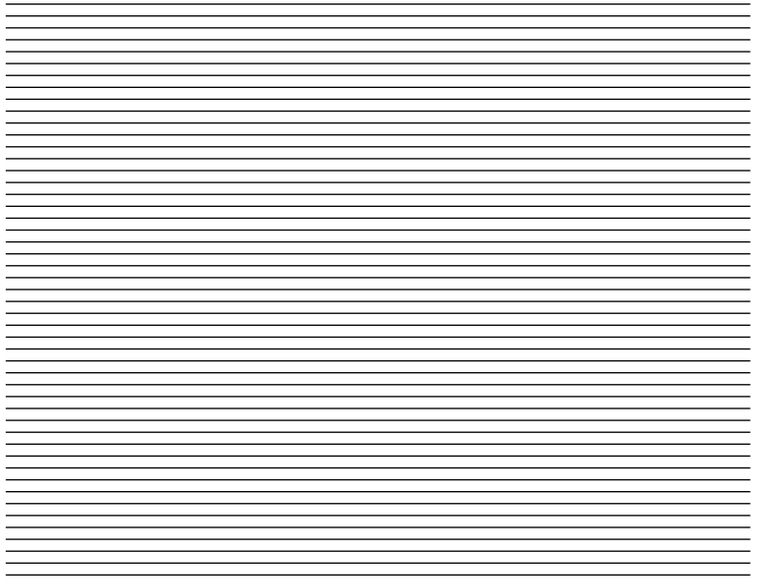
CHAPTER I

Introduction to the endocannabinoid system as a target for drug development



Mechanisms and functions of the endocannabinoid system

EVOLUTION OF THE ENDOCANNABINOID SYSTEM

Although a very ancient biological system, the endocannabinoid system was only discovered and explored over the previous five decades. Named after the plant *Cannabis sativa* L, which produces over 60 cannabinoid compounds, the system is widely distributed phylogenetically: it appears in very ancient, primitive invertebrate species, such as hydras, and in the most evolved mammals, such as humans. Already a few billion years ago the endocannabinoid precursor phosphatidylethanolamine (PEA) was expressed by the cytoplasmic membranes of bacteria. From there, the first molecules with cannabinoid receptor affinity were produced by cyanobacteria, which diverged from eukaryotes at least 2 billion years ago. After the cyanobacteria, endocannabinoids were produced by brown algae which diverged 1.5 billion years ago, again followed by sponges which diverged about 930 million years ago (for a review, see MacPartland (2004)). In absence of specific cannabinoid receptors the endocannabinoids initially had various other targets including 5-HT3A receptors and ion channels. About 790 million years ago, the primordial cannabinoid specific binding place evolved. The development of the endocannabinoid system has accompanied the evolution from monocellular organisms to higher animals, which is mirrored by its widespread involvement in intra- and intercellular signalling.

CANNABIS AND THC

Cannabis sativa L (or cannabis) is the most commonly illicit drug of abuse world-wide. Its major uses are for recreational and medicinal purposes, and the earliest evidence of cannabis use go back as far as 3000 years b.c. (World Health Organisation, 2013; Mechoulam, 1986). Δ^9-tetrahydrocannabinol (THC) is the most well-known active compound from cannabis and is generally held responsible for the well-known effects such as 'the munchies', a term used for hunger pangs after cannabis use, and central effects on consciousness, such as feeling high and altered time perception (Zuurman et al., 2009; Zuurman et al., 2008; Mathew et al., 1998; Plasse et al., 1991; Foltin et al., 1988). As a pharmaceutical substance, THC is mostly referred to as dronabinol, which is the generic name. Cannabis also contains many other cannabinoids such as cannabidiol, but for most of these compounds the pharmacologically activity is still unclear.

FUNCTIONS OF THE ENDOCANNABINOID SYSTEM

Currently, two cannabinoid receptors have been identified: CB_2 and CB_2 receptors, which have different functions and localisation patterns. CB_1 receptors are abundantly present in the nervous system, mostly located in cortical and limbic regions of the brain, as well as the cerebellum (Herkenham et al., 1991). In addition to the nervous system, CB_2 receptor MRNA has been found in the adrenal gland, bone marrow, heart, liver, kidney lung, prostate, ovary, and testicles of different species including humans (for review, see Pertwee (1997)). The CB_2 receptor is less widely expressed than the CB_2 receptor, and its MRNA is mainly present in various parts of the immune system, such as tonsils, spleen, thymus, bone marrow, and in B lymphocytes, monocytes, macrophages, mast cells and microglia in several species, including humans (for review, see Pertwee (1997)). CB_2 receptors are also expressed at lower densities in the brain, mainly on microglia (Gong et al., 2006; Nunez et al., 2004) (for an overview of the distribution of CB_2 and CB_2 receptors, see Figure 1).
The cannabinoid system mainly has a modulatory role in the regulation of complex physiological systems, such as metabolism (including digestive and endocrine systems), and the nervous system and immune system (for a review, see Melamede (2005)). Under normal physiological conditions, the endocannabinoid system is thought to generally have a low

activity, whereas the system can become overactive in pathological conditions or during stress. As earlier suggested by the late Ester Fride, this could be related to the numerous observations of biphasic cannabinoid effects (Fride, 2002). A clear example of biphasic characteristics following pharmacological intervention, include effects on anxiety (Rey et al., 2012): high doses of THC can induce panic attacks, whereas lower levels generally have a relaxing effect. This widespread involvement of endocannabinoids provides numerous opportunities for the development of new medicines for metabolic, neural or immune disorders, including Alzheimer's disease, multiple sclerosis, rheumatoid arthritis, diabetes mellitus, dyslipidemia and movement disorders.

PHARMACOLOGY OF THE ENDOCANNABINOID SYSTEM

In various mammal species, including humans, the endocannabinoid system includes two subtypes of G protein coupled cannabinoid receptors (CB_1 and CB_2) and endogenous messengers. The two most important messengers are anandamide (AEA) and 2-arachidonoyl glycerol (2-AG) (Figure 2) (Matsuda et al., 1990; Munro et al., 1993). AEA acts as a partial agonist with stronger binding affinity (Ki) and efficacy at the CB_1 receptor (Ki = 61-543 nM) compared to CB_2 (Ki = 279-1940 nM) (Pertwee, 2005). 2-AG has shown higher efficacy with similar affinities, and acts as a full agonist on both CB_1 and CB_2 receptors (Ki = 58-472 nM and 145-1400 nM respectively) (Pertwee, 2005). AEA and 2-AG are synthesised by membrane components (arachidonic acid) and released 'on demand' (Di Marzo et al., 1994). AEA and 2-AG are broken down by the enzymes fatty acid amidohydrolase (FAAH) and monoglyceride lipase (MAGL) respectively (Cravatt et al., 1996; Dinh et al., 2002).

Endocannabinoids regulate a variety of cellular effects via inter- (paracrine) and intracellular (autocrine) communication. The endogenous ligands bind to the v or CB_2 receptor, which affects ion channels or second messenger signalling pathways (Bosier et al., 2008; Prather et al., 2000; Su and Vo, 2007; Mackie et al., 1995; Twitchell et al., 1997). The exact pathway depends on the receptor subtype that is activated (Figure 3). CB_1 receptors in the nervous system are located on the pre-synapse. In this way, endocannabinoids act as retrograde synaptic messengers (Figure 4). The receptors are able to regulate activation and inhibition of the post-synaptic cell by stimulating the release of neurotransmitters like GABA and glutamate (Twitchell et al., 1997; Guo and Ikeda, 2004; Binzen et al., 2006).

PATHOLOGY OF THE ENDOCANNABINOID SYSTEM

Because of its essential basic physiological functions and its widespread presence throughout the body, the endocannabinoid system might be involved with many different pathological conditions. Although many findings are still controversial, studies in animal models and patients demonstrated changes in the endocannabinoid system activity in certain diseases or disease models, such as increased AEA levels in the CSF of schizophrenic patients (for example, see Richardson et al. (2008)). However, whether a deregulated system is a cause or a result of the disorder remains to be investigated and only little is known about the pathophysiology of the cannabinoid system.

PSYCHIATRY AND NEUROLOGY – Due to the clear psychotomimetic effects of cannabis consumption, the pathophysiology of the endocannabinoid system in psychiatric and neurologic disorders is relatively well studied. Many studies have led to the theory that chronic cannabis consumption can contribute to schizophrenia (for a review, see Ferretjans, Moreira, Teixeira, & Salgado (2012)). Several labs studied the endocannabinoid system in schizophrenia pathology, however, no consistency could be found regarding CB_1 expression in the brain, or blood and tissue concentrations of the major endocannabinoids as outlined in a review

by Ferretjans, Moreira, Teixeira, & Salgado (2012). It has been suggested, however, that schizophrenia is associated with polymorphisms of the CNR1 gene, which is responsible for encoding the CB_1 receptor (Ujike et al., 2002), although many other genes have also been implicated. Variations of the CNR1 gene are also associated with major depression and with the mediation of antidepressant drug effects (Mitjans et al., 2013).

Other evidence regarding the pathophysiology of the endocannabinoid system in minor and major depression and suicide is contradictory (as outlined in a review by Micale, Di Marzo, Sulcova, Wotjak & Drago (2013)). It is more certain, however, that the endocannabinoid system plays a gate-keeper role with regard to activation of the hormonal hypothalamic-pituitary-adrenal (HPA) axis, which has a major role in controlling reactions to stress. Stress has a large influence on cognition, anxiety and mood, and chronic stress can lead to depression-like symptoms. Endocannabinoids regulate the neurotransmitter release leading to hormonal release by retrograde messaging, which is mostly related to downregulation of excitatory, glutamatergic transmission.

Also for other brain regions, there are possible relationships between the endocannabinoid system and pathology. However, the exact role in pathological states remains still unclear. For example, in a rat model of autism the endocannabinoid system showed downregulation of 2-AG degrading enzymes in certain brain areas and higher tissue concentrations of endocannabinoids following social exposure (Kerr et al., 2013), but no conclusions can be drawn regarding the pathophysiology of the endocannabinoid system in autism. Also, due to the expression of CB_1 receptors on inhibitory GABAergic as well as excitatory glutamatergic neurons, cannabinoids can be both pro- and anti-convulsive, but their role in epilepsy is not well studied. A study by Sagredo et al. (2007) found that the CB_1 receptor is downregulated in early stages of Parkinson's disease, and the cannabinoid system becomes overactive in advanced stages of the disease (Sagredo et al., 2007). Although previous studies reported beneficial effects of cannabinoids on symptoms of Alzheimer's disease, including mood, sleep and cognitive decline, and on neuroprotection, the exact role of the endocannabinoid system in Alzheimer's disease is unknown (for review, see Orgado, Fernandez-Ruiz, & Romero (2009)). The endocannabinoid system is involved in the majority of the processes that occur before, during and after ischemia, and result in vasodilatation, neuroprotection, immunomodulation and antioxidation (Orgado et al., 2009; Martinez-Org et al., 2007). Also, the endocannabinoid system is involved in nociception, chronic inflammatory and neuropathic pain (Zogopoulos et al., 2013). Recently, it has come to light that some metabolites of AEA and 2-AG can either exacerbate or inhibit nociceptive signalling (Rani et al., 2012). The exact role of the endocannabinoid system in ischemia and pain modulation is still under investigation at several labs.

The major function of the endocannabinoid system is believed to be the regulation of the feeding system (De Petrocellis et al., 1999). This applies to both the feeling of hunger and the direct involvement in energy regulation. An obvious example includes getting 'the munchies' or a craving for high caloric food after cannabis use. Also, endocannabinoid activity directs towards energy storage, for example by stimulating adipogenesis and gluconeogenesis (for review, see Silvestri & Di Marzo (Silvestri and Di Marzo, 2013) and Osei-Hyiaman et al. (2008)). This inspired academy and industry to investigate the possibilities of the endocannabinoid system in the light of eating disorders such as obesity and anorexia. However, studies on the potential therapeutic validity of cannabinoids in eating disorders are scarce and inconclusive. The same counts for substance abuse, in which no conclusions can be drawn on the exact mechanisms. However, it has been found that CB_1 contributes to the motivational and reinforcing properties of ethanol, and chronic consumption alters endocannabinoid transmitter levels and CB_1 expression in brain addiction pathways (Pava and Woodward, 2012). Also, several studies associated polymorphisms in the CNR1 and FAAH genes with drug-related behaviours (Lopez-Moreno et al., 2012).

IMMUNOLOGY – Immunologic disorders for which the endocannabinoid system has been investigated include multiple sclerosis, arthritis, sepsis, inflammatory bowel disease, pancreatitis, uveitis and peridontitis. Studies performed *in vitro*, preclinically and in humans showed an upregulation of the endocannabinoid system in inflammation (Richardson et al., 2008) (for an overview of the studies in multiple sclerosis, see the review by Pertwee (2007)). For example, AEA and 2-AG have been found in synovial fluid of arthritic patients, whereas in the synovial fluid of healthy volunteers, no cannabinoids were detected (Richardson et al., 2008). In post-mortem lesioned brain tissue from patients with chronic multiple sclerosis, the concentration of anandamide was significantly elevated compared to brain tissue from healthy controls (Eljaschewitsch et al., 2006). These examples suggest a protective role of the endocannabinoid system in inflammation.

ENDOCRINOLOGY – Several studies demonstrated that the upregulation of endocannabinoids and CB_1 and CB_2 stimulation increases food intake, obesity-related inflammation and adipogenesis (Gamage and Lichtman, 2012) (for an overview, see review by Cluny, Reimer, & Sharkey (2012) and Faurholt Bennetzen (2010)). Clinical studies found that obese subjects have a decreased subcutaneous CB_1 expression compared to lean subjects, and that the endocannabinoid system reduction is normalised with weight loss (Faurholt Bennetzen, 2010). This could imply a reactive compensation in obese patients.

In line with these observations, mice lacking the CB_1 receptor in hepatocytes, although still susceptible to diet-induced obesity, are protected against liver steatosis, hyperglycemia, dyslipidemia, and insulin resistance (Osei-Hyiaman et al., 2008). Blocking CB_1 function is associated with alleviation of hyperglycemia and dyslipidemia. In line with these findings, several studies indicate that endocannabinoids have negative effects on glucose tolerance and insulin secretion (for review, see Doyle (2011)). Studies in patients with advanced diabetic nephropathy and in mice, suggested that CB_2 signalling was impaired (Barutta et al., 2011). The exact role of the endocannabinoid system in the pathophysiology of diabetes, however, still needs to be investigated.

CARDIOVASCULAR – The endocannabinoid system affects heart and arterial performance in pathological conditions, including regulation of vessel contractility and atherogenesis. This happens directly or indirectly via alteration of cardiometabolic risk factors and CB_1 and CB_2 receptors often seem to act in opposing ways (for a review, see Montecucco & Di Marzo (2012)).

GLAUCOMA – A study in patients showed lower COX-2 expression and lower PGE2 concentration in aqueous humor compared to healthy individuals (Maihofner et al., 2001). As COX-2 and PGE2 can be increased by cannabinoids and glaucoma can be treated by cannabinoids, it has been suggested that the endocannabinoid system might contribute to the control of processes leading to glaucoma (for review, see Nucci et al. (2008)).

ONCOLOGY – Endocannabinoids might represent one of the many adaptive responses aimed at counteracting tumour cell growth. Several studies demonstrated that cannabinoids exert anti-proliferative and apoptotic effects (for review, see Hermanson & Marnett (2011)). Also, increased endocannabinoid signalling is found in some human malignancies compared with the corresponding healthy tissues, as well as in human cancer cells with a high degree of invasiveness (see review by Di Marzo, Bifulco, & De Petrocellis (2004)). However, over-expression of CB_2 receptors on haematopoietic precursor cells has been suggested to be associated with, and possibly a causative factor of, human acute myeloid leukaemia.

In summary, we can conclude that endocannabinoid changes accompany a wide variety of disorders, although many changes are still controversial. This is largely due to the physiological complexity of the endo-

cannabinoid system, which often involves feedback mechanisms at a local tissue level, or indirect influences on processes that are also regulated by other systems. Changes in signalling sometimes represent an attempt to counteract a pathological process, and in other instances could be one of the causative factors underlying the disease or its symptoms. Although it is premature to view endocannabinoids as markers of pathological states, a general conclusion from previous studies is that, endocannabinoids seem to have a protective or ameliorating role in many cases.

Complexities of cannabinoid drug development

Endocannabinoids are involved in complex physiological systems that play an important role in a huge number of diseases in almost all areas of medicine. In principle, this makes them appealing targets for drug development. However, because of this complexity and the relatively recent discovery of the endocannabinoid system, endocannabinoid research is still in a premature stage. Cannabinoid research and drug development is complicated further by a number of factors which are summarised in the following paragraphs.

LIMITED SUBTYPE SPECIFICITY

The limited number of receptor subtypes and the limited number of endogenous ligands and their ubiquitous presence makes it difficult to identify the exact local steering processes. For example, in contrast, the GABA-A receptor has at least half a dozen subtypes and the serotonin-system has over a dozen of 5-HT receptor subtypes. This creates ample opportunities for the development of highly selective compounds as research tools or potential drugs, or to develop genetic knock-in or knock-out models to study the functional role of a specific receptor subtype. In the case of the endocannabinoid system, such models and interventions generally affect many systems at the same time. The number of enzymes

that are involved in endocannabinoid synthesis and degradation is also limited. Consequently, there is a shortage of good pharmacological interventions to manipulate the endogenous cannabinoids, such as inhibitors of degradation but also of reuptake or transport.

WIDESPREAD DISTRIBUTION

The endocannabinoid system is one of the most widely distributed pharmacological systems in the body (for review, see Pertwee (1997)). This complicates systemic or organ-specific targeting. By trying to target a specific location, the ubiquitous presence of the system easily causes unnecessary or undesirable effects elsewhere, which limits the development of therapeutically specific drugs.

HIGH LIPOPHILICITY

To optimise specific targeting of cannabinoids to those parts of the body that are involved in a disease, pharmacokinetics of endogenous and exogenous cannabinoids can be modified, for example by changing administration routes, dosing quantity and time intervals, or by differentiating peripheral and central drug distribution. However, pharmacokinetic optimisation is limited by the strong lipophilic character of exogenous and endogenous cannabinoids (e.g. Log P values for anandamide and 2-AG are 6.31 and 8.01 respectively (Stanton et al., 2005). Although lipophilic compounds are generally well absorbed gastro-intestinally, they carry the risk not to be optimally distributed systemically, due to the rapid diffusion from the blood to fatty organs, such as adipose tissue, liver and brain. As a consequence, a relatively large concentration is located at specific sites, whereas other sites are much less exposed to the compound. Also, very lipophilic compounds are often only slowly redistributed from fatty organs back into the blood, as a result of which the compounds accumulate and remain detectable in the blood for long time periods after

dosing. Furthermore, lipophilic compounds can be rapidly metabolised, resulting in fast metabolite exposure. They generally have a high protein binding, resulting in a low free drug fraction and thereby more variable drug exposure. Also lipophilic compounds generally have a somewhat limited specificity, i.e. 'pharmacological promiscuity'. Consequently, the lipophilicity of cannabinoids creates a large complexity for specific dosing in terms of target and time frame.

COMPLEX PHYSIOLOGICAL INTEGRATION

Due to the ancient phylogeny of the endocannabinoid system and its involvement in primitive systems, it is deeply embedded in basic functions and complex physiological systems. Locally, these systems can have very diverse signalling pathways, cellular messaging and functions (Figure 3). Most systems in which endocannabinoids are involved, such as the central nervous system or immune system) form highly integrated networks, with many layers of feedback and regulation. This makes it enormously difficult for pharmacology to precisely interfere with one specific signalling pathway. For the same reasons there are also many uncertainties regarding the exact role of endocannabinoids in pathophysiology, which in most diseases has not been unequivocally demonstrated. For the few diseases in which consistent involvement of the endocannabinoid system has been found, it is still unclear to what extent a deregulation is part of the cause or merely a consequence or sign of dysfunction.

COMPLICATED EFFECT MEASUREMENTS

The integration of the endocannabinoid system at subcellular levels of complex multicascadic physiological mechanisms and the wide range of effects create a major challenge for measurement of changes in their activity, which is essential in drug development. The methodology currently used in clinical research is unable to track all drug- or disease-induced changes. Therefore, it is easy to miss relevant effects. This can be the case in acute single dosing studies, where the, very often subtle, changes in homeostasis can be easily overlooked.

Optimisation of early cannabinoid development

In spite of the complexity of endocannabinoids, many efforts have been made to develop drugs that are targeted on this system. In general, several options are available to overcome the pharmacologic limitations and the problems with effect measurements that are described in the previous sections. This section deals with these options, and how they were approached in this thesis.

DRUG DESIGN OPTIMISATION

PHARMACOLOGICAL OPTIMISATION – To act on pathological conditions, which are often very local or limited to a single physiological system, receptor subtypes should be targeted as specifically as possible. thc is the most well-known cannabinoid and is generally used as an experimental compound in CB_1 agonist studies and THC-challenge studies and is a major compound in various registered and experimental medical formulations, including medicinal cannabis, Sativex® and Marinol®. However, THC lacks cannabinoid receptor specificity and exhibits its effects as a partial agonist on both the CB_1 and the CB_2 receptor (for a review, see Pertwee (2008)). Also, THC is very lipophilic (log P = 6.97) and accordingly, after administration, THC is very quickly distributed to the peripheral fatty tissues including lungs, adipose tissue and the brain (Thomas et al., 1990; Lemberger et al., 1970; Ryrfeldt et al., 1973; Brunet et al., 2010). Besides the option of exogenous targeting of the cannabinoid receptors, the endogenous cannabinoid levels could be manipulated.

Options for manipulation of the endogenous cannabinoid levels include influencing synthesis, transport, release, and degradation. The

most well-known example of current investigation of this type of manipulation is the development of FAAH-inhibitors, which inhibit the metabolism of AEA. Clinical studies with FAAH-inhibitors for the indications of several pain and inflammation states are still ongoing. Studies with inhibitors of monoacylglycerol lipase (MAGL) and transporters are still in pre-clinical *in vitro* phase (ICRS, 2012). Despite these attempts, the options are very limited due to the small number of available compounds influencing endogenous cannabinoids and are unlikely to allow enough pharmacological selectivity for a wide array of disease-specific cannabinoid-targeted treatments.

Another way to improve the therapeutic window of drugs with limited pharmacological selectivity is to control their action site penetration. This can be achieved by pharmacokinetic optimisation of drug levels and tissue penetration.

PHARMACOKINETIC OPTIMISATION

REDUCING SYSTEMIC VARIABILITY: ADMINISTRATION ROUTE – The administration route can influence pharmacokinetic aspects such as time of drug absorption or peak concentration and distribution, and thereby time of effect onset, and the number and magnitude of concentration-related therapeutic and undesirable effects. The most common administration route is the oral route. Oral administration is generally very easy and convenient, however, pharmacokinetically, there are some risks with this administration route. Oral administration could result in variable plasma concentrations, as absorption to the blood is dependent on GI tract activity, pH variations and food interactions. Also, the compounds reach the liver before they reach the systemic blood circulation, resulting in metabolism and possible modification of the activity of compounds that are metabolised by CYP450 enzymes. These enzymes are also situated in the gut wall, and their activity can vary due to genetic variations and interactions with foods and drugs.

In order to avoid gut- and liver metabolism, drugs can be administered intravenously, directly into the blood stream (i.e. 100% bioavailability). This administration route is limited by its invasiveness. Non-invasive ways of avoiding hepatic metabolism are for example intrapulmonal, sublingual and transdermal administrations. These routes are not suitable for each compound. They may give less variable pharmacokinetics compared to oral administration, but the administration routes are less practical. Another way to enhance the bioavailability of a compound is by galenic manipulation. Changing the formulation can improve the resorption of a compound and affect the exposure profile.

In this thesis, we investigated the pharmacokinetics and pharmacodynamics of several different administration routes of THC. In previous studies by Zuurman et al. we have optimized the intrapulmonary administration of THC, using a vaporizer and pure THC rather than the more usual method of smoking cannabis extracts (Zuurman et al., 2008). Although inhalation of vaporized pure THC produces reliable pharmacokinetic profiles, it is a less convenient mode of administration, which gives little control over the exposure profile. This can be improved by the so called repeated paced puffing protocol, which uses predetermined dosages and times to achieve a desired exposure profile, however with relatively variable results (Chapter 3, 4, 5 and 6).

In Chapter 2, we investigate different oral and sublingual administrations of Namisol, a new tablet containing THC. Namisol is manufactured with Alitra™, a novel lipophilic compound delivery technology that has an improved absorption of poor water soluble compounds in the human blood, thereby improving bioavailability with reduced variability. Th most favourable administration route was chosen for further development of Namisol for the indication of pain and spasms in multiple sclerosis.

PHARMACOKINETIC CONTROL OF THE THERAPEUTIC WINDOW: DOSAGE AND TIME INTERVAL – Besides the route of administration, the actual

dose given and the time interval between multiple doses are essential factors to maintain drug concentrations within the therapeutic window for the required time periods. Overdosing could lead to unwanted (side) effect profiles and even to toxicological effects. It is essential therefore to accurately predict the optimal therapeutic window for a new drug. This may be problematic if the effects are difficult to measure; either because they are part of an integrated system with many homeostatic mechanisms; or because specific tests are lacking; or because the beneficial effects are chronic (e.g. weight reduction or cardiovascular risk) or infrequent (*e.g.* epileptic seizures or exacerbations of multiple sclerosis). This was a problem with the first cannabinoid antagonists like rimonabant, where traditional methods like preclinical dose predictions and maximum tolerability levels in healthy subjects were used to determine the doses for clinical trials (Cohen, 2010). Although this approach led to the registration of rimonabant for obesity, the drug was withdrawn soon after launch because of unacceptable psychiatric side effects in a minority of patients. It is important therefore to determine the concentration range that has an optimal effect on the right pharmacological target: not too much or too little inhibition or stimulation; and not at action sites that are not involved in the disease. The determination of dose- or concentration-effect relationships for different mechanisms of action of cannabinoids is an important part of this thesis. In Chapter 2 a first in human study with the novel THC tablet Namisol aimed to find the optimal dosage for single dose administration by evaluating pharmacokinetics and pharmacodynamic effects. In Chapters 4-6 we try to establish the concentration-effect relationships for different cannabinoid agonists and antagonists, with the aim of establishing a dosing regimen with an optimal pharmacological effect.

TARGET SPECIFICITY – Options for improving specificity in drug development are limited due to the limited number of cannabinoid receptor subtypes (i.e. two) and their presence all throughout the body. Another option to improve specificity for a specific target or location would be to improve the delivery of the compound to a specific location. For example, manipulation of the compound's permeability for the blood brain barrier could keep a compound outside of the central nervous system. In Chapter 5, we actually tested TM38837, a compound that showed peripheral restriction in preclinical studies with the aim to demonstrate peripheral activity without central activity. In this study, we compared TM38837 with the centrally and peripherally active antagonist rimonabant, using biomarkers of peripheral and central CB_1-activity that have been previously identified (i.e. feeling high and heart rate) (Zuurman et al., 2009). TM38837 is under development for treatment of peripherally associated disorders (including hepatic disorders and obesity) with reduced central side-effects.

METHODOLOGICAL OPTIMISATION

General challenges in drug development are to precisely and accurately detect and measure relevant (side) effects, and to ensure translatability of drug responses from preclinical animals and healthy volunteers to patients and vice versa. These challenges particularly apply to the development of cannabinoid drugs.

ACCURATE EFFECT MEASUREMENTS – The endocannabinoid system is deeply embedded within a variety of physiological networks. When endocannabinoid changes are induced in the network, these changes can be quickly modified by other homeostatic processes. The complex interactions between the networks and their eventual results are not always immediately measurable. Consequently, results from acute dosing studies cannot always be extrapolated to multiple dose studies in which more chronic effects are studied.

Cannabinoids can induce a wide palette of effects which makes measuring the relevant effects related to various physiological networks

quite challenging. It should be tested whether the compound reaches the target site and other sites and to what magnitude and in what time frame the effects take place. With the availability of a broad range of tests, especially of the central nervous system (CNS), an adequate set of tests should be chosen in order not to miss any relevant effects.

TEST SELECTION – Zuurman et al. performed a systematic literature review on biomarkers for the effects of cannabis and THC in healthy volunteers (Zuurman et al., 2009). Consistently, she found increases of heart rate frequency and feeling high, and decreases of motor control. This trio of consistent effect measurements are considered as the basic cannabinoid responses that were measurable in a consistent way, and were therefore applied in all studies in this thesis.

For the detection of these effects in healthy volunteers, the Centre for Human Drug Research (CHDR) developed the NeuroCart test battery. This test battery includes all of the functions mentioned, using non-invasive tests with a short duration and limited learning effect, which allows for repeated measures. The NeuroCart battery include visual analogue scales of mood, alertness and calmness by Bond & Lader (1974), psychedelic effects by Bowdle et al. (1998), and body sway measurement, and was applied to the studies reported in Chapters 2, 4, 5 and 6 and partially in Chapter 3.

Even with a battery of tests, effects of the endocannabinoid system can be easily missed. There is a need therefore, for methods that provide integrated representations of functional activities, which are highly sensitive and specific to pharmacological effects. For the immune system and metabolism, biochemical analyses have been developed to examine specific functional arrays (immunoarrays and metabolic arrays), and the more general 'omic' approaches (proteomics, metabolomics) can provide broad screens of functional changes on an individual level (Powanda and Beisel, 2003; Ahmed et al., 2013). Biochemical effects are more difficult to determine in the central nervous system, but network analysis could provide sensitive indications for a wide range of CNS effects. In Chapter 3, we examined the novel technique of pharmacological resting state functional magnetic resonance imaging (RS-FMRI). This technique seems very valuable for clinical phases of drug development; however, it has not yet been applied for this purpose. Besides better understanding the pharmacodynamics of THC, we aimed to bring RS-FMRI one step closer towards application in drug development.

OPTIMAL STUDY DESIGNS

Pharmacological therapies try to achieve a correction of homeostasis (i.e. healthy state) by artificially interfering with the disturbed elements in a disordered biological system (e.g. stress or pathology). Since early phase clinical research investigates cannabinoids in healthy humans, one should find possibilities for translating the effects seen in healthy volunteers to clinically relevant outcomes in patients. The latter is a specific challenge if the acute effects of a pharmacological manipulation are not measurable in healthy subjects. It is difficult for instance to show the effects of a pharmacological stimulus (e.g. a receptor agonist), if the target system is already maximally active. Such ceiling effects are well-known for cognitive enhancers in healthy students. It is also challenging to show effects of pharmacological inhibition (e.g. a receptor antagonist) in case of 'floor effects', when the endogenous system is dormant under physiological conditions. The low basic activity of the endocannabinoid system may be the reason why cannabinoid antagonists do not show any effect in healthy volunteers at doses that are clearly effective in various disease states (Rodriguez de Fonseca et al., 1999). In such cases, pharmacological or functional challenge tests can be used to perturb the target system in such a way, that it is possible to show correction by the drug. For example, a scopolamine-challenge causes cognitive deterioration, which can be improved by procognitive drugs (Snyder et al., 2005). To enable detection and quantification of

effects of cannabinoid antagonists, CHDR developed the THC challenge test (Zuurman et al., 2008; Zuurman et al., 2010). This test allows indirect quantification of agonistic effects by measuring the antagonistic inhibition of THC-induced effects. In practice, this means that on one study occasion an agonist is used to induce acute effects (e.g. feeling high), whereas on another occasion the agonist is dosed together with an antagonist, which can now be shown to reduce the agonistic effects. This provides unequivocal proof that the antagonist has reached pharmacologically active concentrations in relevant parts of the body, which is an essential prerequisite for therapeutic activity. Obviously, these relevant body parts need to be represented by the measurements that are used in the study.

The THC challenge test has been developed as a standard method that has been applied in over ten studies, including both antagonist studies and studies investigating THC effects only (Zuurman et al., 2008; Zuurman et al., 2010) and it was applied for Chapters 4, 5 and 6.

CONCENTRATION-EFFECT MODELLING

Modelling is a very powerful tool to simulate and predict pharmacokinetics (PK) and pharmacodynamic effects (PD). These models allow the optimisation of a study design by predicting effective dosages and concentrations and relevant effects, but they can also be applied to 'translate' data from experimental animals and healthy volunteers to patient groups.

The mathematical models that relate PK and PD are referred to as PK-PD models. These models are data driven mathematical models that best describe the relationship between the plasma concentration and a particular pharmacodynamic effect, based on a relatively simple underlying function (usually an E_{MAX}-model). During recent years, the field of modelling underwent major improvements with the development of new theoretical concepts, including the receptor theory and dynamical systems analysis, which takes into account the specific physiological characteristics of a body system (such as blood flow and lipophilicity). Also, statistical and technical improvements led to the more widespread application of visual predictive checks and objective assessments of model complexity (minimal value of objective function), thereby improving the quality of model predictions. Previously, CHDR developed PK-PD models for THC effects (Strougo et al., 2008). These models were used for the design of all THC challenge studies described in this thesis. In this thesis, we tried to expand these models with new PK-PD models for the CB_1 antagonists drinabant (AVE1625), surinabant (SR147778) and TM38837 and CB_1 inverse agonist rimonabant (SR141716) based on inhibition of THC-induced effects (Chapter 6).

In summary, the aim of this thesis is to improve cannabinoid drug development in early phase clinical research, by investigating new cannabinoid compounds and new formulations to improve pharmacological effects, experimenting with new methodologies to optimise effect measurement, and applying new concentration-effect models to improve the simulation and prediction of optimal dosing regimens of cannabinoid agents for future studies.

REFERENCES

7TM Pharma A/S (2009) Investigator's Brochure - TM38837, a CB1 receptor antagonist/inverse agonist.

Ahmed MU, Saaem I, Wu PC, Brown AS (2013) Personalized diagnostics and biosensors: a review of the biology and technology needed for personalized medicine. Crit Rev Biotechnol.

Alexander SP, Mathie A, Peters JA (2008) Guide to Receptors and Channels (GRAC), 3rd edition. Br J Pharmacol 153 Suppl 2:S1-209.

Aronne LJ, Tonstad S, Moreno M, Gantz I, Erondu N, Suryawanshi S, Molony C, Sieberts S, Nayee J, Meehan AG, Shapiro D, Heymsfield SB, Kaufman KD, Amatruda JM (2010) A clinical trial assessing the safety and efficacy of taranabant, a CB1R inverse agonist, in obese and overweight patients: a high-dose study. Int J Obes (Lond) 34:919-935.

Baker D, Pryce G, Croxford JL, Brown P, Pertwee RG, Huffman JW, Layward L (2000) Cannabinoids control spasticity and tremor in a multiple sclerosis model. Nature 404:84-87.

Barutta F, Piscitelli F, Pinach S, Bruno G, Gambino R, Rastaldi MP, Salvidio G, Di M, V, Cavallo PP, Gruden G (2011) Protective role of cannabinoid receptor type 2 in a mouse model of diabetic nephropathy. Diabetes 60:2386-2396.

Beck AT, Steer RA, Ball R, Ranieri W (1996) Comparison of Beck Depression Inventories -IA and -II in psychiatric outpatients. J Pers Assess 67:588-597.

Beckmann CF, DeLuca M, Devlin JT, Smith SM (2005) Investigations into resting-state connectivity using independent component analysis. Philos Trans R Soc Lond B Biol Sci 360:1001-1013.

Beckmann CF, Smith SM (2005) Tensorial extensions of independent component analysis for multisubject FMRI analysis. Neuroimage 25:294-311.

Benowitz NL, Jones RT, Lerner CB (1976) Depression of growth hormone and cortisol response to insulin-induced hypoglycemia after prolonged oral delta-9-tetrahydrocannabinol administration in man. J Clin Endocrinol Metab 42:938-941.

Bermudez-Silva FJ, Suarez J, Baixeras E, Cobo N, Bautista D, Cuesta-Munoz AL, Fuentes E, Juan-Pico P, Castro MJ, Milman G, Mechoulam R, Nadal A, Rodriguez de FF (2008) Presence of functional cannabinoid receptors in human endocrine pancreas. Diabetologia 51:476-487.

Bermudez-Silva FJ, Viveros MP, McPartland JM, Rodriguez de FF (2010) The endocannabinoid system, eating behavior and energy homeostasis: the end or a new beginning? Pharmacol Biochem Behav 95:375-382.

Bhattacharyya S, Fusar-Poli P, Borgwardt S, Martin-Santos R, Nosarti C, O'Carroll C, Allen P, Seal ML, Fletcher PC, Crippa JA, Giampietro V, Mechelli A, Atakan Z, McGuire P (2009) Modulation of mediotemporal and ventrostriatal function in humans by Delta9-tetrahydrocannabinol: a neural basis for the effects of Cannabis sativa on learning and psychosis. Arch Gen Psychiatry 66:442-451.

Bhattacharyya S, Morrison PD, Fusar-Poli P, Martin-Santos R, Borgwardt S, Winton-Brown T, Nosarti C, O' Carroll CM, Seal M, Allen P, Mehta MA, Stone JM, Tunstall N, Giampietro V, Kapur S, Murray RM, Zuardi AW, Crippa JA, Atakan Z, McGuire PK (2010) Opposite effects of delta-9-tetrahydrocannabinol and cannabidiol on human brain function and psychopathology. Neuropsychopharmacology 35:764-774.

Binzen U, Greffrath W, Hennessy S, Bausen M, Saaler-Reinhardt S, Treede RD (2006) Co-expression of the voltage-gated potassium channel Kv1.4 with transient receptor potential channels (TRPV1 and TRPV2) and the cannabinoid receptor CB1 in rat dorsal root ganglion neurons. Neuroscience 142:527-539.

Birn RM (2012) The role of physiological noise in resting-state functional connectivity. Neuroimage Epub ahead of print.

Birn RM, Smith MA, Jones TB, Bandettini PA (2008) The respiration response function: the temporal dynamics of fMRI signal fluctuations related to changes in respiration. Neuroimage 40:644-654.

Biswal BB, et al. (2010) Toward discovery science of human brain function. Proc Natl Acad Sci U S A 107:4734-4739.

Bocker KB, Hunault CC, Gerritsen J, Kruidenier M, Mensinga TT, Kenemans JL (2010) Cannabinoid modulations of resting state EEG theta power and working memory are correlated in humans. J Cogn Neurosci 22:1906-1916.

Bond A, Lader M (1974) The use of analogue scales in rating subjective feelings. Br J Med Psychol 47:211-218.

Borgwardt SJ, Allen P, Bhattacharyya S, Fusar-Poli P, Crippa JA, Seal ML, Fraccaro V, Atakan Z, Martin-Santos R, O'Carroll C, Rubia K, McGuire PK (2008) Neural basis of Delta-9-tetrahydrocannabinol and cannabidiol: effects during response inhibition. Biol Psychiatry 64:966-973.

Bosier B, Lambert DM, Hermans E (2008) Reciprocal influences of CB1 cannabinoid receptor agonists on ERK and JNK signalling in N1E-115 cells. FEBS Lett 582:3861-3867.

Bossong MG, van Berckel BN, Boellaard R, Zuurman L, Schuit RC, Windhorst AD, van Gerven JM, Ramsey NF, Lammertsma AA, Kahn RS (2009) Delta 9-tetrahydrocannabinol induces dopamine release in the human striatum. Neuropsychopharmacology 34:759-766.

Bowdle TA, Radant AD, Cowley DS, Kharasch ED, Strassman RJ, Roy-Byrne RP (1998) Psychedelic effects of ketamine in healthy volunteers. Relationship to steady-state plasma concentrations. Anesthesiology82-88.

Brunet B, Hauet T, Hebrard W, Papet Y, Mauco G, Mura P (2010) Postmortem redistribution of THC in the pig. Int J Legal Med 124:543-549.

Buccellato E, Carretta D, Utan A, Cavina C, Speroni E, Grassi G, Candeletti S, Romualdi P (2010) Acute and chronic cannabinoid extracts administration affects motor function in a CREAE model of multiple sclerosis. J Ethnopharmacol.

Cahill K, Ussher MH (2011) Cannabinoid type1 receptor antagonists for smoking cessation. Cochrane Database Syst Rev CD005353.

Callaway J, Schwab U, Harvima I, Halonen P, Mykkanen O, Hyvonen P, Jarvinen T (2005) Efficacy of dietary hempseed oil in patients with atopic dermatitis. J Dermatolog Treat 16:87-94.

Carbuto M, Sewell RA, Williams A, Forselius-Bielen K, Braley G, Elander J, Pittman B, Schnakenberg A, Bhakta S, Perry E, Ranganathan M, D'Souza DC (2011) The safety of studies with intravenous Delta (9)-tetrahydrocannabinol in humans, with case histories. Psychopharmacology (Berl).

Carter RM, O'Doherty JP, Seymour B, Koch C, Dolan RJ (2006) Contingency awareness in human aversive conditioning involves the middle frontal gyrus. Neuroimage 29:1007-1012.

Centre for Reviews and Dissemination (2004) Health Information Resources.

Chang AE, Shiling DJ, Stillman RC, Goldberg NH, Seipp CA, Barofsky I, Simon RM, Rosenberg SA (1979) Delata-9-tetrahydrocannabinol as an antiemetic in cancer patients receiving high-dose methotrexate. A prospective, randomized evaluation. Ann Intern Med 91:819-824.

Chang C, Cunningham JP, Glover GH (2009) Influence of heart rate on the BOLD signal: the cardiac response function. Neuroimage 44:857-869.

Chen CL, Willis BA, Mooney L, Ong GK, Lim CN, Lowe SL, Tauscher-Wisniewski S, Cutler GB, Jr., Wiss SD (2010) Cortisol response to individualised graded insulin infusions: a reproducible biomarker for CNS compounds inhibiting HPA activation. Br J Clin Pharmacol 70:886-894.

Chiang CN, Rapaka RS (1987) Pharmacokinetics and disposition of cannabinoids. NIDA Res Monogr 79:173-188.

Cluny NL, Reimer RA, Sharkey KA (2012) Cannabinoid signalling regulates inflammation and energy balance: the importance of the brain-gut axis. Brain Behav Immun 26:691-698.

Cluny NL, Vemuri VK, Chambers AP, Limebeer CL, Bedard H, Wood JT, Lutz B, Zimmer A, Parker LA, Makriyannis A, Sharkey KA (2010) A novel peripherally restricted cannabinoid receptor antagonist, AM6545, reduces

food intake and body weight, but does not cause malaise, in rodents. Br J Pharmacol 161:629-642.
Cohen AF (2010) Developing drug prototypes: pharmacology replaces safety and tolerability? Nat Rev Drug Discov 9:856-865.
Cohen C, Perrault G, Griebel G, Soubrie P (2005) Nicotine-associated cues maintain nicotine-seeking behavior in rats several weeks after nicotine withdrawal: reversal by the cannabinoid (CB1) receptor antagonist, rimonabant (SR141716). Neuropsychopharmacology 30:145-155.
Cohen C, Perrault G, Voltz C, Steinberg R, Soubrie P (2002) SR141716, a central cannabinoid (CB (1)) receptor antagonist, blocks the motivational and dopamine-releasing effects of nicotine in rats. Behav Pharmacol 13:451-463.
Cole DM, Beckmann CF, Long CJ, Matthews PM, Durcan MJ, Beaver JD (2010) Nicotine replacement in abstinent smokers improves cognitive withdrawal symptoms with modulation of resting brain network dynamics. Neuroimage 52:590-599.
Committee for medicinal products for human use (CHMP) (2010) Guideline on the investigation of bioequivalence.
Corchero J, Fuentes JA, Manzanares J (1999) Chronic treatment with CP-55,940 regulates corticotropin releasing factor and proopiomelanocortin gene expression in the hypothalamus and pituitary gland of the rat. Life Sci 64:905-911.
Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB (1996) Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. Nature 384:83-87.
Crockett D, Klonoff H, Clark C (1976) The effects of marijauna on verbalization and thought processes. J Pers Assess 40:582-587.
Curran HV, Brignell C, Fletcher S, Middleton P, Henry J (2002) Cognitive and subjective dose-response effects of acute oral Delta 9-tetrahydrocannabinol (THC) in infrequent cannabis users. Psychopharmacology (Berl) 164:61-70.
Dalton WS, Martz R, Lemberger L, Rodda BE, Forney RB (1976) Influence of cannabidiol on delta-9-tetrahydrocannabinol effects. Clin Pharmacol Ther 19:300-309.
Damoiseaux JS, Rombouts SA, Barkhof F, Scheltens P, Stam CJ, Smith SM, Beckmann CF (2006) Consistent resting-state networks across healthy subjects. Proc Natl Acad Sci U S A 103:13848-13853.
Davis MP (2008) Oral nabilone capsules in the treatment of chemotherapy-induced nausea and vomiting and pain. Expert Opin Investig Drugs 17:85-95.
Daw ND, O'Doherty JP, Dayan P, Seymour B, Dolan RJ (2006) Cortical substrates for exploratory decisions in humans. Nature 441:876-879.
De Luca M, Beckmann CF, De Stefano N, Matthews PM, Smith SM (2006) fMRI resting state networks define distinct modes of long-distance interactions in the human brain. Neuroimage 29:1359-1367.
De Petrocellis L, Melck D, Bisogno T, Milone A, Di Marzo V (1999) Finding of the endocannabinoid signalling system in Hydra, a very primitive organism: possible role in the feeding response. Neuroscience 92:377-387.
de Visser SJ, van der Post J, Pieters MS, Cohen AF, van Gerven JM (2001) Biomarkers for the effects of antipsychotic drugs in healthy volunteers. Br J Clin Pharmacol 51:119-132.
Desmond JE, Glover GH (2002) Estimating sample size in functional MRI (fMRI) neuroimaging studies: statistical power analyses. J Neurosci Methods 118:115-128.
Devane WA, Dysarz FA, III, Johnson MR, Melvin LS, Howlett AC (1988) Determination and characterization of a cannabinoid receptor in rat brain. Mol Pharmacol 34:605-613.
Dewey WL, Peng TC, Harris LS (1970) The effect of 1-trans-delta 9-tetrahydrocannabinol on the hypothalamo-hypophyseal-adrenal axis of rats. Eur J Pharmacol 12:382-384.
Di Marzo V, Bifulco M, De Petrocellis L (2004) The endocannabinoid system and its therapeutic exploitation. Nat Rev Drug Discov 3:771-784.
Di Marzo V, Fontana A, Cadas H, Schinelli S, Cimino G, Schwartz JC, Piomelli D (1994) Formation and inactivation of endogenous cannabinoid anandamide in central neurons. Nature 372:686-691.
Dinh TP, Freund TF, Piomelli D (2002) A role for monoglyceride lipase in 2-arachidonoylglycerol inactivation. Chem Phys Lipids 121:149-158.
Doyle ME (2011) The role of the endocannabinoid system in islet biology. Curr Opin Endocrinol Diabetes Obes 18:153-158.
du Boisgueheneuc F, Levy R, Volle E, Seassau M, Duffau H, Kinkingnehun S, Samson Y, Zhang S, Dubois B (2006) Functions of the left superior frontal gyrus in humans: a lesion study. Brain 129:3315-3328.
Eggan SM, Lewis DA (2007) Immunocytochemical distribution of the cannabinoid CB1 receptor in the primate neocortex: a regional and laminar analysis. Cereb Cortex 17:175-191.
Eljaschewitsch E, Witting A, Mawrin C, Lee T, Schmidt PM, Wolf S, Hoertnagl H, Raine CS, Schneider-Stock R, Nitsch R, Ullrich O (2006) The endocannabinoid anandamide protects neurons during CNS inflammation by induction of MKP-1 in microglial cells. Neuron 49:67-79.
Emrich HM, Weber MM, Wendl A, Zihl J, von ML, Hanisch W (1991) Reduced binocular depth inversion as an indicator of cannabis-induced censorship impairment. Pharmacol Biochem Behav 40:689-690.
Engeli S, Bohnke J, Feldpausch M, Gorzelniak K, Janke J, Batkai S, Pacher P, Harvey-White J, Luft FC, Sharma AM, Jordan J (2005) Activation of the peripheral endocannabinoid system in human obesity. Diabetes 54:2838-2843.
Faurholt Bennetzen M (2010) Investigations of the Endocannabinoid System in Adipose Tissue.
Ferretjans R, Moreira FA, Teixeira AL, Salgado JV (2012) The endocannabinoid system and its role in schizophrenia: a systematic review of the literature. Rev Bras Psiquiatr 34 Suppl 2:S163-S177.
Ferron G, Klumpers LE, van Gerven JMA, Roy C (2008) PK and PK/PD modeling of CB1 blocker antagonism of THC induced CNS and Heart Rate effect.
Filippini N, MacIntosh BJ, Hough MG, Goodwin GM, Frisoni GB, Smith SM, Matthews PM, Beckmann CF, Mackay CE (2009) Distinct patterns of brain activity in young carriers of the APOE-epsilon4 allele. Proc Natl Acad Sci U S A 106:7209-7214.
Foltin RW, Fischman MW, Brady JV, Bernstein DJ, Capriotti RM, Nellis MJ, Kelly TH (1990) Motivational effects of smoked marijuana: behavioral contingencies and low-probability activities. J Exp Anal Behav 53:5-19.
Foltin RW, Fischman MW, Byrne MF (1988) Effects of smoked marijuana on food intake and body weight of humans living in a residential laboratory. Appetite 11:1-14.
Fox MD, Snyder AZ, Vincent JL, Raichle ME (2007) Intrinsic fluctuations within cortical systems account for intertrial variability in human behavior. Neuron 56:171-184.
Fride E (2002) Endocannabinoids in the central nervous system-an overview. Prostaglandins Leukot Essent Fatty Acids 66:221-233.
Galiegue S, Mary S, Marchand J, Dussossoy D, Carriere D, Carayon P, Bouaboula M, Shire D, Le FG, Casellas P (1995) Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. Eur J Biochem 232:54-61.
Gamage TF, Lichtman AH (2012) The endocannabinoid system: role in energy regulation. Pediatr Blood Cancer 58:144-148.
Garrett ER, Hunt CA (1977) Pharmacokinetics of delta9-tetrahydrocannabinol in dogs. J Pharm Sci 66:395-407.
Gomez R, Navarro M, Ferrer B, Trigo JM, Bilbao A, Del A, I, Cippitelli A, Nava F, Piomelli D, Rodriguez de FF (2002) A peripheral mechanism for CB1 cannabinoid receptor-dependent modulation of feeding. J Neurosci 22:9612-9617.
Gong JP, Onaivi ES, Ishiguro H, Liu QR, Tagliaferro PA, Brusco A, Uhl GR (2006) Cannabinoid CB2 receptors: immunohistochemical localization in rat brain. Brain Res 1071:10-23.
Goodwin RS, Baumann MH, Gorelick DA, Schwilke E, Schwope DM, Darwin WD, Kelly DL, Schroeder JR, Ortemann-Renon C, Bonnet D, Huestis MA (2011) CB1 - Cannabinoid Receptor Antagonist Effects on Cortisol in Cannabis-Dependent Men. Am J Drug Alcohol Abuse.
Greicius MD, Srivastava G, Reiss AL, Menon V (2004) Default-mode network activity

distinguishes Alzheimer's disease from healthy aging: evidence from functional MRI. Proc Natl Acad Sci U S A 101:4637-4642.
Gronewold A, Skopp G (2011) A preliminary investigation on the distribution of cannabinoids in man. Forensic Sci Int 210:e7-e11.
Grotenhermen F (2003) Pharmacokinetics and pharmacodynamics of cannabinoids. Clin Pharmacokinet 42:327-360.
Guo J, Ikeda SR (2004) Endocannabinoids modulate N-type calcium channels and G-protein-coupled inwardly rectifying potassium channels via CB_1 cannabinoid receptors heterologously expressed in mammalian neurons. Mol Pharmacol 65:665-674.
Guy G, Robson P (2003) A phase I, open label, four-way crossover study to compare the pharmacokinetic profiles of a single dose of 20 mg of a cannabis based medicine extract (CBME) administered on 3 difference areas of the buccal mucosa and to investigate the pharmacokinetics of CBME per oral in healthy male and female volunteers. J Cannabis Ther 3:79-120.
Harvey DJ, Brown NK (1991) Comparative in vitro metabolism of the cannabinoids. Pharmacol Biochem Behav 40:533-540.
Hazekamp A, Bastola K, Rashidi H, Bender J, Verpoorte R (2007) Cannabis tea revisited: a systematic evaluation of the cannabinoid composition of cannabis tea. J Ethnopharmacol 113:85-90.
Hazekamp A, Ruhaak R, Zuurman L, van Gerven J, Verpoorte R (2006) Evaluation of a vaporizing device (Volcano) for the pulmonary administration of tetrahydrocannabinol. J Pharm Sci 95:1308-1317.
Herkenham M (1992) Cannabinoid receptor localization in brain: relationship to motor and reward systems. Ann N Y Acad Sci 654:19-32.
Herkenham M, Lynn AB, Johnson MR, Melvin LS, de Costa BR, Rice KC (1991) Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. J Neurosci 11:563-583.
Hermanson DJ, Marnett LJ (2011) Cannabinoids, endocannabinoids, and cancer. Cancer Metastasis Rev 30:599-612.
Hine ML (1918) The recovery of fields of vision in concussion injuries of the occipital cortex. Br J Ophthalmol 2:12-25.
Huestis MA, Boyd S, Heishman SJ, Preston KL, Bonnet D, Le Fur G, Gorelick DA (2007) Single and multiple doses of rimonabant antagonize acuteeffects of smoked cannabis in male cannabis users. Psychopharmacology 194:505-515.
Huestis MA, Gorelick DA, Heishman SJ, Preston KL, Nelson RA, Moolchan ET, Frank RA (2001) Blockade of effects of smoked marijuana by the CB_1-selective cannabinoid receptor antagonist SR141716. Arch Gen Psychiatry 58:322-328.
Huestis MA, Henningfield JE, Cone EJ (1992) Blood cannabinoids. I. Absorption of THC and formation of 11-OH-THC and THCCOOH during and after smoking marijuana. J Anal Toxicol 16:276-282.
Hunt CA, Jones RT (1980) Tolerance and disposition of tetrahydrocannabinol in man. J Pharmacol Exp Ther 215:35-44.
ICRS (2012) 22nd annual symposium of the international cannabinoid research society.
Ishac EJ, Jiang L, Lake KD, Varga K, Abood ME, Kunos G (1996) Inhibition of exocytotic noradrenaline release by presynaptic cannabinoid CB_1 receptors on peripheral sympathetic nerves. Br J Pharmacol 118:2023-2028.
Jenkins AJ, Oblock J (2008) Phencyclidine and cannabinoids in vitreous humor. Leg Med (Tokyo) 10:201-203.
Jha AP, Ranucci MB, Giuliani NR (2006) Organization of mnemonic and response operations within prefrontal cortex. Brain Res 1097:133-141.
Karler R, Turkanis SA (1987) Different cannabinoids exhibit different pharmacological and toxicological properties. NIDA Res Monogr 79:96-107.
Karschner EL, Darwin WD, Goodwin RS, Wright S, Huestis MA (2011) Plasma Cannabinoid Pharmacokinetics following Controlled Oral (Delta)9-Tetrahydrocannabinol and Oromucosal Cannabis Extract Administration. Clin Chem 57:66-75.
Kerr DM, Downey L, Conboy M, Finn DP, Roche M (2013) Alterations in the endocannabinoid system in the rat valproic acid model of autism. Behav Brain Res 249C:124-132.
Khalili-Mahani N, Zoethout RM, Beckmann CF, Baerends E, de Kam ML, Soeter RP, Dahan A, van Buchem MA, van Gerven JM, Rombouts SA (2011) Effects of morphine and alcohol on functional brain connectivity during "resting state":A placebo-controlled crossover study in healthy young men. Hum Brain Mapp.
Kim W, Doyle ME, Liu Z, Lao Q., Shin YK, Carlson OD, Kim HS, Thomas S, Napora JK, Lee EK, Moaddel R, Wang Y, Maudsley S, Martin B, Kulkarni RN, Egan JM (2011) Cannabinoids inhibit insulin receptor signaling in pancreatic beta-cells. Diabetes 60:1198-1209.
Kipnes MS, Hollander P, Fujioka K, Gantz I, Seck T, Erondu N, Shentu Y, Lu K, Suryawanshi S, Chou M, Johnson-Levonas AO, Heymsfield SB, Shapiro D, Kaufman KD, Amatruda JM (2010) A one-year study to assess the safety and efficacy of the CB_1R inverse agonist taranabant in overweight and obese patients with type 2 diabetes. Diabetes Obes Metab 12:517-531.
Kirilly E, Gonda X, Bagdy G (2012) CB_1 receptor antagonists: new discoveries leading to new perspectives. Acta Physiol (Oxf) 205:41-60.
Klausner HA, Dingell JV (1971) The metabolism and excretion of delta 9-tetrahydrocannabinol in the rat. Life Sci I 10:49-59.
Koechlin E, Basso G, Pietrini P, Panzer S, Grafman J (1999) The role of the anterior prefrontal cortex in human cognition. Nature 399:148-151.
Koethe D, Gerth CW, Neatby MA, Haensel A, Thies M, Schneider U, Emrich HM, Klosterkotter J, Schultze-Lutter F, Leweke FM (2006) Disturbances of visual information processing in early states of psychosis and experimental delta-9-tetrahydrocannabinol altered states of consciousness. Schizophr Res 88:142-150.
Kolmel HW (1988) Pure homonymous hemiachromatopsia. Findings with neuro-ophthalmologic examination and imaging procedures. Eur Arch Psychiatry Neurol Sci 237:237-243.
Laird AR, Fox PM, Eickhoff SB, Turner JA, Ray KL, McKay DR, Glahn DC, Beckmann CF, Smith SM, Fox PT (2011) Behavioral interpretations of intrinsic connectivity networks. J Cogn Neurosci 23:4022-4037.
Lemberger L (1973) Tetrahydrocannabinol metabolism in man. Drug Metab Dispos 1:461-468.
Lemberger L, Crabtree RE, Rowe HM (1972) 11-hydroxy- 9 -tetrahydrocannabinol: pharmacology, disposition, and metabolism of a major metabolite of marihuana in man. Science 177:62-64.
Lemberger L, Martz R, Rodda B, Forney R, Rowe H (1973) Comparative pharmacology of Delta9-tetrahydrocannabinol and its metabolite, 11-OH-Delta9-tetrahydrocannabinol. J Clin Invest 52:2411-2417.
Lemberger L, Silberstein SD, Axelrod J, Kopin IJ (1970) Marihuana: studies on the disposition and metabolism of delta-9-tetrahydrocannabinol in man. Science 170:1320-1322.
Lemberger L, Tamarkin NR, Axelrod J, Kopin IJ (1971) Delta-9-tetrahydrocannabinol: metabolism and disposition in long-term marihuana smokers. Science 173:72-74.
Leuschner JT, Harvey DJ, Bullingham RE, Paton WD (1986) Pharmacokinetics of delta 9-tetrahydrocannabinol in rabbits following single or multiple intravenous doses. Drug Metab Dispos 14:230-238.
Lindgren JE, Ohlsson A, Agurell S, Hollister L, Gillespie H (1981) Clinical effects and plasma levels of delta 9-tetrahydrocannabinol (delta 9-THC) in heavy and light users of cannabis. Psychopharmacology (Berl) 74:208-212.
Lopez-Moreno JA, Echeverry-Alzate V, Buhler KM (2012) The genetic basis of the endocannabinoid system and drug addiction in humans. J Psychopharmacol 26:133-143.
Mackie K, Lai Y, Westenbroek R, Mitchell R (1995) Cannabinoids activate an inwardly rectifying potassium conductance and inhibit Q-type calcium currents in AtT20 cells transfected with rat brain cannabinoid receptor. J Neurosci 15:6552-6561.
Maihofner C, Schlotzer-Schrehardt U, Guhring H, Zeilhofer HU, Naumann GO, Pahl A, Mardin C, Tamm ER, Brune K (2001) Expression of cyclooxygenase-1 and -2 in normal and glaucomatous human eyes. Invest Ophthalmol Vis

Sci 42:2616-2624.
Martinez J, Fabre D, Kanamaluru V (2007) Population pharmacokinetics of rimonabant in obesity.
Martinez-Org, Fernandez-Lopez D, Lizasoain I, Romero J (2007) The seek of neuroprotection: introducing cannabinoids. Recent Pat CNS Drug Discov 2:131-139.
Mathew RJ, Wilson WH, Chiu NY, Turkington TG, DeGrado TR, Coleman RE (1999) Regional cerebral blood flow and depersonalization after tetrahydrocannabinol administration. Acta Psychiatr Scand 100:67-75.
Mathew RJ, Wilson WH, Turkington TG, Coleman RE (1998) Cerebellar activity and disturbed time sense after THC. Brain Res 797:183-189.
Mathew RJ, Wilson WH, Turkington TG, Hawk TC, Coleman RE, DeGrado TR, Provenzale J (2002) Time course of tetrahydrocannabinol-induced changes in regional cerebral blood flow measured with positron emission tomography. Psychiatry Res 116:173-185.
Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI (1990b) Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346:561-564.
Mattes RD, Engelman K, Shaw LM, Elsohly MA (1994) Cannabinoids and appetite stimulation. Pharmacol Biochem Behav 49:187-195.
McDonald J, Schleifer L, Richards JB, de WH (2003) Effects of THC on behavioral measures of impulsivity in humans. Neuropsychopharmacology 28:1356-1365.
McPartland JM (2004) Phylogenomic and chemotaxonomic analysis of the endocannabinoid system. Brain Res Brain Res Rev 45:18-29.
Mechoulam R (1986) The pharmacohistory of Cannabis sativa. In: Cannabinoids as therapeutic agents (Boca Raton (FL), ed), pp 1-19. CRC Press.
Mechoulam R, Gaoni J (1965) A total synthesis of dl-delta-1-tetrahydrocannabinol, the active constituent of hashish. J Am Chem Soc 87:3273-3275.
Melamede R (2005) Harm reduction-the cannabis paradox. Harm Reduct J 2:17.
Mennes M, Kelly C, Zuo XN, Di MA, Biswal BB, Castellanos FX, Milham MP (2010) Inter-individual differences in resting-state functional connectivity predict task-induced BOLD activity. Neuroimage 50:1690-1701.
Merck & Co. I (2008) Merck discontinues development of investigational medicine taranabant for obesity.
Micale V, Di M, V, Sulcova A, Wotjak CT, Drago F (2013) Endocannabinoid system and mood disorders: priming a target for new therapies. Pharmacol Ther 138:18-37.
Miller EK, Cohen JD (2001) An integrative theory of prefrontal cortex function. Annu Rev Neurosci 24:167-202.
Milman G, Barnes AJ, Schwope DM, Schwilke EW, Darwin WD, Goodwin RS, Kelly DL, Gorelick DA, Huestis MA (2010) Disposition of cannabinoids in oral fluid after controlled around-the-clock oral THC administration. Clin Chem 56:1261-1269.
Milman G, Schwope DM, Schwilke EW, Darwin WD, Kelly DL, Goodwin RS, Gorelick DA, Huestis MA (2011) Oral Fluid and Plasma Cannabinoid Ratios after Around-the-Clock Controlled Oral (Delta)9-Tetrahydrocannabinol Administration. Clin Chem.
Mitjans M, Serretti A, Fabbri C, Gasto C, Catalan R, Fananas L, Arias B (2013) Screening genetic variability at the CNR1 gene in both major depression etiology and clinical response to citalopram treatment. Psychopharmacology (Berl) 227:509-519.
Montecucco F, Di Marzo V (2012) At the heart of the matter: the endocannabinoid system in cardiovascular function and dysfunction. Trends Pharmacol Sci 33:331-340.
Morrison MF, Ceesay P, Gantz I, Kaufman KD, Lines CR (2010) Randomized, controlled, double-blind trial of taranabant for smoking cessation. Psychopharmacology (Berl) 209:245-253.
Morrison PD, Zois V, McKeown DA, Lee TD, Holt DW, Powell JF, Kapur S, Murray RM (2009) The acute effects of synthetic intravenous Delta9-tetrahydrocannabinol on psychosis, mood and cognitive functioning. Psychol Med 39:1607-1616.
Munro S, Thomas KL, Abu-Shaar M (1993) Molecular characterization of a peripheral receptor for cannabinoids. Nature 365:61-65.

Naef M, Curatolo M, Petersen-Felix S, Arendt-Nielsen L, Zbinden A, Brenneisen R (2003) The analgesic effect of oral delta-9-tetrahydrocannabinol (THC), morphine, and a THC-morphine combination in healthy subjects under experimental pain conditions. Pain 105:79-88.
Naef M, Russmann S, Petersen-Felix S, Brenneisen R (2004) Development and pharmacokinetic characterization of pulmonal and intravenous delta-9-tetrahydrocannabinol (THC) in humans. J Pharm Sci 93:1176-1184.
Nakazawa K, Costa E (1971) Metabolism of delta 9 -tetrahydrocannabinol by lung and liver homogenates of rats treated with methylcholanthrene. Nature 234:48-49.
Nichols TE, Holmes AP (2002) Nonparametric permutation tests for functional neuroimaging: a primer with examples. Hum Brain Mapp 15:1-25.
Nogueiras R, Veyrat-Durebex C, Suchanek PM, Klein M, Tschop J, Caldwell C, Woods SC, Wittmann G, Watanabe M, Liposits Z, Fekete C, Reizes O, Rohner-Jeanrenaud F, Tschop MH (2008) Peripheral, but not central, CB_1 antagonism provides food intake-independent metabolic benefits in diet-induced obese rats. Diabetes 57:2977-2991.
NONMEM project group (1992) NONMEM users guide.
Nucci C, Bari M, Spano A, Corasaniti M, Bagetta G, Maccarrone M, Morrone LA (2008) Potential roles of (endo)cannabinoids in the treatment of glaucoma: from intraocular pressure control to neuroprotection. Prog Brain Res 173:451-464.
Nunez E, Benito C, Pazos MR, Barbachano A, Fajardo O, Gonzalez S, Tolon RM, Romero J (2004) Cannabinoid CB_2 receptors are expressed by perivascular microglial cells in the human brain: an immunohistochemical study. Synapse 53:208-213.
Nyberg L, Kim AS, Habib R, Levine B, Tulving E (2010) Consciousness of subjective time in the brain. Proc Natl Acad Sci U S A 107:22356-22359.
Ohlsson A, Lindgren JE, Wahlen A, Agurell S, Hollister LE, Gillespie HK (1980) Plasma delta-9 tetrahydrocannabinol concentrations and clinical effects after oral and intravenous administration and smoking. Clin Pharmacol Ther 28:409-416.
Ohlsson A, Lindgren JE, Wahlen A, Agurell S, Hollister LE, Gillespie HK (1982) Single dose kinetics of deuterium labelled delta 1-tetrahydrocannabinol in heavy and light cannabis users. Biomed Mass Spectrom 9:6-10.
Orgado JM, Fernandez-Ruiz J, Romero J (2009) The endocannabinoid system in neuropathological states. Int Rev Psychiatry 21:172-180.
Orgado JM, Fernandez-Ruiz J, Romero J (2009) The endocannabinoid system in neuropathological states. Int Rev Psychiatry 21:172-180.
Orr LE, McKernan JF, Bloome B (1980) Antiemetic effect of tetrahydrocannabinol. Compared with placebo and prochlorperazine in chemotherapy-associated nausea and emesis. Arch Intern Med 140:1431-1433.
Osei-Hyiaman D, Liu J, Zhou L, Godlewski G, Harvey-White J, Jeong WI, Batkai S, Marsicano G, Lutz B, Buettner C, Kunos G (2008) Hepatic CB_1 receptor is required for development of diet-induced steatosis, dyslipidemia, and insulin and leptin resistance in mice. J Clin Invest 118:3160-3169.
Pan C, Yoo HJ, Ho LT (2011) Perspectives of CB_1 Antagonist in Treatment of Obesity: Experience of R10-Asia. J Obes 2011:957268.
Pava MJ, Woodward JJ (2012) A review of the interactions between alcohol and the endocannabinoid system: implications for alcohol dependence and future directions for research. Alcohol 46:185-204.
Perez-Reyes M, Wall ME (1982) Presence of delta9-tetrahydrocannabinol in human milk. N Engl J Med 307:819-820.
Pertwee RG (1997) Pharmacology of cannabinoid CB_1 and CB_2 receptors. Pharmacol Ther 74:129-180.
Pertwee RG (2005) Pharmacological actions of cannabinoids. Handb Exp Pharmacol1-51.
Pertwee RG (2007) Cannabinoids and multiple sclerosis. Mol Neurobiol 36:45-59.
Pertwee RG (2008) The diverse CB_1 and CB_2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. Br J Pharmacol 153:199-215.

Pertwee RG (2009) Emerging strategies for exploiting cannabinoid receptor agonists as medicines. Br J Pharmacol 156:397-411.
Pijlman FT, Rigter SM, Hoek J, Goldschmidt HM, Niesink RJ (2005) Strong increase in total delta-THC in cannabis preparations sold in Dutch coffee shops. Addict Biol 10:171-180.
Plasse TF, Gorter RW, Krasnow SH, Lane M, Shepard KV, Wadleigh RG (1991) Recent clinical experience with dronabinol. Pharmacol Biochem Behav 40:695-700.
Post TM, Freijer JI, Ploeger BA, Danhof M (2008) Extensions to the visual predictive check to facilitate model performance evaluation. J Pharmacokinet Pharmacodyn 35:185-202.
Powanda MC, Beisel WR (2003) Metabolic effects of infection on protein and energy status. J Nutr 133:322S-327S.
Prather PL, Martin NA, Breivogel CS, Childers SR (2000) Activation of cannabinoid receptors in rat brain by WIN 55212-2 produces coupling to multiple G protein alpha-subunits with different potencies. Mol Pharmacol 57:1000-1010.
Proietto J, Rissanen A, Harp JB, Erondu N, Yu Q, Suryawanshi S, Jones ME, Johnson-Levonas AO, Heymsfield SB, Kaufman KD, Amatruda JM (2010) A clinical trial assessing the safety and efficacy of the CB_1R inverse agonist taranabant in obese and overweight patients: low-dose study. Int J Obes (Lond) 34:1243-1254.
Quigley M, Cordes D, Turski P, Moritz C, Haughton V, Seth R, Meyerand ME (2003) Role of the corpus callosum in functional connectivity. AJNR Am J Neuroradiol 24:208-212.
Ramaekers JG, Kauert G, Theunissen EL, Toennes SW, Moeller MR (2009) Neurocognitive performance during acute THC intoxication in heavy and occasional cannabis users. J Psychopharmacol 23:266-277.
Ranganathan M, Braley G, Pittman B, Cooper T, Perry E, Krystal J, D'Souza DC (2009) The effects of cannabinoids on serum cortisol and prolactin in humans. Psychopharmacology (Berl) 203:737-744.
Rani SD, Burston JJ, Woodhams SG, Chapman V (2012) Dynamic changes to the endocannabinoid system in models of chronic pain. Philos Trans R Soc Lond B Biol Sci 367:3300-3311.
Ravinet TC, Arnone M, Delgorge C, Gonalons N, Keane P, Maffrand JP, Soubrie P (2003) Anti-obesity effect of SR141716, a CB_1 receptor antagonist, in diet-induced obese mice. Am J Physiol Regul Integr Comp Physiol 284:R345-R353.
Rey AA, Purrio M, Viveros MP, Lutz B (2012) Biphasic effects of cannabinoids in anxiety responses: CB_1 and GABA (B) receptors in the balance of GABAergic and glutamatergic neurotransmission. Neuropsychopharmacology 37:2624-2634.
Richardson D, Pearson RG, Kurian N, Latif ML, Garle MJ, Barrett DA, Kendall DA, Scammell BE, Reeve AJ, Chapman V (2008) Characterisation of the cannabinoid receptor system in synovial tissue and fluid in patients with osteoarthritis and rheumatoid arthritis. Arthritis Res Ther 10:R43.
Rinaldi-Carmona M, Barth F, Heaulme M, Shire D, Calandra B, Congy C, Martinez S, Maruani J, Neliat G, Caput D, . (1994) SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. FEBS Lett 350:240-244.
Rodriguez de Fonseca F, Roberts AJ, Bilbao A, Koob GF, Navarro M (1999) Cannabinoid receptor antagonist SR141716A decreases operant ethanol self administration in rats exposed to ethanol-vapor chambers. Zhongguo Yao Li Xue Bao 20:1109-1114.
Romero J, Hillard CJ, Calero M, Rabano A (2002) Fatty acid amide hydrolase localization in the human central nervous system: an immunohistochemical study. Brain Res Mol Brain Res 100:85-93.
Rose JE, McClernon FJ, Froeliger B, Behm FM, Preud'homme X, Krystal AD (2011) Repetitive Transcranial Magnetic Stimulation of the Superior Frontal Gyrus Modulates Craving for Cigarettes. Biol Psychiatry.
Ryrfeldt A, Ramsay CH, Nilsson IM, Widman M, Agurell S (1973) Whole-body autoradiography of 1-tetrahydrocannabinol and 1(6)-tetrahydrocannabinol in mouse. Pharmacokinetic aspects of 1-tetrahydrocannabinol and its metabolites. Acta Pharm Suec 10:13-28.
Sagredo O, Garcia-Arencibia M, de LE, Finetti S, Decio A, Fernandez-Ruiz J (2007) Cannabinoids and neuroprotection in basal ganglia disorders. Mol Neurobiol 36:82-91.
Sallan SE, Zinberg NE, Frei E, III (1975) Antiemetic effect of delta-9-tetrahydrocannabinol in patients receiving cancer chemotherapy. N Engl J Med 293:795-797.
sanofi (2008) Summary of Product Characteristics Rimonabant.
sanofi-aventis (2006) A clinical study to investigate the tolerability and pharmacokinetic parameters of SR147778 after repeated oral administrations (soft gelatin capsules) in young healthy male subjects; CSRCO-TDR5736-EN-E^01 - Data on file.
sanofi-aventis (2006) Determination of the Cytochrome P450 (CYP) Isoforms Involved in the Oxidative Metabolism of SR147778 in vitro, CMPKCO-MIH0135-EN-E^01 - Data on file.
sanofi-aventis (2006c) Excretion balance, pharmacokinetics, metabolites profiling and identification after single oral administration of SR147778 using [14C]-labeled compound in healthy male subjects. Open, non-randomized and single center study, CSRCO-BEX4499-EN-E^01 - Data on file.
Schatz AR, Lee M, Condie RB, Pulaski JT, Kaminski NE (1997) Cannabinoid receptors CB_1 and CB_2: a characterization of expression and adenylate cyclase modulation within the immune system. Toxicol Appl Pharmacol 142:278-287.
Schwilke EW, Schwope DM, Karschner EL, Lowe RH, Darwin WD, Kelly DL, Goodwin RS, Gorelick DA, Huestis MA (2009) Delta9-Tetrahydrocannbinol (THC), 11-Hydroxy-THC, and 11-NOR-9-CARBOXY-THC Plasma Pharmacokinetics during and after Continuous High-Dose oral THC. Clinical Chemistry 55:2180-2189.
Silvestri C, Di Marzo V (2013) The endocannabinoid system in energy homeostasis and the etiopathology of metabolic disorders. Cell Metab 17:475-490.
Smith SM, Fox PT, Miller KL, Glahn DC, Fox PM, Mackay CE, Filippini N, Watkins KE, Toro R, Laird AR, Beckmann CF (2009) Correspondence of the brain's functional architecture during activation and rest. Proc Natl Acad Sci U S A 106:13040-13045.
Smith SM, Nichols TE (2009) Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. Neuroimage 44:83-98.
Snyder PJ, Bednar MM, Cromer JR, Maruff P (2005) Reversal of scopolamine-induced deficits with a single dose of donepezil, an acetylcholinesterase inhibitor. Alzheimers Dement 1:126-135.
Solvay Pharmaceuticals (2004) Summary of Product Characteristics Marinol, http://www.fda.gov/ohrms/dockets/dockets/05n0479/05N-0479-emc0004-04.pdf.
Speth H (2004) A Linux cluster for population pharmacokinetic analyses. Int J Clin Pharmacol Ther 42:189-190.
Stanton P, Bramham C, Sharfman HE (2005) Synaptic Plasticity and Transsynaptic Signaling. Springer.
Stokes PR, Egerton A, Watson B, Reid A, Breen G, Lingford-Hughes A, Nutt DJ, Mehta MA (2010) Significant decreases in frontal and temporal [11C]-raclopride binding after THC challenge. Neuroimage 52:1521-1527.
Stoodley CJ, Schmahmann JD (2009) Functional topography in the human cerebellum: a meta-analysis of neuroimaging studies. Neuroimage 44:489-501.
Strougo A, Zuurman L, Roy C, Pinquier JL, van Gerven JM, Cohen AF, Schoemaker RC (2008) Modelling of the concentration-effect relationship of THC on central nervous system parameters and heart rate - insight into its mechanisms of action and a tool for clinical research and development of cannabinoids. J Psychopharmacol 22:717-726.
Su JY, Vo AC (2007) 2-Arachidonylglyceryl ether and abnormal cannabidiol-induced vascular smooth muscle relaxation in rabbit pulmonary arteries via receptor-pertussis toxin sensitive G proteins-ERK1/2 signaling. Eur J Pharmacol 559:189-195.
Sugiura T, Kondo S, Sukagawa A, Nakane S, Shinoda A, Itoh K, Yamashita A, Waku K (1995) 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. Biochem Biophys Res Commun 215:89-97.
Tam J, Cinar R, Liu J, Godlewski G, Wesley D, Jourdan T, Szanda G, Mukhopadhyay B, Chedester L, Liow JS, Innis RB, Cheng K, Rice KC,

Deschamps JR, Chorvat RJ, McElroy JF, Kunos G (2012) Peripheral cannabinoid-1 receptor inverse agonism reduces obesity by reversing leptin resistance. Cell Metab 16:167-179.
The European Medicines Agency (EMEA) (2008) Questions and answers on the recommendation to suspend the marketing authorisation of Acomplia (rimonabant).
Thomas BF, Compton DR, Martin BR (1990) Characterization of the lipophilicity of natural and synthetic analogs of delta 9-tetrahydrocannabinol and its relationship to pharmacological potency. J Pharmacol Exp Ther 255:624-630.
Tinklenberg JR, Kopell BS, Melges FT, Hollister LE (1972) Marihuana and alcohol, Time production and memory functions. Arch Gen Psychiatry 27:812-815.
Tonstad S, Aubin HJ (2012) Efficacy of a dose range of surinabant, a cannabinoid receptor blocker, for smoking cessation: a randomized controlled clinical trial. J Psychopharmacol 26:1003-1009.
Turpault S, Kanamaluru V, Lockwood GF, Bonnet D, Newton J (2006) *Rimonabant pharmacokinetics in healthy and obese subjects*. pp 50.
Twitchell W, Brown S, Mackie K (1997) Cannabinoids inhibit N- and P/Q-type calcium channels in cultured rat hippocampal neurons. J Neurophysiol 78:43-50.
Ujike H, Takaki M, Nakata K, Tanaka Y, Takeda T, Kodama M, Fujiwara Y, Sakai A, Kuroda S (2002) CNR1, central cannabinoid receptor gene, associated with susceptibility to hebephrenic schizophrenia. Mol Psychiatry 7:515-518.
Ungerleider JT, Andyrsiak T, Fairbanks L, Ellison GW, Myers LW (1987) Delta-9-THC in the treatment of spasticity associated with multiple sclerosis. Adv Alcohol Subst Abuse 7:39-50.
Valeant Pharmaceuticals International (2006) Cesamet Package Insert.
Van Gaal LF, Rissanen AM, Scheen AJ, Ziegler O, Rossner S (2005) Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study. Lancet 365:1389-1397.
Van Gaal LF, Scheen AJ, Rissanen AM, Rossner S, Hanotin C, Ziegler O (2008) Long-term effect of CB_1 blockade with rimonabant on cardiometabolic risk factors: two year results from the RIO-Europe Study. Eur Heart J 29:1761-1771.
van Hell HH, Bossong MG, Jager G, Kristo G, van Osch MJ, Zelaya F, Kahn RS, Ramsey NF (2011) Evidence for involvement of the insula in the psychotropic effects of THC in humans: a double-blind, randomized pharmacological MRI study. Int J Neuropsychopharmacol1-12.
Venkatraman V, Rosati AG, Taren AA, Huettel SA (2009) Resolving response, decision, and strategic control: evidence for a functional topography in dorsomedial prefrontal cortex. J Neurosci 29:13158-13164.
Wall ME, Perez-Reyes M (1981) The metabolism of delta 9-tetrahydrocannabinol and related cannabinoids in man. J Clin Pharmacol 21:178S-189S.
Wall ME, Sadler BM, Brine D, Taylor H, Perez-Reyes M (1983) Metabolism, disposition, and kinetics of delta-9-tetrahydrocannabinol in men and women. Clin Pharmacol Ther 34:352-363.
Watanabe K, Yamaori S, Funahashi T, Kimura T, Yamamoto I (2007) Cytochrome P450 enzymes involved in the metabolism of tetrahydrocannabinols and cannabinol by human hepatic microsomes. Life Sci 80:1415-1419.
Wathion N (2009) Public statement on Acomplia (rimonabant): Withdrawal of the marketing authorisation in the European Union.
Wenger T, Fernandez-Ruiz JJ, Ramos JA (1999) Immunocytochemical demonstration of CB_1 cannabinoid receptors in the anterior lobe of the pituitary gland. J Neuroendocrinol 11:873-878.
Widman M, Nordqvist M, Dollery CT, Briant RH (1975) Metabolism of delta1-tetrahydrocannabinol by the isolated perfused dog lung. Comparison with in vitro liver metabolism. J Pharm Pharmacol 27:842-848.
Wilson RS, May EL (1975) Analgesic properties of the tetrahydrocannabinols, their metabolites, and analogs. J Med Chem 18:700-703.
Winton-Brown TT, Allen P, Bhattacharyya S, Borgwardt SJ, Fusar-Poli P, Crippa JA, Seal ML, Martin-Santos R, Ffytche D, Zuardi AW, Atakan Z, McGuire PK (2011) Modulation of auditory and visual processing by delta-9-tetrahydrocannabinol and cannabidiol: an FMRI study. Neuropsychopharmacology 36:1340-1348.
Wise RG, Preston C (2010) What is the value of human fMRI in CNS drug development? Drug Discov Today 15:973-980.
World Health Organisation (2013) Management of substance abuse - Cannabis.
World Health Organization (2011) Obesity and overweight - Fact sheet.
Yamamoto I, Watanabe K, Narimatsu S, Yoshimura H (1995) Recent advances in the metabolism of cannabinoids. Int J Biochem Cell Biol 27:741-746.
Zajicek J, Fox P, Sanders H, Wright D, Vickery J, Nunn A, Thompson A (2003) Cannabinoids for treatment of spasticity and other symptoms related to multiple sclerosis (CAMS study): multicentre randomised placebo-controlled trial. Lancet 362:1517-1526.
Zajicek JP, Sanders HP, Wright DE, Vickery PJ, Ingram WM, Reilly SM, Nunn AJ, Teare LJ, Fox PJ, Thompson AJ (2005) Cannabinoids in multiple sclerosis (CAMS) study: safety and efficacy data for 12 months follow up. J Neurol Neurosurg Psychiatry 76:1664-1669.
Zhang L, Beal SL, Sheiner LB (2003) Simultaneous vs. sequential analysis for population PK/PD data I: best-case performance. J Pharmacokinet Pharmacodyn 30:387-404.
Zhang Y, Brady M, Smith S (2001) Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm. CAMS Trans Med Imaging 20:45-57.
Zogopoulos P, Vasileiou I, Patsouris E, Theocharis SE (2013) The role of endocannabinoids in pain modulation. Fundam Clin Pharmacol 27:64-80.
Zuurman L, Ippel AE, Moin E, van Gerven JMA (2009) Biomarkers for the effects of cannabis and THC in healthy volunteers. Br J Clin Pharmacol 67:5-21.
Zuurman L, Roy C, Schoemaker R, Amatsaleh A, Guimaeres L, Pinquier JL, Cohen AF, van Gerven JMA (2010) Inhibition of THC-induced effects on the central nervous system and heart rate by a novel CB_1 receptor antagonist AVE1625.2010 Mar; 24 (3):363-71. Epub 2008 Sep 18. J Psychopharmacol 24:363-371.
Zuurman L, Roy C, Schoemaker R, Hazekamp A, den Hartigh J, Bender J, Verpoorte R, Pinquier J, Cohen A, van Gerven J (2008) Effect of intrapulmonary tetrahydrocannabinol administration in humans. J Psychopharmacol 22:707-716.

BOX – THC PHARMACOKINETICS

As the most well known constituent of cannabis, THC is often used as a cannabinoid (partial) agonist in pharmacological studies (Pijlman et al., 2005). Also, THC is only one of the few cannabinoid agonists available for clinical use. Therefore, we chose THC as the agonist of preference in this thesis. THC is very lipophilic and its pharmacokinetics are complex. In this box, we give an overview of the most important aspects of THC pharmacokinetics in clinical trials.

ADMINISTRATION

INTRAVENOUS – Intravenous THC administration is a very uncommon and merely experimental administration route that was only applied in a limited number of clinical experiments (Bhattacharyya et al., 2010; Carbuto et al., 2011; Lemberger et al., 1973). Intravenous administration has a bioavailability of 100%, and it thereby allows assessment of absolute bioavailablity when compared to other formulations.

INHALATION – One of the most common administration routes of THC is via smoking cannabis. This has several methodological disadvantages in addition to the problems of smoking. Cannabis is usually smoked in combination with tobacco, resulting in the inhalation of a varied mixture of (noxious) compounds which could influence the THC-induced effects. When smoked as pure cannabis, the mixture of cannabinoids could as well influence the THC-induced effects. For example, cannabidiol (CBD) abolishes the well-known THC-induced 'feeling high' effect (Dalton et al., 1976). Moreover, the lack of dosage control makes smoking less suitable for clinical research; the exact amount of THC that is inhaled cannot be controlled due to partial combustion of the THC at times when the cigarette smoke is not inhaled. Moreover, efficiency of smoking is dependent on the experience of cannabis users.

To avoid these disadvantages, a THC inhalation method using a vaporiser was developed at the Centre for Human Drug Research (CHDR, Leiden). Pure THC diluted in 100% ethanol is applied on the Volcano® device. Hot air from the vaporiser vaporises the THC dilution into a balloon that is attached to the vaporiser. The balloon is closed with a valve that opens when the content is inhaled. Using a paced puffing protocol, volunteers inhale an exactly known amount of THC from vapour in the balloon (Hazekamp et al., 2006; Zuurman et al., 2008). This inhalation method is used in several chapters of this thesis. An overview of the average loss of THC during the THC administration using the vaporiser, and the quantity of THC that is inhaled is given in Figure 5.

ORAL – Oral THC administration is another very commonly used method for both recreational and clinical usage. Cannabis could be processed into baked products, such as biscuits and cakes, or decocted and served as 'tea'. The disadvantages of oral administration are the variation of cannabinoid composition and the late onset and unpredictable magnitude of effects. The cannabinoid composition is dependent on way the cannabis is processed. For example, due to the lipophilic character of some cannabinoids such as THC, the composition of cannabinoids in tea shifts to relatively lower concentrations of THC and higher concentrations of THC-acid (THCA) (Hazekamp et al., 2007). Also, the temperature during processing is of relevance for cannabinoid composition due to conversion of cannabinoid acids (Hazekamp et al., 2007).

To avoid the problems of variable cannabinoid composition, several oral formulations of cannabis derived medicines (CDM) and THC have been developed containing predefined amounts of cannabinoids. These formulations include Marinol®, a capsule with a synthetic form of THC dissolved in sesame oil, and Cesamet®, a capsule containing THC analogue nabilone.

OROMUCOSAL AND SUBLINGUAL – Sativex® is a CDM that, besides its major compounds THC and cannabidiol (CBD), contains a mixture of several other cannabinoids (presented during the 20th and 21st Symposiums of the International Cannabinoid Research Society, 2010 and 2011). At present, Sativex® is the only registered CDM that is applied for oromucosal and sublingual administration. Sativex® is administered by spraying into the oral cavity.

OTHER – Other THC and CDM administration routes such as dermal and rectal have been applied as well in clinical trials (Mattes et al., 1994; Callaway et al., 2005). These administration routes are not applied for currently registered cannabinoid medicines, and are beyond the scope of this thesis.

ABSORPTION

Plasma concentration profiles of THC for different administration routes are given in Figure 6. THC profile after inhalation of pure THC is comparable to the profile after intravenous administration, with an instant time to peak plasma concentration (T_{MAX}) within 3 min and a steep decline of plasma concentration (Ohlsson et al., 1980). Although the oral administration route is more practical, THC absorption is less favourable compared to intravenous or intrapulmonary administration routes. The oral T_{MAX} lies between 60 to 90 min after eating of a 20 mg THC-containing chocolate cookie (Ohlsson et al., 1980) and between 2.8 to 3 h for 5-20 mg Marinol® (Schwilke et al., 2009; Karschner et al., 2011). An oromucosal THC-CBD dosage, administered as a spray, gives a relatively late THC T_{MAX} of 4 h (Karschner et al., 2011).

Previous pharmacokinetic studies reported that bioavailability of THC inhalation was between 10 and 28.7% on average. Frequent cannabis users had higher THC plasma levels compared to infrequent users after smoking (Ohlsson et al., 1982; Lindgren et al., 1981). However, a study by Ohlsson et al. (1982) found that intravenous THC administration resulted in only small plasma concentration differences between infrequent and frequent users. This indicates that a substantial amount of THC from cigarettes is not absorbed and that the amount of THC intake is relatively variable. However, the intrapulmonary administration route has advantages over intravenous administration with regards to familiarity and its non-invasive character. Therefore, the Centre for Human Drug Research developed a standardised THC inhalation protocol that was reported by Zuurman et al. This protocol was applied for studies with repeated measurements for the assessment of concentration-effect relationship modelling, and in challenge tests (Zuurman et al., 2008; Strougo et al., 2008; Zuurman et al., 2008).

Oral bioavailability is relatively small, varying on average from 6 to 20% (Ohlsson et al., 1980; Wall et al., 1983). The relative bioavailability of oral THC was 87.2% when compared to sublingual THC+CBD, and 93.9% when compared to buccal THC+CBD administration (Guy and Robson, 2003). A study with oromucosal THC+CBD administration (both sublingual and buccal) found a 92.6 to 98.8% bioavailability of oral THC (Karschner et al., 2011).

DISTRIBUTION

Although extensive data are available from studies in animals, only little is reported on the distribution of cannabinoids in humans. Due to its lipophilic nature, THC is distributed to peripheral tissues, such as lungs, adipose tissue and kidneys. This happens very quickly after central absorption, as can be seen by the steep concentration decline in Figure 6 (Lemberger et al., 1970; Ryrfeldt et al., 1973; Brunet et al., 2010). Gronewold and Skopp (2011) investigated distribution of THC and its metabolites in five human post mortem cases (Gronewold and Skopp, 2011). Bile contained high concentrations of THC and metabolites and muscle tissue also contained high concentrations of THC, although metabolites could hardly be detected. In the liver, THC had low concentrations or was even undetectable, while 11-NOR-9-CARBOXY-THC glucuronide (THC-

COOglu) had appreciably concentrations in both liver and kidney. Furthermore, THC was present in lung specimens. Metabolites were largely absent in brain tissue, with 11-HYDROXY-THC (11-OH-THC) being completely absent. Gronewold and Skopp (2011) suggested that muscle tissue serves as a matrix for detection of cannabis use, and that retention from muscle tissue, in addition to retention in fat, could be a source of the prolonged elimination period of cannabinoids (Gronewold and Skopp, 2011). Findings from bile supported extensive enterohepatic recirculation of THC-COOglu (Gronewold and Skopp, 2011). The role of enterohepatic circulation in the distribution pattern of THC has also been described in animal studies (Garrett and Hunt, 1977; Klausner and Dingell, 1971). In daily cannabis users, a previous study on cannabinoids in oral fluid described the abundant presence of THC-COOH in 98.2% of the samples (Milman et al., 2010). Conversely, 11-OH-THC was not detected in any sample, whereas THC was present in only 20.7% of plasma samples. Previous studies also described the distribution and determination of THC in detail in vitreous humour, oral fluid, breast milk and foetuses (Jenkins and Oblock, 2008; Milman et al., 2011; Perez-Reyes and Wall, 1982). These aspects are beyond the scope of this thesis, and are therefore not described.

METABOLISM

In humans, THC is predominantly metabolised by hydroxylation and oxidation via cytochrome P450 (CYP) enzymes (Yamamoto et al., 1995). CYP2C9 and to a lesser extend CYP2C19 play the major roles in humans (Watanabe et al., 2007). Metabolism mainly takes place in the liver, and to lesser extend in the heart and lungs, as reported from animal studies (Nakazawa and Costa, 1971; Widman et al., 1975). Many pre-clinical studies reported on metabolic rates, but extrapolation of the results is limited by interspecies differences that could be explained by differences in CYP profiles (Harvey and Brown, 1991). The major metabolism pathway is visualised in Figure 2. The ratios at which the metabolites occur after human administration, is largely dependent on the administration route.

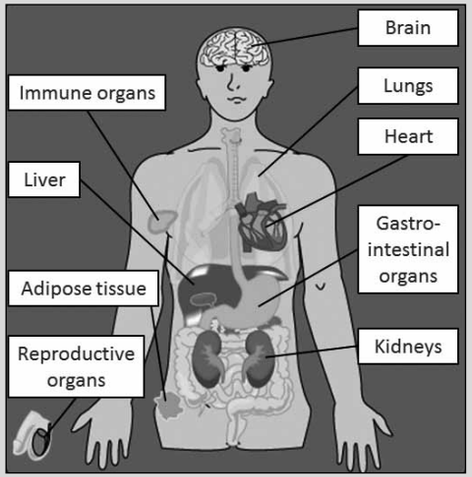
ELIMINATION

After reaching the maximum concentration (C_{MAX}) for THC inhalation and right after intravenous administration, THC plasma concentration has a steep decline until the concentration reaches a second phase, resembling an equilibrium (Figure 6). This equilibrium occurs between approximately 20 minutes and 6 hours after THC administration. After 6 hours a third phase is reached in which the plasma concentration has a flatter slope compared to the second phase. The exact course of elimination phases in humans is unknown, but preclinical studies reported up to 6 phases (Leuschner et al., 1986).

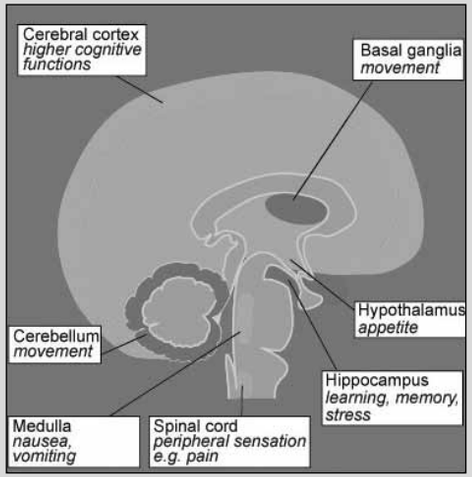
The steep decline in the first phase, which could be attributed by a combination of rapid distribution and metabolism, has a half-life (initial half life or $t_{1/2}^{INIT}$) of 30 min (Lemberger et al., 1970). In the second and third phase, equilibriums between plasma and tissue are reached (Chiang and Rapaka, 1987; Lemberger et al., 1970). The terminal plasma $t_{1/2}^{TERM}$ was calculated up to 57 hours (Lemberger et al., 1971). It should be noted that the actual $t_{1/2}$ calculation is difficult and is limited by difficulties in the quantitative analysis of very low plasma concentrations that are found in phase 3. The clearance of THC in the third phase is between 0.0033 and 0.06 L/h, while the maximum clearance at t = 100 min was reported to be 1.2 L/h (Ohlsson et al., 1982; Wall et al., 1983; Hunt and Jones, 1980). The slow elimination of THC from the plasma could be explained by redistribution from peripheral tissues, such as the adipose tissue, into the blood compartment.

About 15-30% of THC is excreted in urine, mainly as acid metabolites with less than 0.05% of unchanged THC. About 30-65% is excreted in faeces, less than 5% of an oral dose as unchanged drug (Lemberger et al., 1970; Hunt and Jones, 1980; Wall et al., 1983). Most of the THC metabolites in urine were excreted as polar acidic metabolites during day 1.

FIGURE 1 Distribution of cannabinoid receptors in the body and brain. CB_1 receptors are widely distributed in areas related to metabolism and energy storage, such as the adipose tissue, digestive tract, liver, brain, but also in the kidney, lungs and reproductive organs (1a). CB_2 receptors are mainly present in the immune system, such as tissues of the spleen, tonsils, and thymus gland, and immune cells including glia cells in the brain, monocytes, macrophages, B-cells and T-cells and hematopoetic stem cells (1a). **FIGURE 1B** shows the location of CB_1 receptors in the brain in more detail. The CB_1 receptor is predominantly found in the hypothalamus (associated with appetite regulation), hippocampus (associated with memory and stress), amygdala (associated with emotion), basal ganglia and cerebellum (associated with coordination and movement), medulla (associated with basal functions, including vomiting), spinal cord (associated with sensations, including pain) and in the cortex (associated with higher cognitive functions).

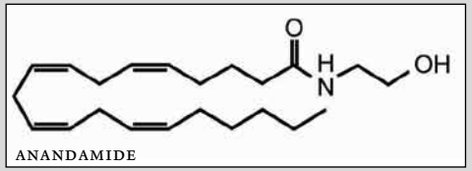


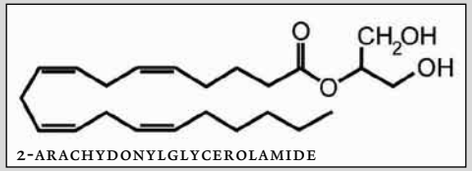
A *(see inside cover for this figure in colour)*



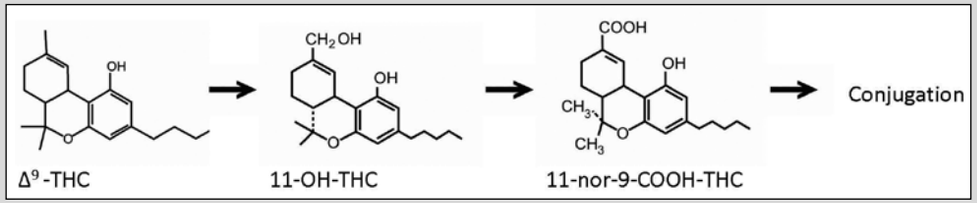
B *(see inside cover for this figure in colour)*

FIGURE 2 Structural formulas of the endocannabinoids anandamide and 2-arachydonylglycerolamide (2a), and the metabolic pathway of the exogenous cannabinoid Δ^9-tetrahydrocannabinol that is metabolised to 11-nor-9-carboxy-THC glucuronide, a water soluble congener which can be more easily excreted by the body (2b).





A



B

FIGURE 3 Complexity of intracellular CB_1 receptor signalling. As for G protein coupled receptors in general, the CB_1 receptor has the ability to activate multiple G proteins. Consequently, different functions are regulated by a variety of pathways. For example, cell survival and cell death are regulated by the MAPK cascades, whereas ion currents are directly involved in the process of neurotransmitter release. The triggering of the variety of intracellular pathways and thereby functional responses elicited by cannabinoid receptors is dependent on several factors, including the type of cells or tissue targeted, the type of ligand and the duration of receptor activation (Sanchez et al., 2001; Galve-Roperh et al., 2000). For example, successive activation might lead to a biphasic concentration-response profile or to tolerance by a molecular switch between G proteins (Asimaki and Mangoura, 2011; Sulcova et al., 1998; Paquette et al., 2007).

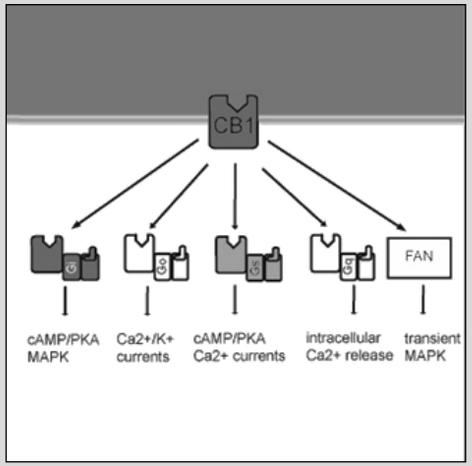


FIGURE 4 Signalling of endocannabinoids on CB_1 receptors located at the axon terminals is via a retrograde pathway. Endocannabinoids, such as anandamide and 2-arachidonoyl glycerol are released post-synaptically. Via the synapse, the molecules migrate to the pre-synaptic cell, where they give feedback via stimulation of the CB_1 receptor. Upon stimulation, a second messenger pathway influences ion channels (e.g. inhibition of calcium) thereby regulating the release of neurotransmitters including glutamate and GABA *(see inside cover for this figure in colour)*.

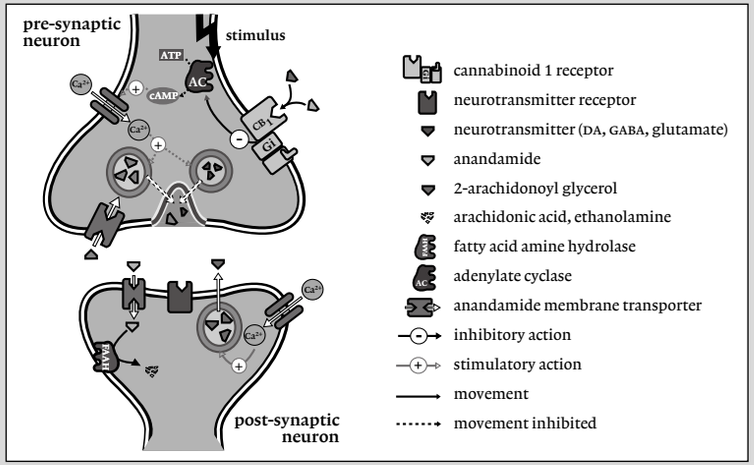


FIGURE 5 Overview of the different steps of the THC administration process where THC loss occurs. The given percentages are mean values. Eventually, 25.8% of the THC dose stays in the lungs. The exact percentage of the THC that actually reaches the blood stream is unknown, since THC metabolising enzymes are present in the lungs. The data are derived from work by Hazekamp et al. (2006).

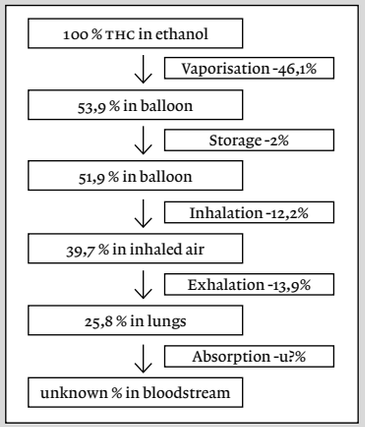


FIGURE 6 Plasma concentration profiles of 10 mg THC after inhalation, intravenous and oral absorption as simulated from a CHDR THC model in 2012 (data on file). With this model, we are able to distinguish three elimination phases: a steep decline of plasma concentration (phase 1) occurs in all administrations and lasts for a few minutes. Subsequently, a less steep decline occurs (phase 2), which changes into a flat phase that could last for over an hour (phase 3).