processen op de causale

route tussen toediening van een geneesmiddel en de respons. Voorbeelden zijn het dopaminerge en noradrenalinerge systeem, die vergelijkbare kenmerken met het serotonerge systeem hebben.

In dit proefschrift zijn verschillende PK, PD en PK/PD modellen ontwikkeld die nauwkeurig de waargenomen effecten konden voorspellen na acute toediening van fluvoxamine. In toekomstige onderzoeken zouden de verschillende effecten na chronische fluvoxamine toediening moeten worden onderzocht omdat er belangrijke tijdsafhankelijkheden lijken te zijn in de therapeutische effecten van SSRIs. De ontwikkelde modellen beschreven in dit proefschrift kunnen voor deze onderzoeken worden gebruikt en verder ontwikkeld.

List of abbreviations

5-HIAA	5-hydroxyindoleacetic acid, serotonin metabolite
5-HT	5-hydroxytryptamine, serotonin
A	Autoreceptor
A_0	total amount of autoreceptor
A_{50}	fluvoxamine amount in deep brain compartment at which 50% of saturation of the active removal flux is reached
A_{DB}	amount of fluvoxamine in deep brain compartment
AP	Anterior-Posterior
A-S	Autoreceptor-Serotonin complex
A_{SP}	amount of fluvoxamine in shallow perfusion-limited compartment
A_T	total amount of fluvoxamine in the brain
AUC	Area Under the Curve
AW	Active Wake
BBB	blood-brain barrier
B_{max}	maximal SERT occupancy
BSA	Bovine Serum Albumin
BW	Body Weight
C_{50}	fluvoxamine concentration in deep brain compartment at which 50% of saturation of the active removal flux is reached
C_B	total fluvoxamine concentration in the brain.
CCK	cholecystokinin
C_{DB}	fluvoxamine concentration in deep brain compartment
C_{FLV}	fluvoxamine plasma concentration
C.I.	Confidence Interval
C_{in}	fluvoxamine concentration entering the brain
$C_{in\text{-}rec}$	fluvoxamine concentration entering microdialysis probe
CL	systemic clearance
C_m	measured median levels of 5-HT or 5-HIAA
C_{max}	maximal concentration
CNS	Central Nervous System
C_{out}	fluvoxamine concentration leaving the brain
$C_{out\text{-}rec}$	fluvoxamine concentration leaving microdialysis probe
C_p	fluvoxamine plasma concentration
$C_{p,\ 5\text{-}HT\ /\ 5\text{-}HIAA}$	population median levels of 5-HT or 5-HIAA predicted by the model
CSF	cerebrospinal fluid
C_{SP}	fluvoxamine concentration in shallow perfusion-limited compartment
C_T	total fluvoxamine concentration in the brain
C.V.	Coefficient of Variation
CYP	cytochrome P450

DA	dopamine
DMSO	dimethylsulfoxide
E	Effect
EC_{50}	concentration at half of maximal effect
ECT	electroconvulsive therapy
EDTA	ethylenediaminetetraacetic acid
EEG	electroencephalogram
$Emax$	maximal effect
EMG	electromyogram
EOG	electro-oculogram
EXC	excitation
FBP	flat body posture
f_{DB}	partition coefficient for deep brain compartment
f_{SP}	partition coefficient for shallow perfusion-limited compartment
FST	forced swim test
FT	forepaw trampling
HPA	hypothalamic-pituitary-adrenal
I	inhibition term on the production of 5-HT in the presynaptic cell
IC_{50}	% levels of 5-HT at which 50% of the inhibition of the 5-HT response was reached
ICSS	intercranial self-stimulation
I_{max}	maximal inhibition of the inhibition of the 5-HT response
IIV	inter-individual variability
IP	intraperitoneal
IS	Intermediate Stage
IV	intravenous
ka	absorption rate constant
k_{in}	zero-order rate constant for production of REM sleep
k_{out}	first-order rate constant for the loss of REM sleep
KCl	potassium chloride
k_{DA}	distribution coefficient
k_{diff}	diffusion rate constant
k_{in}	influx rate constant in the brain
k_{out}	efflux rate constant from the brain
L	Lateral
LC-MS/MS	liquid chromatography-mass spectrometry/mass spectrometry
M	metabolite (5-HIAA)
MAOI	monoamine oxidase inhibitor
m-CPP	*m*-chlorophenylpiperazine
MPR	amount of M in the presynaptic cell
MRP	Multidrug Resistance Protein

MVOF	Minimum Value of Objective Function
NA	noradrenaline
NaOH	sodium hydroxide
N_{CL}	rate of release of 5-HT from the presynaptic cell into the synaptic cleft
N_{max}	maximal active removal flux
NONMEM	nonlinear mixed effects modeling
N_S	rate of formation of 5-HT or 5-HIAA in the presynaptic cell
$N_{SP\text{-}DB}$	net mass exchange between shallow perfusion-limited compartment and deep brain compartment
OCD	Obsessive-Compulsive Disorder
P	partition coefficient
PCA	*para*-chloroamphetamine
PET	Positron Emission Tomography
Pgp	P-glycoprotein
PK/PD	pharmacokinetics/pharmacodynamics
PVP	polyvinylpyrrolidone
PW	Passive Wake
Q, Q_2, Q_3	inter-compartmental clearances
Q_B	effective plasma perfusion rate
QC	Quality Control
R	postsynaptic Receptor
REM	Rapid Eye Movement
REM_{max}	fraction determining the value of kin
$REMS$	REM sleep state describing the relationship between fluvoxamine dose and REM sleep
$R\text{-}S$	Receptor-Serotonin complex
S	Serotonin
$S\text{-}A$	Serotonin-Autoreceptor complex
SAD	Seasonal Affective Disorder
SCL	amount of 5-HT in the synaptic cleft
S.E.	Standard Error
SERT	serotonin reuptake transporter
SPECT	Single Photon Emission Computed Tomography
S_{PR}	amount of 5-HT in the presynaptic cell
SSRI	Selective Serotonin Reuptake Inhibitor
ST	Serotonin-Transporter complex
S_{VE}	amount of 5-HT stored in the vesicles of the presynaptic cell
SWS1	Light Slow Wave Sleep
SWS2	Deep Slow Wave Sleep
T	Transporter (SERT)
$t_{1\ (x\ mg/kg\ FLV)}$	onset of the increase in REM sleep, dependent on fluvoxamine dose
t_2	offset of the increase in REM sleep

TCA	tricyclic antidepressant
T_{max}	peak time corresponding to maximal concentration
T-S	Transporter-Serotonin complex
TST	Tail Suspension Test
V	Vertical
V_1	central volume of distribution
V_2, V_3	peripheral volumes of distribution
V_{DB}	volume in deep brain compartment
V_{SP}	volume in shallow perfusion-limited compartment
V_T	brain volume
WHO	World Health Organization

Nawoord

Het moment is nu toch echt eindelijk daar, mijn proefschrift is afgerond! De afgelopen jaren hebben veel personen op verschillende manieren een belangrijke bijdrage geleverd aan de totstandkoming van dit proefschrift. Een aantal mensen zou ik hierbij graag in het bijzonder willen noemen.

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Marian

Curriculum Vitae

Marian Geldof werd geboren op 30 oktober 1977 te Vlaardingen. Na het behalen van het diploma VWO in 1996 aan de S.G. Groen van Prinsterer te Vlaardingen, begon zij de studie Bio-Farmaceutische Wetenschappen aan de Universiteit Leiden, waar zij in 1997 het propedeutisch diploma verkreeg. Tijdens de doctoraal-fase heeft zij haar hoofdvakstage gedaan op de afdeling Farmaceutische Technologie, onder begeleiding van Dr. B.I. Florea, Dr. G. Borchard en Prof. Dr. H.E. Junginger. Zij ontving voor haar stageverslag, getiteld “Chitosan and chitosan derivatives as safe transfection vectors for non-viral gene delivery in cystic fibrosis", de derde Organon Young Research Talent Prijs. Vervolgens heeft zij een onderzoeksstage gedaan op de afdeling niet-klinische farmacokinetiek bij Johnson & Johnson, Pharmaceutical Research and Development in Beerse, België, onder begeleiding van K. Wuyts en Drs. L. v. Beijsterveldt. Hier heeft ze onderzoek gedaan naar de chronische cannulatie van de poortader in de rat. In 2001 werd het doctoraal diploma Bio-Farmaceutische Wetenschappen behaald (cum laude).

Van 2001 tot en met 2005 werkte zij als onderzoeker in opleiding bij de afdeling Farmacologie van het Leiden/Amsterdam Center for Drug Research onder begeleiding van Prof. Dr. M. Danhof, alwaar het in dit proefschrift beschreven onderzoek werd uitgevoerd. In het kader van dit onderzoek werd samengewerkt met verschillende afdelingen van Johnson & Johnson, Pharmaceutical Research and Development in België.

Sinds 2005 is zij werkzaam als Scientist Drug Metabolism & Pharmacokinetics bij Johnson & Johnson, Pharmaceutical Research and Development in België.

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