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Pharmacological aspects of corticotrophinergic and vasopressinergic function tests for HPA axis activation
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PHARMACOLOGICAL ASPECTS OF CORTICOTROPHINERGIC AND
VASOPRESSINERGIC FUNCTION TESTS FOR HPA AXIS ACTIVATION

PHARMACOLOGICAL ASPECTS OF CORTICOTROPHINERGIC AND VASOPRESSINERGIC FUNCTION TESTS FOR HPA AXIS ACTIVATION

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LIST OF ABBREVIATIONS

| | | | |
|------------------|---|------------------|---|
| 5-HT | 5-hydroxytryptamine; serotonin | MRS | Proton Magnetic Resonance spectroscopy |
| 5-HTP | 5-hydroxytryptophan | NAA | N-acetyl-aspartate |
| 7T | 7-Tesla | NA | Noradrenaline |
| ACTH | Adreno-corticotrophic hormone | NRI | Noradrenaline reuptake inhibitor |
| ADH | Antidiuretic hormone | OGTT | Oral glucose tolerance test |
| AE | Adverse event | PD | Pharmacodynamic |
| ANCOVA | Analysis of covariance | PK | Pharmacokinetic |
| ANS | Autonomic nervous system | POMC | Pro-opiomelanocortin |
| ATD | Acute tryptophan depletion | PTSD | Post-traumatic stress disorder |
| AUC | Area under the plasma drug concentration-time curve | PVN | Paraventricular nucleus |
| AVP | Arginine-vasopressin | SAM | sympatho-adrenal-medullary |
| BBB | Blood-brain-barrier | SBP | systolic blood pressure |
| BOLD | Blood oxygen level dependent | SCL-90 | symptoms check List |
| CAMP | Cyclic adenosine monophosphate | SSRI | selective serotonin reuptake inhibitor |
| CAR | cortisol awakening response | SON | supraoptic nucleus |
| CBD | Carbidopa | STEAM | single voxel stimulated echo acquisition mode |
| CHDR | centre for Human Drug Research | T _{1/2} | elimination half life |
| Chol | choline | TCA | Tricyclic antidepressant drug |
| CL | Total clearance of drug from plasma | TE | Echo time |
| CNS | central nervous system | THC | Tetrahydrocannabinol |
| CORT | cortisol | T _{MAX} | Time to maximal plasma drug concentration |
| C _{MAX} | Maximal mean plasma concentration | TM | Mixing time |
| creat | creatinine | TR | repetition time |
| CRH ₁ | CRH ₁ receptor | TSST | Trier social stress test |
| CRH | corticotrophin-releasing hormone | V ₂ | vasopressin-2 receptor |
| CTZ | chemoreceptor trigger zone | V ₃ | vasopressin-3 receptor |
| D ₂ | Dopamine-2 receptor | VAS | visual analogue scale |
| DA | Dopamine | VOI | volume-of-interest |
| DBP | Diastolic blood pressure | VWF | von willebrand factor |
| dDAVP | Desmopressin | | |
| DEX | Dexamethasone | | |
| DEX/CRH | Dexamethasone/corticotrophin test | | |
| ECG | Electrocardiogramme | | |
| fMRI | Functional Magnetic resonance imaging | | |
| GABA | γ-aminobutyric acid | | |
| Glx | Glutamate/glutamine | | |
| GR | Glucocorticoid receptor | | |
| HR | Heart rate | | |
| hCRH | Corticotrophin | | |
| HPA | Hypothalamus-pituitary-adrenal | | |
| LSM | Least squared means | | |
| LUMC | Leiden University Medical Centre | | |
| mCPP | m-chlorophenylpiperazine | | |
| MAOI | Mono-amine oxidase inhibitor | | |
| MCP | Metoclopramide | | |
| MDD | Major depressive disorder | | |
| MDMA | 3,4-methylenedioxymethamphetamine | | |
| MPFC | Medial prefrontal cortex | | |
| MR | Mineralocorticoid receptor | | |
| mRNA | Messenger ribonucleic acid | | |

CHAPTER 1

Introduction

Introduction

The hypothalamus is the principle integrating centre between central neurotransmitter circuits involved in affective processing and the peripherally situated pituitary and adrenal glands (de Kloet et al., 2005; Holsboer and Ising, 2008). Stimuli from the environment activate affective neurotransmitter circuits in the medial prefrontal cortex (MPFC), the limbic system and the autonomic brainstem (Gratton and Sullivan, 2005; Reul and Droste, 2009). Afferent neurotransmitter projections originating from these circuits ultimately terminate in the hypothalamus (Aguilera and Rabadan-Diehl, 2000; de Kloet et al., 2005; Dinan and Scott, 2005; Holsboer and Ising, 2008). There, rapid gene transcription products modulate the production of the neuropeptides corticotrophin-releasing hormone (CRH) and arginine-vasopressin (AVP) (DeBold et al., 1984; Favrod-Coune et al., 1993; Scott and Dinan, 1998). CRH and AVP in turn induce the production and release of ACTH from the anterior pituitary (Holsboer and Barden, 1996). ACTH is the main stimulatory HPA axis neuroendocrine hormone and is responsible for the increased synthesis and release of the endogenous glucocorticoid cortisol (CORT) from the adrenal cortices (de Kloet et al., 2005; Dinan and Scott, 2005; Pariente and Lightman, 2008). CORT facilitates systemic behavioural- and metabolic adaptation to environmental stimuli. At the same time it inhibits the neuroendocrine response via negative feedback that ultimately shuts down the affective neuroendocrine response (de Kloet et al., 2005; Keck, 2006). Together, these organs and their respective effectors form a functional-anatomical system known as the hypothalamus-pituitary-adrenal (HPA) axis.

HPA axis activation in health

The paraventricular nucleus (PVN) of the hypothalamus is intimately involved in the coordination of HPA axis function. It maintains the physiological tone of the HPA axis and plays a crucial role in the integration and translation of stress signals (Herman et al., 2005). Following affective stimuli, the PVN is activated by serotonin (5-hydroxytryptamine – 5-HT) and noradrenaline (NA) that projects from distinct nuclei situated in the brainstem (Ulrich-Lai and Herman, 2009; Herman et al., 2005) and by stimulatory pathways from the (medial nuclei) of the amygdala (Ulrich-Lai and Herman, 2009). Conversely, the PVN is inhibited by γ -aminobutyric acid (GABA)-producing cells in the peri-paraventricular region of the hypothalamus and by a relatively specific population of neurons in the subiculum of the hippocampus (Ulrich-Lai and Herman, 2009). The (central) hypothalamic PVN induces (peripheral) endocrine activation by releasing the neuropeptides CRH and AVP into the pituitary portal circulation. CRH is considered the major HPA axis secretagogue under physiological conditions, while AVP co-activates the HPA axis together with CRH under conditions of acute stress (Aguilera and Rabadan-Diehl, 2000; Dinan and Scott, 2005; Holsboer et al., 1984a; Pariente and Lightman, 2008; Keck, 2006). CRH is primarily produced by the parvocellular neurons of the hypothalamic PVN and acts specifically at the CRH₁ receptors on the anterior pituitary to release ACTH into the systemic circulation (corticotrophinergic activation) (Holsboer et al., 1984a; von Bardeleben and Holsboer, 1988; Dinan and Scott, 2005). AVP, otherwise known as antidiuretic hormone (ADH), is produced in both the magnocellular- and parvocellular neurons of the PVN and the supraoptic nucleus (SON) of the hypothalamus (Ring, 2005). AVP secreted by the magnocellular cells is transported via the medial eminence to the posterior pituitary where it is stored and released systemically in response to (physiological) stimuli such as hypotension, hypoglycemia, hypovolemia and hyperosmolality (Dinan and Scott, 2005; Keck, 2006; Pariente and Lightman, 2008; Ring, 2005). On the other hand, AVP originating from the hypothalamic parvocellular neurosecretory cells is released into the pituitary portal system following acute (affective) stress, enabling AVP to stimulate the vasopressin 3 receptor (V₃ or V_{1B}) on the anterior pituitary (Ring, 2005; Ryckmans, 2010). There,

AVP transiently mobilizes calcium into the cytoplasm and subsequently potentiates CRH₁-associated ACTH release by enhancing its production via proteolysis of the ACTH precursor pro-opiomelanocortin (POMC). (DeBold et al., 1984; Dinan and Scott, 2005; Favrod-Coune et al., 1993). AVP synergizes ACTH release in the presence of increased CRH, culminating in the enhanced release of CORT from the adrenal cortices into the systemic circulation (vasopressinergic co-activation) (Dinan and Scott, 2005; Holsboer and Barden, 1996; Pariante and Lightman, 2008; Scott and Dinan, 1998; Tichomirowa et al., 2005). In turn, CORT induces concentration-dependent affective-, cognitive- and metabolic effects that facilitate adequate adaptive behavioural responses to (stressful) environmental stimuli. In parallel, CORT inhibits the neuroendocrine response via a complex feedback mechanism that involves the glucocorticoid receptors (GR) and mineralocorticoid receptors (MR) in the pituitary, hypothalamus and hippocampus (Figure 1) (Ulrich-Lai and Herman, 2009; de Kloet et al., 2005). Thus, physiological HPA axis activity is the consequence of a dynamic balance between corticotrophinergic activation and CORT feedback, while a stress-induced increase of this neuroendocrine activity is determined by both corticotrophinergic activation and vasopressinergic co-activation on the one hand and CORT feedback on the other hand (Pariante and Lightman, 2008; Tichomirowa et al., 2005; Aguilera and Rabadan-Diehl, 2000; de Kloet et al., 2005).

Pathological HPA axis function

Abnormal HPA axis function is associated with different forms of stress-related psychopathology, which include chronic fatigue syndrome, post-traumatic stress disorder (PTSD) and major depressive disorder (MDD) (Bao et al., 2008; Dinan and Scott, 2005; Holsboer et al., 1984a; Holsboer and Ising, 2008; von Bardeleben and Holsboer, 1988). Specifically, hyperactivity of the HPA axis is the most consistently reported finding in MDD and is especially present in individuals that develop more severe forms of the disease (Bao et al., 2008; Dinan and Scott, 2005; Pariante and Lightman, 2008; Tichomirowa et al., 2005). Untreated depressed patients with melancholic and/or psychotic features are likely to display increased CRH levels in the cerebrospinal fluid (Nemeroff et al., 1984), adrenal

gland enlargement (Nemeroff et al., 1992; Rubin et al., 1995), disturbed ACTH- and CORT secretory bursts (Tichomirowa et al., 2005; Carroll et al., 2007), increased basal CORT levels and urinary free CORT excretion (Carroll et al., 2007; Pariante and Miller, 2001), and increased CORT release after the administration of exogenous ACTH (Dinan et al., 1999; Dinan et al., 2004; Amsterdam et al., 1983). Mechanistically, both disturbed neurotransmitter/neuropeptide function (leading to increased activation) and dysfunctional GR/MR feedback (leading to decreased inhibition) have been implicated in MDD-associated HPA axis hyperactivity (Carroll et al., 2007; Pariante and Miller, 2001; Pariante and Lightman, 2008; Carroll, 1982b). Increased HPA axis activation in MDD has traditionally been ascribed to increased CRH production or increased sensitivity of the CRH₁ receptors (Nemeroff et al., 1984; Nemeroff et al., 1988). However, recent observations have also implicated AVP in MDD-associated HPA hyperactivity. In MDD, CRH₁ receptors undergo downregulation in response to the chronically elevated CORT levels, peripheral AVP levels seem to be elevated (Dinan et al., 1999; Dinan et al., 2004; van Londen et al., 1997) and an increased expression of hypothalamic vasopressinergic-secreting neurons has been found in suicide victims (Meynen et al., 2006; Purba et al., 1996). Also, chronically elevated CORT levels in rats enforce the AVP system by increasing the expression of AVP-receptor mRNA and the AVP: CRH mRNA ratio in the PVN (Aguilera and Rabadan-Diehl, 2000). Taken together, these findings suggest that either increased V₃ receptor expression, increased central AVP levels or a combination of these factors leads to sustained vasopressinergic co-activation and subsequent HPA axis hyperactivity in severe MDD (Figure 2). The individual contributions of corticotrophinergic activation and vasopressinergic co-activation to HPA axis pathology have not been clearly defined. This is not surprising considering that HPA activation and -inhibition are dynamically intertwined. Also, AVP is a co-activator of the HPA axis, and is therefore expected to interact significantly with CRH in HPA axis disturbances. Although these corticotrophinergic and vasopressinergic factors are difficult to disentangle in observational studies, they may play distinct roles in the pathophysiology of different forms of MDD and other psychiatric disorders (Pariante and Lightman, 2008; Swaab et al., 2005). This distinction may also have an impact on prognosis and

treatment, particularly for new therapies that are specifically designed to correct one essential step in the various abnormal feedback loops of the dysregulated HPA axis. Reliable clinical laboratory tools that functionally dissect corticotrophinergic and vasopressinergic aspects of HPA axis activation and/or feedback would clearly be advantageous.

Clinical laboratory tools for the HPA axis: pharmacological function tests

Function tests are clinical laboratory tools that are applied to characterize physiological systems or - functions that are not readily subject to examination, due to their anatomical location and/or complex interconnectivity with other systems. Typically, function tests are developed and technically perfected in healthy individuals after which validation is undertaken in (different) patient groups. Function tests have been in use for decades in both clinical practice and research settings and are still widely applied across different medical specialties. These vary from the oral glucose tolerance test (OGTT) to the exertional electrocardiogram and various allergen provocation tests. In psychiatry, function tests are frequently applied in the research setting to examine the functionality of neurotransmitter systems and/or HPA axis function (Gijsman, 2002; Gijsman et al., 2004). Often, the stimuli are psychological as in the case of the Trier social stress test (TSST) (Kirschbaum et al., 1993) or physiological with measurement of the early morning CORT awakening response (CAR) (Clow et al., 2004). However, research on the pharmacology of neurotransmitter- and neuropeptide systems has enabled the development of pharmacological function tests to characterize and quantify the functionality of the neurotransmitters and neuropeptides involved in HPA axis function. A pharmacological function test consists of an oral (p.o.) or intravenous (i.v.) dose of a drug with a relevant and well-characterized pharmacological mechanism (Gijsman et al., 2004; van Gerven, 2005). The administered drug leads to quantifiable pharmacodynamic (PD) responses that reflect the function of the stimulated neurotransmitter system and its associated central nervous system (CNS) functions. Most pharmacological CNS function tests rely on the principle of pharmacological stimulation and are the result of

either (1) agonism at excitatory (mainly postsynaptic) receptors, (2) increase of a (direct) precursor to a neurotransmitter or neuropeptide (3), antagonism at inhibitory (mainly presynaptic axodendritic) receptors, (4) release stimulation of a neurotransmitter or neuropeptide, or (5) reuptake inhibition of a neurotransmitter (Gijsman et al., 2004). Pharmacological CNS function tests induce a wide range of PD effects that vary from (neuro)physiological or -psychological effects such as changes in cognition or in subjective mood or anxiety, to neuroendocrine HPA activation and the release of ACTH, prolactin, CORT and/or growth hormone.

Pharmacological function tests: application in HPA axis function assessment

Many different pharmacological systems are involved in HPA axis activation, which reflects its central role in the stress system that is essential for survival. Consequently, a number of different drugs have been used as pharmacological function tests of the HPA axis in the past (Table 1) (Gijsman et al., 1998; Gijsman et al., 2002b; Gijsman et al., 2002a; Gijsman, 2002). A range of serotonergic drugs have been used to study the links between the HPA axis and psychiatric disorders like depression and anxiety, in which both serotonergic and stress systems are involved (Gijsman, 2002; Van der Does, 2001). The most direct way of investigating the corticotrophinergic and vasopressinergic aspects of HPA axis activation is by administration of (synthetic forms of) the neuropeptides AVP and CRH. Exogenous i.v. administration of corticotrophin-releasing hormone (corticotropin-releasing hormone - hCRH) alone induces corticotrophinergic activation by directly stimulating the peripheral pituitary CRH₁ receptors. Similarly, direct stimulation of the pituitary V₃ receptors with i.v. administration of desmopressin (DDAVP) allows for the examination of vasopressinergic co-activation. The integrity of HPA axis feedback can be assessed with the synthetic glucocorticoid dexamethasone (DEX), which is administered p.o. either alone (DEX suppression test) or in combination with i.v. hCRH (DEX/CRH test).

In this chapter, a short (non-exhaustive) overview of some of the most frequently applied pharmacological function tests in HPA axis research will be provided. Conceptually, these tests are best approached by artificially classifying them in tests that

assess HPA axis feedback integrity and those addressing HPA axis activation (major corticotrophinergic activation and vasopressin-ergic co-activation) (Figure 3).

Assessment of HPA axis feedback

ORAL DEXAMETHASONE (DEX) TEST

The oral DEX test consists of measuring early morning CORT concentrations after the oral administration of a single dose of DEX (usually 1.5 mg) on the preceding evening (Carroll, 1982c; Carroll, 1982a). DEX is a synthetic glucocorticoid with relatively specific affinity for the GR. Contrary to CORT, DEX does not cross the blood-brain-barrier (BBB) and hence does not mimic the endogenous hypothalamic CORT feedback. The DEX excess however does cause feedback inhibition of ACTH production on the level of the pituitary, which suppresses the physiological morning ACTH - and CORT surge in healthy individuals. In depressive illness this so-called “DEX suppression” is decreased, meaning that CORT levels are elevated after DEX administration compared to healthy controls (Heuser et al., 1994; Holsboer, 1983). This phenomenon is interpreted as a sign of reduced feedback inhibition of the HPA axis in depression, probably by an abnormality at the level of GR/MR-receptor function (Carroll et al., 2007; Holsboer, 1983). However, the DEX suppression test typically consists of a fixed dose of DEX and two CORT measurements, which is a relatively simple way to measure a complex regulatory process. Hence, the variability is quite large and the sensitivity of this test to distinguish patients with mood disorder from the healthy population is too low to be of much clinical value (Ising et al., 2005). In most cases, the DEX suppression test is also too imprecise for a more detailed evaluation of HPA axis dysregulation.

DEX/HCRH TEST

In an attempt to enhance the DEX test's reliability, hCRH was administered after DEX which resulted in the combined DEX/hCRH test. The DEX/hCRH test consists of the administration of an evening dose of a fixed DEX dose (1.5mg), followed by the administration of hCRH (usually 100µg i.v.) the following afternoon (Heuser et al., 1994; Ising et al., 2005). hCRH is a synthetic polypeptide with selective affinity for the CRH₁ receptor. In healthy individuals, the

administration of DEX reduces the release of ACTH and CORT by hCRH, much as it suppresses the early morning activation of the HPA axis without exogenous hCRH. In depressed patients however, the DEX/hCRH test is associated with an enhanced release of ACTH and CORT compared to both healthy individuals and depressed patients receiving 100µg hCRH alone, again similar to non-suppression following the DEX-suppression test and another sign of reduced negative feedback sensitivity (Heuser et al., 1994; Ising et al., 2007; Watson et al., 2006). A possible explanation is that the fall in brain CORT levels after DEX reduces the occupation of limbic MR- and GR-receptors, which are usually occupied due to increased CORT production in depression. The disoccupation of MR- and GR-receptors disinhibits the blunting of the HPA axis after hCRH administration without prior DEX treatment in depression. Such exaggerated response may involve AVP release since the decrease in CORT levels due to DEX pretreatment leads to a compensatory increase in AVP release which in turn enhances the effect of hCRH administered i.v. (Aguilera and Rabadan-Diehl, 2000). Thus, the next afternoon these changes culminate in an increased sensitivity to CRH, which is reflected by the enhanced CORT response to hCRH in depressed patients, the so-called “DEX/hCRH non-suppression”.

Assessment of HPA axis activation

CORTICOTROPHINERGIC ACTIVATION USING hCRH

100µg hCRH i.v. induces a blunted ACTH response in patients with depression compared to healthy volunteers (Holsboer et al., 1984b; Holsboer et al., 1984a; von Bardeleben and Holsboer, 1988). This phenomenon is assumed to be due to the downregulation and/or desensitization of CRH₁ receptors secondary to chronic hypercortisolism (Pariante and Lightman, 2008; von Bardeleben and Holsboer, 1988). Such an assumption is supported by the fact that CORT (but not ACTH) release is similar between patients and healthy individuals after the administration of 100µg hCRH (Holsboer et al., 1984b; Holsboer et al., 1984a; von Bardeleben and Holsboer, 1988). Taken together, these findings indicate that more CRH is needed in patients to induce CORT release similar to that in healthy individuals. Thus, the hCRH function test is able to demonstrate that the setpoint of the HPA axis in depression is increased, as it were.

VASOPRESSINERGIC ACTIVATION USING dDAVP

Vasopressinergic HPA axis activation can be quantified dose-dependently by the administration of 5µg and 10µg dDAVP i.v. and the subsequent measurement of ACTH and CORT (Scott et al., 1999). dDAVP is a partially specific vasopressin receptor agonist exhibiting pharmacological activity at the V_3 as well as the vasopressin 2 receptors (V_2) (Lethagen, 1994). Since endogenous CRH concentrations are low under non-pathological situations and AVP acts as co-activator of the HPA together with CRH, HPA axis activation associated with dDAVP administration in healthy individuals is small. In depressed patients, 10µg dDAVP i.v. induces an exaggerated ACTH response compared to that in healthy volunteers, indicating vasopressinergic (receptor) hyperactivity in depression (Dinan et al., 2004; Dinan and Scott, 2005; Scott and Dinan, 1998). This has been confirmed by an experiment combining both dDAVP and hCRH in patients. As alluded to earlier, 100µg hCRH i.v. induces a “blunted” ACTH response in depressed patients compared to healthy individuals. However, when 100µg hCRH and 10µg dDAVP are administered concomitantly, the blunting associated with hCRH in the depressed patients is abolished and the ACTH response of depressed individuals is identical to that of healthy volunteers (Dinan et al., 1999). These findings are strongly indicative of AVP acting as principle HPA axis activator in depression as opposed to CRH in health.

ALTERNATIVE FUNCTION TESTS TO hCRH AND dDAVP

hCRH and dDAVP modulate HPA axis activation on the level of the pituitary. By focusing on the pituitary, the crucial role of the more “proximal” structures such as the hypothalamus and important central neurotransmitter contributions originating from the MPFC and limbic circuits are not addressed. HPA axis dysregulation in depression is more likely to be associated with central than pituitary or adrenal mechanisms. Although changes in any part of the different release chains and feedback loops of the HPA axis will inevitably also change the other parts, such an “exogenous” approach clearly has its limitations. In contrast, “endogenous” function tests targeting the hypothalamus or limbic neurotransmitters would have the advantage of relying on rate-limiting physiological processes such as central neuropeptide/transmitter

release or enzymatic conversion rather than direct pituitary receptor stimulation. Such an approach might therefore reflect HPA axis functionality in depression more accurately and may have less potentially (indirect) confounding effects than the traditional “exogenous” function tests. In this context, the release of endogenous CRH and/or AVP instead of directly stimulating CRH₁ receptors with hCRH and v₃ receptors with dDAVP has been proposed. Acute increases of serotonin (5-HT) stimulate the release of CRH via 5-HT_{2A} and/or 5-HT_{2C} receptors in the PVN to induce corticotrophinergic activation of the HPA axis (Gartside and Cowen, 1990; Lowry, 2002; Zhang et al., 2002). Central 5-HT concentrations can be increased by the administration of the direct 5-HT precursor 5-hydroxytryptophan (5-HTP). Similarly, an agent that releases endogenous AVP from the hypothalamus or pituitary would be able to induce vasopressinergic co-activation without directly interacting with v₃ receptors. In this context, the anti-emetic D₂ receptor antagonist metoclopramide has been reported to activate the HPA axis by releasing AVP. 5-HTP might therefore be an alternative to hCRH to quantify corticotrophinergic HPA activation, and metoclopramide to dDAVP in assessing vasopressinergic co-activation.

CORTICOTROPHINERGIC ACTIVATION USING 5-HTP

The role of the neurotransmitter serotonin/5-hydroxytryptamine (5-HT) in the regulation of affective circuits of the brain is widely accepted. Moreover, dysfunction in the serotonergic circuits is implicated in the pathophysiology of depression according to its monoamine hypothesis (Pariante and Lightman, 2008). 5-HT is produced in the serotonergic dorsal raphe nuclei which are located in the brain stem. From its source in the brain stem, 5-HT is projected into several different functional brain circuits, including the limbic system and the hypothalamus (Dinan, 1996; Leonard, 2005; Sotty et al., 2009). A pharmacological function test that enhances serotonergic neurotransmission would therefore also activate the HPA axis and induce a neuroendocrine response. In the past, several serotonergic agents such as the direct 5-HT precursor L-5-hydroxytryptophan (5-HTP), subtype selective 5-HT agonists and 5-HT reuptake inhibitors have been applied as function test models for this

purpose (Gijsman, 2002; Gijsman et al., 2004). Notably, orally administered 5-HTP activates the HPA axis as a precursor drug for endogenous 5-HT, which stimulates the postsynaptic 5-HT_{2A} and 5-HT_{2C} receptors in the hypothalamic PVN (Lowry, 2002; Zhang et al., 2002; Gartside and Cowen, 1990). Corticotrophinergic HPA axis activation with 5-HTP therefore reflects (central) hypothalamic HPA activation as opposed to (peripheral) pituitary activation with administration of hCRH. Although such an approach is attractive, the use of oral 5-HTP has been associated with technical problems. Variable kinetics, little standardization and a narrow window between PD effects and side-effects have hampered its application in HPA axis research (Gijsman et al., 2002a). Further development and fine tuning of the 5-HTP test was therefore needed. In this context, the addition of the peripheral monoamine decarboxylase inhibitor carbidopa to 5-HTP increased the availability of 5-HTP for central conversion to 5-HT and rendered 5-HTP pharmacokinetics (PK) more predictable. However, the frequent occurrence nausea and vomiting with 5-HTP remains an obstacle to its further development and validation.

VASOPRESSINERGIC ACTIVATION USING METOCLOPRAMIDE

The anti-emetic metoclopramide has previously been shown to activate the HPA axis (Chiodera et al., 1986; Seki et al., 1997; Walsh et al., 2005). Metoclopramide is a substituted benzamide and acts as D₂- and 5-HT₃ receptor antagonist on the one hand and 5-HT₄ receptor agonist on the other hand. It is hypothesized to release endogenous AVP from the hypothalamus and/or the pituitary, leading to vasopressinergic co-activation through pharmacological mechanisms that have not been clearly identified. However, it is an unknown test and has not been applied frequently as function test for vasopressinergic HPA axis activation. Also, methodological issues in previous trials examining its effects limit interpretation of the results (Chiodera et al., 1986; Seki et al., 1997; Walsh et al., 2005). AVP release from the hypothalamus by metoclopramide would be confirmed by a reliable AVP assay detecting AVP in peripheral blood and/or most importantly, would demonstrate co-activation of the HPA axis in the presence of increased endogenous CRH.

Shortcomings of pharmacological function tests for HPA axis activation

In the context of the pharmacological function test paradigm, the neurotransmitter system or CNS function under investigation can be conceived of as a “black box” (van Gerven, 2005; Gijssman, 2002). A pharmacological agent with a known pharmacological profile is applied to induce a pharmacological effect via specific receptors within the “black box”, which leads to different objectively quantifiable PD effects. Reliable quantification of such effects and characterization of the agent’s pharmacokinetic (PK) properties can thus be used to describe the process inside the “black box” in terms of the relationship between the PK properties and PD effects. An important assumption in this model is that differences between groups of healthy individuals or patients are not due to inherent variation in the test itself, but to functional differences in the system under investigation. However, inherent variability is often introduced unintentionally when applying pharmacological function tests. For instance, the administration of different doses of function agent and little or no attention for the pharmacological agent’s PK characteristics is an important source of variability. Also, pharmacological characteristics and PD endpoints are not always unequivocal and potential confounding PD effects are often disregarded. Usually, the PD effects are dependent on the dose of function agent and exhibit changes over time, which makes it essential to correct the PD effects for different concentrations of the function agent over a certain time period. Therefore, too infrequent or once-off sampling of PD parameters does not allow for adequate assessment of effects over time and prevents the clarification of PK-PD relationships. Clearly, such issues add confusion to an already complex research field and at the same time hamper the further development and wider application of the function test paradigm. Investigating and minimizing inherent variability should ideally be an important step in the development of any given pharmacological function test. In this context, certain principles have been proposed to minimize variability including (1) a well-known and -characterized pharmacological mechanisms; (2) little or no confounding pharmacological effects; (3) inducing PD effects that are easily and reliably measurable; (4) a robust physiological

model of the investigated system, based on the PK/PD-characteristics of the function test; (5) limited and acceptable adverse effects; (6) a wide window between PD effects and undesirable effects; (7) PD effects that can be plausibly linked to the system or function under investigation; (8) practical to administer and (9) an ethical balance between the information obtained with the function test and the discomfort it causes for patients or healthy volunteers (van Gerven, 2005). The application of these principles to pharmacological function tests is expected to reduce their inherent variability and increase their reliability. However, most existing function tests have not been exposed to such systematic scrutiny. Consequently, many questions regarding the interpretation of HPA axis function in MDD and other stress-related disorders still remain. It is expected that the systematic application of these principles to existing function tests will address such issues and contribute to their wider application in CNS research. The main objective of this thesis was to address some of the major shortcomings of several different corticotrophinergic and vasopressinergic HPA axis function tests (Table 2, Chapters 2 - 6). The (functional) characteristics of the HPA axis are almost always derived from changes in ACTH and CORT levels. The pharmacokinetic properties of these hormones vary considerably between subjects, and release rates change with time due to feedback inhibition. Therefore, it is very difficult to construct a quantitative physiological model of the HPA axis based on these instable parameters, and hence to comply with criterion number four mentioned above. It would be helpful to directly quantify the activation of the hypothalamus, which drives the HPA axis after central stimulation. To this end, a neuroimaging technique was applied to investigate pharmacologically-induced hypothalamic changes in Chapter 7.

SHORTCOMINGS OF hCRH AND dDAVP

The pharmacological characteristics of hCRH and dDAVP are well-characterized and fairly specific, which makes them suitable as function tests for the HPA axis. However, their interpretation is often thwarted by variable responses due to a lack of standardization and variable PK properties, and there are confounding pharmacodynamic (PD) effects that may cause safety concerns in vulnerable patients. Previous studies with dDAVP have focused on the magnitude of v_3 modulated neuroendocrine PD effects with

minimal attention for systemic effects that may cause secondary HPA axis activation. Specifically, dDAVP may cause blood pressure reduction that may lead to ACTH release by activation of the autonomic nervous system (ANS). Also, dDAVP enhances the coagulation cascade, which is particularly relevant if the dDAVP function test is to be applied in patient populations with comorbid cardiovascular disease. We therefore investigated the relation between neuroendocrine, cardiovascular, pro-coagulatory, anti-diuretic and non-specific stress effects of dDAVP applied as pharmacological function test for assessing vasopressinergic co-activation of the HPA in healthy volunteers (Chapter 2).

The individual effects of corticotrophinergic activation (with hCRH) and vasopressinergic co-activation (with dDAVP) and their interactions are required for a proper understanding of HPA axis activation. Although it is possible to induce HPA axis activation with dDAVP, it is difficult to derive quantitative measures of co-activation from this response without knowing the prevailing level of CRH activity. Therefore, we examined whether concomitant administration of dDAVP and hCRH could provide more informative vasopressinergic co-activation of the HPA axis than dDAVP alone in healthy volunteers (Chapter 3). In the literature, corticotrophinergic activation and vasopressinergic co-activation of the HPA axis have been determined on different study days, which gives rise to inter-session variability and is unpractical for patient studies. So far, there was no practical function test that is able to examine both activation routes on a single short study occasion. Independent evaluations of each route would logically require an adequate washout period, to ensure that v_3 stimulation does not affect subsequent CRH_1 stimulation (or vice versa), but it is unknown how long the carry-over effects remain. Since the plasma half-life of dDAVP is only 90 to 180 minutes, we determined the feasibility of a practical function test of both corticotrophinergic activation and vasopressinergic co-activation of the HPA axis on a single short laboratory visit, with a washout period of two hours (Chapter 3).

5-HTP

5-HTP is a direct 5-HT precursor used to assess central serotonergic function. In the past, its use has been limited by a narrow window between neuroendocrine changes and side effects, and variable kinetics related to inconsistent administration modes (Gijssman

et al., 2002a). The addition of the peripheral monoamine decarboxylase inhibitor carbidopa to 5-HTP increased the availability of 5-HTP for central conversion to 5-HT and rendered 5-HTP PK more predictable (Gijsman et al., 2002a). However, the dose-response relationship and the pharmacokinetic properties of various oral doses of 5-HTP, their tolerability and subjective (adverse) effects, and their secondary impact on HPA axis activation are still unclear. We investigated the feasibility of the combined 5-HTP/CBD function test as candidate test for examining endogenous HPA activation. To this end, we examined the dose- and concentration effect relationship of different doses of orally administered 5-HTP in combination with CBD in healthy volunteers (Chapter 4) and we attempted to suppress the most confounding systematic serotonergic side-effects (nausea and vomiting) without influencing the neuroendocrine response, using a subtype selective serotonin receptor antagonist (Chapter 5).

METOCLOPRAMIDE

Metoclopramide is hypothesized to lead to vasopressinergic co-activation of the HPA axis. It would have the advantages of releasing endogenous AVP from the hypothalamus or pituitary and of inducing minimal confounding effects. However, there is a paucity of data regarding its potency to co-activate the HPA axis, and methodological issues in previous trials preclude adequate interpretation of metoclopramide's effects on HPA axis activation. Therefore, endogenous vasopressinergic co-activation using metoclopramide was investigated under conditions of low and enhanced endogenous CRH-release in healthy volunteers (Chapter 6).

HYPOTHALAMIC NEUROIMAGING

In the studies described in Chapters 2-6, activity of the HPA axis is derived from neuroendocrine changes measured in peripheral blood. These hormonal responses are the product of complex interacting phenomena, such as central and peripheral amplification and co-activation, negative feedback mechanisms, and pharmacokinetic clearance. More direct measurements of drug-induced neuronal activity in the brain using neuroimaging techniques could lead to a better understanding of the CNS processes involved in the stimulation of neurotransmitter systems. Functional neuroimaging of pharmacological CNS-processes often use functional

magnetic resonance imaging (fMRI) to demonstrate regional shifts in blood oxygen level dependent (BOLD) signals. However, the application of BOLD-MRI is limited by factors such as drift of the MRI signal with time, changes due to nonspecific confounding factors such as (drug-induced) vascular reactivity, and substantial intersession variance in cross-over designs. These factors limit the application of fMRI in the quantification of drug-induced neuronal activity. Alternatively, functional proton resonance magnetic spectroscopy (MRS) could be a candidate neuroimaging technique to directly quantify drug-induced neurotransmission. In vivo proton magnetic resonance spectroscopy (MRS) of the brain determines relative and absolute concentrations of protons from tissue chemicals other than water, such as glutamate-glutamine (Glx), N-acetyl-aspartate (NAA), myo-inositol, lactate, choline and creatine. In this context, NAA and Glx are generally regarded as surrogate MRS markers for neuronal activity, and Chol is considered a metabolic marker of membrane density and integrity. Although MRS is typically applied in neurodegenerative disease and malignant brain tumours, recent findings suggests that MRS might also be able to demonstrate and quantify changes in neuronal biochemistry during (prolonged) pharmacological treatment with CNS-active drugs (Taylor et al., 2008). We studied the effects of a combined 5-HTP/CBD/granisetron function test on hypothalamic levels of glutamate/glutamine (Glx), choline, N-acetyl-aspartate (NAA) and creatine using 7-Tesla (7T) MRS, and related these effects to 5-HTP-induced ACTH and CORT in peripheral blood in healthy volunteers (Chapter 7).

This thesis focuses on the most important pharmacological short-coming of hCRH and 5-HTP (for corticotrophinergic activation) and dDAVP and metoclopramide (for vasopressinergic co-activation), in an attempt to contribute to the validation of these drugs as clinical laboratory tools in HPA axis research. At the same time, these function tests are expected to contribute to a better understanding of HPA axis function and to further clarify the respective contributions of CRH and AVP in its activation. Hopefully, such information will be helpful in the future application of these function tests to investigate aberrant HPA axis activation in different patient groups and as clinical tools in the development of novel drugs targeting the major HPA axis neuropeptide systems.

Table 1

Drugs that have been used as function tests to assess HPA axis activation: a summary of their respective characteristics and shortcomings (adapted from Gijsman 2002).

| Drug | Pharmacological characteristics | Shortcomings |
|-------------------------------------|--|--|
| meta-Chlorophenyl-piperazine (mCPP) | 5-HT _{2C} receptor agonist; 5-HT _{2B} receptor antagonist; strongly binds to alpha-2 receptors | Autonomic shortcomings and emotional disturbance; large interindividual variability in PK; CYP P450 inhibitors; limited test-retest variability. |
| Dexfenfluramine | Stimulates 5-HT release from axons in the frontal cortex, hippocampus and striatum; 5-HT _{2A} and 5-HT _{2C} receptor agonist. | Reproducibility not investigated; few side-effects; neurotoxicity after a single dose possible. |
| 5-hydroxytryptophan (5-HTP) | Direct precursor of the neurotransmitter serotonin (5-HT); decarboxylated into 5-HT by 5-HT decarboxylase; available for non-specific actions at pre- and postsynaptic serotonergic receptors. | Test-retest variability not investigated; PK characteristics unclear; potentially confounding and bothersome side-effects |

Table 2

Summary of the corticotrophinergic (hCRH and 5-HTP/carbidopa) and vasopressinergic (dDAVP and metoclopramide) function tests to assess HPA axis activation and their correspondence to the proposed principles of reliable pharmacological function tests

| Characteristic | Corticotrophinergic activation | | Vasopressinergic co-activation | |
|---|---|--|---|--|
| | hCRH | 5-HTP/carbidopa | dDAVP | Metoclopramide |
| Pharmacological mechanism | Agonism at the CRH ₁ receptor in the anterior pituitary | Conversion of 5-HTP to 5-HT in the raphé nuclei followed by agonism at 5-HT _{2A} or 5-HT _{2C} receptors in the PVN | Agonism at the v ₃ (v _{1B}) receptor of the anterior pituitary | Unclear, maybe D ₂ antagonism |
| Confounding effects | Blood pressure reduction leading to autonomic nervous system activation | Nausea and vomiting leading to autonomic nervous system activation | Blood pressure reduction leading to autonomic nervous system activation | None known |
| Measurable PD effects | ACTH, cortisol, prolactin | ACTH, cortisol, prolactin | ACTH, cortisol, prolactin | ACTH, cortisol, prolactin |
| Robust physiological model | Convincingly demonstrated | Not convincingly demonstrated | Convincingly demonstrated | Never demonstrated |
| Safe/few adverse effects | Few effects | Few effects, nausea and vomiting potentially the most disturbing | Few effects | Few effects |
| Wide window between PD – and undesirable effects | Probably | Narrow window between PD effect and side-effects | Probably, ceiling effects seem likely | Unknown |
| Plausibility | Abnormalities in the CRH system have been associated with MDD | Abnormalities in the 5-HT system are implicated according to the monoamine hypothesis of depression | Abnormalities in the AVP system have been associated with MDD | Unclear |
| Practical | Easy to administer (i.v.) | Administration complicated by carbidopa pretreatment (p.o) | Easy to administer (i.v.) | Easy to administer (i.v.) |
| Ethical | Yes | Yes | Yes | Yes |

Figure 1

Graphic representation of HPA axis function in health: light gray arrows signify activation and dark gray arrows signify inhibition via negative feedback (CRH: corticotrophin-releasing hormone; AVP: vasopressin; ACTH: adrenocorticotrophic hormone; GR: glucocorticoid receptor; MR: mineralocorticoid receptor).

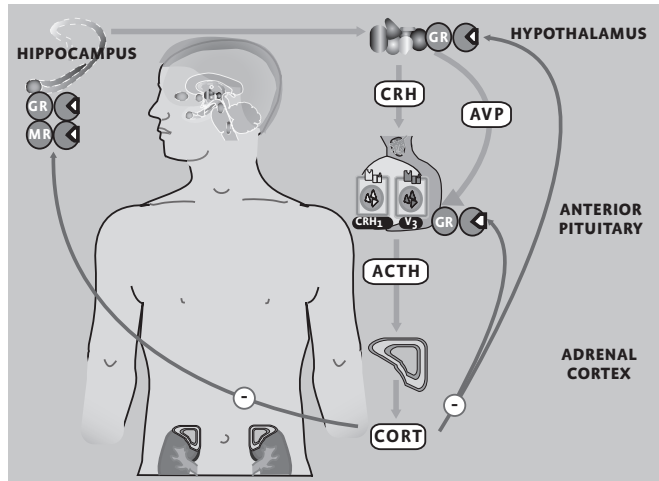


Figure 2

Graphic representation of HPA axis function in severe major depressive disorder (MDD): thick arrows signify hyperactivation and dotted arrows signify decreased inhibition via negative feedback (CRH: corticotrophin-releasing hormone; AVP: vasopressin; ACTH: adrenocorticotrophic hormone; GR: glucocorticoid receptor; MR: mineralocorticoid receptor).

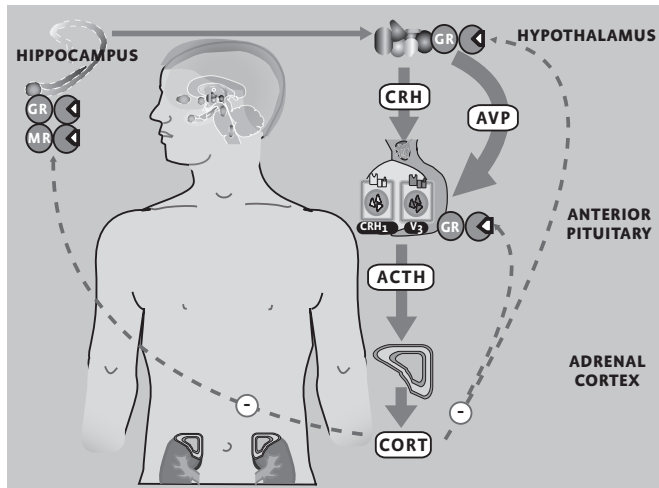
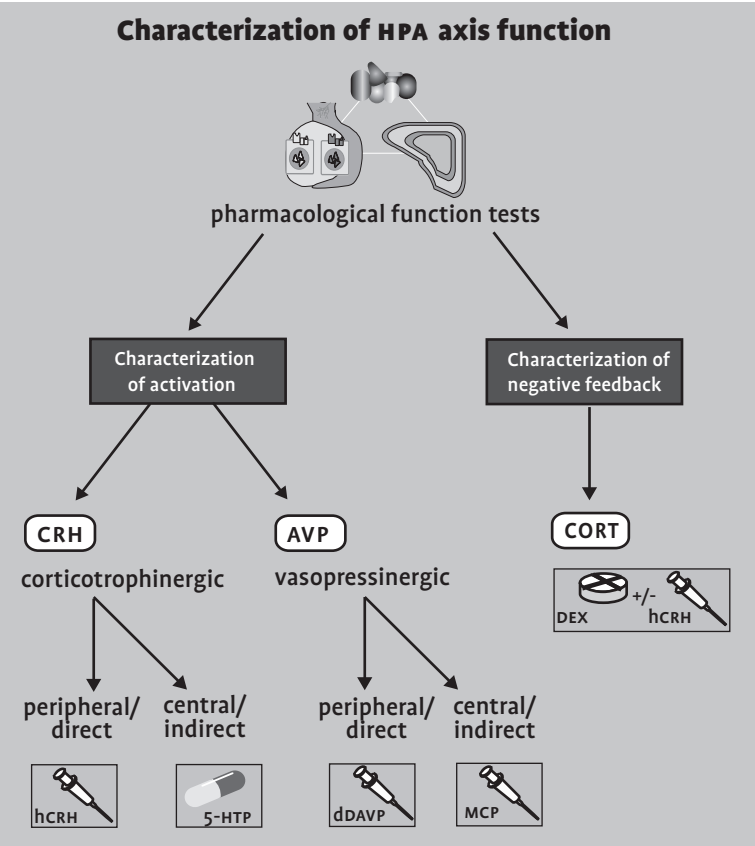


Figure 3

Graphic representation of the characterization of HPA axis function: proposed classification of pharmacological function tests (CRH: corticotrophin-releasing hormone; AVP: vasopressin; hCRH: corticorelin; 5-HTP: 5-hydroxytryptophane; dDAVP: desmopressin; MCP: metoclopramide; DEX: dexamethasone).



REFERENCE LIST

- Aguilera G, Rabadan-Diehl C (2000) Vasopressinergic regulation of the hypothalamic-pituitary-adrenal axis: implications for stress adaptation. *Regul Pept* 96: 23-29.
- Amsterdam JD, Winokur A, Abelman E, Lucki I, Rickels K (1983) Cosyntropin (ACTH alpha 1-24) stimulation test in depressed patients and healthy subjects. *Am J Psychiatry* 140: 907-909.
- Bao AM, Meynen G, Swaab DF (2008) The stress system in depression and neurodegeneration: focus on the human hypothalamus. *Brain Res Rev* 57: 531-553.
- Carroll BJ (1982a) Clinical applications of the dexamethasone suppression test for endogenous depression. *Pharmacopsychiatry* 15: 19-25.
- Carroll BJ (1982b) The dexamethasone suppression test for melancholia. *Br J Psychiatry* 140: 292-304.
- Carroll BJ (1982c) Use of the dexamethasone suppression test in depression. *J Clin Psychiatry* 43: 44-50.
- Carroll BJ, Cassidy F, Naftolowitz D, Tatham NE, Wilson WH, Iranmanesh A, Liu PY, Veldhuis JD (2007) Pathophysiology of hypercortisolism in depression. *Acta Psychiatr Scand Suppl* 90-103.
- Chiodera P, Volpi R, Delsignore R, Marchesi C, Salati G, Camellini L, Rossi G, Coiro V (1986) Different effects of metoclopramide and domperidone on arginine-vasopressin secretion in man. *Br J Clin Pharmacol* 22: 479-482.
- Clow A, Thorn L, Evans P, Hucklebridge F (2004) The awakening cortisol response: methodological issues and significance. *Stress* 7: 29-37.
- de Kloet ER, Joels M, Holsboer F (2005) Stress and the brain: from adaptation to disease. *Nat Rev Neurosci* 6: 463-475.
- DeBold CR, Sheldon WR, DeCherney GS, Jackson RV, Alexander AN, Vale W, Rivier J, Orth DN (1984) Arginine vasopressin potentiates adrenocorticotropin release induced by ovine corticotropin-releasing factor. *J Clin Invest* 73: 533-538.
- Dinan TG (1996) Serotonin and the regulation of hypothalamic-pituitary-adrenal axis function. *Life Sci* 58: 1683-1694.
- Dinan TG, Lavelle E, Scott LV, Newell-Price J, Medbak S, Grossman AB (1999) Desmopressin normalizes the blunted adrenocorticotropin response to corticotropin-releasing hormone in melancholic depression: evidence of enhanced vasopressinergic responsivity. *J Clin Endocrinol Metab* 84: 2238-2240.
- Dinan TG, O'Brien S, Lavelle E, Scott LV (2004) Further neuroendocrine evidence of enhanced vasopressin VC receptor responses in melancholic depression. *Psychol Med* 34: 169-172.
- Dinan TG, Scott LV (2005) Anatomy of melancholia: focus on hypothalamic-pituitary-adrenal axis overactivity and the role of vasopressin. *J Anat* 207: 259-264.
- Favrod-Coune C, Raux-Demay MC, Proeschel MF, Bertagna X, Girard F, Luton JP (1993) Potentiation of the classic ovine corticotrophin releasing hormone stimulation test by the combined administration of small doses of lysine vasopressin. *Clin Endocrinol (Oxf)* 38: 405-410.
- Gartside SE, Cowen PJ (1990) Mediation of ACTH and prolactin responses to 5-HTP by 5-HT₂ receptors. *Eur J Pharmacol* 179: 103-109.
- Gijsman HJ (2002) Pharmacological aspects of challenge tests of the serotonergic system of the brain.
- Gijsman HJ, Cohen AF, van Gerven JM (2004) The application of the principles of clinical drug development to pharmacological challenge tests of the serotonergic system. *J Psychopharmacol* 18: 7-13.
- Gijsman HJ, van Gerven JM, de Kam ML, Schoemaker RC, Pieters MS, Weemaes M, de Rijk R, van der PJ, Cohen AF (2002a) Placebo-controlled comparison of three dose-regimens of 5-hydroxytryptophan challenge test in healthy volunteers. *J Clin Psychopharmacol* 22: 183-189.
- Gijsman HJ, van Gerven JM, Tieleman MC, Schoemaker RC, Pieters MS, Ferrari MD, Cohen AF, van Kempen GM (1998) Pharmacokinetic and pharmacodynamic profile of oral and intravenous meta-chlorophenylpiperazine in healthy volunteers. *J Clin Psychopharmacol* 18: 289-295.
- Gijsman HJ, van Gerven JM, Verkes RJ, Schoemaker RC, Pieters MS, Pennings EJ, Hessing TJ, Cohen AF (2002b) Saccadic peak velocity and EEG as end-points for a serotonergic challenge test. *Hum Psychopharmacol* 17: 83-89.
- Gratton A, Sullivan RM (2005) Role of prefrontal cortex in stress responsivity. In: *Handbook of stress and the brain* (Steckler T, Kalin NH, Reul JMHM, eds), pp 807-817. Elsevier.
- Herman JP, Ostrander MM, Mueller NK, Figueiredo H (2005) Limbic system mechanisms of stress regulation: hypothalamo-pituitary-adrenocortical axis. *Prog Neuropsychopharmacol Biol Psychiatry* 29: 1201-1213.
- Heuser I, Yassouridis A, Holsboer F (1994) The combined dexamethasone/CRH test: a refined laboratory test for psychiatric disorders. *J Psychiatr Res* 28: 341-356.

- Holsboer F (1983) The dexamethasone suppression test in depressed patients: clinical and biochemical aspects. *J Steroid Biochem* 19: 251-257.
- Holsboer F, Barden N (1996) Antidepressants and hypothalamic-pituitary-adrenocortical regulation. *Endocr Rev* 17: 187-205.
- Holsboer F, Gerken A, Steiger A, Benkert O, Muller OA, Stalla GK (1984a) Corticotropin-releasing-factor induced pituitary-adrenal response in depression. *Lancet* 1: 55.
- Holsboer F, Ising M (2008) Central CRH system in depression and anxiety--evidence from clinical studies with CRH₁ receptor antagonists. *Eur J Pharmacol* 583: 350-357.
- Holsboer F, von Bardeleben U, Gerken A, Stalla GK, Muller OA (1984b) Blunted corticotropin and normal cortisol response to human corticotropin-releasing factor in depression. *N Engl J Med* 311: 1127.
- Ising M, Horstmann S, Kloiber S, Lucae S, Binder EB, Kern N, Kunzel HE, Pfennig A, Uhr M, Holsboer F (2007) Combined dexamethasone/corticotropin releasing hormone test predicts treatment response in major depression - a potential biomarker? *Biol Psychiatry* 62: 47-54.
- Ising M, Kunzel HE, Binder EB, Nickel T, Modell S, Holsboer F (2005) The combined dexamethasone/CRH test as a potential surrogate marker in depression. *Prog Neuropsychopharmacol Biol Psychiatry* 29: 1085-1093.
- Keck ME (2006) Corticotropin-releasing factor, vasopressin and receptor systems in depression and anxiety. *Amino Acids* 31: 241-250.
- Kirschbaum C, Pirke KM, Hellhammer DH (1993) The 'Trier Social Stress Test'--a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology* 28: 76-81.
- Leonard BE (2005) The HPA and immune axes in stress: the involvement of the serotonergic system. *Eur Psychiatry* 20 Suppl 3: S302-S306.
- Lethagen S (1994) Desmopressin (dDAVP) and hemostasis. *Ann Hematol* 69: 173-180.
- Lowry CA (2002) Functional subsets of serotonergic neurones: implications for control of the hypothalamic-pituitary-adrenal axis. *J Neuroendocrinol* 14: 911-923.
- Meynen G, Unmehopa UA, van Heerikhuizen JJ, Hofman MA, Swaab DF, Hoogendijk WJ (2006) Increased arginine vasopressin mRNA expression in the human hypothalamus in depression: A preliminary report. *Biol Psychiatry* 60: 892-895.
- Nemeroff CB, Krishnan KR, Reed D, Leder R, Beam C, Dunnick NR (1992) Adrenal gland enlargement in major depression. A computed tomographic study. *Arch Gen Psychiatry* 49: 384-387.
- Nemeroff CB, Owens MJ, Bissette G, Andorn AC, Stanley M (1988) Reduced corticotropin releasing factor binding sites in the frontal cortex of suicide victims. *Arch Gen Psychiatry* 45: 577-579.
- Nemeroff CB, Widerlov E, Bissette G, Walleus H, Karlsson I, Eklund K, Kilts CD, Loosen PT, Vale W (1984) Elevated concentrations of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients. *Science* 226: 1342-1344.
- Pariente CM, Lightman SL (2008) The HPA axis in major depression: classical theories and new developments. *Trends Neurosci* 31: 464-468.
- Pariente CM, Miller AH (2001) Glucocorticoid receptors in major depression: relevance to pathophysiology and treatment. *Biol Psychiatry* 49: 391-404.
- Purba JS, Hoogendijk WJ, Hofman MA, Swaab DF (1996) Increased number of vasopressin- and oxytocin-expressing neurons in the paraventricular nucleus of the hypothalamus in depression. *Arch Gen Psychiatry* 53: 137-143.
- Reul JMHM, Droste SK (2009) The hypothalamic-pituitary-adrenal axis as a dynamically organized system: lessons from exercising mice. In: *Handbook of stress and the brain* (Steckler T, Kalin NHRJMHM, eds), pp 95-112. Elsevier.
- Ring RH (2005) The central vasopressinergic system: examining the opportunities for psychiatric drug development. *Curr Pharm Des* 11: 205-225.
- Rubin RT, Phillips JJ, Sadow TF, McCracken JT (1995) Adrenal gland volume in major depression. Increase during the depressive episode and decrease with successful treatment. *Arch Gen Psychiatry* 52: 213-218.
- Ryckmans T (2010) Modulation of the vasopressin system for the treatment of CNS diseases. *Curr Opin Drug Discov Devel* 13: 538-547.
- Scott LV, Dinan TG (1998) Vasopressin and the regulation of hypothalamic-pituitary-adrenal axis function: implications for the pathophysiology of depression. *Life Sci* 62: 1985-1998.
- Scott LV, Medbak S, Dinan TG (1999) ACTH and cortisol release following intravenous desmopressin: a dose-response study. *Clin Endocrinol (Oxf)* 51: 653-658.
- Seki K, Kato T, Sekiya S (1997) Corticotrophin and vasopressin responses to metoclopramide in patients with hypothalamic amenorrhoea. *Clin Endocrinol (Oxf)* 46: 203-207.
- Sotty F, Folgering JH, Brennum LH, Hogg S, Mork A, Hertel P, Cremers TI (2009) Relevance of dorsal

- raphe nucleus firing in serotonin 5-HT_{2C} receptor blockade-induced augmentation of SSRIs effects. *Neuropharmacology* 57: 18-24.
- Swaab DF, Bao AM, Lucassen PJ (2005) The stress system in the human brain in depression and neurodegeneration. *Ageing Res Rev* 4: 141-194.
- Tichomirowa MA, Keck ME, Schneider HJ, Paez-Pereda M, Renner U, Holsboer F, Stalla GK (2005) Endocrine disturbances in depression. *J Endocrinol Invest* 28: 89-99.
- Ulrich-Lai YM, Herman JP (2009) Neural regulation of endocrine and autonomic stress responses. *Nat Rev Neurosci* 10: 397-409.
- Van der Does AJ (2001) The effects of tryptophan depletion on mood and psychiatric symptoms. *J Affect Disord* 64: 107-119.
- van Gerven JM (2005) Het benevelde brein: de Delyside-challenge-test van Sandoz.
- van Londen L, Goekoop JG, van Kempen GM, Frankhuijzen-Sierevogel AC, Wiegant VM, van der Velde EA, De Wied D (1997) Plasma levels of arginine vasopressin elevated in patients with major depression. *Neuropsychopharmacology* 17: 284-292.
- von Bardeleben U, Holsboer F (1988) Human corticotropin releasing hormone: clinical studies in patients with affective disorders, alcoholism, panic disorder and in normal controls. *Prog Neuropsychopharmacol Biol Psychiatry* 12 Suppl: S165-S187.
- Walsh P, Spelman L, Sharifi N, Thakore JH (2005) Male patients with paranoid schizophrenia have greater ACTH and cortisol secretion in response to metoclopramide-induced AVP release. *Psychoneuroendocrinology* 30: 431-437.
- Watson S, Gallagher P, Smith MS, Ferrier IN, Young AH (2006) The dex/CRH test--is it better than the DST? *Psychoneuroendocrinology* 31: 889-894.
- Zhang Y, Damjanoska KJ, Carrasco GA, Dudas B, D'Souza DN, Tetzlaff J, Garcia F, Hanley NR, Scripathirathan K, Petersen BR, Gray TS, Battaglia G, Muma NA, Van de Kar LD (2002) Evidence that 5-HT_{2A} receptors in the hypothalamic paraventricular nucleus mediate neuroendocrine responses to (-)DOI. *J Neurosci* 22: 9635-9642.

Desmopressin as a pharmacological tool in vasopressinergic hypothalamus–pituitary–adrenal (HPA) axis modulation: neuroendocrine, cardiovascular and coagulatory effects

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Abstract

- BACKGROUND** Arginine-vasopressin (AVP) is a physiological co-activator of the hypothalamus-pituitary-adrenal-axis (HPA), together with corticotrophin-releasing hormone (CRH). A synthetic analogue of AVP, desmopressin (dDAVP) is often used as pharmacological tool to assess co-activation in health and disease. The relation between dDAVP's neuroendocrine, cardiovascular, pro-coagulatory, anti-diuretic and non-specific stress effects has not been studied.
- OBJECTIVES** A randomized, double-blind, placebo-controlled, three-way cross-over study was performed in 12 healthy male and female volunteers (6: 6). dDAVP was administered intravenously as a 10µg bolus (over one minute) or a 30µg incremental infusion (over 60 minutes). Neuroendocrine, cardiovascular, pro-coagulatory, anti-diuretic effects and adverse events (AE's) were recorded, and autonomic nervous system (ANS) activation evaluated.
- RESULTS** The incremental infusion reached 1.8 fold higher dDAVP concentrations than the bolus. Neuroendocrine effects were similar for the 10µg dDAVP bolus and the 30µg incremental infusion, while cardiovascular- and coagulatory effects were greater with the 30µg dose. Osmolality and ANS activity remained uninfluenced. AE's corresponded to dDAVP's side-effect profile.
- CONCLUSIONS** The neuroendocrine effects of a 10µg dDAVP bolus administered over one minute are similar to those of a 30µg incremental infusion administered over one hour, despite higher dDAVP concentrations after the infusion. Cardiovascular and coagulatory effects showed clear dose-related responses. A 10µg dDAVP bolus is considered a safe vasopressinergic function test at which no confounding effects of systemic or autonomic stress were seen.

Introduction

Arginine-vasopressin (AVP) is the nonapeptide modulator of the human vasopressinergic system. It acts as neurotransmitter within the central nervous system (CNS) and as neuroendocrine hormone in the peripheral circulation (Ring, 2005). AVP produced by hypothalamic parvocellular neurones of the medial paraventricular nucleus (PVN) is not only released into the CNS but also secreted into the pituitary portal circulation (Aguilera and Rabadan-Diehl, 2000; Scott and Dinan, 2002). Centrally acting AVP is believed to play a role in the regulation of learning and memory, social behaviours, circadian rhythmicity and thermoregulation (Ring, 2005). Furthermore, following acute psychological and/or physical stress, it acts as co-activator of the hypothalamus-pituitary-adrenal (HPA) axis by inducing ACTH release via the V_3 receptor (V_3 or V_{1B}) on the anterior pituitary (Dinan and Scott, 2005; Scott and Dinan, 1998). Co-activation is believed to occur in the presence of the main HPA axis activator corticotrophin-releasing hormone (CRH) (Scott and Dinan, 2002). Subsequently, the stress hormone cortisol is released into the circulation from the adrenal cortices, causing various systemic and metabolic effects in peripheral tissues, and different behavioural and cognitive effects in the CNS. In addition to these actions on the HPA-axis, AVP originating from magnocellular neurons of the supraoptic nucleus and PVN of the hypothalamus is secreted peripherally via the posterior pituitary (Ring, 2005). Peripheral release is triggered by hypo-osmolality, hypovolemia, reduced blood pressure, hemorrhage, hypoglycemia, fever and pain. After release, it acts principally at V_{1A} receptors of the renal tubular cells restoring plasma volume and osmolality (Ring, 2005).

Hyperactivity of the AVP system of the anterior pituitary has been implicated in HPA axis dysregulation during chronic stress-related psychopathology (Pariante and Lightman, 2008; Scott and Dinan, 2002). Preclinical data indicate that AVP sustains HPA axis hyperactivity under conditions of chronic stress (Spiga et al., 2008). Humans with (subtypes of) depressive disorder display hypercortisolism, indicating chronic HPA axis hyperactivation. Also, these patients have an enhanced neuroendocrine response to the combined dexamethasone-corticotrophin release hormone (DEX/hCRH) function test. This potentiation of the response to hCRH is

predominantly ascribed to pre-existing hypersensitivity of the AVP system (Holsboer, 1983; Holsboer, 2000). However, the exact role of AVP in the pathophysiology of different (sub)types of affective disorders or other psychiatric conditions has not been clearly defined.

The lack of a better understanding of the role of AVP in psychopathology is in part related to the lack of validated function tests of vasopressinergic HPA axis regulation. A reliable pharmacological vasopressinergic function test would not only be useful to examine AVP in HPA axis regulation in health and (psychiatric) disease in more detail, but it would also be helpful to investigate innovative drugs targeting this system. Modulation of the AVP system by V_3 antagonists could for instance be clearly demonstrated in an early stage of drug development by suppression of the HPA-effects induced by a validated AVP function test. Such a function test can also be used as functional diagnostic and/or prognostic tool in patients suffering from HPA axis associated psychiatric disorders. Previously, intravenous (i.v.) administration of desmopressin (dDAVP), a synthetic analogue of AVP, has been proposed as pharmacological function test of vasopressinergic HPA axis function (Dinan and Scott, 2005; Scott and Dinan, 1998). dDAVP is a partially specific vasopressin receptor agonist exhibiting pharmacological activity at the V_3 as well as the vasopressin 2 receptors (V_2) (Craighead et al., 2008). It has an elimination half-life of 0.9 to 3.8 hours and is excreted unchanged by the kidneys (Rembratt et al., 2004). In the past, a number of important studies have utilized dDAVP to help understand AVP's role in the (patho)physiology of HPA axis regulation (Dinan et al., 1999; Dinan et al., 2004; Scott et al., 1999a; Scott et al., 1999b). However, to a large extent these studies have focussed on the magnitude of V_3 modulated neuroendocrine pharmacodynamic (PD) effects with minimal attention for systemic effects that may confound HPA axis activation by non-specific stress, or affect the safety of this test. Specifically, the influence of blood pressure reduction induced by dDAVP via the V_2 and its potential subsequent influence on ACTH release via the autonomic nervous system (ANS) remains unclear. There is also very little information on the procoagulant and anti-diuretic adverse effects of i.v. administered 10 μ g dDAVP via the V_2 . These effects are particularly relevant if the dDAVP function test is to be applied in depressed patient populations, who have an increased risk for

comorbid cardiovascular disease or (components of) the metabolic syndrome (Evans et al., 2005; Musselman et al., 2003; Ramasubbu, 2002).

We performed a study that simultaneously investigated the neuroendocrine-, cardiovascular-, anti-diuretic-, pro-coagulant- and (indirect) ANS effects of dDAVP, and their concentration- and infusion rate-dependence. Two different doses of dDAVP were administered i.v. using two different administration modes: (1) 10µg dDAVP was administered as a bolus over 60 seconds and (2) 30µg dDAVP was administered incrementally over a period of 60 minutes. The 10µg dose was previously shown to be the minimum dose able to induce vasopressinergic HPA axis activation, whereas an incremental dose of 30µg was expected to produce comparable peak dDAVP plasma levels (C_{MAX}) (Agerso et al., 2004). In this regard, it is important to note that the predictability of this approach is limited since previous pharmacokinetic analyses have reported more than 2-fold differences in clearance and 100-fold differences in C_{MAX} of i.v. administered dDAVP (Callreus et al., 1999; Fjellestad-Paulsen et al., 1996; Odeberg et al., 2004; Rembratt et al., 2004). At any rate, the use of two different infusion paradigms was to provide an impression on how the different peripheral and central effects of dDAVP were influenced by concentrations and infusion rates.

Methods

Study design

A randomized, double-blind, double-dummy infusion, placebo-controlled, three-way crossover study was performed in 12 healthy volunteers. The study protocol was approved by the Medical Ethics Committee of Leiden University Medical Centre (LUMC) and performed according to Good Clinical Practice and International Conference on Harmonisation guidelines.

Main outcome measures

The main pharmacodynamic (PD) outcome measures were dDAVP's (1) neuroendocrine effects (serum ACTH, cortisol)

and (2) cardiovascular effects (systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR)). Furthermore, dDAVP's effect on coagulation was assessed by measuring plasma Von Willebrand factor (vWF) and its antidiuretic effects were investigated by measuring serum osmolality. dDAVP-modulated ANS activation was assessed indirectly by measuring serum prolactin and saliva alpha amylase. Adverse events (AE's) were recorded throughout the trial.

Drug administration

The incremental infusion consisted of cumulative i.v. doses of dDAVP 30µg over 60 minutes (0min to 60min). The infusion rate was increased every five minutes for the first 30 minutes up an infusion rate of 40µg/h, after which the infusion rate remained constant at 40µg/h for the remaining 30 minutes. At 50min an intravenous bolus of 10µg dDAVP was administered over 60 seconds.

Volunteers

Twelve healthy volunteers (six male and six female) participated in the study. After obtaining written informed consent, the subjects underwent a full medical screening to assess eligibility. Volunteers were excluded from study participation if (1) using more than 4 units alcohol on average per day, (2) smoking more than 5 cigarettes per day, (3) using any drug (except oral contraceptives for females) or substance within one week before the first dosing, (4) using any drug or substance known to influence the metabolism of dDAVP in the month preceding the trial (5) demonstrating an ADAMTS-13 deficiency or presenting with a personal or family history of hypercoagulability as reflected by a diagnosis of coagulation factor deficiency (eg. Factor V Leiden mutation, APC resistance) or previous pulmonary embolism, deep venous thrombosis or other (arterial) cardiovascular disease, (6) presenting with a personal or family history of renal disease including diabetes insipidus and symptoms such as polydipsia, (7) presenting with a personal or a first degree family history of a clinical significant psychiatric disorder according to DSM-IV. Xanthine containing foods or

beverages, tobacco or alcohol were not allowed during the stay on the research unit. Concomitant medication other than paracetamol was not permitted during the study period.

dDAVP

DDAVP (Octostim ®) and placebo (0.9% NaCl) were prepared for administration by Profil GmbH, Neuss, Germany and were identical in appearance.

Study days

Volunteers arrived at the Centre for Human Drug Research (CHDR) on the evening before the study day. On admission urinary screening was performed for drugs of abuse using the OnCall™ Test, ACON laboratories, Inc. Rapid Assays for Drug Abuse (Instruchemie Hilversum B.V. the Netherlands) and qualitative color immunochromatographic urinary hCG was performed in all female subjects using the 'On Call' test device (Acon Laboratories Inc, San Diego, CA 92121, USA). Volunteers went to bed at 23.00 and were woken up around 8.00 the next morning after which a standardized breakfast was served. Two cannulas were inserted into the antecubital vein of each arm for blood sampling and intravenous administration of dDAVP. This occurred at least 1 hour preceding the first PD blood sampling to allow any potential procedure-related HPA axis activation to return to baseline. Fluid intake was restricted to 900 ml water administered at fixed time points and subjects remained supine on bed for the first four hours after start of the incremental infusion. At 0min either 30µg dDAVP or placebo was infused incrementally for 30 minutes followed by a constant infusion rate for 30 minutes. At 0min and 50min dDAVP or placebo was administered as described above. Administration of the i.v. infusions was performed under hospital conditions and a research physician attended all study occasions. At the end of the study day, volunteers were discharged from the research unit only after having produced urine. Adverse events were registered from spontaneous reports and hourly inquiries.

Biochemical measurements

Venous blood (1.2ml; prechilled EDTA tubes) was collected for the determination of plasma ACTH on -20, -10 before and 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 100, 110, 120, 140, 160, 180, 210, 240, 270 and 480 minutes after start of the incremental infusion. Samples were immediately placed on ice, processed within 30 minutes and stored at -80°C. Samples were analyzed within six weeks using the Immulite 2500 Analyzer Assay (EURO/DCP, United Kingdom) at the Central Laboratories of LUMC. Venous blood (1.2ml; serum tubes) for the assay of serum cortisol and prolactin was taken at -20, -10 before and 10, 20, 25, 30, 35, 40, 45, 55, 60, 65, 70, 75, 80, 90, 100, 120, 140, 160, 180, 210, 240, 270 and 480 minutes after start of the incremental infusion. Samples were stored for 30 – 45 minutes at ambient temperature to allow coagulation, subsequently centrifuged for 15 minutes at 2000 x g and stored at -20 °C. Samples were analyzed using Perkin Elmer AutoDelfia Testkits at Organon Development GmbH, Department of Bioanalytics, Waltrop, Germany. Venous blood (2 ml; EDTA tubes) was obtained for plasma dDAVP on -10 before and 10, 20, 25, 30, 35, 40, 45, 55, 60, 65, 70, 75, 80, 90, 120, 180, 240, and 480 after start of the incremental infusion. Samples were inverted, centrifuged within 15 min after collection for 15 minutes between 2000 – 3000 x g and stored at -20 °C. dDAVP was analyzed using LC-MS/MS at Xendo Drug Development BV, Groningen, The Netherlands. It appeared that the stability of dDAVP in human plasma, under the storage conditions used, was not demonstrated to cover the length of time from sample collection to analysis (accuracy against value at 0 min was >15%). Therefore, only relative and no absolute dDAVP concentrations are reported. 1.2ml venous blood was collected in citrate-0.106mol/l collection tubes for von Willebrand Factor (vWF) on -10 before and 30, 60, 90, 120 and 240 min after start of the incremental infusion. Samples were centrifuged within 30 minutes for 20 minutes at 2000xg and 4°C, frozen according to the “snap freeze” method and stored at -40°C. Samples were analyzed at the Laboratory of tno Pharma, Leiden, the Netherlands. 1.2ml blood was collected in serum clotting activator tubes for serum osmolality on -10, 30, 60, 90, 120, 150, 180, 240 min after start of the incremental

infusion. Samples were stored for 30 – 45 minutes at ambient temperature to allow coagulation, subsequently centrifuged for 10 minutes at 2000xg and 4°C and stored refrigerated until analysis at the end of the collection day. Samples were analyzed by molecular freezing-point depression with a Osmometer OM-6050 of Arkry (Japan) at the Central Laboratories of LUMC.

Vital signs

Blood pressure and pulse rate were measured continuously from 15 minutes prior until 4 h after start infusion using the Finapres methodology (Finapres Medical Systems BV, Amsterdam The Netherlands) and Nihon-Kohden (BSM-1100) or Colin (Pressmate BP-8800) blood pressure apparatus. Electrocardiogram (ECG) recordings were made at -30min and 180 min.

Data analysis

Neuroendocrine parameters (ACTH, cortisol), cardiovascular parameters (systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR)), serum osmolality, vWF and indirect ANS effects (prolactin, saliva alpha amylase) were analyzed by mixed model analyses of variance (using SAS PROC MIXED) with treatment, time, gender, gender-by-treatment and treatment-by-time as fixed effects, with subject and subject-by-time as random effect, and with the average baseline value as covariate. The neuroendocrine parameters, prolactin and serum osmolality were log-transformed prior to analysis. The contrasts between placebo and the bolus and between placebo and the incremental infusion were calculated over different time periods: for the incremental infusion over the period 0 to 180min, and for the bolus infusion over 50 to 180min since it was administered from 50min to 51min. Contrasts were reported as placebo vs. bolus infusion; placebo vs. incremental infusion and bolus infusion vs. incremental infusion. Results were presented as average differences compared to placebo (in percentages when back transformed from log-transformed variables) with 95% confidence intervals. The pharmacokinetic analysis of dDAVP consisted of determining the maximal mean plasma concentration (C_{MAX}).

Results

Subject disposition and demographic data

Eighteen volunteers were screened after having provided informed consent. Four subjects did not comply with the in- and exclusion criteria and were excluded from participation. Twelve volunteers received study medication of which one dropped out due an AE not related to study medication. The mean age of the volunteers was 21 years (range 18-27 years) and was similar for males and females. Females had a mean weight of 65kg (60-67kg) and mean height of 1.73m (range 1.68-1.84m). Males had a mean weight of 80kg (range 63-99kg) and mean height of 1.88 (range 1.83-1.90m).

Adverse events

All AE's were of mild to moderate intensity, transitory in nature and had mostly dissipated within 12 hours after drug administration. AE's were predictable based on the side-effect profile of dDAVP and no subjects discontinued participation directly due to related adverse effects. The most commonly occurring AE's were light-headedness (2/12 subjects), headache (2/12 subjects) and fatigue (2/12 subjects) for placebo; facial flushing(5/12 subjects), headache (5/12 subjects), fatigue (3/12 subjects) and dizziness (2/12 subjects) for the incremental infusion and facial flushing (8/11 subjects), dizziness (4/11 subjects) and headache (4/11 subjects) for the bolus infusion respectively.

Pharmacokinetics

In contrast to the expectation, a 1.8-fold higher maximal dDAVP concentration was reached during the incremental infusion compared to the bolus infusion. The maximal dDAVP concentration was reached 55 minutes after start of the incremental infusion and 5 minutes after the start of the bolus infusion, ie. at 55 minutes for both administration modes.

Neuroendocrine pharmacodynamic effects

Mean ACTH release was statistically significant for both the bolus +26.1 (5.8, 50.3)% and incremental +30.9 (11.1, 54.2)% infusions compared to placebo, but the difference between the two infusion regimens was not significant (Table 1). Maximal mean ACTH concentrations were also comparable with 15.85 ng/l and 15.02 ng/l for the bolus and incremental infusions respectively. The maximal mean ACTH concentration was attained at 55 min, 5 minutes after the C_{MAX} of dDAVP for both the bolus and incremental infusions. Mean cortisol levels increased with +18.9 (4.1, 35.8)% for the bolus and +17.7 (3.5, 33.8)% for the incremental infusion compared to placebo (Table 1). The maximal mean concentrations of cortisol, attained 15 minutes after those of ACTH, were very similar at 160.22 ng/ml and 158.25 ng/ml for the bolus and incremental infusions respectively. The effects on ACTH and cortisol are illustrated in figures 1 and 2.

Pro-coagulant – and anti-diuretic effects

vWF was released after dDAVP administration, with higher levels attained with the 30µg incremental infusion. Compared to placebo, the incremental infusion led to an increase of +104% (84,124). A smaller increase of +64.0% (41,86) was observed with the bolus infusion (Table 2). Although the levels of vWF-Ag tended to decrease from 120min onwards, they had not returned to baseline at the end of the measurement period (480min). Furthermore, serum osmolality was not altered by either the bolus or the incremental infusion.

Cardiovascular pharmacodynamic effects

The incremental infusion had larger blood pressure lowering effects than the bolus infusion. Heart rate increased compensatory with both infusions. These effects are illustrated best for DBP and HR in figures 3 and 4. After the bolus, rapid but short lasting circulatory effects were recorded. Similar maximum cardiovascular changes occurred during the incremental infusion, but these effects appeared and disappeared more gradually than after the bolus. Compared to placebo, the bolus infusion decreased average

SBP by -3.2 (-6.7, 0.3) mmHg and DBP by -7.2 (-10.2, -4.3) mmHg, and increased HR by +6.0 (1.2, 10.7) bpm. These effects were somewhat smaller than after the incremental infusion, which decreased average SBP by -5.3 (-8.6, -2.0) mmHg and DBP by -8.5 (-11.2, -5.8) mmHg, and increased HR by +9.7 (5.1, 14.4) bpm (Table 2).

Measures of dDAVP-induced ANS activation

Serum prolactin was not consistently affected by either dDAVP infusion. The incremental infusion decreased prolactin significantly by -15.4 (-23.5, -6.4) % while the bolus infusion increased prolactin non-significantly by +6.1 (-5.2, 18.8)% compared to placebo. Salivary alpha-amylase increased non-significantly by +11.4 (-14.5, 45.2)% following the incremental infusion and by +13.6 (-13.9, 50.0)% after the bolus infusion.

Discussion

This study aimed to concomitantly investigate neuroendocrine-, cardiovascular-, antidiuretic-, procoagulatory- and (indirect) ANS effects of i.v. dDAVP, administered using two different dosing regimens. dDAVP could be a useful pharmacological function tests of vasopressinergic activation of the HPA axis to study the involvement of this system in normal or abnormal (psycho)physiology and during treatment. The study showed differences in neuroendocrine, coagulatory and cardiovascular effects between a slow incremental infusion of 30µg and a rapid intravenous bolus of 10µg in healthy volunteers.

AVP is a physiological co-activator of the HPA-axis, mediated by V_3 -receptors on anterior pituitary corticotrophes and requiring the presence of endogenously available CRH to release ACTH (Dinan and Scott, 2005; Ring, 2005). We found small but statistically significant increases in ACTH and cortisol release after administering either 10µg dDAVP over one minute or 30µg dDAVP over 60 minutes. Both administration modes induced comparable elevations of ACTH and cortisol, despite an almost two-fold higher maximum dDAVP concentration reached with the incremental infusion compared to the bolus. The maximum serum cortisol concentrations of roughly 160 ng/ml after dDAVP were only 50 to 70% of the levels

attained previously with serotonergic and corticotrophinergic function tests. Meta-chlorophenylpiperazine (mCPP) 0.5 mg/kg administered i.v. lead to a cortisol C_{MAX} 228.4ng/ml (Gijssman et al., 1998), 5-hydroxytryptophan (5-HTP) 300mg administered orally induced a cortisol C_{MAX} of 218.9ng/ml (Smarius et al., 2008) and 100µg human CRH (hCRH) i.v. produced maximal serum cortisol concentrations of 210ng/ml (Dinan et al., 1999; Scott et al., 1999b). This indicates a higher maximum release of ACTH and cortisol following corticotrophinergic stimulation than after vasopressinergic co-activation. This is also illustrated in trials where dDAVP induced a dose-dependent ACTH release for 5µg and 10µg but not for 15µg dDAVP (Scott et al., 1999a) and CRH augmented the vasopressin-induced V_3 effects when administered concomitantly with dDAVP (Favrod-Coune et al., 1993). Such limited vasopressinergic co-activation may be determined by low levels of endogenous CRH, since this study was performed late in the morning and early afternoon, when circadian activity of the HPA-axis is relatively low.

The HPA-axis can also be activated by homeostatic perturbations, like hypotension causing an indirect stress responses via the sympatho-medullary route. dDAVP-induced blood pressure reduction seems to be dependent on V_2 stimulation, causing prostaglandin release from vascular endothelium (Aldasoro et al., 1997; Medina et al., 1999). A reduction in peripheral resistance can activate sympathetic outflow via fibres descending from the vasoconstrictor regions of the medulla oblongata. Concomitantly, direct stimulation of sympathetic outflow fibres terminating on the adrenal cortex can release adrenaline, which in turn might induce ACTH release via AVP neurons (Aldasoro et al., 1997; Scott and Dinan, 2002). On average, 10µg dDAVP reduced diastolic blood pressure (DBP) by 7.2 mmHg, and the incremental 30µg infusion by 8.5 mmHg in the current study. Heart rate showed average compensatory increases of 6.0 bpm after the bolus and 9.7 bpm with the infusion. It could be argued that even these small cardiovascular changes signify an activation of the autonomous nervous system (ANS), inducing ACTH release via alternative routes. However, indirect stress measures (saliva alpha amylase and serum prolactin) that indicate catecholamine-mediated ANS activity via the sympathoadrenal medullary route (Aldasoro et al., 1997; Rohleder et al., 2004) remained unaffected by either dDAVP administration mode.

Thus, dDAVP's effects on the HPA-axis do not seem to be mediated by peripheral stress effects, at least if subjects are kept supine during the test to minimize blood pressure lowering effects of dDAVP.

dDAVP displayed a differential concentration-effect relationship for the neuroendocrine parameters on the one hand and cardiovascular and coagulatory parameters on the other. dDAVP's v_2 -mediated effects (HR and vWF) were concentration dependent, which was not the case for its v_3 -mediated effects (ACTH and cortisol release). The maximal plasma concentrations (C_{MAX}) attained with 30 μ g dDAVP were nearly twice (1.8-fold) as large as those reached with 10 μ g dDAVP. This difference was reflected by the concentration-dependence of the cardiovascular and coagulatory effects, but not of the neuroendocrine responses, which were similar for the two doses.

The results suggest that 10 μ g dDAVP is a safe pharmacological function test in healthy volunteers and patients, at least if some potential restrictions are considered. Neither 10 μ g dDAVP nor 30 μ g dDAVP affected serum osmolality with fluid restriction, but dDAVP can cause a reduced osmolality if liberal water consumption is allowed. dDAVP clearly induced von Willebrand factor (vWF) release, but this is unlikely to produce coagulatory complications. The intravenous administration of dDAVP stimulates the release of vWF and factor VIII from the vascular endothelium via v_2 receptors (Lethagen, 1994). At the same time, dDAVP releases tissue plasminogen activator (tPA), which mitigates the prothrombotic effects of vWF (Burggraaf et al., 1994). vWF itself is degraded by the vWF cleaving protease ADAMTS13. Also, a twice higher dose of dDAVP compared to the present trial (0,3 μ g/kg vs. 0,13 μ g/kg) lead to (maximal) vWF release (Lethagen, 1994) without the occurrence of coagulation-related AE's in healthy volunteers. The prothrombotic effects of 10 μ g dDAVP thus appear to be self-limiting, at least in healthy volunteers. Susceptible patients however may be at an increased risk of thrombo-embolic events following dDAVP-induced vWF-elevations, although this is only suggested by rare case reports of patients who developed an acute myocardial infarction after administration of dDAVP for their hemophilia or Von Willebrand's disease (Follenius et al., 1982). Therefore, it is probably sensible to avoid higher doses of dDAVP, and to exclude patients with multiple cardiovascular risk factors or a (past) history of

ischemic heart disease or a (congenital) ADAMTS13 deficiency. In this study, dDAVP had its maximum neuroendocrine effects at a dose of 10µg, with limited coagulatory and cardiovascular manifestations. At this dose, the risk of thrombotic complications seems very small in patients who suffer from depression but have no clearly elevated cardiovascular risk.

In conclusion, administering 10µg dDAVP intravenously over one minute is an effective pharmacological tool to induce vasopressinergic HPA axis activation in healthy volunteers. Under low corticotrophinergic conditions, this function test produces a clear but limited co-activation of pituitary ACTH-release via the v_3 . A 10µg dDAVP bolus does not cause much systemic effects, and avoids non-specific stress responses. The effects on serum osmolality and coagulation are limited, although the safety in specific risk populations remains to be established. Higher doses of dDAVP are not expected to produce much more informative vasopressinergic co-activation, and could induce adverse and confounding v_2 -mediated effects.

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Table 1

Pharmacodynamic (PD) parameters for the period 50 min to 3 hours for the bolus infusion and over 3 hours for the incremental infusion and: Estimated means (back transformed Least Square Means) for plasma ACTH (ng/l), serum cortisol (ng/ml), serum prolactin (ng/ml) and saliva alpha-amylase (U/ml) for placebo, 10µg dDAVP bolus infusion and 30µg dDAVP incremental infusion. Estimated difference (%) with 95% confidence interval from placebo for 10µg dDAVP bolus infusion and 30µg dDAVP incremental infusion and the estimated difference (%) with 95% confidence interval between 10µg dDAVP bolus infusion and 30µg dDAVP incremental infusion.

| PD parameter | Back transformed Least Square Means (LSM) | | | Estimated difference with 95% Confidence interval (%) | | | |
|-----------------------------|---|----------------------------------|--|---|--|---|--------------------------------|
| | Placebo (n=12) | 10µg dDAVP bolus infusion (n=11) | 30µg dDAVP incremental infusion (n=12) | Treatment p-value | Bolus infusion vs placebo 50 – 180 min | Incremental infusion vs placebo 0 – 180 min | Bolus vs incremental |
| Plasma ACTH (ng/L) | 50 – 180 min 9.62 | 0 – 180 min 12.13 | 0 – 180 min 12.73 | 0.011 | +26.1 (5.8, 50.3) p=0.013 | +30.9 (11.1, 54.2) p=0.003 | +13.0 (-5.0, 34.4) p=0.155 |
| Serum cortisol (ng/mL) | 117.91 | 125.46 | 147.62 | 0.036 | +18.9 (4.1, 35.8) p=0.014 | +17.7 (3.5, 33.8) p=0.016 | +2.9 (-9.9, 17.5) p=0.66 |
| Serum prolactin (ng/mL) | 4.33 | 4.54 | 3.85 | 0.001 | +6.1 (-50.2, 18.8) p=0.281 | -15.4 (-23.5, -6.4) p=0.03 | -19.0 (-27.5, -9.5) p=0.001 |
| Saliva alpha-amylase (U/mL) | 110.71 | 107.56 | 119.81 | 0.689 | +13.6 (-13.9, 50.0) p=0.350 | +11.4 (-14.5, 45.2) p=0.404 | +7.9 (-18.0, 50.0) p=0.568 |

Table 2

Cardiovascular, coagulatory and anti-diuretic parameters for the period 50 min to 3 hours for the bolus infusion and over 3 hours for the incremental infusion: Estimated difference (%) with 95% confidence interval from placebo for 10µg dDAVP bolus infusion and 30µg dDAVP incremental infusion and the estimated difference (%) with 95% confidence interval between 10µg dDAVP bolus infusion and 30µg dDAVP incremental infusion for systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), von Willebrand factor (vWF) and serum osmolality (mOsmol/kg)

| Parameter | Estimated difference with 95% Confidence interval (%) | | |
|------------------------------|---|---|---------------------------|
| | Bolus infusion vs placebo 50 – 180 min | Incremental infusion vs placebo 0 – 180 min | Bolus vs incremental |
| SBP (mmHg) | -3.2 (-6.7, 0.3) p=0.0715 | -5.3 (-8.6, -2.0) p=0.0036 | -2.3 (-5.7, 1.1) p=0.1722 |
| DBP (mmHg) | -7.2 (-10.2, -4.3) p<.0001 | -8.5 (-11.2, -5.8) p<.0001 | -2.1 (-5.0, .08) p=0.1473 |
| HR (bpm) | +6.0 (1.2, 10.7) p=0.016 | +9.7 (5.1, 14.4) p=0.000 | +5.7 (1.0, 10.4) p=0.020 |
| vWF (%) | +64.0 (41, 86) p<0.000 | +104.0 (84, 124) p<0.000 | +58.0 (36, 80) p<0.000 |
| Serum osmolality (mOsmol/kg) | -0.2 (-0.6, 0.3) p=0.446 | -0.2 (-0.6, 0.1) p=0.199 | -0.1 (-0.6, 0.4) p=0.644 |

Figure 1

Average concentration time profile of plasma adrenocorticotrophic hormone (ACTH) with SD error bars (closed circle: Placebo; open circle: Incremental infusion; open square: Bolus infusion)

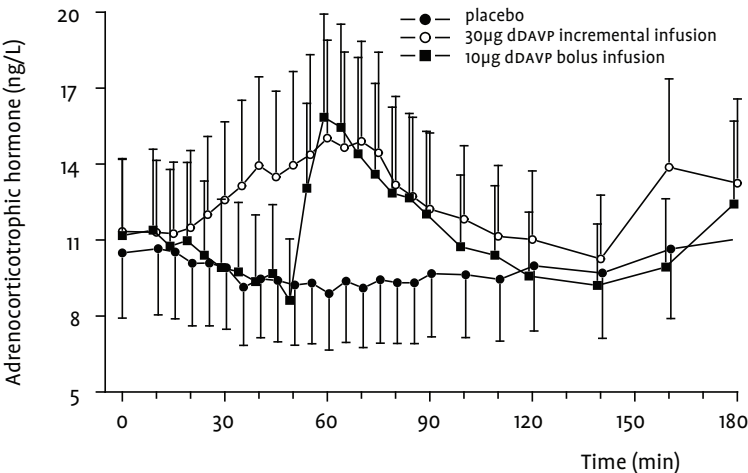


Figure 2

Average concentration time profile of serum cortisol with SD error bars (closed circle: Placebo; open circle: Incremental infusion; open square: Bolus infusion)

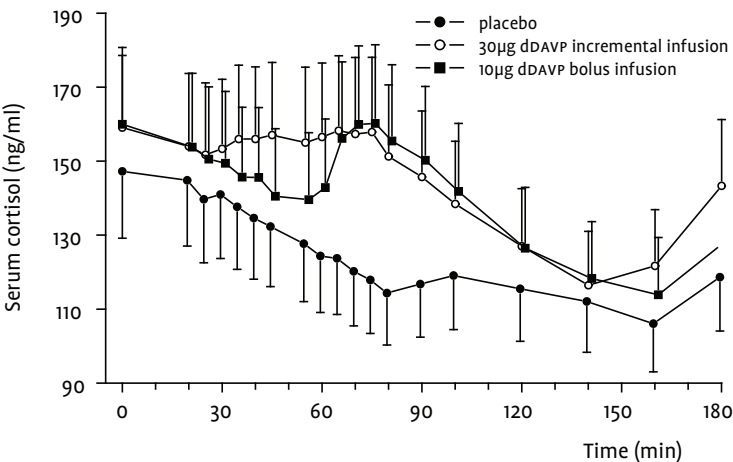


Figure 3

Average concentration time profile of diastolic blood pressure (DBP) with SD error bars (closed circle: Placebo; open circle: Incremental infusion; open square: Bolus infusion)

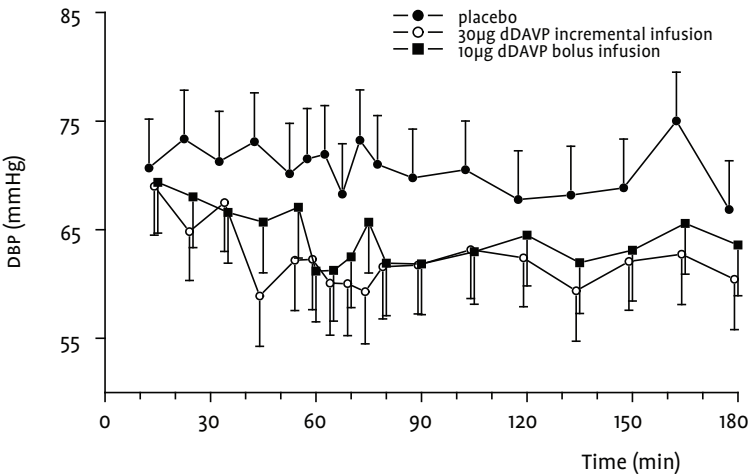
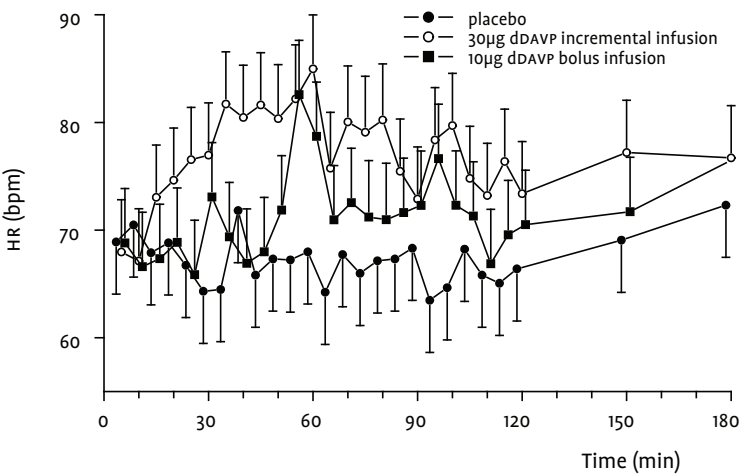


Figure 4

Average concentration time profile of heart rate (HR) with SD error bars (closed circle: Placebo; open circle: Incremental infusion; open square: Bolus infusion)



REFERENCE LIST

- Agerso H, Seiding LL, Riis A, Lovgren U, Karlsson MO, Senderovitz T (2004) Pharmacokinetics and renal excretion of desmopressin after intravenous administration to healthy subjects and renally impaired patients. *Br J Clin Pharmacol* 58: 352-358.
- Aguilera G, Rabadan-Diehl C (2000) Vasopressinergic regulation of the hypothalamic-pituitary-adrenal axis: implications for stress adaptation. *Regul Pept* 96: 23-29.
- Aldasoro M, Medina P, Vila JM, Otero E, Martinez-Leon JB, Lluch S (1997) Endothelium-dependent relaxation of human saphenous veins in response to vasopressin and desmopressin. *J Vasc Surg* 25: 696-703.
- Burggraaf J, Schoemaker HC, Kroon JM, Huisman L, Kluit C, Cohen AF (1994) Influence of 1-desamino-8-D-vasopressin on endogenous fibrinolysis, haemodynamics and liver blood flow in healthy subjects. *Clin Sci (Lond)* 86: 497-503.
- Callreus T, Odeberg J, Lundin S, Hoglund P (1999) Indirect-response modeling of desmopressin at different levels of hydration. *J Pharmacokinet Biopharm* 27: 513-529.
- Craighead M, Milne R, Campbell-Wan L, Watson L, Presland J, Thomson FJ, Marston HM, Macsweeney CP (2008) Characterization of a novel and selective V_{3B} receptor antagonist. *Prog Brain Res* 170: 527-535.
- Dinan TG, Lavelle E, Scott LV, Newell-Price J, Medbak S, Grossman AB (1999) Desmopressin normalizes the blunted adrenocorticotropin response to corticotropin-releasing hormone in melancholic depression: evidence of enhanced vasopressinergic responsivity. *J Clin Endocrinol Metab* 84: 2238-2240.
- Dinan TG, O'Brien S, Lavelle E, Scott LV (2004) Further neuroendocrine evidence of enhanced vasopressin V_3 receptor responses in melancholic depression. *Psychol Med* 34: 169-172.
- Dinan TG, Scott LV (2005) Anatomy of melancholia: focus on hypothalamic-pituitary-adrenal axis overactivity and the role of vasopressin. *J Anat* 207: 259-264.
- Evans DL, et al. (2005) Mood disorders in the medically ill: scientific review and recommendations. *Biol Psychiatry* 58: 175-189.
- Favrod-Coune C, Raux-Demay MC, Proeschel MF, Bertagna X, Girard F, Luton JP (1993) Potentiation of the classic ovine corticotrophin releasing hormone stimulation test by the combined administration of small doses of lysine vasopressin. *Clin Endocrinol (Oxf)* 38: 405-410.
- Fjellestad-Paulsen A, d'Agay-Abensour L, Hoglund P, Rambaud JC (1996) Bioavailability of 1-deamino-8-D-arginine vasopressin with an enzyme inhibitor (aprotinin) from the small intestine in healthy volunteers. *Eur J Clin Pharmacol* 50: 491-495.
- Follenius M, Brandenberger G, Hietter B, Simeoni M, Reinhardt B (1982) Diurnal Cortisol Peaks and Their Relationships to Meals. *Journal of Clinical Endocrinology and Metabolism* 55: 757-761.
- Gijssman HJ, van Gerven JM, Tieleman MC, Schoemaker RC, Pieters MS, Ferrari MD, Cohen AF, van Kempen GM (1998) Pharmacokinetic and pharmacodynamic profile of oral and intravenous meta-chlorophenylpiperazine in healthy volunteers. *J Clin Psychopharmacol* 18: 289-295.
- Holsboer F (1983) The dexamethasone suppression test in depressed patients: clinical and biochemical aspects. *J Steroid Biochem* 19: 251-257.
- Holsboer F (2000) The corticosteroid receptor hypothesis of depression. *Neuropsychopharmacology* 23: 477-501.
- Lethagen S (1994) Desmopressin (DDAVP) and hemostasis. *Ann Hematol* 69: 173-180.
- Medina P, Segarra G, Vila JM, Chuan P, Domenech C, Lluch S (1999) V_2 -receptor-mediated relaxation of human renal arteries in response to desmopressin. *Am J Hypertens* 12: 188-193.
- Musselman DL, Betan E, Larsen H, Phillips LS (2003) Relationship of depression to diabetes types 1 and 2: epidemiology, biology, and treatment. *Biol Psychiatry* 54: 317-329.
- Odeberg JM, Callreus T, Lundin S, Roth EB, Hoglund P (2004) A pharmacokinetic and pharmacodynamic study of desmopressin: evaluating sex differences and the effect of pre-treatment with piroxicam, and further validation of an indirect response model. *J Pharm Pharmacol* 56: 1389-1398.
- Pariante CM, Lightman SL (2008) The HPA axis in major depression: classical theories and new developments. *Trends Neurosci* 31: 464-468.
- Ramasubbu R (2002) Insulin resistance: a metabolic link between depressive disorder and atherosclerotic vascular diseases. *Med Hypotheses* 59: 537-551.
- Rembratt A, Graugaard-Jensen C, Senderovitz T, Norgaard JP, Djurhuus JC (2004) Pharmacokinetics and pharmacodynamics of desmopressin administered orally versus intravenously at daytime versus night-time in healthy men aged 55-70 years. *Eur J Clin Pharmacol* 60: 397-402.
- Ring RH (2005) The central vasopressinergic system: examining the opportunities for psychiatric drug development. *Curr Pharm Des* 11: 205-225.

- Rohleder N, Nater UM, Wolf JM, Ehler U, Kirschbaum C (2004) Psychosocial stress-induced activation of salivary alpha-amylase: an indicator of sympathetic activity? *Ann N Y Acad Sci* 1032: 258-263.
- Scott LV, Dinan TG (1998) Vasopressin and the regulation of hypothalamic-pituitary-adrenal axis function: implications for the pathophysiology of depression. *Life Sci* 62: 1985-1998.
- Scott LV, Dinan TG (2002) Vasopressin as a target for antidepressant development: an assessment of the available evidence. *J Affect Disord* 72: 113-124.
- Scott LV, Medbak S, Dinan TG (1999a) ACTH and cortisol release following intravenous desmopressin: a dose-response study. *Clin Endocrinol (Oxf)* 51: 653-658.
- Scott LV, Medbak S, Dinan TG (1999b) Desmopressin augments pituitary-adrenal responsiveness to corticotropin-releasing hormone in subjects with chronic fatigue syndrome and in healthy volunteers. *Biol Psychiatry* 45: 1447-1454.
- Smarius LJ, Jacobs GE, Hoeberechts-Lefrandt DH, de Kam ML, van der Post JP, de Rijk R, van Pelt J, Schoemaker RC, Zitman FG, van Gerven JM, Gijsman HJ (2008) Pharmacology of rising oral doses of 5-hydroxytryptophan with carbidopa. *J Psychopharmacol* 22: 426-433.
- Spiga F, Harrison L, Macsweeney C, Thomson F, Craighead M, Lightman S (2008) Effect of vasopressin 1b receptor blockade on the hypothalamic-pituitary-adrenal response of chronically stressed rats to a heterotypic stressor. *J Endocrinol.* 2009 Mar;200(3):285-91. Epub 2008 Dec 12.

A pharmacological tool to assess vasopressinergic co-activation of the hypothalamus–pituitary–adrenal (HPA) axis more integrally in healthy volunteers

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Abstract

BACKGROUND Pharmacological function tests consisting of 100µg hCRH (corticotropin-releasing hormone) and 10µg dDAVP (desmopressin) mimic endogenous HPA-axis activation. However, physiological CRH concentrations preclude informative vasopressinergic co-activation (using dDAVP) and independent quantification of both corticotrophinergic (using hCRH) and vasopressinergic (using dDAVP) activation is limited due to administration on separate occasions.

OBJECTIVES This randomized, double-blind, placebo-controlled, partial five-way crossover study in healthy males and females (six: six) examined whether (1) concomitant administration of dDAVP and hCRH provides more informative vasopressinergic co-activation than dDAVP alone and (2) whether the administration of dDAVP followed two hours later by hCRH can quantify both vasopressinergic and corticotrophinergic activation on a single test day.

RESULTS 10µg dDAVP combined with 10µg and 30µg hCRH caused dose-related ACTH- and cortisol-release which was larger than with 10µg dDAVP alone and respectively comparable to and greater than that induced by 100µg hCRH. 10µg dDAVP alone demonstrated limited ACTH-release while the effects of 100µg hCRH two hours later were three times as large. ACTH and cortisol released by 10µg dDAVP returned to baseline prior to 100µg hCRH administration and dDAVP did not influence the response to subsequent hCRH administration.

CONCLUSIONS Dose-related vasopressinergic co-activation of the HPA-axis was induced by combining 10µg dDAVP with 10µg and 30µg hCRH. 10µg dDAVP combined with 10µg hCRH induced the potentially most informative vasopressinergic co-activation since it is not restricted by ceiling- or flooring-effects. The hCRH-response was not affected by prior dDAVP, allowing for a practical function test examining both HPA-activation routes on the same day.

Introduction

The hypothalamic neuropeptides corticotrophic release hormone (CRH) and vasopressin (AVP) modulate activation of the hypothalamus-pituitary-adrenal axis (HPA), releasing adrenocorticotrophic hormone (ACTH) from pituitary corticotrophes and subsequently cortisol from the adrenal cortices. Under physiological circumstances, CRH acts as major neuroendocrine secretagogue via pituitary CRH₁ receptors (CRH₁) (inducing corticotrophinergic activation) while AVP has weak neuroendocrine properties at pituitary vasopressin 3 receptors (V₃) (inducing vasopressinergic co-activation) (Ring, 2005; Scott and Dinan, 1998). However, following acute psychological and/or physical stress, AVP synergistically releases ACTH in the presence of increased levels of CRH, inducing an up to 30 times higher ACTH release compared to the effects of CRH alone (vasopressinergic co-activation) (DeBold et al., 1984; Favrod-Coune et al., 1993; Lamberts et al., 1984). After disappearance of a stressful insult, the HPA axis returns to basal activity via cortisol feedback and AVP again resumes its role as minor activator (de Kloet et al., 2005; Holsboer, 2000). In some stress-related psychiatric disorders, failure of the HPA axis to “reset” after stress has been attributed to chronic vasopressinergic hyperactivity or sustained vasopressinergic co-activation (Ring, 2005; Scott and Dinan, 1998; Holsboer et al., 1984; Holsboer and Ising, 2008; Scott et al., 1999b). A pharmacological function test quantifying vasopressinergic co-activation would therefore be useful to examine functional vasopressinergic disturbances of the HPA axis.

The synthetic analogue of AVP, desmopressin (dDAVP), is frequently used as a pharmacological function test for vasopressinergic functionality (Craighead et al., 2008). Infusing 10 µg dDAVP over 60 seconds induces a neuroendocrine response at the V₃ receptor, without causing clinically relevant confounding effects via other AVP receptors (Jacobs et al., 2009). However, ACTH and cortisol release induced by dDAVP alone is small and (frequently) variable (Dinan and Scott, 2005; Scott et al., 1999b; Jacobs et al., 2009). Without addition of hCRH, 10 µg dDAVP administered as a bolus over one minute induces a maximal V₃-mediated ACTH release of 16 ng/l, which is virtually equal to the increase (15 ng/l) induced by 30 µg dDAVP administered incrementally over one hour (Jacobs et

al., 2009). These increases are small compared to corticotrophinergic HPA activation with intravenous (i.v.) human corticotrophic release hormone (hCRH) or oral administration of the serotonergic precursor agent 5-hydroxytryptophan (5-HTP) (Dinan et al., 1999; Scott et al., 1999b) (Smarius et al., 2008). At maximally tolerated doses, these interventions induce maximal serum cortisol concentrations (C_{MAX}) that are roughly 90 – 100% higher than those induced by 10 μ g dDAVP alone. In these experiments, the effects of dDAVP alone seem to be limited by the low levels of endogenous CRH. Thus, since AVP is a CRH-dependent co-activator of the HPA axis, it might be better to study vasopressinergic co-activation by administration of dDAVP in combination with hCRH.

Corticotrophinergic (via CRH_1) and vasopressinergic (via v_3) HPA axis activation have been quantified previously, most frequently by investigating the effects of dDAVP and hCRH separately on three (including placebo) different study days (Dinan et al., 1999; von Bardeleben and Holsboer, 1988). Since such an approach would be unfeasible in most studies in (depressed) patients, the current study examined whether it would be possible to address the most important aspects of pituitary ACTH-release (vasopressinergic co-activation and corticotrophic responsiveness alone) in a single short study occasion. Independent evaluations of each route would require an adequate washout period, to ensure that v_3 stimulation does not affect subsequent CRH_1 stimulation (or vice versa). A minimal washout period after a dDAVP challenge would at least require a full return of ACTH levels to baseline, which according to previous studies occurs after 90 minutes (Jacobs et al., 2009; Scott et al., 1999a).

To examine these issues, we performed a single trial consisting of two separate experiments divided by two hours. In experiment A we examined whether vasopressinergic co-activation could be quantified by simultaneously adding small amounts of hCRH (either 10 μ g or 30 μ g hCRH) to a vasopressinergic function test consisting of 10 μ g dDAVP in healthy volunteers. Experiment B examined the effects of hCRH alone, and potential interaction effects of prior dDAVP administration on corticotrophinergic activation by administration of 100 μ g hCRH two hours later. To compare the two routes of ACTH-activation, the ACTH-release by 100 μ g hCRH alone in experiment B was used as benchmark for the effects of the combinations in experiment A. This dose was chosen since 100 μ g

hCRH has often been used in the literature, whereas the effects of lower doses (such as those used in experiment A in combination with dDAVP) were unknown. This attempt to characterize both routes of ACTH-stimulation on the same day had several practical reasons. The design restricted the burden for subjects and the amount of blood sampling, and is anticipated to be more acceptable than separate study days for patients in future clinical trials.

Methods

Study design

The study protocol was approved by the Medical Ethics Committee of Leiden University Medical Centre and performed according to Good Clinical Practice and International Conference on Harmonisation guidelines. A randomized, double blind, placebo-controlled, partial five-way crossover trial was performed in 12 healthy volunteers. Study medication was administered intravenously over 30 seconds, at two time points separated by two hours ($t=0\text{min}$ and $t=120\text{min}$). In this way, two different experiments could be performed on the same day (*Figure 1*). Experiment A examined modulation of vasopressinergic co-activation by concomitant administration of different doses of hCRH with dDAVP at $t=0\text{min}$. Experiment B examined the effect of corticotrophinergic activation alone, and any carry-over effects of vasopressinergic co-activation after two hours, by first administering dDAVP or placebo followed by hCRH at $t=120\text{min}$. The main pharmacodynamic (PD) outcome measures for both experiments were changes in the concentrations of serum ACTH, cortisol and prolactin. Also, adverse events (AE's) were recorded to assess the safety and tolerability of concomitantly administering dDAVP and hCRH.

Volunteers

Twelve healthy volunteers (six male and six female) participated in the study. After providing their written informed consent volunteers received a full medical examination during a pre-study screening. Volunteers were excluded from study participation if (1) using more than 4 units alcohol on average per day, (2) smoking

more than 5 cigarettes per day, (3) using any drug or substance within one week before the first dosing, (4) using any drug or substance known to influence the metabolism of dDAVP in the month preceding the trial (5) demonstrating an ADAMTS-13 deficiency or presenting with a personal or family history of hypercoagulability as reflected by a diagnosis of coagulation factor deficiency (eg. Factor V Leiden mutation, APC resistance) or previous pulmonary embolism, deep venous thrombosis or other (arterial) cardiovascular disease, (6) presenting with a personal or family history of renal disease including diabetes insipidus and symptoms such as polydipsia, (7) presenting with a personal or a first degree family history of a clinical significant psychiatric disorder according to DSM-IV. No xanthine containing foods or beverages, tobacco or alcohol were allowed during the stay on the research unit. Concomitant medication other than paracetamol was not permitted during the study period.

dDAVP and hCRH

Identical syringes containing either dDAVP (Octostim®) or 0.9% NaCl as placebo were prepared for administration by the Department of Clinical Pharmacy and Toxicology of the Leiden University Medical Centre (LUMC). hCRH (Ferring CRH®) was provided by the Department of Clinical Pharmacy and Toxicology of the LUMC and identical syringes containing either hCRH or hCRH solvent as placebo were prepared for administration on-site at CHDR by trained staff not directly involved in the trial. Due to hCRH's rapid degradation after preparation, a maximal limit of 6 minutes was allowed between its preparation and administration to the volunteers. If any delay caused transgression of this limit, hCRH was prepared again and administered within 6 minutes.

Study days

Volunteers arrived at the Centre for Human Drug Research (CHDR) on the evening preceding each study day. On admission urinary screening was performed for drug use on all volunteers (tetrahydrocannabinol (THC), cocaine, morphine, benzodiazepines, 3,4-methylenedioxymethamphetamine (MDMA)/metamphetamine and

amphetamines) using the OnCall™ Test, ACON laboratories, Inc. Rapid Assays for Drug Abuse (Instruchemie Hilversum B.V. the Netherlands). In all females hCG was qualitatively measured in urine using the 'On Call' test device (Acon Laboratories Inc, San Diego, CA 92121, USA). Volunteers went to bed at 23.00 and were woken up around 8.00 the next morning after which a standardized breakfast was served. Two cannulas were inserted into the antecubital vein of each arm for blood sampling and intravenous administration of study medication. This occurred at least 1 hour preceding the first PD blood sampling to allow any puncture-induced HPA axis activation to return to baseline. Fluid intake was restricted to a total of 900 ml until the first urination infusion and only water was administered on fixed time points during the study day. Administration of the intravenous infusions was performed under hospital conditions and a research physician attended all study days. At the end of a study day, volunteers were discharged from the research unit only after having produced urine.

Biochemical measurements and vital signs on study days

1.2ml venous blood was collected in prechilled EDTA collection tubes for the determination of plasma ACTH on -60, -10 before and 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 75, 90, 120, 125, 130, 135, 140, 145, 150, 155, 160, 170, 180, 195, 210, 240, 270, 300, 360, 390, 420 and 510 minutes after the administration of the infusion at $t=0h$. Samples were immediately placed on ice, processed within 30 minutes and stored at $-80^{\circ}C$. Samples were analyzed within six weeks using the Immulite 2500 Analyzer (Siemens, Germany) at the Laboratory for Clinical Chemistry, Leiden University Medical Centre (LUMC), Leiden, the Netherlands. 1.2ml venous blood was collected in serum collection tubes for the determination of serum cortisol and prolactin on -60, -10 before and 10, 20, 30, 40, 50, 60, 75, 90, 120, 130, 140, 150, 160, 180, 195, 210, 240, 300, 360, 390, 420 and 510 minutes after start of the incremental infusion. Samples were stored for 30 – 45 minutes at ambient temperature to allow coagulation, subsequently centrifuged for 15 minutes at $2000 \times g$ and stored at $-20^{\circ}C$. Samples were analyzed using Perkin Elmer AutoDelfia at Organon Development GmbH, Department of Bioanalytics, Waltrop, Germany. Blood pressure and pulse rate were measured at fixed

timepoints using the Nihon-Kohden (BSM-1100) or Colin (Pressmate BP-8800) blood pressure apparatus. Electrocardiogram (ECG) recordings were made at -30 and 180 min after infusion.

Pharmacodynamic (PD) analysis

The PD endpoints (ACTH, cortisol, prolactin) for 12 volunteers were log-transformed and analyzed by mixed model analyses of variance (using SAS PROC MIXED) with treatment, time, gender, gender-by-treatment and treatment-by-time as fixed effects, with subject and subject-by-time as random effect, and with the average baseline value as covariate. Since the trial consisted of two experiments, the PD effects were analyzed over two different time periods. To examine the effects of 10 µg and 30 µg hCRH on 10 µg dDAVP-induced vasopressinergic HPA axis activation (experiment A), PD effects were analyzed over the period 0 to 90 min. This analysis period was based on a previous investigation of the PD effects of 10 µg dDAVP, showing that ACTH and cortisol had returned to placebo levels within 90 minutes after its administration. To examine the effect of 100 µg hCRH on corticotrophinergic HPA axis activation and the effect of vasopressinergic HPA axis activation using 10 µg dDAVP two hours earlier (experiment B), PD effects were analyzed over the period 120 to 510 min since 100 µg hCRH was administered at $t=120$ min. To put the vasopressinergic-corticotrophinergic co-activation in perspective, the mean maximal concentrations (C_{MAX}) of ACTH and cortisol of 100 µg hCRH in experiment B were compared to those of 10 µg and 30 µg hCRH with 10 µg dDAVP in experiment A. Neuroendocrine results were presented as estimates of percentual difference from placebo, with 95% confidence intervals and mean maximal concentrations (C_{MAX}). Adverse events were registered from spontaneous reports and hourly inquiries.

Results

Subject disposition and demographic data

Thirteen volunteers were screened after having provided informed consent. Twelve volunteers received study medication of which one dropped out due an AE not related to study medication. The mean

age of the volunteers was 21 years (range 18-27 years), which was similar for males and females. Females had a mean weight of 65kg (60-67kg) and mean height of 1.73m (range 1.68-1.84m). Males had a mean weight of 80kg (range 63-99kg) and mean height of 1.88 (range 1.83-1.90m).

Adverse events

All AE's were of mild to moderate intensity, transitory in nature and had dissipated within 12 hours after drug administration. AE's were predictable based on the side-effect profiles of hCRH and dDAVP and no subjects discontinued participation directly due to related adverse effects. Since the side-effects of both drugs are very much alike, it was not possible to readily identify the causating agent when dDAVP and hCRH were administered simultaneously. At any rate, the administration of dDAVP and hCRH together, either simultaneously or sequentially did not influence the intensity or occurrence of AE's. The most commonly occurring AE's were headache (2/12 subjects) for placebo; facial flushing (10/12 subjects), palpitations (4/12 subjects), dizziness (2/12 subjects) and headache (1/12 subjects) for 100 µg hCRH; facial flushing (8/12 subjects), palpitations (3/12 subjects), dizziness (2/12 subjects) and headache (2/12 subjects) for 100 µg hCRH preceded by 10 µg dDAVP; facial flushing (6/12 subjects), palpitations (1/12 subjects) and headache (1/12 subjects) for 10 µg dDAVP combined with 10 µg hCRH and facial flushing (8/12 subjects), headache (3/12 subjects), palpitations (2/12 subjects) and dizziness (1/12 subjects) for 10 µg dDAVP combined with 30 µg hCRH.

Neuroendocrine effects

In experiment A, ACTH increased by +34.6(8.7, 66.7, $p=0.0075$)%, ($C_{MAX}19.3\text{ng/l}$) with 10 µg dDAVP; by +154 (105, 213, $p<0.0001$)%, ($C_{MAX}56.7\text{ng/l}$) after 10 µg dDAVP combined with 10µg CRH; and by +242(177, 322, $p<0.0001$)%, ($C_{MAX}75.2\text{ng/l}$) following 10 µg dDAVP combined with 30µg hCRH (Table 1; Figures 2 and 3). Cortisol increased similarly by +18.2(-2.4, 42.9)%, ($C_{MAX}114.2\text{ ng/ml}$) with 10 µg dDAVP; by +76.7(46.1, 114)%, ($C_{MAX}172\text{ ng/ml}$) after 10 µg dDAVP combined with 10µg hCRH; and by +113(76.5, 158)%,

(C_{MAX} 192 ng/ml) following 10 µg dDAVP combined with 30µg hCRH. (Table 1; Figure 4).

In experiment B, ACTH increased by +88.0(58.4, 145), $p<0.0001$)% (C_{MAX} 59.2 ng/l) after 100µg hCRH, and by +104(70.5, 145., $p<0.0001$)% (C_{MAX} 70.4 ng/l) with 100 µg hCRH preceded by 10 µg dDAVP. The difference between these two responses was not statistically significant [+8.7(-9.0, 29.9)% $p=0.340$)] (Table 2; Figures 2 and 5). Cortisol increased similarly by +40.0(25.5, 56.1), $p<0.0001$)% (C_{MAX} 196 ng/ml) following 100 µg hCRH and by +45.7 (30.2, 63.1), $p<0.0001$)% (C_{MAX} 209 ng/ml) after 100 µg hCRH preceded by 10 µg dDAVP. This difference was also not statistically significant [+4.1 (-6.7, 16.1), $p=0.425$)] (Table 2; Figure 6).

The ACTH increases after 10 µg or 30 µg hCRH combined with 10 µg dDAVP of respectively 154% and 242%, were larger than the increases of 88.0% and 104% following 100 µg hCRH.

Discussion

AVP is believed to enhance CRH₁-associated cAMP production in pituitary corticotrophes by stimulating v_3 , potentiating the effect of CRH and leading to a synergistic release in ACTH and subsequently cortisol (Scott and Dinan, 1998; DeBold et al., 1984; Favrod-Coune et al., 1993). A full pharmacological characterisation of this co-activation, including a quantitative description of the individual and (supra)additive components of the system, would have required a complete assessment of all interactions between dDAVP and hCRH, including different doses. Although undoubtedly useful, such a study would be very difficult to perform, even in healthy volunteers and much more so in patients. The current study was not designed for these purposes, but it provided clear indications for a dose-related co-activation, and showed that the effects of low doses of hCRH are boosted by dDAVP to the levels of a much higher dose of hCRH alone. Administration of 10 µg dDAVP alone induced a maximal ACTH concentration of 19.3 ng/l. The addition of 10 µg hCRH produced an ACTH-peak of 56.7 ng/l, which did not differ much from the effect of a ten-fold higher dose of 100 µg hCRH alone (59.2 ng/l). The combination with 30 µg hCRH caused a C_{MAX} of 75.2 ng/l, which was on average even larger than with 100 µg hCRH. Very similar results were found for cortisol, where the maximal

levels induced by dDAVP alone or combined with 10 µg and 30 µg hCRH were 60%, 90% and 98% of those produced by 100 µg hCRH alone. This clearly shows that dDAVP potentiates the pituitary release of ACTH caused by exogenous hCRH in a dose-related manner. The results also show that the effects of 10 µg dDAVP alone are limited to a C_{MAX} of 19.3 ng/l, possibly reflecting the relatively low endogenous hCRH levels in healthy volunteers during the morning.

Instead of administering 100 µg hCRH in a separate treatment arm, it was given two hours later than 10 µg dDAVP. This relatively short washout period was mainly chosen to investigate whether a vasopressinergic and corticotrophinergic function test could be combined in half a day, in order to reduce the burden for patients in future clinical trials. Previous studies showed that ACTH-release following dDAVP alone return to baseline within 90 min (Jacobs et al., 2009; Scott et al., 1999a), and this was confirmed in the current study for dDAVP combined with hCRH. A longer washout duration could also have introduced differences related to diurnal fluctuations, which were probably less important within this late morning two-hour period. The results show that the vasopressinergic effects of a single dose of 10 µg dDAVP are small and have disappeared after two hours. Such a dose does not have a significant interaction with 100 µg hCRH administered 120min later in healthy volunteers. This allows for a fairly independent assessment of the corticotrophic component of ACTH-stimulation, after determination of the vasopressinergic co-activation using a dDAVP-challenge earlier the same day. The difference in ACTH- and cortisol release induced by hCRH with or without preceding dDAVP was on average less than 10%. Although this difference is statistically non-significant, a small augmentation of the hCRH effect by administering dDAVP two hours earlier is not fully precluded. However, this potential carry-over effect is no more than a fraction of the effects of the concomitant administration of dDAVP and hCRH. It should be realized however that the optimal challenge interval to avoid confounding carry-over effects still needs to be confirmed in patients. In patients with vasopressinergic hyperactivity the effects of dDAVP alone will be determined by both V_3 receptor hypersensitivity and (increased) endogenous CRH-levels. Under these circumstances, the carry-over effects may be larger and therefore might require a longer wash-out period between

subsequent administrations. On the other hand, co-administration of dDAVP and hCRH in the present trial pharmacologically mimicked

a vasopressinergic hypersensitivity analogous to that expected in patients. Despite a clear augmentation of the maximal ACTH and cortisol responses in experiment A, their return to baseline levels remained unaffected and there was no detectable influence on the effects of hCRH in part B. In practice therefore, 10 µg dDAVP with 10 µg or 30 µg hCRH followed by hCRH alone after two hours, is also expected to provide useful information both on vasopressinergic and corticotrophinergic activity of the HPA-axis in patients. Moreover, the application of a function test in this form in patients could be used to detect unexpected interactions between v_3 and CRH_1 by comparing baseline values and normative responses.

An important goal of this study programme is to develop a reliable and practical pharmacological function test to quantify vasopressinergic co-activation. Such a function test could be used to demonstrate treatment effects of novel pharmacological agents modulating the v_3 receptor (Spiga et al., 2008b; Spiga et al., 2008a), and ultimately it can be used to detect functional differences between (patient) groups. In this regard, it is important to induce co-activation that is neither too small nor too large (Gijsman et al., 2004). If a function test would produce suboptimal stimulation, it would be difficult to detect differences between patient groups or treatment effects. On the other hand, maximal or supramaximal stimulation may obscure changes or differences in vasopressinergic sensitivity by means of ceiling effects. The ideal dose combination to quantify vasopressinergic/corticotrophinergic co-activation can only be determined from the full dose-response curves of hCRH and dDAVP and their combinations. Although such data are not available, our studies provide indications that 10 µg dDAVP with 10 µg hCRH may actually constitute a reasonable combination. We showed that the 10 µg dDAVP function test (in the presence of physiological CRH concentrations) induces small maximal cortisol concentrations of 120 ng/ml in the present study and 140 ng/ml in a previous study (Jacobs et al., 2009). A dose of 30 µg hCRH administered alone has previously been reported to induce maximal cortisol concentrations of 134 ng/ml (Oelkers et al., 1988). Considering the C_{MAX} of 196 ng/ml after the 100 µg dose in the current study,

50 µg hCRH should produce a readily detectable HPA-activation above baseline, but clearly lower than that caused by 100 µg. Addition of 10 µg hCRH to 10 µg dDAVP increases cortisol levels to 172 ng/ml, which is still below E_{MAX} -values in healthy volunteers found with other pharmacological function tests. For example, the administration of 100 µg hCRH previously demonstrated maximal serum cortisol concentrations around 210 ng/ml (Dinan et al., 1999; Scott et al., 1999b). Also, the non-specific serotonin agonist meta-chlorophenylpiperazine (mCPP) 0.5 mg/kg administered i.v. lead to a cortisol C_{MAX} 228.4 ng/ml (Gijssman et al., 1998) and the direct serotonin precursor agent 5-hydroxytryptophan (5-HTP) 300mg administered orally induced a cortisol C_{MAX} of 219 ng/ml (Smarius et al., 2008). Higher doses of hCRH have not been administered due to undesirable aspecific stress effects, while mCPP and 5-HTP are not tolerated at higher doses due to serotonergic side-effects. Cortisol release (at least via the CRH_1 receptor) therefore appears to be limited to approximately 210 - 230 ng/ml in this setting. Thus, a combination of 10µg dDAVP with 10 µg hCRH is expected to constitute a good function test to assess co-activation. The vasopressinergic contribution to the HPA-response can be determined by comparing the ACTH- and cortisol-effects of the combination of 10 µg dDAVP and 10 µg hCRH to those of 50 µg hCRH alone, administered two hours later. This being said, it should be borne in mind that our recommendation is putative and the application of pharmacokinetic-pharmacodynamic (PK-PD) modelling techniques in future will have to point out the validity of this approach.

In conclusion, we demonstrated dose-dependent vasopressinergic co-activation when combining hCRH with dDAVP. These findings form a basis for the further development of a pharmacological function test to assess vasopressinergic co-activation of the HPA-axis. This tool should be able to establish functional differences between different patient groups, and to demonstrate modulatory effects of innovative drug such as V_3 -antagonists. Furthermore, administering dDAVP followed by hCRH at least two hours later presents a practical procedure to independently examine vasopressinergic co-activation and corticotrophinergic activation of the HPA axis.

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Table 1

Average plasma ACTH (ng/l) and serum cortisol (ng/ml) for the period 120–510 min for 100 mg hCRH and for 100 mg hCRH preceded by 10 mg dDAVP: estimated means (back transformed least square means [LSM]) for placebo, 100 mg hCRH and 100 mg hCRH preceded by 10 mg dDAVP. Estimated difference (%) with 95% confidence interval from placebo for 100 mg hCRH and 100 mg hCRH preceded by 10 mg dDAVP; and estimated difference (%) with 95% confidence interval from 100 mg hCRH for 100 mg hCRH preceded by 10 mg dDAVP

| Parameter | Back transformed least Square Means (LSM) | | | | Estimated difference with 95% Confidence interval (%) | | | | | |
|------------------------------|---|---|--|--------------------------|---|--|---|---|---|---|
| | Placebo (n=12) | 10µg dDAVP combined with 10 µg hCRH (n=12) | 10µg dDAVP combined with 30µg hCRH (n=12) | 100 µg hCRH (n=12) | 10µg dDAVP vs placebo | 10µg dDAVP combined with 10 µg hCRH vs placebo | 100 µg hCRH (120-510min) vs placebo (120-510min) | 10µg dDAVP combined with 10 µg hCRH vs 10µg dDAVP | 10µg dDAVP combined with 30 µg hCRH vs 10µg dDAVP | 10µg dDAVP combined with 30 µg hCRH vs 10µg dDAVP |
| Plasma ACTH (ng/l) | 0- 90 min | 120-510 min | 0- 90 min | 0- 90 min | 10µg dDAVP vs placebo | 10µg dDAVP combined with 10 µg hCRH vs placebo | 100 µg hCRH (120-510min) vs placebo (120-510min) | 10µg dDAVP combined with 10 µg hCRH vs 10µg dDAVP | 10µg dDAVP combined with 30 µg hCRH vs 10µg dDAVP | 10µg dDAVP combined with 30 µg hCRH vs 10µg dDAVP |
| | 11.2 | 13.4 | 28.4 | 38.3 | 25.3 | 153.5 (105.1, 213.4) p<0.0001 | 88.0 (58.4, 145.1) p<0.0001 | 88.4 (48.0, 139.7) p<0.0001 | 241.9 (177.1, 321.8) p<0.0001 | 154.0 (99.4, 223.5) p<0.0001 |
| Serum cortisol (ng/ml) | 77.3 | 80.4 | 136.6 | 164.9 | 18.2 (-2.4, 42.9) p=0.0849 | 76.7 (46.1, 113.6) p<0.0001 | 40.0 (25.5, 56.1) p<0.0001 | 49.6 (20.4, 85.9) p=0.0006 | 113.4 (76.5, 158.0) p<0.0001 | 80.7 (45.3, 124.7) p<0.0001 |
| | 5.1 | 5.3 | 4.9 | 5.1 | 5.2 | -2.6 (-12.6, 8.5) p=0.622 | -1.9 (-15.4, 13.7) p=0.790 | 3.0 (-9.1, 16.8) p=0.633 | 0.7 (-9.6, 12.1) p=0.899 | 6.5 (-5.9, 20.6) p=0.309 |

Table 2

Average plasma ACTH (ng/l) and serum cortisol (ng/ml) for the period 120 to 510 min for 100 µg hCRH and for 100 µg hCRH preceded by 10 µg dDAVP: Estimated means (back transformed least Square Means) for placebo, 100µg hCRH and 100 µg hCRH preceded by 10µg dDAVP. Estimated difference (%) with 95% confidence interval from placebo for 100µg hCRH and 100µg hCRH preceded by 10 µg dDAVP; and estimated difference (%) with 95% confidence interval from 100 µg hCRH for 100µg hCRH preceded by 10 µg dDAVP.

| Parameter | Back transformed least Square Means (LSM) | | | Estimated difference with 95% Confidence interval (%) | | |
|------------------------|---|--------------------|--|---|--|--|
| | Placebo (n=12) | 100 µg hCRH (n=12) | 100 µg hCRH preceded by 10 µg dDAVP (n=12) | 100 µg hCRH vs placebo | 100 µg hCRH preceded by 10 µg dDAVP vs placebo | 100 µg hCRH vs 100 µg hCRH preceded by 10 µg dDAVP |
| Plasma ACTH (ng/l) | 13.4 | 25.3 | 27.5 | 88.0 (58.4, 145.1)% p<0.0001 | 104.4 (70.5, 145.1) p<0.0001 | 8.7 (-9.0, 29.9) P=0.340 |
| Serum cortisol (ng/ml) | 80.4 | 112.5 | 117.2 | 40.0 (25.5, 56.1)% p<0.0001 | 45.7 (30.2, 63.1) p<0.0001 | 4.1 (-6.7, 16.1) P=0.452 |

Figure 1 Schematic representation of the study design: a five-way crossover study consisting of two different experiments.

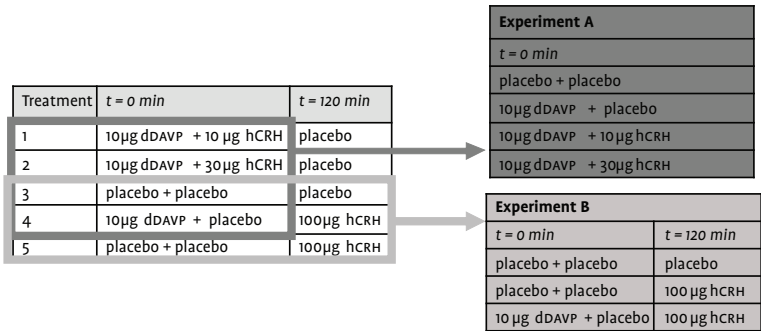


Figure 2 Average concentration time profile of plasma adrenocorticotrophic hormone (ACTH) with SD error bars (ng/l) for experiments A and B combined (closed circle: placebo followed by placebo; open triangle: placebo followed by 100 mg hCRH; open circle: 10 mg dDAVP followed by 100 mg hCRH; closed square: 10 mg dDAVP combined with 10 mg hCRH followed by placebo; open square: 10 mg dDAVP combined with 30 mg hCRH followed by placebo).

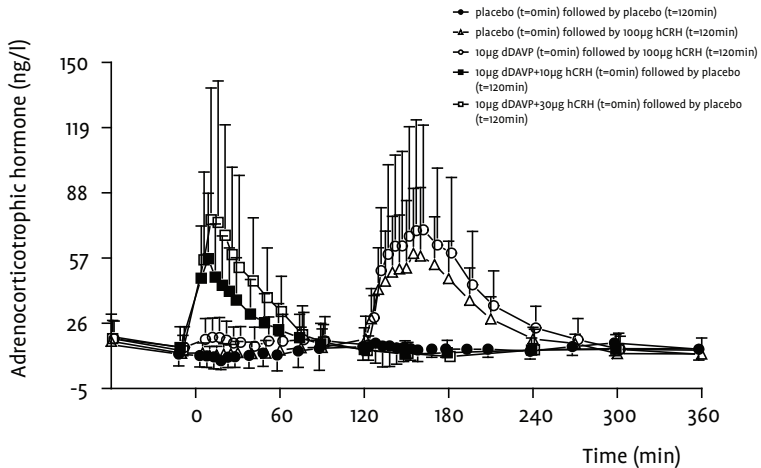
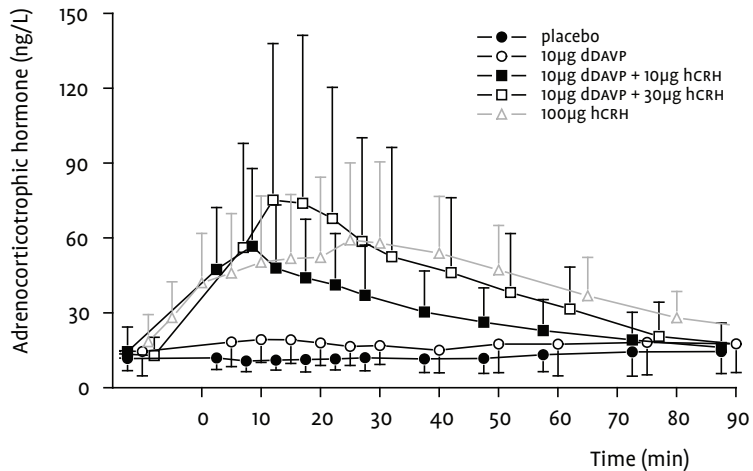


Figure 3

Average concentration time profile of plasma adrenocorticotrophic hormone (ACTH) with SD error (ng/l) bars for experiment A (closed circle: placebo; open circle: 10 mg dDAVP; closed square: 10 mg dDAVP combined with 10 mg hCRH; open square: 10 mg dDAVP combined with 30 mg hCRH; grey open triangle: 100 mg hCRH superimposed from administration point at $t = 120$ min in experiment B).

**Figure 4**

Average concentration time profile of serum cortisol with SD error (ng/ml) bars for experiment A (closed circle: placebo; open circle: 10 mg dDAVP; closed square: 10 mg dDAVP combined with 10 mg hCRH; open square: 10 mg dDAVP combined with 30 mg hCRH; grey open triangle: 100 mg hCRH superimposed from administration point at $t = 120$ min in experiment B).

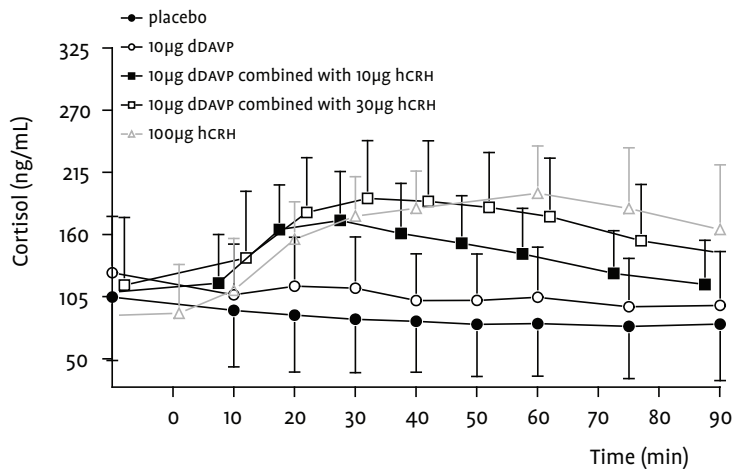


Figure 5

Average concentration time profile of plasma adrenocorticotrophic hormone (ACTH) with SD error (ng/l) bars for experiment B (closed circle: placebo followed by placebo; open triangle: placebo followed by 100 mg hCRH; open circle: 10 mg dDAVP followed by 100 mg hCRH).

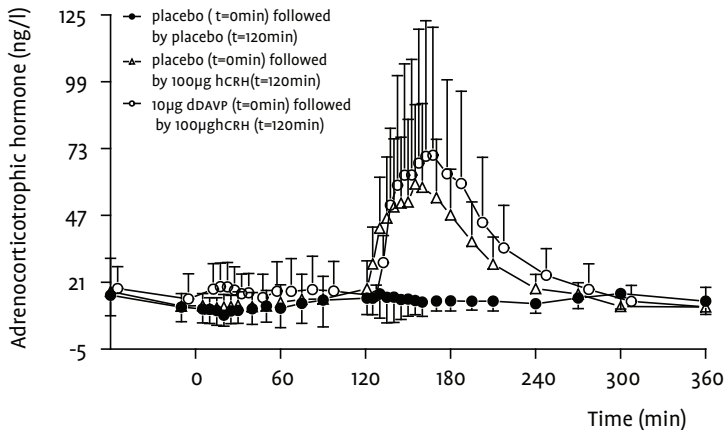
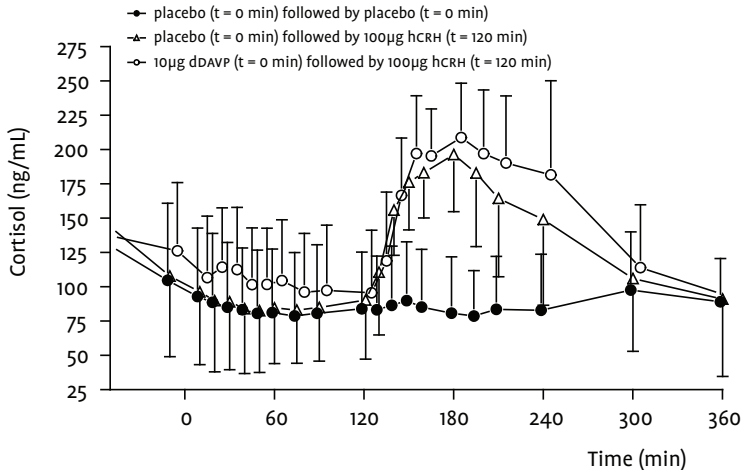


Figure 6

Average concentration time profile of serum cortisol with SD error (ng/mL) bars for experiment B (closed circle: placebo followed by placebo; open triangle: placebo followed by 100 mg hCRH; open circle: 10 mg dDAVP followed by 100 mg hCRH).



REFERENCE LIST

- Craighead M, Milne R, Campbell-Wan L, Watson L, Pre-sland J, Thomson FJ, Marston HM, Macsweeney CP (2008) Characterization of a novel and selective VAB receptor antagonist. *Prog Brain Res* 170: 527-535.
- de Kloet ER, Joels M, Holsboer F (2005) Stress and the brain: from adaptation to disease. *Nat Rev Neurosci* 6: 463-475.
- DeBold CR, Sheldon WR, DeCherney GS, Jackson RV, Alexander AN, Vale W, Rivier J, Orth DN (1984) Arginine vasopressin potentiates adrenocorticotropin release induced by ovine corticotropin-releasing factor. *J Clin Invest* 73: 533-538.
- Dinan TG, Lavelle E, Scott LV, Newell-Price J, Medbak S, Grossman AB (1999) Desmopressin normalizes the blunted adrenocorticotropin response to corticotropin-releasing hormone in melancholic depression: evidence of enhanced vasopressinergic responsiveness. *J Clin Endocrinol Metab* 84: 2238-2240.
- Dinan TG, Scott LV (2005) Anatomy of melancholia: focus on hypothalamic-pituitary-adrenal axis overactivity and the role of vasopressin. *J Anat* 207: 259-264.
- Favrod-Coune C, Raux-Demay MC, Proeschel MF, Bertagna X, Girard F, Luton JP (1993) Potentiation of the classic ovine corticotrophin releasing hormone stimulation test by the combined administration of small doses of lysine vasopressin. *Clin Endocrinol (Oxf)* 38: 405-410.
- Gijsman HJ, Cohen AF, van Gerven JM (2004) The application of the principles of clinical drug development to pharmacological challenge tests of the serotonergic system. *J Psychopharmacol* 18: 7-13.
- Gijsman HJ, van Gerven JM, Tieleman MC, Schoemaker RC, Pieters MS, Ferrari MD, Cohen AF, van Kempen GM (1998) Pharmacokinetic and pharmacodynamic profile of oral and intravenous meta-chlorophenylpiperazine in healthy volunteers. *J Clin Psychopharmacol* 18: 289-295.
- Holsboer F (2000) The corticosteroid receptor hypothesis of depression. *Neuropsychopharmacology* 23: 477-501.
- Holsboer F, Gerken A, Steiger A, Benkert O, Muller OA, Stalla GK (1984) Corticotropin-releasing-factor induced pituitary-adrenal response in depression. *Lancet* i: 55.
- Holsboer F, Ising M (2008) Central CRH system in depression and anxiety--evidence from clinical studies with CRH₁ receptor antagonists. *Eur J Pharmacol* 583: 350-357.
- Jacobs GE, Hulskotte EGJ, Burggraaf J, Zuurman HH, Ellassais-Schaap J, Peeters PAM, van Gerven JM, Ruigt G (2009) Safety of intravenously administered desmopressin for HPA-axis characterization in healthy volunteers.
- Lamberts SW, Verleun T, Oosterom R, de JF, Hackeng WH (1984) Corticotropin-releasing factor (ovine) and vasopressin exert a synergistic effect on adrenocorticotropin release in man. *J Clin Endocrinol Metab* 58: 298-303.
- Oelkers W, Boelke T, Bahr V (1988) Dose-response relationships between plasma adrenocorticotropin (ACTH), cortisol, aldosterone, and 18-hydroxycorticosterone after injection of ACTH-(1-39) or human corticotropin-releasing hormone in man. *J Clin Endocrinol Metab* 66: 181-186.
- Ring RH (2005) The central vasopressinergic system: examining the opportunities for psychiatric drug development. *Curr Pharm Des* 11: 205-225.
- Scott LV, Dinan TG (1998) Vasopressin and the regulation of hypothalamic-pituitary-adrenal axis function: implications for the pathophysiology of depression. *Life Sci* 62: 1985-1998.
- Scott LV, Medbak S, Dinan TG (1999a) ACTH and cortisol release following intravenous desmopressin: a dose-response study. *Clin Endocrinol (Oxf)* 51: 653-658.
- Scott LV, Medbak S, Dinan TG (1999b) Desmopressin augments pituitary-adrenal responsiveness to corticotropin-releasing hormone in subjects with chronic fatigue syndrome and in healthy volunteers. *Biol Psychiatry* 45: 1447-1454.
- Smarius IJ, Jacobs GE, Hoeberechts-Lefrandt DH, de Kam ML, van der Post JP, de Rijk R, van Pelt J, Schoemaker RC, Zitman FG, van Gerven JM, Gijsman HJ (2008) Pharmacology of rising oral doses of 5-hydroxytryptophan with carbidopa. *J Psychopharmacol* 22: 426-433.
- Spiga F, Harrison L, Macsweeney C, Thomson F, Craighead M, Lightman S (2008a) Effect of vasopressin 1b receptor blockade on the hypothalamic-pituitary-adrenal response of chronically stressed rats to a heterotypic stressor. *J Endocrinol*.
- Spiga F, Harrison L, Wood S, Knight D, Macsweeney C, Thomson F, Craighead M, Lightman S (2008b) Blockade of the V_{1B} receptor reduces ACTH, but not corticosterone, secretion induced by stress without effecting basal HPA axis activity. *J Endocrinol*.
- von Bardeleben U, Holsboer F (1988) Human corticotropin releasing hormone: clinical studies in patients with affective disorders, alcoholism, panic disorder and in normal controls. *Prog Neuropsychopharmacol Biol Psychiatry* 12 Suppl: S165-S187.

Pharmacology of rising oral doses of 5-hydroxytryptophan with carbidopa

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Abstract

- BACKGROUND** 5-hydroxytryptophan (5-HTP) is a direct 5-hydroxytryptamine (5-HT) precursor used to assess central serotonergic function. Its use has been limited by a narrow window between neuroendocrine changes and side effects, and variable kinetics related to inconsistent administration modes. By combining 5-HTP with carbidopa (CBD), increased bioavailability for brain penetration, and decreased peripheral side effects would be expected, due to reduced peripheral decarboxylation of 5-HTP to 5-HT.
- OBJECTIVES** A double blind, placebo-controlled, single rising dose, four-way crossover trial with placebo randomization was performed in fifteen healthy male volunteers to investigate the neuroendocrine dose-response relationship at various 5-HTP levels, the tolerability and subjective effects of oral 5-HTP at 100, 200 and 300 mg combined with CBD and the pharmacokinetic properties of the 5-HTP/CBD-challenge.
- RESULTS** Dose-dependent increases in average cortisol concentrations were observed. Mean response (AUC) over the first 4 hours (SD): 172.0 nmol/l (22.3) for placebo; 258.3 nmol/l (72.6) for 100 mg; 328.47 nmol/l (84.6) for 200 mg; and 387.3 nmol/l (82.4) for 300 mg 5-HTP. Similar dose-dependent increases for prolactin were seen while ACTH-response was more variable. 5-HTP kinetics were adequately described using a one-compartment model with first order absorption and a lag time (mean oral clearance 28L/hr \pm inter-individual coefficient of variation 31%). Nausea and vomiting occurred dose-dependently as most frequent side effects, resulting in dose-related dropout of 6.6% at 100 mg and 45.5% at 300 mg 5-HTP.
- CONCLUSIONS** Orally administered 5-HTP combined with CBD is an effective serotonergic challenge test, exhibiting dose-related plasma concentrations and neuroendocrine responsiveness. Frequent occurrence of nausea and vomiting limits the applicability of this challenge at 5-HTP doses above 100 mg.

Introduction

Pharmacological stimulation tests, using challenges with receptor agonists, release stimulators or re-uptake inhibitors, are used to quantify the integrity and function of serotonergic pathways in the central nervous system (CNS) (Gijsman et al., 2002b). These challenges may provide a tool for understanding the mechanisms underlying psychiatric disorders, or to predict the efficacy of novel therapies with a direct or indirect effect on serotonergic systems (Meibohm and Derendorf, 1997). One of the more frequently used serotonergic challenge tests consists of a single dose of L-5-Hydroxytryptophane (5-HTP), the immediate precursor of serotonin. Its decarboxylation into serotonin in raphé neurons with hypothalamic projections induces increases in cortisol and prolactin in peripheral blood, which are frequently used neuroendocrine endpoints of serotonergic challenges. However, there is little standardisation of 5-HTP-challenge tests, and their use has been hampered by a narrow window between neuroendocrine responses and side effects, mainly nausea and vomiting. The doses used in 5-HTP challenge tests vary extensively, and include oral doses of 10, 20 and 40 mg (den Boer and Westenberg, 1990), 60 mg (van Vliet et al., 1996), 100 mg (Vlasses et al., 1989), 200 mg (Maron et al., 2004; Schruers et al., 2002b; Schruers et al., 2002a), and intravenous doses of 0.8 mg/kg (Birmaher et al., 1997; Ryan et al., 1992). These doses were established by trial and error. In some studies, oral 5-HTP has been administered together with the peripheral L-aromatic decarboxylase inhibitor carbidopa (CBD). The rationale of CBD co-administration is that it prevents peripheral decarboxylation of 5-HTP, leading to more 5-HTP being available to enter the brain, and less peripheral serotonin being formed to cause systemic side effects. In practice, different doses of CBD have been used, varying from one single dose to three daily doses for one week before the challenge test (Birmaher et al., 1997; Magnussen and Nielsen-Kudsk, 1979; Magnussen and Engbaek, 1978; van Vliet et al., 1996; Vlasses et al., 1989). Recently, the pharmacokinetics and pharmacodynamics of a 5-HTP challenge test, consisting of two different doses of 5-HTP with or without pre-treatment with CBD, were studied (Gijsman et al., 2002a). A dose of 100 mg 5-HTP plus CBD (100 mg before and 50 mg after 5-HTP administration) induced a significant cortisol

and prolactin response, and the elimination half-life of 5-HTP was doubled compared to 5-HTP 100 mg without CBD. However, the neuroendocrine effects, which are known to be relatively sensitive to serotonergic challenges, were smaller after 5-HTP 100 mg with CBD than with usual challenge doses of dexfenfluramine or mCPP. 5-HTP 100 mg with CBD produced few clinically significant adverse events in this study (3 out of 12 cases of nausea of which 1 vomited) compared to mCPP. This suggests that the 100 mg dose of 5-HTP may be too low to exert a full serotonergic response, despite its combination with CBD. The optimal dosing regimen of 5-HTP that leads to a simple, reliable and tolerable challenge test of pre-synaptic serotonergic transmission thus remains to be established. Therefore, the current study was aimed at examining the neuroendocrine effects and the tolerability of single doses of 5-HTP with CBD co treatment, in a rising dose design. A second aim was to reproduce some of the findings of the previous 5-HTP-study, to develop an impression of the intersubject variability and to establish the pharmacokinetic properties of the 5-HTP/CBD-challenge test.

Methods

This study was a double blind, placebo controlled, four-way cross-over, single rising dose investigation of oral 5-HTP with co-administration of carbidopa (CBD) with interspersed placebo randomization. The study protocol was approved by the Medical Ethics Committee of Leiden University Medical Centre (LUMC) and all subjects gave written informed consent. The four dose regimens were placebo (including CBD placebo); 5-HTP 100 mg; 5-HTP 200 mg; and 5-HTP 300 mg, the last three with co-administration of 100 mg CBD 3 hours before and 50 mg CBD 3 hours after administration of 5-HTP. Based on the pharmacokinetic characteristics of CBD, we expected this regimen to result in a relatively constant level of CBD during the first hours after administration of 5-HTP.

Subjects

Twelve healthy, male volunteers were recruited from the CHDR database and from the local student population by means of

advertisements. Dropouts were to be replaced with subjects assigned to the same treatment randomisation order. A Dutch translation of the Structured Clinical Interview for DSM-IV axis I (SCID I), was used to exclude any subject with a past or present psychiatric disorder, including substance abuse (Groenestijn MAC and Akkerhuis GW, 1999). Other exclusion criteria were abnormalities on physical or laboratory examination or ECG; participation in a clinical study within three months prior to the study, or in (more than) two studies in the past year; blood donation within three months before the study period; a self-admitted chance of a HIV positive state; the use of medication other than occasional paracetamol in the last months; smoking of more than 5 cigarettes per day; an average intake of xanthene containing fluids of more than 3 units per day; current average use of alcohol of more than 3 units per day; or an irregular day-night-rhythm.

Study days

Volunteers arrived at the Centre for Human Drug Research (CHDR) at 8.00 in a fasted state, and remained on site for the following 12 hours. On arrival a urine drug screen (morphine, benzodiazepines, cocaine, amphetamine, THC, methamphetamines including MDMA) was performed. Subjects with a negative drug screen received the first oral dose of CBD or placebo ($t=-3$ hr). This was followed by a standardized breakfast, which, like all other supplied meals and snacks during their stay, did not contain tryptophan rich foods. Subsequently, a cannula was inserted into the antecubital vein of one arm to allow withdrawal of blood, an ECG was mounted and all baseline measurements were made. At 11.00 hours ($t=0$ hr), 5-HTP or its placebo was administered and the second dose of CBD was given at $t=3$ hr. At $t=2$ hr the volunteers received a glucose drink, after CBD administration ($t=3$ hr) a light lunch and at $t=7$ hr a dinner. All challenges were performed under hospital conditions. A research physician attended all study occasions. Vital signs were monitored at 1 hour before and at $t=1, 2, 3, 4, 5$ and 6 hours after 5-HTP administration. Blood pressure was measured using a non-invasive oscillometric system, the Nihon Kohden Lifescope EC BSM-1101J/K (Nihon Kohden Co., Oss). For Electrocardiography (12 lead) a Nihon Kohden Cardiofax with ECaps 12 software (Nihon Kohden Co.,

Japan) was used. Oral temperature was measured with a digital thermometer (Terumo Corporation, Tokyo, Japan. Occasions were separated by a washout period of at least 6 days.

5-HTP and CBD

5-HTP and CBD were obtained from BUFA b.v. (Uitgeest, The Netherlands) and prepared by the Department of Clinical Pharmacy of the Leiden University Medical Centre. Capsules containing 100 mg 5-HTP and capsules containing 50 mg CBD were made with matching placebos.

Biochemical measurements

On every challenge day at 1 hour before and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6 and 8 hours after 5-HTP administration, 9 ml whole blood was drawn in a Greiner Vacuette EDTA-tube, tilted, and immediately stored on ice water (4°C). Within one hour blood was centrifuged at 4°C for 10 minutes at 2000G. Plasma was divided into four portions, with two portions of at least 1.0 ml for the 5-HTP assay, at least 1.0 ml for cortisol and prolactin and 0.5 ml for ACTH. Plasma was stored directly at -20°C. The 5-HTP assay was performed at the Biochemical Laboratory of the Rijngestgroep, Oegstgeest, The Netherlands. After addition of buffer and internal standard, a solution with 1.55 mol/l trichloroacetic acid, 13.4 mmol/l EDTA, and 50 mmol/l sodium bisulphite (Na₂S₂O₅) was added to remove proteins. After centrifuging at 3,000 g for 32 minutes, the supernatant was assayed by high-pressure liquid chromatography (HPLC) with electrochemical detection using 650 millivolts. The HPLC contained a Merck LiChroSpher 60, RP Select B5 _m, 125 _4 mm internal diameter plus 1 cm guard column. For the mobile phase, we used a solution (pH 3.60) with 50 mmol/l sodium acetate (NaAc), 50 mmol/l citric acid, 0.27 mmol/l EDTA, 1.17 mmol/l 1-octanesulfonic acid sodium salt, and 1.5% volume- to-volume ratio acetonitrile. The lower limits of detection and quantification were 0.5 ng/ml and 1.7 ng/ml, respectively. The coefficients of variability for precision and reliability were 2.6% and 7.9%, respectively. Cortisol and prolactin were analysed by an electrochemiluminescent immunoassay (ECLIA) on the Roche Elecsys 1010, and analysis of ACTH

was performed by radio-immuno-assay, according to methods described previously (Gijsman et al., 2002a).

Side-effects

Adverse events were registered from spontaneous reports and hourly inquiries. For each symptom, the relationship (definite, probable, possible, unknown, definitely not) with the treatment was assessed by the research physician. Only side effects judged definitely, probably or possibly related to the treatment were considered in the analyses. Side effects did not necessarily lead to dropout from the study. If deemed safe by the research physician, subjects who experienced side effects were allowed to participate in the following occasion.

Self report questionnaires

The volunteers scored self report questionnaires at 1 hour before and 1, 2, 3, 4, 5, 6 and 8 hours after 5-HTP administration. The short version of the Dutch translation of the Profile of Mood States (POMS) (McNair DM et al., 1971; Wald FDM and Mellenbergh GJ, 1990), the Dutch version of the State scale of the Spielberger State-Trait Anxiety Inventory (STAI-DY-1) (Spielberger CD et al., 1983; Wald FDM and Mellenbergh GJ, 1990), the somatization-subscale of the Dutch Translation and adaptation of the Symptom Check List (SCL-90) (Arrindell WA, 1986; Derogatis et al., 1973) and Visual Analogue Scales (as originally described by Norris, for the three factors corresponding to alertness, mood and calmness (Bond A and Lader M, 1974; Norris, 1971) were used for follow up of subjective effects. All Visual Analogue Scales were practised three times during the pre-study screening.

Pharmacodynamic analysis

Occasions on which subjects vomited were excluded from pharmacodynamic analysis, since this would have interfered with the neuroendocrine treatment effects. Analyses were performed on subjects of whom the data of at least two non-vomiting occasions were available. For each individual, the AUC over 4 hour and 8-hour

periods were calculated and divided by the corresponding time-span for all parameters. The resulting time-weighted average effect was analyzed using ANOVA with factors subject, treatment and occasion. Contrasts of all treatments with placebo were estimated within the ANOVA design and presented with 95% confidence intervals. The 4 hour period was chosen based on the results of a previous study, indicating that most of the effects of 5-HTP 100 mg with CBD had dissipated after 4 hours (Gijssman et al., 2002a). All variables were analyzed untransformed.

Pharmacokinetic analysis

The 5-HTP data were analysed using nonlinear mixed effect modelling as implemented in the NONMEM program (Version 5, Globomax LLC, Hanover, MD, USA). This ‘population approach’ allows the description of the entire data set using a common structural model, with allowance for interindividual kinetic variability. Based on the population estimates, individual empirical Bayes estimates can be generated by NONMEM that can be used to generate individual concentration time profiles. First order conditional estimation was used throughout. Data were described using a one-compartment model with first order absorption and a lag-time. Various models and parameterisations were attempted. In the final model absorption half-life, elimination half-life, oral clearance (Cl/F) and lag-time were estimated. Inter-individual variability was modelled using a multiplicative model with a correlation between absorption and elimination half-life. An additive residual error model best described the data.

Occasions on which subjects vomited were excluded from primary pharmacokinetic analysis. Analysis was performed on subjects for whom the data of at least one non-vomiting occasion were available.

Results

Demographic data

Twenty-three volunteers were recruited originally, eight of whom were excluded at screening. To replace dropouts, three volunteers

were recruited in addition to the anticipated 12 subjects. It was decided not to recruit more replacements, when blinded interim safety analyses suggested that the dropout rate approached 50% at the highest dose. The mean age of the 15 participants was 23.2 years (range 19 – 29 years).

Subject disposition

Study dropouts occurred solely due to nausea and vomiting, at different stages of the study. Of 15 subjects who started, 1/15 dropped out after 100 mg (6,6%), 4/14 after 200 mg (28,6%), and 5/11 after 300 mg (45,5%) 5-HTP respectively. Two subjects opted to continue the study despite having vomited on the 100 mg and 200 mg occasions respectively, and dropped out after the next higher dose. In total nine out of 15 subjects commenced all four occasions, six of whom completed all doses without vomiting. The subject disposition is represented in *Figure 1*. Different numbers of subjects per dosing group were evaluable for the different analyses. For analyses of safety and tolerability, all subjects who started with a dose were included. Vomiting may have had an impact on both the pharmacokinetics of study medication (since the absorption of 5-HTP and CBD could have been influenced, also considering that in eight out of 10 cases of vomiting subjects did not receive their second dose of CBD), and on neuroendocrine responses (enhanced pharmacokinetic variability, stress-related cortisol increase and nausea-induced prolactin elevation). Therefore, subject occasions on which a subject vomited were excluded from the primary pharmacokinetic and pharmacodynamic endocrine analyses. A secondary analysis was performed to investigate whether subjects who vomited were more susceptible to the effects of 5-HTP or had higher drug levels. For this, the neuroendocrine and pharmacokinetic results at 100 mg were compared between subjects who never vomited at all (Subgroup A), and those who did not vomit at 100 mg but did at a higher dose (Subgroup B). This approach eliminated the direct influence of vomiting on the neuroendocrine reactions or plasma concentrations, but allowed us to identify inherent differences in drug sensitivity or pharmacokinetic characteristics between subjects who were prone to vomiting and those who were not.

Adverse events

No side effects were reported during the placebo occasion. During all 5-HTP occasions, nausea occurred most frequently (6/15; 12/14; 9/11 occasions for 100, 200 and 300 mg respectively). Vomiting occurred less frequently but in a dose-related manner (1/15; 4/14; 5/11 occasions respectively). The main other reported side effects were epigastric discomfort (5/15; 5/14; 7/11 occasions respectively), dizziness/lightheadedness (1/15; 4/14; 4/11 occasions respectively) and drowsiness/sleepiness/tiredness (4/15; 2/14; 0/11 occasions respectively). On two occasions during an episode of nausea and vomiting at 300 mg, mild disorientation in place and euphoria with *déjà-vu*'s occurred respectively.

Self report questionnaires

There were no significant effects on the STAI, POMS and visual analogue scales, except for a significant decrease on the POMS vigor scale ($p=0.008$), in the 4-hour period after the 200 mg dose. The SCL-90 subscale somatization showed a significant effect on the nausea score for 100 mg ($p=0.045$), 200 mg ($p=0.002$), and 300 mg ($p=0.040$) in the 4-hour period. There also was a significant effect on the warm/cold score for the 300 mg dose for the 4-hour period ($p<0.001$).

Pharmacokinetics

The results from the NONMEM model as described in the Methods section are shown in *Table 1*. The average concentration-time-curves for the three different doses of 5-HTP are displayed in *Figure 2*. 5-HTP has dose-proportional pharmacokinetics. The absorption and elimination half-lives, which are highly correlated, show a large variability. However, these parameters do not determine the level of exposure of 5-HTP as much as mean oral clearance (Cl/F), which has a limited inter-individual variability of 31%. Subjects who vomited at higher doses of 5-HTP may have had inherently different pharmacokinetic characteristics, leading to higher plasma levels at the 100 mg dose, although this dose did not yet lead to vomiting. To examine this possibility, we performed a secondary

comparison of the 5-HTP concentrations at 100 mg between Subgroup A (n=6) and Subgroup B (n=6), at 4 and 8 hours. No significant differences in concentrations could be found.

Pharmacodynamic effects

There was a significant dose effect on cortisol and prolactin. The AUC of cortisol differed significantly from placebo on all three occasions for the 4-hour period, which covered the main part of the response (Table 2). Figure 3 shows the plasma cortisol responses (nmol/l) after the four dose regimens. The effect of ACTH was short lasting and only statistically significant after 200 mg in the 4-hour period (Figure 4). The prolactin response reached statistical significance only for 100 mg and 200 mg in the 4-hour period, but not in the small number of subjects who completed the 300 mg dose (Figure 5). To examine the possibility that vomiting subjects were more sensitive to the effects of 5-HTP, the pharmacodynamic responses at the 100 mg dose were compared between Subgroup A (n=6) and Subgroup B (n=6). Vomiting subjects showed a larger average increase in the AUC of the SCL Nausea score for the 8-hour period after the 100 mg dose, but there were no relationships with the cortisol or prolactin responses.

Discussion

The lack of a standardized serotonergic challenge procedure hampers the application of this potentially useful paradigm in biological psychiatry and CNS drug development (Gijsman et al., 2004). The variability of different serotonergic challenge tests also contributes to the confusion that surrounds many aspects of the role of serotonergic systems in psychiatric disease. We have therefore set up a series of experiments, with the aim of identifying a reproducible and practical serotonergic challenge procedure. Such a procedure would have to be well tolerated, show little pharmacokinetic variability, and cause clear reproducible dose-related responses of meaningful functions of the central nervous system (CNS) (Gijsman et al., 2004).

One of the main aims of this study was to determine the pharmacokinetic (PK) properties of the 5-HTP/CBD-challenge. The study

also allowed us to create an impression of the inter- and intra-subject variability of the challenge. Although the study did not systematically evaluate all aspects of reproducibility, it did allow an assessment of dose-proportionality, and inter- and intra-subject variability of different pharmacokinetic properties. The 5-HTP/CBD-challenge displays clear dose proportional increases in plasma concentrations, and the variability of the main pharmacokinetic parameter that determines drug exposure, mean oral clearance (Cl/F), shows an acceptable inter-individual coefficient of variation (IICV) of 31%. Another aspect of the reliability of the challenge can be derived from a comparison with a previous study in which a lower dose range of 5-HTP was examined, which also included 100 mg 5-HTP combined with CBD (Gijsman et al., 2002a). Both studies showed quite similar pharmacokinetic characteristics for this challenge: Mean oral clearance (Cl/F) was 36 L/hr with a 30% IICV in the first study, and 28 L/hr with 31% IICV in the current one. The average elimination $T_{1/2}$ was 3.0 hr in both studies. After oral administration, absorption and elimination of 5-HTP run in parallel and cannot be reliably separated by the PK models used in this study. Consequently, these two parameters were highly correlated, and their added intersubject variability was considerable. It could not be assessed whether variability was largest for absorption or for elimination. An inter-individual kinetic variability of about 30% seems acceptable for a challenge test, particularly since a non-invasive challenge will be more acceptable for depressed patients than an intravenous infusion, which may also cause stress-related cortisol increases.

It seems reasonable to assume that vomiting may have reduced drug absorption, also because this usually interfered with the ingestion of the second carbidopa-dose. Subjects who vomited were therefore excluded from the primary pharmacokinetic analyses. It is possible that subjects who vomited after 5-HTP had higher plasma levels or were more sensitive to the drug's effects. To explore this possibility, we compared the effects of 5-HTP 100 mg between subjects who never vomited at any dose of 5-HTP (n=6), and those who did not vomit at 100 mg but only at a higher dose (n=6). Although both subgroups are too small for statistical comfort, they showed quite similar pharmacokinetic profiles of 5-HTP at the 100 mg dose. Neuroendocrine responses were also very

comparable. However, subjects who vomited at a higher dose had statistically significant larger nausea scores at the 100 mg dose, which had not yet caused vomiting. This suggests that nausea and vomiting in these subjects are not due to higher plasma levels, but to a larger sensitivity of the pertaining gastrointestinal and/or medullar chemoreceptors. This does not seem to be accompanied by an increased sensitivity of the other (central) serotonergic systems that are involved in the release of cortisol or prolactin, since these neuroendocrine responses appeared very similar in these small subgroups. Before vomiting occurs, nausea does not seem to have an influence on the pharmacokinetics of 5-HTP. This contributes to the pharmacokinetic reproducibility of the different dose regimens of the 5-HTP/CBD-challenge.

5-HTP 100, 200 and 300 mg plus CBD caused dose-related increases in cortisol release, the most reliable response parameter for serotonergic challenges. A comparable estimated difference from placebo of 91 nmol/l was obtained with the 100 mg dose of 5-HTP plus CBD in the 4 hour period in a previous study (Gijsman et al., 2002a). Thus, it seems that the pharmacodynamic reproducibility of the 5-HTP/CBD-challenge is adequate for 100 mg, but this remains to be proven for the higher dose range.

The ACTH response for the 5-HTP 200 mg treatment was statistically significant compared to placebo. However, at 300 mg the average response was lower and the increase did not reach statistical significance. This could signify a lack of statistical power, due to dropouts at the highest dose; an assay variability that was higher than for cortisol; and somewhat conservative estimates of treatment effects (ie. overall AUCs rather than individual timepoints, relatively few measurements etc.). It is unlikely that 5-HTP 200 mg approached its ceiling effects on CRH-ACTH activation, since this would have led to a congruent reduction in cortisol at the highest dose. Alternatively, a dose-related cortisol-increase despite a lack of a dose-proportional ACTH-increase could indicate that 5-HTP-induced cortisol-release is partly independent from CRH-ACTH activation; ie. that at higher doses it occurs at the level of the adrenal cortex. This cannot be fully excluded from this study, but there are several arguments from previous studies pointing to the contrary. ACTH-release was shown to be induced by 5-HTP alone or combined with CBD (Imura et al., 1973; Lee et al., 1991), although not all studies

confirmed this finding (Gijsman et al., 2002a). Several pharmacological studies in experimental animals and healthy humans suggest that serotonergic stimulation of cortisol-release involves activation of hypothalamic 5-HT₂ (5-HT_{1C}) receptors (Gartside and Cowen, 1990; Jorgensen et al., 2002). In all these studies, the ACTH-response preceded the cortisol-response, whereas a primary adrenal cortisol-release would have resulted in an ACTH-reduction. Nonetheless, our results cannot fully exclude that the slightly lower average ACTH-response at the highest dose of 5-HTP-induced was due to negative feedback from an ensuing 5-HTP-induced adrenal cortisol release. The data itself and most of the literature results are compatible with a mixed hypothalamic/pituitary and peripheral/adrenal effect for 5-HTP, and more research is needed to separate these two potentially concomitant processes

The average maximum cortisol level caused by the highest 5-HTP dose was 609.5 nmol/l. This approaches the effect of a (close to maximum tolerated) intravenous mCPP dose of 0.5 mg/kg, which leads to an average maximum concentration of around 630 nmol/l (Gijsman et al., 1998). Thus, it seems that the 5-HTP/CBD-challenge with doses of 100-300 mg covers a major part of the maximum effect range for cortisol release that can be attained in healthy volunteers. The administration of higher doses of both mCPP and 5-HTP was unfortunately impeded by adverse events, including nausea and vomiting. These side effects prevent a wider use of the 5-HTP dosing regimens employed in this study, although these otherwise satisfy many of the methodological criteria of an adequate challenge test. Only the lowest dose of 100 mg 5-HTP plus CBD seems to be tolerated well enough to be of practical use. Dose related nausea and vomiting are also the most frequently reported side effects in the literature (den Boer and Westenberg, 1990; Mag-nussen and Nielsen-Kudsk, 1979; Westenberg et al., 1982). Generally, it seems that plasma concentrations above 1000 mg/l cause high rates of nausea and vomiting, which clearly limits the applicability of the 5-HTP/CBD-challenge at doses at or above 200 mg.

In practice, the current 5-HTP/CBD-challenge is limited to a maximum oral dose of 100 mg. Although this produces cortisol responses with an acceptable reproducibility, these are quite limited compared to the maximum response that is generated by other (less practical or less well tolerated) serotonergic challenge

tests. In principle, a challenge does not always have to produce a maximum response to show treatment effects or differences between patient groups. In fact, (supra)maximal challenges may even obscure changes or differences in serotonergic sensitivity between different groups by means of ceiling effects or unintended neuroendocrine activation through other (less specific or adverse effect related) mechanisms. On the other hand, it is also difficult to detect differences between patient groups or treatment effects, if the challenge test produces a suboptimal stimulation. Therefore, it would be preferable to have a challenge test with a larger therapeutic window, covering the main part of the dose response curve without unacceptable side effects. One possibility to achieve this may be to combine a high-dose 5-HTP/CBD-challenge with an anti-emetic to suppress nausea and vomiting. The most appropriate anti-emetic agent needs to be established, because several different neurotransmitter systems are involved in drug-induced nausea and vomiting, notably serotonin and dopamine, which are also involved in the neuroendocrine challenge-responses. Not every anti-emetic may be efficacious, and others may interfere with the desired serotonergic effects. It remains important to develop a serotonergic challenge with all the proper methodological characteristics, to provide a reliable tool for the study of serotonergic functions in health and disease. So far, 5-HTP 100 mg combined with carbidopa 100+50 mg seems to be the best tolerated and most reproducible oral serotonergic challenge test.

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Table 1**Population pharmacokinetic parameters of 5-HTP**

| Parameter | Mean | SEM | IICV |
|-----------------------------|-------|--------|-------|
| Oral clearance (Cl/F, l/hr) | 28.0 | 2.06 | 31% |
| Absorption half-life (hr) | 0.358 | 0.0963 | 22%* |
| Elimination half-life (hr) | 3.02 | 0.222 | 122%* |
| lag-time (hr) | 0.407 | 0.0353 | 19% |
| Residual variability (SD) | 142 | | |

*Correlation coefficient between absorption and elimination; half-life = -0.75; Mean = population average;
SEM = standard error of the population average; IICV= interindividual coefficient of variation.

Table 2

Pharmacodynamic effects over 4 hours: Least square mean (SD) AUC over 4 hours of cortisol (nmol/l), prolactin (μ U/ml), ACTH (ng/l) and SCL-90 Nausea score and estimated difference from placebo with 95% confidence interval for placebo, 100 mg 5-HTP, 200 mg 5-HTP and 300 mg 5-HTP.

| Pharmacodynamic parameter | | placebo n=10 | 100 mg n=12 | 200 mg n=10 | 300 mg n=6 | Overall treatment effect |
|---------------------------|--|---------------------------------|--------------------------------|---------------------------------|---------------|--------------------------------|
| Cortisol (nmol/l) | LSM AUC (SD) | 187.0 (22.5) | 272 (28.1) | 345 (26.4) | 350.1 (40.38) | p < 0.001 |
| | Estimated difference from placebo (95% CI) | 84 (17, 151) p=0.016 | 157 (89, 226) p=0.000 | 162 (57, 267) p=0.004 | | |
| Prolactin (mU/l) | LSM AUC (SD) | 246 (18.33) | 306 (22.9) | 330 (21.5) | 284 (32.9) | p= 0.021 |
| | Estimated difference from placebo (95% CI) | 60 (6,114) p=0.032 | 85 (29,140) p=0.004 | 38 (-47,123) p=0.370 | | |
| ACTH (ng/l) | LSM AUC (SD) | 23.31 (7.13) | 34.1 (8.9) | 47.5 (8.4) | 38.5 (12.8) | p= 0.159 |
| | Estimated difference from placebo (95% CI) | 10.8 (-10.3,31.9) p=0.300 | 24.2 (2.5,45.8) p=0.031 | 15.2 (-18.0,48.4) p=0.350 | | |
| SCL-90 Nausea | LSM AUC (SD) | 1.1 (0.10) | 1.4 (0.13) | 1.6 (0.12) | 1.6 (0.18) | p=0.008 |
| | Estimated difference from placebo (95% CI) | 0.3 (0.01,.60) p=0.05 | 0.51 (0.21,0.82) p=0.002 | 0.49 (0.02,0.96) p=0.04 | | |

Figure 1 Subject disposition for dropout rate, primary and secondary pharmacokinetic analysis, primary and secondary neuroendocrine analyses and safety analyses.

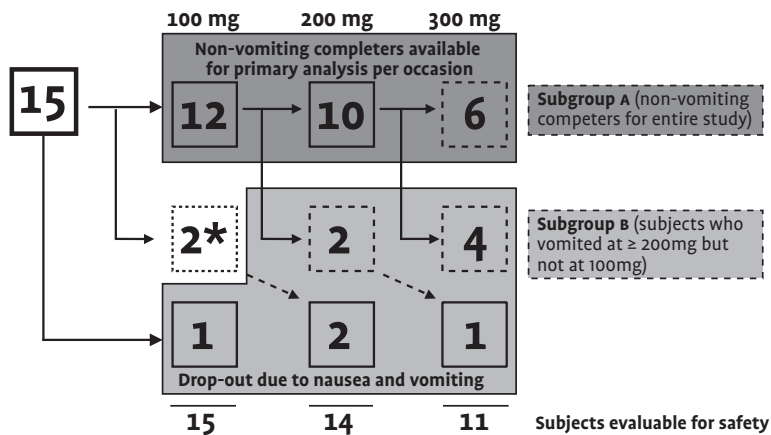


Figure 2 Average graph of predicted and observed 100 mg (n=12), 200 mg (n=10) and 300 mg (n=6) 5-HTP (ng/ml) and CBD co-treatment with SD error bars

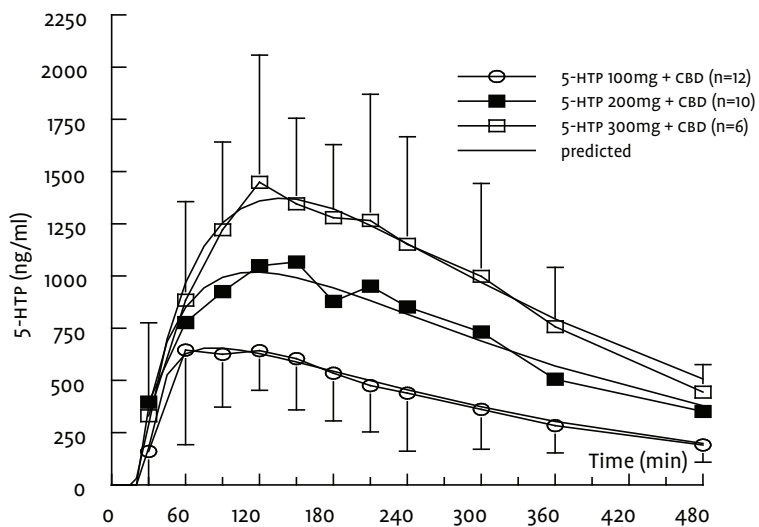


Figure 3

Average plasma cortisol (nmol/l) time-course profile after administration of placebo (n=10), 100 mg (n=12), 200 mg (n=10) and 300 mg (n=6) 5-HTP and CBD co-treatment with SD error bars, and least square mean AUC's over 4 hours.

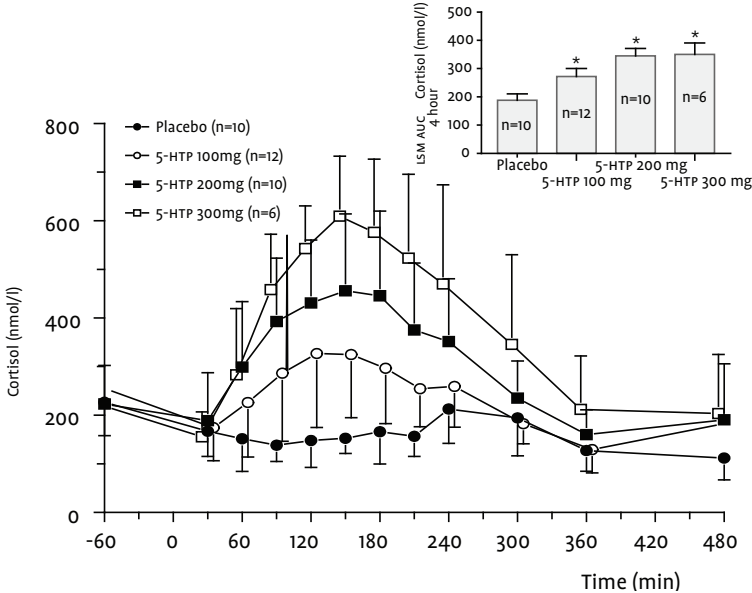


Figure 4

Average plasma ACTH (ng/l) time-course profile after administration of placebo (n=10), 100 mg (n=12), 200 mg (n=10) and 300 mg (n=6) 5-HTP and CBD co-treatment with SD error bars, and least square mean AUC's over 4 hours.

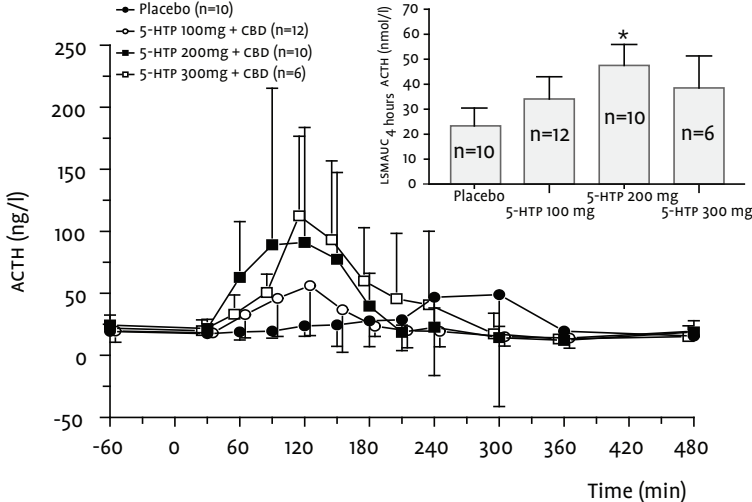
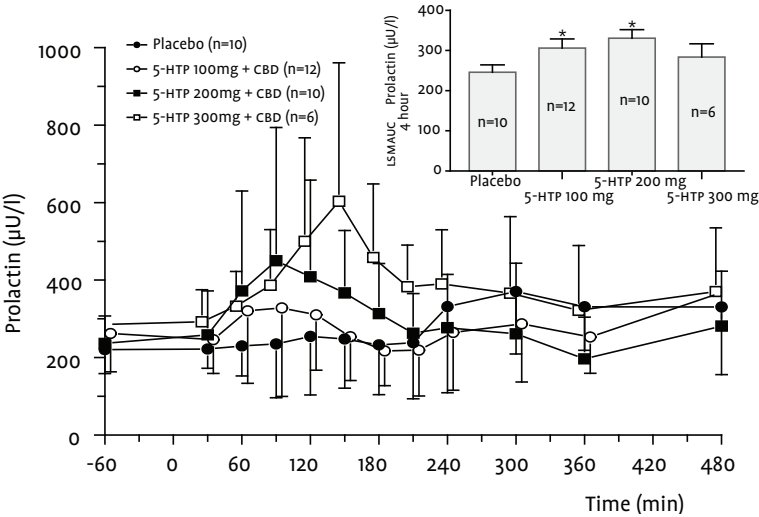


Figure 5

Average plasma prolactin ($\mu\text{U/l}$) time-course profile after administration of placebo ($n=10$), 100 mg ($n=12$), 200 mg ($n=10$) and 300 mg ($n=6$) 5-HTP and CBD co-treatment with SD error bars, and least square mean AUC's over 4 hours.



* correlation coefficient between absorption and elimination half-life: -0.75 / Mean= Population Average / SEM= Standard Error of the Population Average / IICV= Inter Individual Coefficient of Variation

REFERENCE LIST

- Arrindell WA EJ (1986) SCL-90. Handleiding bij een multidimensionele psychopathologie-indicator. Lisse.
- Birmaher B, Kaufman J, Brent DA, Dahl RE, Perel JM, al Shabbout M, Nelson B, Stull S, Rao U, Waterman GS, Williamson DE, Ryan ND (1997) Neuroendocrine response to 5-hydroxy-L-tryptophan in prepubertal children at high risk of major depressive disorder. *Arch Gen Psychiatry* 54: 1113-1119.
- Bond A, Lader M (1974) The use of analogue scale in rating subjective feelings. *Br J Med Psychol* 47: 211-218.
- den Boer JA, Westenberg HG (1990) Behavioral, neuroendocrine, and biochemical effects of 5-hydroxytryptophan administration in panic disorder. *Psychiatry Res* 31: 267-278.
- Derogatis LR, Lipman RS, Covi L (1973) SCL-90: an outpatient psychiatric rating scale--preliminary report. *Psychopharmacol Bull* 9: 13-28.
- Gartside SE, Cowen PJ (1990) Mediation of ACTH and prolactin responses to 5-HTP by 5-HT₂ receptors. *Eur J Pharmacol* 179: 103-109.
- Gijsman HJ, Cohen AF, Van Gerven JM (2004) The application of the principles of clinical drug development to pharmacological challenge tests of the serotonergic system. *J Psychopharmacol* 18: 7-13.
- Gijsman HJ, Van Gerven JM, Tieleman MC, Schoemaker RC, Pieters MS, Ferrari MD, Cohen AF, Van Kempen GM (1998) Pharmacokinetic and pharmacodynamic profile of oral and intravenous meta-chlorophenylpiperazine in healthy volunteers. *J Clin Psychopharmacol* 18: 289-295.
- Gijsman HJ, van Gerven JMA, de Kam ML, Schoemaker RC, Pieters MSM, Weemaes M, de Rijk R, van der Post J, Cohen AF (2002a) Placebo-controlled comparison of three dose-regimens of 5-hydroxytryptophan challenge test in healthy volunteers. *Journal of Clinical Psychopharmacology* 22: 183-189.
- Gijsman HJ, van Gerven JMA, Verkes RJ, Schoemaker RC, Pieters MSM, Pennings EJM, Hessing TJ, Cohen AF (2002b) Saccadic peak velocity and EEG as endpoints for a serotonergic challenge test. *Human Psychopharmacology-Clinical and Experimental* 17: 83-89.
- Groenestijn MAC, Akkerhuis GW KRSNNW (1999) Structured Clinical Interview for DSM-IV Axis I Disorders. Lisse, The Netherlands: Swets Test Publishers.
- Imura H, Nakai Y, Yoshimi T (1973) Effect of 5-hydroxytryptophan (5-HTP) on growth hormone and ACTH release in man. *J Clin Endocrinol Metab* 36: 204-206.
- Jorgensen H, Knigge U, Kjaer A, Moller M, Warberg J (2002) Serotonergic stimulation of corticotropin-releasing hormone and pro-opiomelanocortin gene expression. *J Neuroendocrinol* 14: 788-795.
- Lee MA, Nash JF, Barnes M, Meltzer HY (1991) Inhibitory effect of ritanserin on the 5-hydroxytryptophan-mediated cortisol, ACTH and prolactin secretion in humans. *Psychopharmacology (Berl)* 103: 258-264.
- Magnussen I, Engbaek F (1978) The effects of aromatic amino acid decarboxylase inhibitors on plasma concentrations of 5-hydroxytryptophan in man. *Acta Pharmacol Toxicol (Copenh)* 43: 36-42.
- Magnussen I, Nielsen-Kudsk F (1979) Pharmacokinetics of intravenously administered L-5-hydroxytryptophan in man. *Acta Pharmacol Toxicol (Copenh)* 44: 308-314.
- Maron E, Toru I, Vasar V, Shlik J (2004) The effect of 5-hydroxytryptophan on cholecystokinin-4-induced panic attacks in healthy volunteers. *J Psychopharmacol* 18: 194-199.
- McNair DM, Lorr M, Droppelman LF (1971) Manual for the profile of mood states. San Diego.
- Meibohm B, Derendorf H (1997) Basic concepts of pharmacokinetic/pharmacodynamic (PK/PD) modelling. *Int J Clin Pharmacol Ther* 35: 401-413.
- Norris H (1971) The action of sedatives on brain stem oculomotor systems in man. *Neuropharmacology* 10: 181-191.
- Ryan ND, Birmaher B, Perel JM, Dahl RE, Meyer V, al Shabbout M, Iyengar S, Puig-Antich J (1992) Neuroendocrine response to L-5-hydroxytryptophan challenge in prepubertal major depression. Depressed vs normal children. *Arch Gen Psychiatry* 49: 843-851.
- Schruers K, van Diest R, Nicolson N, Griez E (2002a) L-5-hydroxytryptophan induced increase in salivary cortisol in panic disorder patients and healthy volunteers. *Psychopharmacology (Berl)* 161: 365-369.
- Schruers K, van Diest R, Overbeek T, Griez E (2002b) Acute L-5-hydroxytryptophan administration inhibits carbon dioxide-induced panic in panic disorder patients. *Psychiatry Res* 113: 237-243.
- Spielberger CD, Gorsuch RL, Lushene RE (1983) STAI manual for the State-Trait Anxiety Inventory. Palo Alto, CA: Consulting Psychologists Press.
- van Vliet IM, Slaap BR, Westenberg HG, den Boer JA (1996) Behavioral, neuroendocrine and biochemical effects of different doses of 5-HTP in panic disorder. *Eur Neuropsychopharmacol* 6: 103-110.

- Vlasses PH, Rotmensch HH, Swanson BN, Clementi RA, Ferguson RK (1989) Effect of repeated doses of L-5-hydroxytryptophan and carbidopa on prolactin and aldosterone secretion in man. *J Endocrinol Invest* 12: 87-91.
- Wald FDM, Mellenbergh GJ (1990) De verkorte versie van de Nederlandse vertaling van de Profile of Mood States (POMS). *Ned T Psychol* 45: 86-90.
- Westenberg HG, Gerritsen TW, Meijer BA, van Praag HM (1982) Kinetics of l-5-hydroxytryptophan in healthy subjects. *Psychiatry Res* 7: 373-385.

Enhanced tolerability of the 5-hydroxytryptophane challenge test combined with granisetron

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Abstract

- BACKGROUND** A recently developed oral serotonergic challenge test consisting of 5-Hydroxytryptophane (5-HTP 200mg) combined with carbidopa (CBD 100mg + 50mg) exhibited dose-related neuroendocrine responsiveness and predictable pharmacokinetics. However, its applicability is limited by nausea and vomiting (Smarius et al.).
- OBJECTIVES** A randomized, double blind, placebo-controlled, four-way cross-over trial was performed in 12 healthy male volunteers. The 5-HTP/CBD-challenge was combined with two oral anti-emetics (granisetron 2mg or domperidone 10mg) to investigate its reliability when side-effects are suppressed. The neuroendocrine response (serum cortisol and prolactin), the side-effect profile (Visual Analogue Scale Nausea (VAS)) and vomiting subjects per treatment were the main outcome measures.
- RESULTS** Compared to 5-HTP/CBD/placebo, 5-HTP/CBD/granisetron had no impact on cortisol [% change with 95% confidence interval: -7.1% (-19; 6.5)] or prolactin levels [-9.6% (-25.1; 9.1)]; 5-HTP/CBD/domperidone increased cortisol [+13.0% (-4.2; 33.4)] and increased prolactin extensively [+336.8% (245.7; 451.9)]. Compared to placebo, VAS Nausea increased non-significantly with granisetron [+7.6mm(-1.3; 16.5)], as opposed to domperidone [+16.2mm(7.2; 25.2)] and 5-HTP/CBD/placebo [+14.7mm(5.5; 23.8)]. No subjects vomited with granisetron, compared to two subjects treated with 5-HTP/CBD/placebo and five subjects with domperidone. Compared to 5-HTP/CBD/placebo, granisetron addition decreased C_{MAX} of 5-HTP statistically significantly (from 1483 ng/ml to 1272 ng/ml) without influencing $AUC_{0-\infty}$.
- CONCLUSIONS** Addition of granisetron to the combined 5-HTP/CBD challenge suppresses nausea and vomiting without influencing the neuroendocrine response or pharmacokinetics, enhancing its clinical applicability in future psychiatric research and drug development.

Introduction

Various pharmacological challenge tests have been utilized to quantify the integrity and function of serotonergic pathways in the human central nervous system (CNS). These may be helpful in innovative central nervous system drug development and in delineating potential biological markers associated with psychiatric disorders. A commonly used serotonergic challenge test encompasses single oral administration of 5-Hydroxytryptophane (5-HTP), the immediate precursor of serotonin (5-HT). 5-HT is formed centrally following carboxylation of 5-HTP in various serotonergic neurons. Subsequently, serotonergic neurons with hypothalamic projections induce cortisol and prolactin release in peripheral blood, probably indirectly via increased corticotrophin-releasing hormone (CRH) release. Based on this assumption, serum cortisol and prolactin are frequently used neuroendocrine endpoints of serotonergic challenges. However, there is little standardisation of 5-HTP-challenge tests, and their use has been hampered by unclear pharmacokinetics, and a narrow window between neuroendocrine responses and side effects (nausea and vomiting). Combined with carbidopa (CBD) to prevent peripheral carboxylation, Smarius *et al.* recently found a 5-HTP (100mg, 200mg and 300mg) challenge test to have a dose-dependent neuroendocrine responsivity and predictable pharmacokinetics (Smarius *et al.*). However, a wider use of this challenge test in psychiatric drug development and research was thwarted by the frequent dose-dependent occurrence of nausea and vomiting. 100mg 5-HTP combined with CBD 100mg + 50mg seemed to be the best tolerated and most reproducible oral serotonergic challenge test, but this causes a suboptimal stimulation of central serotonergic pathways that may be too small to show differences between patient groups or treatment effects. It would therefore be preferable to have a challenge test with a larger window between neuroendocrine effects and side-effects.

To investigate this objective, two pharmacologically distinct anti-emetics were combined with a challenge test consisting of 200mg 5-HTP administered together with CBD in the present study.

Methods

Study design

This study is a randomized, double blind, placebo controlled, four-way crossover study with the oral administration of 5-HTP with carbidopa (CBD) co-treatment, combined with two different anti-emetics with washout periods of at least 4 days. The study protocol was approved by the Medical Ethics Committee of Leiden University Medical Centre and performed according to Good Clinical Practice and International Conference on Harmonisation guidelines.

Drug administration

The four treatments were:

1. 5-HTP_{200 MG}/CBD_{100+50 MG} and placebo anti-emetic (5-HTP/CBD/placebo);
2. 5-HTP_{200MG}/CBD_{100+50 MG} and 10 mg domperidone orally (5-HTP/CBD/domperidone);
3. 5-HTP_{200 MG}/CBD_{100+50MG} and 2 mg granisetron orally (5-HTP/CBD/granisetron);
4. matched double-dummy placebo.

The neuroendocrine responses (serum cortisol and serum prolactin) and the side-effect profile (Visual Analogue Scale Nausea, Somatic subscale of the Symptoms Check List and number of vomiting subjects per occasion) were the main outcome measures.

Subjects

Twelve healthy, male volunteers participated in the study. After providing their written informed consent subjects received a full medical examination during a pre-study screening. A Dutch translation of the Structured Clinical Interview for DSM-IV axis I (SCID I), was used to exclude any subject with a past or present psychiatric disorder, including substance abuse (Groenestijn MAC et al.). Subjects with a current average use of alcohol of more than 3 units a

day or smoking more than 5 cigarettes a day were not allowed to participate in this study. No xanthine or tryptophan containing foods or beverages, tobacco or alcohol were allowed during the stay in the research unit. Concomitant medication other than paracetamol was not permitted during the study period.

5-HTP and CBD

5-HTP and CBD were obtained from BUFA b.v. (Uitgeest, The Netherlands). Granisetron and domperidone were obtained from the Department of Clinical Pharmacy of the Leiden University Medical Centre. All medication was prepared by the Department of Clinical Pharmacy of the Leiden University Medical Centre, including capsules containing 100 mg 5-HTP and capsules containing 50 mg CBD were made with matching placebos.

Study days

Volunteers arrived at the Centre for Human Drug Research (CHDR) in the morning after an overnight fast. Subjects received the first oral dose of CBD (100 mg) or placebo ($t=-3$ hr), which was followed by a standardized breakfast. Subsequently, a cannula was inserted into the antecubital vein of one arm for blood sampling. Administration of either granisetron or domperidone, or its placebo, took place at 10.00 hours ($t=-1$ hr). At 11.00 hours ($t=0$ hr), 5-HTP or placebo was administered. The second dose of CBD (50 mg) was given at $t=3$ hr after which volunteers received a light lunch and dinner ($t=7$ hr). All challenges were performed under hospital conditions and a research physician attended all study occasions.

Vital signs and biochemical measurements

Vital signs were monitored at 1 hour before and at 1, 2, 3, 4, 5 and 6 hours after 5-HTP administration. Blood pressure was measured using a non-invasive oscillometric system, the Nihon Kohden Lifescoop EC BSM-1101J/K (Nihon Kohden Co., Tokyo, Japan). For Electrocardiography (12 lead) a Nihon Kohden Cardiofax with ECaps 12 software (Nihon Kohden Co., Tokyo, Japan) was used. Oral temperature

was measured with a digital thermometer (Terumo Corporation, Tokyo, Japan). On each study day at 1 hour, 30 minutes and 5 minutes before and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6 and 8 hours after 5-HTP administration, 6 ml whole blood was drawn in a Greiner Vacuette EDTA-tube, tilted, and immediately stored on ice water (0°C). Within one hour the blood was centrifuged at 4°C for 10 minutes at 2000 g. Plasma was divided into three portions, with at least 0.5 ml for cortisol and prolactin and at least 1 ml for the 5-HTP assay. Any remaining plasma is stored as reserve. Plasma was stored directly at -20°C. The determination of cortisol in serum was performed with a competitive electrochemiluminescence immunoassay (ECLIA) using a Roche Elecsys 1010 immunoassay analyzer (Mannheim, Germany). The sample volume was 20 µL. The detection limit was 0.5 nmol/l and the total precision in the measuring range was 2%. The prolactin assay was performed in serum by electrochemiluminescence immunoassay (ECLIA) on a Roche Elecsys 1010 immunoassay analyzer (Mannheim, Germany). The assay employed two monoclonal antibodies specifically directed against prolactin. One of the antibodies carried a ruthenium complex label and the other was biotinylated. The 5-HTP assay was performed at the Biochemical Laboratory of the Rijn-geestgroep, Oegstgeest, the Netherlands according to methods described previously (Gijssman et al. 183-89).

Side-effects

Adverse events were registered from spontaneous reports and hourly inquiries. Only side effects judged definitely, probably or possibly related to the treatment were considered in the analyses. Side effects did not necessarily lead to dropout from the study. If deemed safe by the research physician, subjects who experienced side effects were allowed to participate in the following study day.

Self report questionnaires

The volunteers scored self report questionnaires at 1 hour before and 1, 2, 3, 4, 5, 6 and 8 hours after 5-HTP administration. The somatization-subscale of the Dutch Translation and adaptation of the

Symptom Check List (SCL-90) (Arrindell WA) and Visual Analogue Scales as originally described by Norris, for the four factors corresponding to alertness, mood, calmness and nausea (Bond A and Lader M 211-18) were used for follow-up of subjective effects. The volunteers practised all four visual Analogue Scales three times during the pre-study screening.

Pharmacodynamic analysis

All pharmacodynamic data were LOG transformed except VAS-Nausea and SCL-90. Within the ANOVA design, estimated means (Least squared means; LSM's) of the neuroendocrine parameters (cortisol and prolactin) and the side-effect profile (VAS-Nausea and SCL-90) were calculated over eight hours using subject, subject by treatment and subject by time as random factors and treatment, time, study day and treatment by time as fixed factors and average pre-value as covariate. Estimated difference from placebo for all treatments were estimated and presented with 95% confidence intervals. Additionally, difference from 5-HTP/CBD/placebo for 5-HTP/CBD/granisetron and 5-HTP/CBD/domperidone was calculated. Since vomiting could have had an effect on the PD neuroendocrine effects, study days on which subjects had vomited were excluded from neuroendocrine analysis. VAS-Nausea and SCL-90 were analyzed including all study days on which subjects had experienced nausea and emesis.

Pharmacokinetic analysis

C_{MAX} , T_{MAX} , $AUC_{0-\infty}$ and the terminal half-life of 5-HTP were calculated over 8 hours with non-compartmental analysis using SAS9.1.2. (SAS Institute Inc., Cary, USA) The hypothesis that anti-emetics had no influence on PK parameters of 5-HTP was tested on $AUC_{0-\infty}$ and C_{MAX} with a mixed model analysis of variance, with treatment as fixed factor and subject as random factor and an unstructured covariance structure. Past experience suggests that $AUC_{0-\infty}$ and C_{MAX} are distributed as log-normal, therefore parameters were LOG transformed before analysis. Since vomiting could have influenced the PK, occasions on which subjects vomited were excluded from pharmacokinetic analysis.

Results

Demographic data and subject disposition

Twelve subjects initially provided informed consent and were included in the study (mean age 24 years; range 18–38 years). One subject dropped out during the 5-HTP/CBD/placebo treatment due to intolerable dizziness and was subsequently replaced. There were no drop-outs due to nausea or vomiting on any occasion.

Adverse events

The most common side-effect during placebo treatment was headache (6/12 study days). Vomiting occurred in 0/13, 5/12 and 2/13 study days for the 5-HTP/CBD/granisetron, 5-HTP/CBD/domperidone and 5-HTP/CBD/placebo treatments, respectively. Nausea occurred more frequently than vomiting (3/13, 8/12 and 6/13 study days, respectively). The main other reported side effects during active treatment were headache (2/13, 5/12 and 5/13 study days, respectively) and dizziness (2/13, 2/12 and 5/13 study days, respectively).

Self report questionnaires

Granisetron seemed effective in suppressing 5-HTP induced nausea. Compared to placebo VAS-Nausea did not increase significantly during the 5-HTP/CBD/granisetron treatment, while this was the case for both 5-HTP/CBD/placebo and 5-HTP/CBD/domperidone treatments (Table 2). Moreover, SCL-90 Nausea was significantly lower during the 5-HTP/CBD/granisetron treatment compared to the 5-HTP/CBD/placebo treatment (Table 2, Figure 4).

Pharmacodynamic effects

Mean cortisol response differed significantly from placebo for all three active treatments. The 5-HTP/CBD/granisetron induced a cortisol response that differed significantly from placebo but did not differ significantly from that induced by 5-HTP/CBD/placebo (Table 2 and Figure 1). Compared with placebo, mean prolactin response

increased significantly for the 5-HTP/CBD/domperidone and 5-HTP/CBD/placebo treatments, but not the 5-HTP/CBD/granisetrone treatment. However, the difference between 5-HTP/CBD/granisetrone and 5-HTP/CBD/placebo was not statistically significant (Table 2 and Figure 2). Moreover, the mean increases in cortisol and prolactin during the 5-HTP/CBD/placebo treatment were comparable to our previous findings on the same challenge test (Smarius et al.).

Pharmacokinetics

Pharmacokinetic parameters for 5-HTP are presented in Table 1 and the average concentration-time-curves for 5-HTP, 5-HTP combined with domperidone and 5-HTP combined with granisetron are displayed in Figure 1.

Discussion

We have demonstrated enhanced tolerability of a serotonergic challenge test consisting of 5-HTP_{200MG}/CBD_{100+50MG} with addition of the selective 5-HT₃ receptor antagonist granisetron. Moreover, the addition of granisetron had no impact on the 5-HTP induced serum cortisol response or pharmacokinetics of 5-HTP.

After its conversion from 5-HTP, serotonin (5-HT) is available for non-specific post-synaptic neurotransmission via the 5-HT_{1C}, 5-HT₂, 5-HT₃ and 5-HT₄ receptors. As a result, nausea and vomiting ensue as the most intolerable untoward effects. 5-HT induced nausea and vomiting are hypothesized to result from stimulation of both peripheral (vagal fibres and chemoreceptor trigger zone (CTZ)) and central (medulla oblongata and solitary tract nucleus) 5-HT receptors (Endo et al. 189-201; Sanger and Andrews 3-16). Granisetron strongly and selectively binds to 5-HT₃ receptors and since it readily crosses the blood-brain-barrier (BBB) it is believed to exert its anti-emetic action both centrally and peripherally (Tan 1563-71). Normally, domperidone does not effectively cross the BBB and predominantly antagonizes D₂ receptors in the pontine vomiting centre in the area postrema, which lies outside the BBB. The dopamine (DA) receptor antagonists are believed to exert their anti-emetic action by antagonism of the D₂-receptor (D₂) in the area postrema (Mitchelson 295-315) but the actual source of dopamine release

modulating nausea and emesis is unknown. Therefore, although there is a clear rationale on how D_2 antagonists prevent emesis evoked by dopamine agonists (eg. L-DOPA in Parkinson's disease), it is not clear how they inhibit emesis associated with other conditions (eg. chemotherapy, post-operative nausea). Domperidone was not effective in curbing nausea and vomiting, indicating that these symptoms are not mediated by (indirect) serotonergic stimulation of pontine dopamine systems.

Compared with previous investigations of our serotonergic challenge (Smarius et al.) the addition of granisetron in this trial to the combined 5-HTP/CBD challenge test did not significantly influence 5-HTP induced serum cortisol release. Also, the mean maximal serum cortisol concentration attained with 5-HTP_{200MG}/CBD_{100+50MG}/granisetron (465.8 nmol/l) was very similar to that attained with 5-HTP_{200MG}/CBD_{100+50MG} without anti-emetic (455.9 nmol/l) in our previous trial (Smarius et al.). This concentration approaches the maximal one attained with a previous (close to maximum tolerated) intravenous meta-chlorophenylpiperazine (mCPP) dose of 0.5 mg/kg, which lead to an average maximum serum cortisol concentration of around 630 nmol/l (Gijsman et al. 289-95). Thus, it seems that the combined 5-HTP/CBD/granisetron challenge covers a major part of the maximum range for serum cortisol release that can be attained in healthy volunteers, without being hampered by nausea and vomiting. Challenge tests are used to show changes in responsivity of a particular system, in this case the HPA-axis, during pathological conditions or treatments. Therefore, it is essential that the level of stimulation of the HPA-axis with this newly developed challenge (5-HTP/CBD/granisetron) is neither too small to show decreases, nor too large to show increases.

The combined 5-HTP/CBD/domperidone challenge was associated with significant hyperprolactinemia in comparison with the 5-HTP/CBD/placebo and 5-HTP/CBD/granisetron treatments. Prolactin release is believed to depend not only on the stimulation of the postsynaptic 5-HT₂ receptors (Van de Kar et al. 203-08), but also on tonic inhibition by the neurotransmitter dopamine (DA). Pre-treatment with the D_2 antagonist domperidone caused a mean increase in prolactin of roughly 400% compared to 32% and 17% for the 5-HTP-challenge with placebo and granisetron respectively. Prolactin release is a well-known effect of D_2 -antagonism, but the

limited (albeit statistically significant) prolactin increase by 5-HTP with or without granisetron demonstrates that prolactin can also be stimulated directly by serotonergic mechanisms.

Compared to 5-HTP/CBD/placebo, pretreatment with granisetron lead to a statistically significant decrease in C_{MAX} of 5-HTP of 14 ng/ml (from 1483 to 1272 ng/ml) without influencing its $AUC_{0-\infty}$. It would perhaps have been expected that an anti-emetic would improve absorption, rather than reduce it. However, the difference was small and it could be spurious or due to a more erratic absorption of 5-HTP without an anti-emetic, as a manifestation of gastrointestinal side-effects.

There is some controversy regarding the pharmacological mechanisms of serotonergic stimulation of the HPA axis. Our studies show that serotonin-induced HPA axis activation is not critically dependent on 5-HT₃-receptors. Furthermore, previous experiments found no effects of granisetron on 5-HTP induced ACTH release (Gartside and Cowen 103-09). Studies in experimental animals and healthy humans suggest that activation of central post-synaptic 5-HT₁ or 5-HT_{1C} receptors is involved (Jorgensen et al. 788-95).

The current challenge is composed of three different medications, to improve the pharmacokinetic stability and tolerability of the test. Despite this complexity, we believe that the improved reproducibility and reduced side effect profile of the combined 5-HTP/CBD/granisetron-test renders it more acceptable as a practical serotonergic challenge test in clinical research. Such a test could be used to show biochemical/neuroendocrine differences between healthy individuals and individuals (at risk of) suffering from HPA-axis related psychiatric disease. It could also be useful in innovative drug development, to study the effects of neuro-modulatory agents that may have few specific pharmacological CNS-effects of their own in healthy subjects, but which are expected to indirectly affect serotonergic functionality. For example, specific serotonin reuptake inhibitors (SSRI's) have few consistent effects in healthy subjects (Dumont et al. 495-510), but cause clear changes in sensitivity to serotonergic challenges. SSRI's like paroxetine (Sargent, Williamson, and Cowen 49-52) or citalopram (Lowe et al. 473-84) cause an increase in 5-HTP-induced cortisol release. Furthermore, this sensitivity changes over time. Initially, 5-HTP-induced cortisol release is augmented in combination with

paroxetine, but this diminishes during three weeks of continued SSRI-treatment (Sargent, Williamson, and Cowen 49-52). These changes may indicate a decrease in serotonergic sensitivity during chronic SSRI-dosing. This may be related to down-regulation of (post synaptic 5HT₂) serotonin receptors, which has also been implicated in the delayed therapeutic action of SSRIs.

An improved serotonergic challenge may be a very useful instrument to investigate the physiology of serotonergic systems in health and disease, and its changes over time under different conditions or treatments. Addition of granisetron to the oral 5-HTP/CBD challenge (Smarius et al.) has enlarged the window between the neuroendocrine pharmacodynamic effects and the adverse side-effects of this serotonergic challenge.

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Table 1

Pharmacokinetic parameters for 5-HTP: Least square mean (SE) of C_{MAX} (ng/ml) and $AUC_{0-\infty}$ (min*ng/ml) with estimated difference from 5-HTP/CBD with 95% confidence interval for 200 mg 5-HTP/CBD/domperidone and 200 mg 5-HTP/CBD/granisetron.

| Pharmacokinetic parameter | | 5-HTP/CBD n=13 | 5-HTP/CBD/ domperidone n=12 | 5-HTP/CBD/ granisetron n=12 |
|------------------------------------|--|-------------------|-----------------------------------|-----------------------------------|
| 5-HTP C_{MAX} (ng/ml) | LSM (SE) Estimated difference from 5-HTP/CBD (95% CI) | 1483 | 1383 -7 (-29;22) p=0.58 | 1272 -14 (-26;0) p=0.05 |
| 5-HTP $AUC_{0-\infty}$ (min*ng/ml) | LSM (SE) Estimated difference from 5-HTP/CBD (95% CI) | 549207 | 495371 -10 (-28;13) p=0.34 | 519492 -5 (-31;29) p=0.70 |

Table 2

Pharmacodynamic parameters over 8 hours: Estimated means (LSM's) of serum cortisol (nmol/l), serum prolactin (ug/l), VAS-Nausea (mm) and SCL-90 Nausea (5 point scale) for the treatments placebo, 5-HTP/CBD/placebo, 5-HTP/CBD/domperidone and 5-HTP/CBD/granisetron. Estimated difference from placebo (%) with 95% confidence interval for placebo, 5-HTP/CBD/placebo, 5-HTP/CBD/domperidone and 5-HTP/CBD/granisetron and estimated difference from 5-HTP/CBD for 5-HTP/CBD/domperidone and 5-HTP/CBD/granisetron.

| Parameter | Least Square Means (vomiting subjects excluded) | | | | | Estimated difference (%) | | | | |
|----------------------------------|---|------------------------|-----------------------|------------------------|----------------------|-------------------------------------|---------------------------------------|------------------------------------|-------------------------------------|------------------------------------|
| | plac n=12 | 5-HTP +plac n=11 | 5-HTP +dom n=7 | 5-HTP +gran n=13 | treatment P-value | 5-HTP + plac vs plac | 5-HTP + dom vs plac | 5-HTP + gran vs plac | 5-HTP + dom vs 5-HTP + plac | 5-HTP + gran vs 5-HTP + plac |
| Serum cortisol (nmol/l) | 167.6 | 315.0 | 356.1 | 292.7 | p < 0.0001 | +88.1 (63.5;116.4) p < 0.0001 | +112.6 (80.7;150.2) p < 0.0001 | +74.8 (53.0;99.7) p < 0.0001 | +13.0 (-4.2;33.4) p=0.1398 | -7.1 (-18.9;6.5) p=0.2775 |
| Serum prolactin (ug/l) | 4.3 | 5.5 | 24.0 | 5.0 | p < 0.0001 | +28.5 (4.9;57.3) p=0.0180 | +461.1 (339.3;616.7) p < 0.0001 | +16.1 (-4.0;40.5) p=0.1162 | +336.8 (245.7;451.9) p<0.0001 | -9.6 (-25.1;9.1) p=0.2750 |
| Parameter | Least Square Means (vomiting subjects included) | | | | | Estimated difference (%) | | | | |
| | plac n=12 | 5-HTP +plac n=12 | 5-HTP +dom n=12 | 5-HTP +gran n=13 | treatment P-value | 5-HTP + plac vs plac | 5-HTP + dom vs plac | 5-HTP + gran vs plac | 5-HTP + dom vs 5-HTP + plac | 5-HTP + gran vs 5-HTP + plac |
| SCL-90 Nausea (5 point scale) | +1.0 | +1.7 | +1.7 | +1.4 | p=0.0002 | +0.7 (0.4;1.0) p=0.0001 | +0.7 (0.4;1.0) p=0.0001 | +0.3 (0.0;0.7) p=0.0389 | 0.0 (-0.3;0.3) p=0.9861 | -0.4 (-0.7;0.0) p=0.0276 |
| VAS-Nausea (mm) | +6.8 | +21.5 | +23.0 | +14.4 | p=0.0034 | +14.7 (5.5;23.8) p=0.0027 | +16.2 (7.2;25.2) p=0.0009 | +7.6 (-1.3;16.5) p=0.0915 | -1.5 (-7.6;10.7) p=0.7343 | -7.1 (-16.0;1.9) p=0.1164 |

plac= Placebo; 5-HTP= 200mg 5-hydroxytryptophane/100mg + 50mg carbidopa; dom= domperidone; gran= granisetron

Figure 1

Average time profile of serum 5-HTP (ng/ml) over 8 hours for the treatments 5-HTP/CBD (n=13), 5-HTP/CBD/domerperidone (n=12) and 5-HTP/CBD/granisetrone (n=12) with SD error bars.

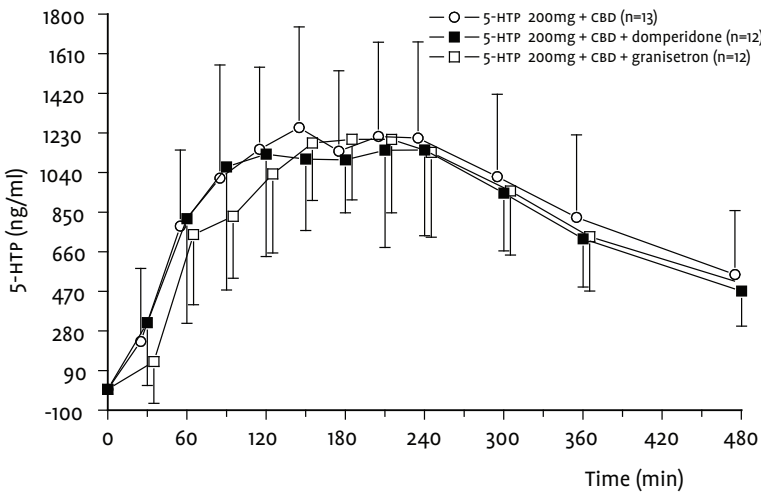


Figure 2

Estimated means (nmol/l) with SD error bars and estimated change from placebo (%) for serum cortisol after administration of placebo (n=12), 5-HTP/CBD (n=11), 5-HTP/CBD/domerperidone (n=7), 5-HTP/CBD/granisetrone (n=13), excluding data of study days on which subjects vomited.

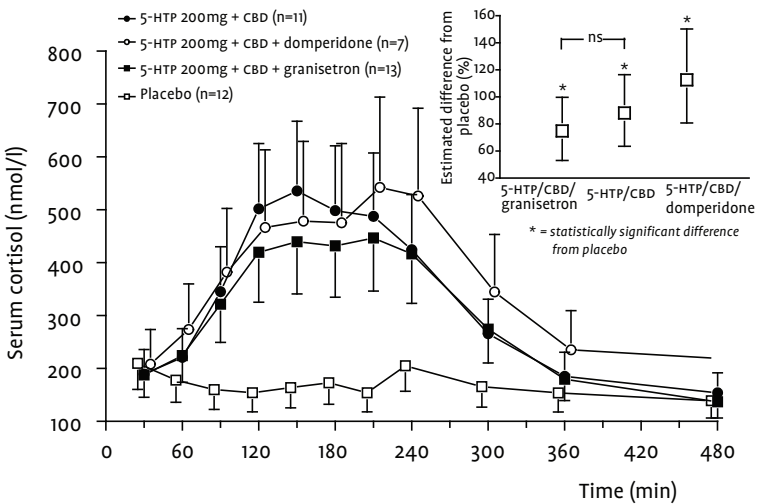


Figure 3

Estimated means ($\mu\text{g/l}$) with SD error bars and estimated change from placebo (%) for serum prolactin after administration of placebo (n=12), 5-HTP/CBD (n=11), 5-HTP/CBD/domperidone (n=7), 5-HTP/CBD/granisetron (n=13), excluding data of study days on which subjects vomited.

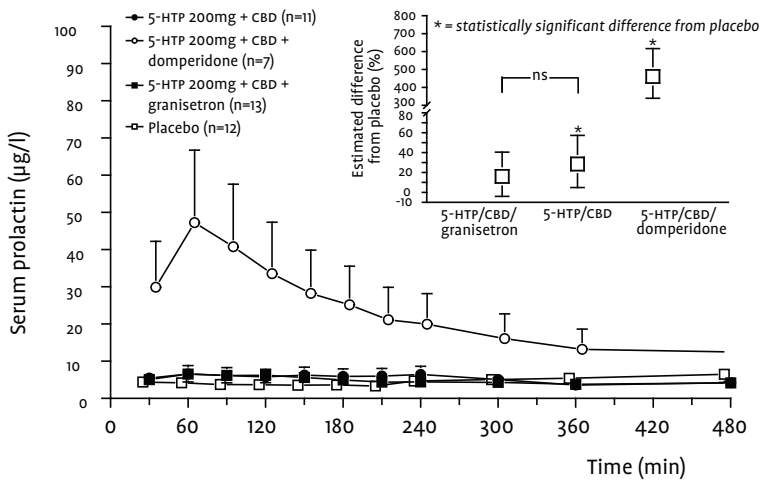
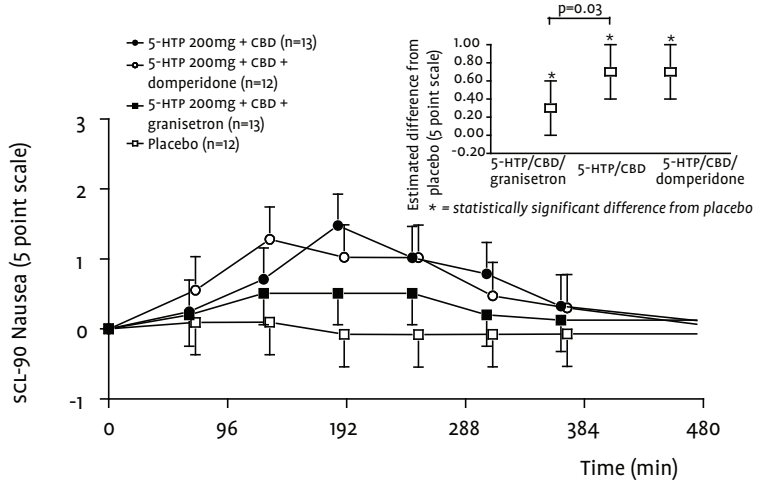


Figure 4

Estimated mean change from baseline (5 point scale) and estimated change from placebo for SCL-90 Nausea after administration of placebo (n=12), 5-HTP/CBD (n=12), 5-HTP/CBD/domperidone (n=12), 5-HTP/CBD/granisetron (n=13) with SD error bars, including all data.



REFERENCE LIST

- Arrindell WA, Ettema JHM. SCL-90. Handleiding Bij Een Multidimensionele Psychopathologie-Indicator. Lisse: 1986.
- Bond A and Lader M. "The use of analogue scale in rating subjective feelings." *Br J Med Psychol* 47 (1974): 211-18.
- Dumont, G. J., et al. "Biomarkers for the effects of selective serotonin reuptake inhibitors (SSRIs) in healthy subjects." *Br J Clin Pharmacol* 59.5 (2005): 495-510.
- Endo, T., et al. "Neurochemistry and neuropharmacology of emesis - the role of serotonin." *Toxicology* 153.1-3 (2000): 189-201.
- Gartside, S. E. and P. J. Cowen. "Mediation of ACTH and prolactin responses to 5-HTP by 5-HT₂ receptors." *Eur J Pharmacol* 179.1-2 (1990): 103-09.
- Gijssman, H. J., et al. "Pharmacokinetic and pharmacodynamic profile of oral and intravenous meta-chlorophenylpiperazine in healthy volunteers." *J Clin Psychopharmacol* 18.4 (1998): 289-95.
- Gijssman, H. J., et al. "Placebo-controlled comparison of three dose-regimens of 5-hydroxytryptophan challenge test in healthy volunteers." *Journal of Clinical Psychopharmacology* 22.2 (2002): 183-89.
- Groenestijn MAC, et al. Structured Clinical Interview for DSM-IV Axis I Disorders. Lisse, The Netherlands: Swets Test Publishers, 1999.
- Jorgensen, H., et al. "Serotonergic stimulation of corticotropin-releasing hormone and pro-opiomelanocortin gene expression." *J Neuroendocrinol.* 14.10 (2002): 788-95.
- Lowe, S. L., et al. "L-5-Hydroxytryptophan augments the neuroendocrine response to a SSRI." *Psychoneuroendocrinology* 31.4 (2006): 473-84.
- Mitchelson, F. "Pharmacological agents affecting emesis. A review (Part I)." *Drugs* 43.3 (1992): 295-315.
- Sanger, G. J. and P. L. Andrews. "Treatment of nausea and vomiting: gaps in our knowledge." *Auton. Neurosci.* 129.1-2 (2006): 3-16.
- Sargent, P. A., D. J. Williamson, and P. J. Cowen. "Brain 5-HT neurotransmission during paroxetine treatment." *Br J Psychiatry* 172 (1998): 49-52.
- Smarius, L. J., et al. "Pharmacology of rising oral doses of 5-hydroxytryptophan with carbidopa." *J Psychopharmacol* (2008).
- Tan, M. "Granisetron: new insights into its use for the treatment of chemotherapy-induced nausea and vomiting." *Expert Opin. Pharmacother.* 4.9 (2003): 1563-71.
- Van de Kar, L. D., et al. "Serotonergic regulation of renin and prolactin secretion." *Behav. Brain Res* 73.1-2 (1996): 203-08.

Metoclopramide as pharmacological tool to assess vasopressinergic co-activation of the hypothalamus–pituitary–adrenal (HPA) axis: a study in healthy volunteers

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Abstract

The synthetic vasopressin (AVP) analogue desmopressin (dDAVP) has been used as pharmacological function test to quantify vasopressinergic co-activation of the hypothalamus-pituitary-adrenal (HPA) axis in the past. Such exogenous vasopressinergic stimulation may induce confounding cardiovascular, pro-coagulatory and anti-diuretic effects and low endogenous corticotrophin-releasing-hormone (CRH) levels may limit its potential to reliably assess co-activation. Alternatively, the dopamine-2-(D₂)-antagonist metoclopramide is believed to induce co-activation indirectly by releasing endogenous AVP. We investigated this indirect co-activation with metoclopramide under conditions of low and enhanced endogenous CRH-release in healthy volunteers. A randomized, double-blind, placebo-controlled, four-way crossover study was performed in 12 healthy males. CRH-release was induced by administering an oral 5-hydroxytryptophan (5-HTP) 200mg function test. Co-activation was investigated by administering metoclopramide 10 mg intravenously around the expected maximal effect of 5-HTP. The neuroendocrine effects were compared to those of metoclopramide alone, the 5-HTP test alone and matching placebo. Metoclopramide safely induced HPA-axis activation by itself, and potentially synergized 5-HTP-induced corticotrophinergic activation of the HPA-axis. These findings are indicative of vasopressinergic co-activation and suggest a role for metoclopramide as a practical function test for co-activation of the HPA-axis. However, its application will be hampered pending clarification of the exact pharmacological mechanism by which metoclopramide induces co-activation of the HPA axis.

Introduction

Corticotrophin-releasing hormone (CRH) and arginine-vasopressin (AVP) are the major neuropeptide activators of the hypothalamus-pituitary-adrenal (HPA) axis (Aguilera and Rabadan-Diehl, 2000; Ring, 2005; Scott and Dinan, 1998). Under physiological circumstances, CRH acts as major neuroendocrine secretagogue that induces corticotrophinergic HPA activation via pituitary CRH₁ receptors (CRH₁), while AVP by itself has weak neuroendocrine properties and induces vasopressinergic co-activation of the HPA at pituitary vasopressin 3 receptors (V₃, also referred to as V_{1b} receptor) (Ring, 2005; Scott and Dinan, 1998; Scott and Dinan, 2002). Following acute stress, AVP releases adrenocorticotrophic hormone (ACTH) synergistically in the presence of increased levels of CRH (DeBold et al., 1984; Favrod-Coune et al., 1993; Lamberts et al., 1984). During chronic stress, either increased AVP synthesis and/or release, increased V₃ responsivity and/or expression (Goekoop and Wiegant, 2009), genetic polymorphisms of the V₃-receptor (Dempster et al., 2007) or a combination of these factors is hypothesized to lead to chronic HPA hyperactivity (Aguilera and Rabadan-Diehl, 2000; Volpi et al., 2004). In this context, sustained vasopressinergic co-activation has been implicated in stress-related psychopathology (Dinan et al., 2004; Dinan and Scott, 2005; Holsboer, 1983; Holsboer et al., 1984b; Volpi et al., 2004). A pharmacological function test that quantifies vasopressinergic co-activation would therefore be a useful tool to study this functional component of the HPA-axis in health and disease, and during treatments directed at the vasopressinergic system.

The synthetic analogue of AVP, desmopressin (dDAVP) is frequently applied as pharmacological function test for vasopressinergic co-activation (Dinan et al., 1999; Dinan et al., 2004). It stimulates the V₃ receptor directly in the presence of (endogenous) CRH, inducing pituitary ACTH and subsequent adrenal cortisol release. Most experiments with dDAVP in healthy volunteers occur in the mid-morning when HPA-axis activity and endogenous CRH levels are relatively low. Since AVP acts in synergy with CRH, 10 µg dDAVP induces small and (frequently) variable co-activation under low CRH activity (Dinan et al., 1999; Dinan et al., 2004; Jacobs et al., 2009). Too small or too variable responses limit the test's

informative value and would not allow for an accurate assessment of conditions or treatments that modulate vasopressinergic co-activation. Recent studies have shown that doses higher than 10 µg dDAVP alone may not produce much more ACTH- or cortisol release during baseline conditions (Jacobs et al., 2009). Moreover, higher dDAVP doses may cause (confounding) cardiovascular, pro-coagulatory and anti-diuretic effects which would limit its tolerability and applicability (Jacobs et al., 2009). Alternative ways of inducing informative vasopressinergic co-activation therefore need to be explored.

One approach would be to stimulate endogenous release of AVP instead of exogenous stimulation of V_3 receptors. The D_2 -receptor antagonist anti-emetic metoclopramide has been shown to activate the HPA-axis (Chiodera et al., 1986; Seki et al., 1997; Walsh et al., 2005). It is hypothesized to produce vasopressinergic co-activation by endogenously releasing AVP from the pituitary and/or hypothalamus, through a hitherto unclear pharmacological mechanism (Chiodera et al., 1986; Nomura et al., 1984). If this were the case, metoclopramide would not affect CRH levels and would induce small neuroendocrine responses that are comparable to those achieved by dDAVP under low CRH activity. Stimulation of the CRH system by exogenous administration of human corticotrophic release hormone (hCRH) induces considerably greater HPA-axis activation (Dinan et al., 1999; Holsboer, 1983; Holsboer et al., 1984a; von Bardeleben and Holsboer, 1988). Alternatively, serotonergic (precursor) agents can be used as endogenous corticotrophinergic stimulants of the HPA-axis (Dinan et al., 1999; Gijsman et al., 1998; Gijsman et al., 2002; Smarius et al., 2008). A serotonergic function test consisting of 5-hydroxytryptophan (5-HTP) has been developed for this purpose (Jacobs et al., 2008a). 5-HTP is the direct precursor of serotonin (5-HT) and is centrally converted into 5-HT. Enhanced 5-HT release stimulates CRH release via postsynaptic 5-HT_{2A} or 5-HT_{2C} receptors in the paraventricular nucleus (PVN) of the hypothalamus (Gartside and Cowen, 1990; Jorgensen et al., 2002). The neuroendocrine response associated with the endogenous release of CRH (by administering 5-HTP) is therefore expected to be synergized by endogenously released AVP (with metoclopramide). Moreover, such an effect would be an (indirect) indication of vasopressinergic co-activation of the HPA-axis with metoclopramide.

We examined metoclopramide's effect on neuroendocrine HPA-axis activation under physiological circumstances (by administering metoclopramide alone) and under enhanced CRH-mediated activation of the HPA-axis (by administering the 5-HTP function test followed by metoclopramide) in healthy male volunteers.

Experimental Procedures

Study design

The study protocol was approved by the Medical Ethics Committee of Leiden University Medical Centre (LUMC) and performed according to Good Clinical Practice and International Conference on Harmonisation (GCP-ICH) guidelines. A randomized, double-blind, double-dummy placebo-controlled, four-way crossover trial was performed in 12 healthy volunteers.

Main outcome measures

The main pharmacodynamic (PD) outcome measures were the neuroendocrine effects (serum ACTH, cortisol, prolactin and AVP) of 10mg metoclopramide, the 200mg 5-HTP function test, and 10mg metoclopramide combined with the 200mg 5-HTP function test. Also, adverse events (AE's) were recorded to assess the safety and tolerability of concomitantly administered metoclopramide and the 5-HTP function test.

Drug administration

The 5-HTP function test was administered according to the procedure described previously (Jacobs et al., 2008a; Smarius et al., 2008). It consisted of the administration of carbidopa 100 mg (at $t=-180$ minutes to reduce peripheral metabolism of 5-HTP) and granisetron 2 mg (at $t=-60$ minutes to prevent nausea and vomiting), followed by a single oral dose of 5-HTP 200 mg (at $t=0$ minutes) and a repeat dose of carbidopa 50 mg (at $t=180$ minutes). Metoclopramide 10 mg was administered intravenously (i.v.) one hour following the administration of 5-HTP, which was expected to coincide with the maximal effect (E_{MAX}) of 5-HTP according to

previous experiments. For all three active drugs, matching double-dummies were administered as placebo. A schematic overview of the four different treatments is provided in table 1.

Volunteers

Twelve healthy, male volunteers participated in the study. After obtaining written informed consent, the volunteers underwent a full medical screening to assess eligibility. Volunteers with a current average use of alcohol of more than four units a day or smoking more than five cigarettes a day were not allowed to participate in this study. Volunteers with a significant personal or family history of psychiatric disorder according to DSM-IV, a history of movement disorder (including movement disorder due to D-antagonists) and with past or present recreational use of methamphetamines, MDMA or 'ecstasy', were excluded from study participation. Xanthine or tryptophan containing foods or beverages, tobacco or alcohol were not allowed during the stay in the research unit. Concomitant medication other than paracetamol was not permitted from two weeks preceding and during the study period.

Study drugs

5-HTP and carbidopa were obtained from BUFA b.v. (Uitgeest, The Netherlands). Granisetron and metoclopramide were obtained from the Department of Clinical Pharmacy of the LUMC. All medications and their respective placebos were prepared by the Department of Clinical Pharmacy of the LUMC, including 100 mg 5-HTP, 50 mg carbidopa and 2mg granisetron in capsules, and syringes containing 10mg metoclopramide.

Study days

Volunteers arrived at the Centre for Human Drug Research (CHDR) on the evening prior to each study day. On admission urinary screening was performed for drug of abuse using the OnCall Test, ACON laboratories, Inc. Rapid Assays for Drug Abuse (Instruchemie Hilversum B.V. the Netherlands). Volunteers went to bed at 23.00h

and were woken up around 8.00h the next morning. Subjects received the first oral dose of carbidopa (100 mg) or placebo ($t=-180$ minutes), which was followed by a standardized breakfast. An intravenous cannula was inserted into the antecubital vein of each arm for blood sampling and intravenous administration of study medication. This occurred at least 1 hour preceding the first PD blood sampling to allow potential procedure-related HPA-axis activation to return to baseline. Administration of granisetron or its placebo took place at $t=-60$ minutes. At $t=0$ minutes 5-HTP or placebo was administered and followed by metoclopramide or placebo at $t=60$ minutes and the second dose of carbidopa (50 mg) at $t=120$ minutes. A standard light lunch was served at $t=130$ minutes and dinner followed at $t=420$ minutes.

Neuroendocrine assays

Venous blood (1.2ml; prechilled EDTA tubes) was collected for the determination of plasma ACTH on -120, -60 before and 1, 30, 55, 70, 80, 90, 105, 120, 150, 180, 240, 300 and 540 minutes after the administration of 5-HTP. Samples were immediately placed on ice, processed within 30 minutes and stored at -80°C . ACTH was determined with a solid-phase, two-site sequential chemiluminescent immuno-metric assay using an Immulite 2500 immunoassay analyzer from DPC (Los Angeles, USA) at the laboratory for clinical chemistry of LUMC. The detection limit for ACTH was 1.1 pmol/l and the total precision in the measuring range was about 5%. Venous blood (1.2ml; serum tubes) for the assay of serum cortisol and prolactin was taken at -120, -60 before and 1, 30, 55, 90, 120, 150, 180, 210, 240, 300, 360, 420 and 540 minutes after the administration of 5-HTP. Samples were stored for 30 – 45 minutes at ambient temperature to allow coagulation, subsequently centrifuged for 15 minutes at $2000 \times g$ and stored at -20°C . Serum cortisol and prolactin were determined using an in-house AutoDELFIA (Perkin Elmer Lifesciences) assay at Organon Development GmbH, Department of Bioanalytics, Waltrop, Germany. Cortisol was measured with a solid phase time-resolved fluoroimmunoassay based on the competitive reaction between Europium-labelled cortisol and sample cortisol. The fluorescence was inversely proportional to the concentrations of cortisol in the samples. Prolactin was measured using a solid

phase, two-site fluoroimmunometric assay based on the direct sandwich technique. The fluorescence was proportional to the concentration of prolactin in the sample.

AVP assay

Venous blood (5ml, prechilled K3 EDTA Aprotinin tubes) was collected for the determination plasma AVP on 1, 30, 55, 70, 80, 90, 105, 120, 150 and 200 minutes after the administration of 5-HTP. Samples were centrifuged directly after homogenisation for 15 minutes at 2000 x g at 4 °C and stored immediately at -20 °C. AVP was isolated from the EDTA plasma by solid phase extraction. The final plasma extract was analyzed by a Radio Immuno Assay (RIA) (Bühlmann Laboratories AG, Schönenbuch, Switzerland) based on the competition between radioactive [125 I]-labelled and non-radioactive vasopressin for a fixed number of antibody binding site by Xendo Drug Development BV, Groningen, the Netherlands

5-HTP assay

Venous blood (2.6ml; serum tubes) for the determination plasma 5-HTP was taken at 1, 30, 55, 90, 120, 150, 180, 210, 240, 270, 300, 340, 420 and 540 minutes after the administration of 5-HTP. Samples were immediately placed on ice, centrifuged for 15 minutes at 2000 x g and stored directly at -20 °C. After addition of buffer and internal standard, a solution with 1.55 mol/l trichloroacetic acid, 13.4 mmol/l EDTA and 50 mmol/l sodium bisulphite ($\text{Na}_2\text{S}_2\text{O}_5$) was added to remove proteins. After centrifuging at 3000g for 32 minutes, the supernatant was assayed by highpressure liquid chromatography (HPLC) with electrochemical detection using 650mV. The HPLC contained a Merck LiChrospher 60 RP-Select B, 5 μ m, 125 \times 4 mm internal diameter plus 1cm guard column. For the mobile phase, we used a solution (pH 3.60) with 50 mmol/l sodium acetate (NaAc), 50 mmol/l citric acid, 0.27 mmol/l EDTA, 1.17 mmol/l 1-octanesulfonic acid sodium salt and 1.5% volume-to-volume ratio acetonitrile. The lower limits of detection and quantification were 0.5 and 1.7 ng/ml, respectively. The coefficients of variability for precision and reliability were 2.6 and 7.9%, respectively. Samples were analyzed at the laboratory for clinical chemistry of LUMC.

Metoclopramide assay

Venous blood (1.8ml; 1.109M citrated tubes) for the determination of plasma metoclopramide was taken at 235, 270, 300, 420 and 600 minutes for subjects 1-6 and 250, 285, 361, 480 and 720 minutes for subjects 7-12 after the administration of 5-HTP. Samples were centrifuged for 10 minutes at 2000 x g at 4°C and 0.5ml plasma was transferred and stored at -20°C. A sensitive and specific HPLC-MS/MS (Waters 2790, Applied Biosystems API-4000) method was developed for the quantitative determination of metoclopramide in human plasma. Plasma samples were prepared by liquid-liquid extraction using ethylether and separated on an Alltima C18 column with 5mM ammonium acetate (pH=5.0): acetonitrile: methanol = 50: 40: 10 (v/v) as the mobile phase. Detection was performed on a triple-quadrupole tandem mass spectrometer using positive electro spray ionization (ESI), and multiple reaction monitoring (MRM) was applied. The lowest limit of quantification of metoclopramide was 0.5ng/ml, and the linear range was 0.5 -200 ng/ml. Accuracies and precisions of all were within $\pm 15\%$. Samples were analyzed at the Clinical Pharmacology Research Centre of Peking Union Medical College Hospital (PUMC), Beijing, China.

Vital signs

Blood pressure and pulse rate were measured using the Nihon-Kohden (BSM-1100) or Colin (Pressmate BP-8800) blood pressure apparatus. Electrocardiogram (ECG) recordings were made at $t=-15$ minutes and $t=175, 230, 300$ and 600 minutes.

Pharmacodynamic (PD) analysis

ACTH, serum cortisol and prolactin were log-transformed prior to analysis to correct for the expected log-normal distribution of the data. Repeatedly measured pharmacodynamic data were analyzed with a mixed model analysis of variance with fixed factors treatment, period, time and treatment by time, random factor subject, subject by treatment and subject by time and the average pre-value as covariate, using SAS for windows V9.1.2 (SAS Institute, Inc., Cary, NC, USA). Initially, the treatments 5-HTP and 5-HTP/meto-

cloramide were planned to be analyzed over the period 0 to 180 minutes. Since metoclopramide was administered at $t=60$ minutes, metoclopramide was to be contrasted for the period 60 to 180 minutes. However, a blinded data review showed that lunch at $t=130$ induced unexpectedly large and highly variable surges in both ACTH and cortisol. It was therefore decided prior to statistical analyses to change the planned analyses for the periods 0 to 180 minutes and 60 to 180 minutes, to 0 to 130 minutes and 60 to 130 minutes. Some volunteers vomited probably due to the serotonergic effects of 5-HTP. Since vomiting could influence the neuroendocrine response, five of the 60 study occasions were excluded *a priori* from the neuroendocrine analyses: three for 5-HTP and two for 5-HTP combined with metoclopramide. Testing for co-activation was complicated by the fact that metoclopramide was administered 60 minutes after 5-HTP or its placebo. At that time, the neuroendocrine values were higher when metoclopramide was administered during the 5-HTP test than after the placebo challenge. Log-transformation of these data would have produced percentual changes from dissimilar pretreatment values. Instead, it was decided to perform an analysis for co-activation based on the untransformed ACTH and cortisol values rather than on Log-transformed data, with the following arguments. If there was no synergism or other form of interaction between metoclopramide and the 5-HTP challenge, each pharmacological intervention would cause an independent absolute increase of ACTH and cortisol. In this case, the null hypothesis is that the absolute effects of metoclopramide added to those of the 5-HTP challenge do not differ from the absolute responses after the combination, even if pretreatment values are not the same. In case of co-activation, the effects of the combination would be larger than the summed effects of the individual tests. Based on this reasoning, the null hypothesis was tested by contrasting the absolute untransformed effects of 5-HTP combined with metoclopramide *minus* 5-HTP alone, with those of metoclopramide alone *minus* placebo.

Pharmacokinetic analysis (PK)

The mean C_{MAX} , T_{MAX} , terminal half life, clearance and $AUC_{0-\infty}$ for metoclopramide and 5-HTP were calculated with noncom-

partmental analysis using WinNonLin Professional for windows V5.0 (Pharsight Corporation, 800 West El Camino Real, Suite 200, Mountain View, CA 94040).

Side-effects

Adverse events were registered from spontaneous reports and hourly inquiries.

Results

Subject disposition and demographic data

Thirteen volunteers were screened after having provided informed consent. One subject did not comply with the inclusion criteria and was excluded from participation. Twelve volunteers received study medication, of which one discontinued due to personal circumstances after having completed the third study period. This subject was not replaced. Participants had a mean age of 25 years (range 19 - 42 years) and a mean BMI of 23.4 kg/m² (range 20 - 27 kg/m²).

Adverse events

All AE's were of mild to moderate intensity, transitory in nature and had mostly dissipated within 12 hours after drug administration. AE's were in line with the side-effect profile described in the Summary of Product Characteristics of 5-HTP and metoclopramide. No subjects discontinued participation directly related to adverse effects. The most commonly occurring AE's were headache (1/12 subjects) for placebo; headache (4/12 subjects), dizziness (4/12 subjects), nausea (8/12 subjects), abdominal discomfort (5/12 subjects) and vomiting (2/12 subjects) for 5-HTP; dizziness (2/12 subjects), drowsiness (2/12 subjects), nausea (5/12 subjects) and vomiting (2/12 subjects) for 5-HTP/metoclopramide; and drowsiness (4/12 subjects) for metoclopramide respectively. One subject experienced a reversible QTc prolongation during the 5-HTP/metoclopramide treatment.

Neuroendocrine effects

The least square means (LSM's) for ACTH and cortisol are presented respectively in figure 1 and figure 2. These changes are expressed as estimates of percentual difference from placebo, with 95% confidence intervals (*table 2*) and maximal mean concentrations (C_{MAX}). ACTH increased by +43.9(23.4, 67.8)% (C_{MAX} 30.2ng/l) with 10mg metoclopramide, by +55.4(31.9, 83.1)% (C_{MAX} 26.2ng/l) after 200mg 5-HTP and by +167(127, 214)% (C_{MAX} 60.6ng/l) following 200mg 5-HTP combined with 10mg metoclopramide. Metoclopramide combined with 5-HTP induced larger ACTH responses than either 5-HTP or metoclopramide alone (*table 2*). The additive effect of metoclopramide combined with 5-HTP was statistically significant [+52.6(27.1, 83.2)%] compared to the effect of 5-HTP alone. The combination of 5-HTP and metoclopramide lead to an increase in ACTH that was significantly greater than the sum of the increases induced by 5-HTP alone and metoclopramide alone [+9.3 (2.8, 15.8)ng/l].

Cortisol increased similarly by +64.0(40.4, 91.6)%, (C_{MAX} 145µg/l) after 10mg metoclopramide, by +48.1(29.2, 69.8)%, (C_{MAX} 191.4 µg/l) with 200mg 5-HTP and by +141(104, 184)%, (C_{MAX} 209µg/l) following 200mg 5-HTP combined with 10mg metoclopramide (*table 2*). Metoclopramide combined with 5-HTP also had a statistically significant effect on cortisol release [+21.8(1.0, 46.9)%] compared to 5-HTP alone.

Prolactin was increased by +995(824, 1197)% after 10mg metoclopramide, +19.0(0.8, 40.5)% with 200mg 5-HTP and by +926(753, 1134)% following 200mg 5-HTP combined with 10mg metoclopramide (*table 2*). Plasma AVP levels remained unaffected by either treatment combination (*table 2*).

Pharmacokinetics (PK)

The oral administration of 5-HTP lead to a mean C_{MAX} of 1319 µg/l while the oral administration of 5-HTP followed by i.v. metoclopramide had a mean C_{MAX} of 1302 µg/l. These mean maximal concentration occurred around 170 minutes after the oral administration of 5-HTP in both instances and did not differ significantly from one another. The mean maximal metoclopramide concentrations (C_{MAX}) were comparable with i.v. administration leading to a mean C_{MAX} of 58.1 µg/l in the absence of 5-HTP and a mean C_{MAX} of 61.1 µg/l

in the presence of oral 5-HTP. Since 5-HTP was not sampled sufficiently until the end of its concentration curve, the terminal half-life was not accurately determinable (*Table 3*).

Discussion

Metoclopramide activated the HPA-axis under physiological circumstances/CRH concentrations: 10 mg administered i.v. induced higher maximum mean ACTH levels (C_{MAX} 30.2 ng/l) than those induced previously with the AVP-analogue dDAVP (C_{MAX} 15.9 ng/l - 19.3 ng/l) (Jacobs et al., 2009), but decisively lower maximum mean ACTH levels than those induced by the CRH-mediated agents 5-HTP (C_{MAX} 55.2 ng/l) in this trial and hCRH (C_{MAX} 80 ng/l - 115 ng/l) in previous trials (Scott and Dinan, 1998; von Bardeleben and Holsboer, 1988). Contrary to previous findings, metoclopramide did not increase (peripheral) AVP levels in this study. Metoclopramide has predominant D_2 receptor antagonist properties and is hypothesized to induce vasopressinergic HPA-axis activation by releasing AVP into the portal system, which results in pituitary V_3 receptor stimulation and ACTH-release. Previously, metoclopramide 10mg administered i.v. induced significant AVP release and subsequent HPA-axis activation in healthy volunteers and a group of schizophrenic patients (Chiodera et al., 1986; Nomura et al., 1984; Seki et al., 1997; Walsh et al., 2005). On the other hand, metoclopramide failed to increase AVP in isolated neuropituitary tissue, indicating that it might also exert its effect in the hypothalamus (Pitzel and Konig, 1984). Although these observations formed the basis of our study, it should be realized that HPA-axis activation with metoclopramide has not unequivocally been shown to be mediated by (portal) AVP-release. Furthermore, variable maximum increases in AVP (varying between 0.2 and 1.9 pg/ml) have been reported previously (Chiodera et al., 1986; Nomura et al., 1984). Such variability in AVP release may be explained by methodological issues, since most previous experiments were not properly placebo controlled. Also, the measurement of peripheral AVP plasma concentrations is technically complicated, and peripheral changes might not necessarily reflect central AVP production because AVP can also be released from other sources. Finally, AVP release in rats following stress is short-lived and AVP returns to basal levels within a question of

minutes (Engelmann et al., 2004). This would imply that our sampling scheme was probably not optimal to detect such changes, at least if AVP release were to be comparable in humans. Notwithstanding these difficulties, we demonstrated that metoclopramide by itself can activate the HPA-axis and is associated with mean ACTH levels that are indicative of vasopressinergic co-activation.

The oral administration of 200 mg 5-HTP alone potentially activated the HPA-axis. The maximal average ACTH concentrations of 46.6 ng/l and cortisol levels of up to 181.4 µg/l were comparable to those induced with other corticotrophinergic function tests in previous studies. For instance, 100µg hCRH i.v. caused maximal ACTH concentration of 59.2 ng/l and maximal cortisol levels of 196.4 µg/l (Accepted – J Psychopharmacol). Also, the present cortisol levels approached those attained previously with (near-maximally tolerated) serotonergic (170 to 230 µg/l) (Jacobs et al., 2008; Smarius et al., 2008) and corticotrophinergic (210 to 230 µg/l) (Dinan and Scott, 2005; Scott et al., 1999) function tests. Taken together, ACTH and cortisol release induced by 200 mg 5-HTP is reconcilable with corticotrophinergic activation of the HPA-axis.

The i.v. administration of 10mg metoclopramide around the E_{MAX} of orally administered 200 mg 5-HTP induced mean ACTH concentrations (167% relative to placebo) roughly four times those induced by metoclopramide alone (44% relative to placebo) and three times those induced by 5-HTP alone (55% relative to placebo). Moreover, the ACTH release induced by metoclopramide in the presence of enhanced CRH levels induced by 5-HTP was synergistic and therefore indicative of vasopressinergic co-activation (9.3 ng/l more than the sum of the individual treatments). Since the administration of metoclopramide was not associated with endogenous AVP release in our experiment, alternative mechanisms by which metoclopramide induces co-activation should be considered. Besides metoclopramide's D_2 receptor antagonist properties, it also acts as 5-HT₃ receptor antagonist and 5-HT₄receptor agonist. Previously, 10 mg metoclopramide i.v. induced ACTH release which was not suppressed by the serotonergic 5-HT₁ and 5-HT₂ receptor antagonists metergoline and ketanserine (Coiro et al., 1989). Also, a recent study has shown that the 5-HT₃-antagonist granisetron does not influence the effects of 5-HTP on HPA-axis activation (Jacobs et al., 2008). In this context, it is not unthinkable that

metoclopramide's co-activatory neuroendocrine effects are mediated, at least in part, by 5-HT₄-agonism rather than mere endogenous AVP release. In summary, metoclopramide is able to co-activate the HPA-axis in the presence of supraphysiological CRH concentrations. Although such an effect is strongly indicative of vasopressinergic co-activation, it still remains unclear whether these effects have endogenous AVP release in common, or (indirect) ACTH release by means of either D₂ antagonism, 5-HT₄ agonism or a combination of both mechanisms.

Our study was complicated by a striking variability of the postprandial ACTH and cortisol surge following lunch. A post hoc analysis showed that this effect was largely suppressed by metoclopramide. HPA activation of this size and variability was unexpected since it had not been observed previously with other antiemetics (like granisetron and domperidone). The diurnal cortisol rhythm has repeatedly been shown to demonstrate a minor surge during midday, which is also present but attenuated during a period of food deprivation. This midday cortisol release is augmented by food-intake only during the afternoon but not during the evening meal (Follenius et al., 1982; Quigley and Yen, 1979). Although the underlying mechanism remains uncertain, food intake is supposed to play a synchronizing role in the circadian periodicity of the HPA (Follenius et al., 1982). Apparently, this food-effect is reduced by metoclopramide, which may be related to the gastrointestinal effects of this compound, which is registered not only as an antiemetic but also for its prokinetic properties. The potential mechanisms for the observed effect of metoclopramide on activation of the HPA-axis by food are unclear. It is not caused by a reduced absorption of 5-HTP, since its plasma kinetics were not affected by the addition of metoclopramide (C_{MAX} 1319 µg/l and 1302 µg/l for 5-HTP alone and 5-HTP combined with metoclopramide, respectively). Whatever the cause, we had to restrict our analysis of metoclopramide's neuroendocrine effects to the period before lunch, after the blinded observation of considerable fluctuations in ACTH- and cortisol levels following the afternoon meal. Since the bulk of the effects of metoclopramide occurred before lunch, we could still detect strong singular and additive HPA-effects over the 70-minute time period between the injection and the meal, as shown in Figure 1. However, in future studies food-

induced fluctuations of ACTH and cortisol should be avoided altogether by not serving food for at least four hours after the administration of 5-HTP.

5-HTP by itself and 5-HTP combined with metoclopramide were associated with bothersome and potentially confounding adverse effects such as dizziness, nausea and vomiting. On the other hand, metoclopramide alone was only associated with moderate drowsiness. In the present study 5-HTP was administered to enhance endogenous CRH release in healthy volunteers, with the purpose of examining metoclopramide's potential to induce co-activation. Since the HPA-axis is expected to be hyperactive in patients suffering from stress-related psychopathology, the exogenous enhancement of endogenous CRH release with 5-HTP is not a necessary consideration. Therefore, metoclopramide administered by itself is expected to sufficiently quantify vasopressinergic co-activation in stress-related psychopathology and is not expected to cause troublesome side-effects since it will not be combined with 5-HTP.

In conclusion, 10 mg metoclopramide causes ACTH release that is larger than exogenous vasopressinergic co-activation with dDAVP, but convincingly smaller than that found with corticotrophinergic function tests like hCRH and 5-HTP. Also, metoclopramide synergizes ACTH release in the presence of supraphysiological CRH concentrations induced by the serotonergic precursor 5-HTP. These results are compatible with the hypothesis that metoclopramide causes vasopressinergic co-activation of the HPA-axis. However, the exact mechanism underlying such co-activation remains elusive since metoclopramide has both D₂ antagonist and 5-HT₄ antagonist properties and increased (peripheral) AVP release with metoclopramide could not be replicated. At any rate, this study shows that metoclopramide may be a useful function test for (vasopressinergic) co-activation of the HPA-axis since it is easy to administer and it has little potentially confounding or bothersome effects when administered alone. Potential future applications may include the detection of depressed individuals with (vasopressinergic) hyperactivity of the HPA axis or to monitor the pharmacodynamic effects of novel antidepressants directed at the v₃ receptor.

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Table 1

A schematic overview of the four different treatments, each consisting of oral (p.o.) and intravenous (i.v.) administration of placebo, carbidopa, granisetron and 5-hydroxytryptophan (5-HTP) on the specified time points (t=).

| Treatment | t=-180 min | t=-60 min | t=0 min | t=60 min | t=180 min |
|-----------|----------------------|----------------------|------------------|--------------------------|---------------------|
| 1 | 100mg carbidopa p.o. | 2mg granisetron p.o. | 200mg 5-HTP p.o. | placebo i.v. | 50mg carbidopa p.o. |
| 2 | placebo p.o. | placebo p.o. | placebo p.o. | 10mg metoclopramide i.v. | placebo p.o. |
| 3 | 100mg carbidopa p.o. | 2mg granisetron p.o. | 200mg 5-HTP p.o. | 10mg metoclopramide i.v. | 50mg carbidopa p.o. |
| 4 | placebo p.o. | placebo p.o. | placebo p.o. | placebo i.v. | placebo p.o. |

Plasma ACTH (ng/L), serum cortisol ($\mu\text{g/L}$), serum prolactin (ng/L) and plasma AVP (ng/L) for the period 60 to 130 min for the treatments 10 mg metoclopramide and 10 mg metoclopramide combined with 200 mg 5-HTP; and for the period 0 to 130 min for the treatments 200 mg 5-HTP: estimated means (back transformed least square means) for placebo, 10 mg metoclopramide, 200 mg 5-HTP and 10 mg metoclopramide combined with 200 mg 5-HTP. Estimated difference (%) with 95% confidence interval from placebo for 10 mg metoclopramide, 200 mg 5-HTP, 10 mg metoclopramide combined with 200 mg 5-HTP; and from 200 mg 5-HTP for 10 mg metoclopramide combined with 200 mg 5-HTP.

| Parameter | Back transformed least Square Means (LSM) | | | | Estimated difference with 95% Confidence interval (% difference) | | | | | | | |
|------------------------------------|---|-----------|---------------------|-------------|--|-------------------|-----------------------------------|-------------------------------|--|--|-----------|----------------------------------|
| | Placebo | | 10mg metoclopramide | 200mg 5-HTP | 10mg metoclopramide combined with 200mg 5-HTP | treatment p-value | 10mg metoclopramide vs placebo | 200mg 5-HTP vs placebo | 10mg metoclopramide combined with 200mg 5-HTP vs placebo | 10mg metoclopramide combined with 200mg 5-HTP vs 200mg 5-HTP | | |
| | n=12 | n=12 | n=9 | n=10 | 60-130min | | 60-130min | 60-130min | 60-130min | 60-130min | | |
| Plasma ACTH (ng/l) | 0-130min | 60-130min | 0-130min | 60-130min | 36.1 | <0.0001 | 43.9 (23.4, 67.8) p<0.0001 | 55.4 (31.9, 83.1) p<0.0001 | 166.8 (126.5, 214.2) p<0.0001 | 60-130min | 60-130min | 52.6 (27.1, 83.2) p<0.0001 |
| | 14.0 | 13.5 | 21.8 | | | | | | | | | |
| Serum cortisol (µg/l) | 85.3 | 76.4 | 126.3 | 183.9 | | <0.0001 | 64.0 (40.4, 91.6) p<0.0001 | 48.1 (29.2,69.8) p<0.0001 | 140.8 (103.9, 184.3) p<0.0001 | | | 21.8 (1.0, 46.9) p=0.0393 |
| Serum prolactin (µg/l) | 4.3 | 4.2 | 5.3 | 42.6 | | <0.0001 | 994.7 (823.8, 1197.2) p<0.0001 | 19.0 (0.8, 40.5) p=0.0170 | 925.5 (752.6, 1133.5) p<0.0001 | | | 657.8 (517.6, 829.8) p<0.0001 |
| Plasma arginine-vasopressin (ng/l) | 1.9 | 2.0 | 2.2 | 2.5 | | p=0.1928 | 8.4 (-10.4, 31.2) p=0.387 | 13.3 (-9.5, 41.8) p=0.258 | 8.4 (-10.4, 31.2) p=0.387 | | | 4.8 (-16.3, 31.4) p=0.666 |

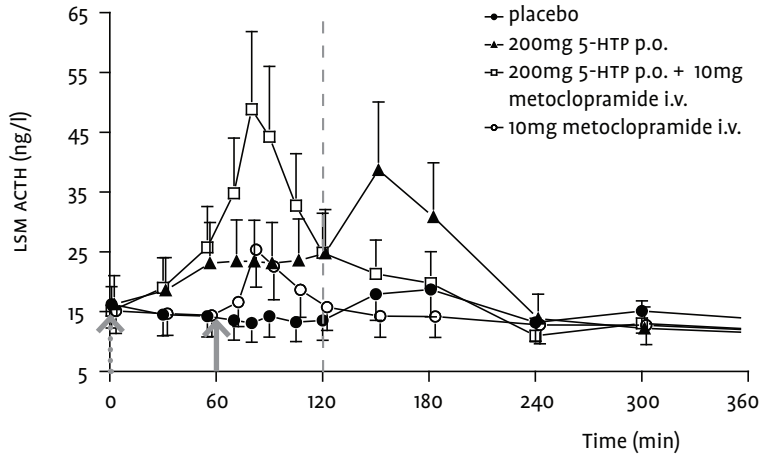
Table 3

Pharmacokinetic (PK) parameters for metoclopramide (MCP) administered as 10 mg i.v. bolus alone and in the presence of 200 mg 5-HTP administered p.o. 60 min earlier; and for 5-HTP administered 200 mg p.o. alone and followed 60 min later by 10 mg metoclopramide as i.v. bolus: Mean (SD) of terminal half life ($t_{1/2}$), T_{MAX} (min), C_{MAX} ($\mu\text{g/L}$) and $AUC_{0-\infty}$ ($\text{min}\cdot\mu\text{g/L}$).

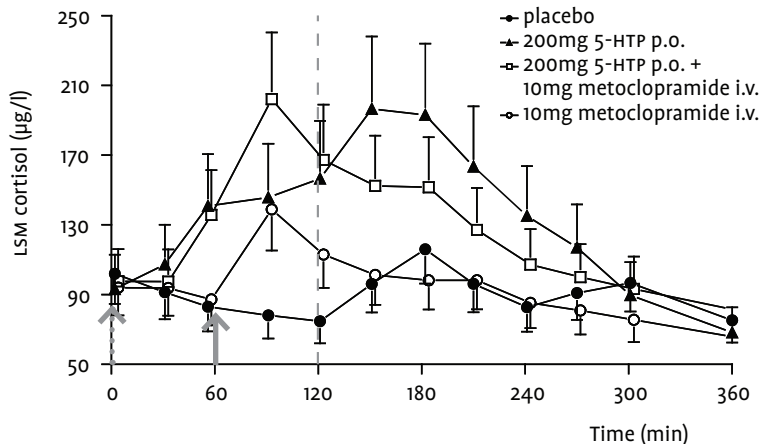
| PK parameter; Mean (SD) | Metoclopramide | | 5-HTP | |
|---|-------------------|--|---------------------|---|
| | administered i.v. | administered i.v. 60min following 200mg 5-HTP p.o. | administered p.o. | administered p.o. 60min preceding 10mg MCP i.v. |
| Terminal half life ($t_{1/2}$) | 270.0 (55.1) | 256.7 (81.6) | 205.6 (95.3) | 212.5 (141.3) |
| T_{MAX} (min) | 46.2 (64.4) | 63.7 (105.2) | 169.8 (54.4) | 171.1 (41.6) |
| C_{MAX} ($\mu\text{g/l}$) | 58.1 (16.6) | 63.7 (25.2) | 1318.6 (141.0) | 1301.7 (155.3) |
| $AUC_{0-\infty}$ ($\text{min}\cdot\mu\text{g/l}$) | 15479.0 (5699.8) | 20825.4 (11258.9) | 566797.7 (113461.7) | 530032.0 (73757.5) |

Figure 1

Least square mean (LSM) of plasma adrenocorticotrophic hormone (ACTH) with 95% confidence interval bars (ng/L) for the period 0–360 min (closed circle: placebo; open circle: 10 mg metoclopramide administered i.v. at t = 60 min; closed triangle: 200 mg 5-HTP administered p.o. at t = 0 min; open square: 200 mg 5-HTP administered at t = 0 min followed by 10 mg metoclopramide administered i.v. at t = 60 min; grey dotted arrow at t = 0 min: administration of 5-HTP; grey arrow at t = 60 min: administration of metoclopramide; grey dotted line at t = 120 min: lunch)

**Figure 2**

Least square mean (LSM) of serum cortisol with 95% confidence interval bars ($\mu\text{g/l}$) for the period 0–360 min (closed circle: placebo; open circle: 10 mg metoclopramide administered i.v. at t = 60 min; closed triangle: 200 mg 5-HTP administered p.o. at t = 0 min; open square: 200 mg 5-HTP administered at t = 0 min followed by 10 mg metoclopramide administered i.v. at t = 60 min; grey dotted arrow at t = 0 min: administration of 5-HTP; grey arrow at t = 60 min: administration of metoclopramide; grey dotted line at t = 120 min: lunch).



REFERENCE LIST

- aan het Rot, Mathew SJ, Charney DS (2009) Neurobiological mechanisms in major depressive disorder. *CMAJ* 180: 305-313.
- Aguilera G, Rabadan-Diehl C (2000) Vasopressinergic regulation of the hypothalamic-pituitary-adrenal axis: implications for stress adaptation. *Regul Pept* 96: 23-29.
- Borowsky B, Kuhn CM (1992) D₁ and D₂ dopamine receptors stimulate hypothalamo-pituitary-adrenal activity in rats. *Neuropharmacology* 31: 671-678.
- Chiodera P, Volpi R, Delsignore R, Marchesi C, Salati G, Camellini L, Rossi G, Coiro V (1986) Different effects of metoclopramide and domperidone on arginine-vasopressin secretion in man. *Br J Clin Pharmacol* 22: 479-482.
- Coiro V, Capretti L, Speroni G, Volpi R, Fagnoni F, Bianconi L, Schianchi L, Caiazza A, Chiodera P (1989) Muscarinic cholinergic, but not serotonergic mediation of arginine vasopressin response to metoclopramide in man. *Clin Endocrinol (Oxf)* 31: 491-498.
- DeBold CR, Sheldon WR, DeCherney GS, Jackson RV, Alexander AN, Vale W, Rivier J, Orth DN (1984) Arginine vasopressin potentiates adrenocorticotropin release induced by ovine corticotropin-releasing factor. *J Clin Invest* 73: 533-538.
- Dempster EL, Burcescu I, Wigg K, Kiss E, Bajji I, Gadoros J, Tamas Z, Kennedy JL, Vetro A, Kovacs M, Barr CL (2007) Evidence of an association between the vasopressin V_{1B} receptor gene (AVPR1B) and childhood-onset mood disorders. *Arch Gen Psychiatry* 64: 1189-1195.
- Dinan TG, Lavelle E, Scott LV, Newell-Price J, Medbak S, Grossman AB (1999) Desmopressin normalizes the blunted adrenocorticotropin response to corticotropin-releasing hormone in melancholic depression: evidence of enhanced vasopressinergic responsiveness. *J Clin Endocrinol Metab* 84: 2238-2240.
- Dinan TG, O'Brien S, Lavelle E, Scott LV (2004) Further neuroendocrine evidence of enhanced vasopressin V₃ receptor responses in melancholic depression. *Psychol Med* 34: 169-172.
- Dinan TG, Scott LV (2005) Anatomy of melancholia: focus on hypothalamic-pituitary-adrenal axis overactivity and the role of vasopressin. *J Anat* 207: 259-264.
- Engelmann M, Landgraf R, Wotjak CT (2004) The hypothalamic-neurohypophyseal system regulates the hypothalamic-pituitary-adrenal axis under stress: an old concept revisited. *Front Neuroendocrinol* 25: 132-149.
- Favrod-Coune C, Raux-Demay MC, Proeschel MF, Bertagna X, Girard F, Luton JP (1993) Potentiation of the classic ovine corticotrophin releasing hormone stimulation test by the combined administration of small doses of lysine vasopressin. *Clin Endocrinol (Oxf)* 38: 405-410.
- Follenius M, Brandenberger G, Hietter B (1982) Diurnal cortisol peaks and their relationships to meals. *J Clin Endocrinol Metab* 55: 757-761.
- Gartside SE, Cowen PJ (1990) Mediation of ACTH and prolactin responses to 5-HTP by 5-HT₂ receptors. *Eur J Pharmacol* 179: 103-109.
- Gijsman HJ, van Gerven JM, de Kam ML, Schoemaker RC, Pieters MS, Weemaes M, de Rijk R, van der PJ, Cohen AF (2002) Placebo-controlled comparison of three dose-regimens of 5-hydroxytryptophan challenge test in healthy volunteers. *J Clin Psychopharmacol* 22: 183-189.
- Gijsman HJ, van Gerven JM, Tieleman MC, Schoemaker RC, Pieters MS, Ferrari MD, Cohen AF, van Kempen GM (1998) Pharmacokinetic and pharmacodynamic profile of oral and intravenous meta-chlorophenylpiperazine in healthy volunteers. *J Clin Psychopharmacol* 18: 289-295.
- Goekoop JG, Wiegant VM (2009) Support for Two Subcategories of Depression with Different Vasopressinergic Mechanisms in the Field of Melancholia. *Current Psychiatry Reviews* 5: 127-136.
- Holsboer F (1983) The dexamethasone suppression test in depressed patients: clinical and biochemical aspects. *J Steroid Biochem* 19: 251-257.
- Holsboer F, Gerken A, Steiger A, Benkert O, Muller OA, Stalla GK (1984a) Corticotropin-releasing-factor induced pituitary-adrenal response in depression. *Lancet* 1: 55.
- Holsboer F, von Bardeleben U, Gerken A, Stalla GK, Muller OA (1984b) Blunted corticotropin and normal cortisol response to human corticotropin-releasing factor in depression. *N Engl J Med* 311: 1127.
- Jacobs G, Kamerling I, de Kam M, Derijk R, van Pelt J, Zitman F, van Gerven J (2008) Enhanced tolerability of the 5-hydroxytryptophan challenge test combined with granisetron. *J Psychopharmacol*.
- Jacobs GE, Hulsbosch EG, van Gerven JMA, Zuurman L, de Kam ML, Ellassais-Schaap J, Ruigt G, van Pelt J, Peeters BWMM, Peeters PAM, Burggraaf J (2009) Desmopressin as a pharmacological tool in vasopressinergic hypothalamus-pituitary-adrenal (HPA) axis modulation: neuroendocrine, cardiovascular and coagulatory effects.

- Jorgensen H, Knigge U, Kjaer A, Moller M, Warberg J (2002) Serotonergic stimulation of corticotropin-releasing hormone and pro-opiomelanocortin gene expression. *J Neuroendocrinol* 14: 788-795.
- Lamberts SW, Verleun T, Oosterom R, de JF, Hackeng WH (1984) Corticotropin-releasing factor (ovine) and vasopressin exert a synergistic effect on adrenocorticotropin release in man. *J Clin Endocrinol Metab* 58: 298-303.
- Moro M, Maraschini C, Cavagnini F (1997) Dopamine infusion enhances the adrenocorticotrophic hormone and cortisol response to metoclopramide in hyperprolactinemic patients but not in normal subjects. *Gynecol Endocrinol* 11: 155-162.
- Nomura K, Kurimoto F, Demura H, Sakurai H, Nomura T, Zibiki K, Naruse M, Kanai N, Shizume K (1984) Effect of metoclopramide in plasma vasopressin in man. *Clin Endocrinol (Oxf)* 21: 117-121.
- Pitzel L, Konig A (1984) Lack of response in the release of oxytocin and vasopressin from isolated neurohypophyses to dopamine, met-enkephalin and leu-enkephalin. *Exp Brain Res* 56: 221-226.
- Quigley ME, Yen SS (1979) A mid-day surge in cortisol levels. *J Clin Endocrinol Metab* 49: 945-947.
- Ring RH (2005) The central vasopressinergic system: examining the opportunities for psychiatric drug development. *Curr Pharm Des* 11: 205-225.
- Scott LV, Dinan TG (1998) Vasopressin and the regulation of hypothalamic-pituitary-adrenal axis function: implications for the pathophysiology of depression. *Life Sci* 62: 1985-1998.
- Scott LV, Dinan TG (2002) Vasopressin as a target for antidepressant development: an assessment of the available evidence. *J Affect Disord* 72: 113-124.
- Scott LV, Medbak S, Dinan TG (1999) Desmopressin augments pituitary-adrenal responsivity to corticotropin-releasing hormone in subjects with chronic fatigue syndrome and in healthy volunteers. *Biol Psychiatry* 45: 1447-1454.
- Seki K, Kato T, Sekiya S (1997) Corticotrophin and vasopressin responses to metoclopramide in patients with hypothalamic amenorrhoea. *Clin Endocrinol (Oxf)* 46: 203-207.
- Smarius LJ, Jacobs GE, Hoeberechts-Lefrandt DH, de Kam ML, van der Post JP, de Rijk R, van Pelt J, Schoemaker RC, Zitman FG, van Gerven JM, Gijssman HJ (2008) Pharmacology of rising oral doses of 5-hydroxytryptophan with carbidopa. *J Psychopharmacol* 22: 426-433.
- Volpi S, Rabadan-Diehl C, Aguilera G (2004) Vasopressinergic regulation of the hypothalamic pituitary adrenal axis and stress adaptation. *Stress* 7: 75-83.
- von Bardeleben U, Holsboer F (1988) Human corticotropin releasing hormone: clinical studies in patients with affective disorders, alcoholism, panic disorder and in normal controls. *Prog Neuropsychopharmacol Biol Psychiatry* 12 Suppl: S165-S187.
- Walsh P, Spelman L, Sharifi N, Thakore JH (2005) Male patients with paranoid schizophrenia have greater ACTH and cortisol secretion in response to metoclopramide-induced AVP release. *Psychoneuroendocrinology* 30: 431-437.

Hypothalamic glutamate levels following serotonergic stimulation: a pilot study using 7-Tesla magnetic resonance spectroscopy in healthy volunteers

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Abstract

INTRODUCTION AND PURPOSE Functional proton magnetic resonance spectroscopy (MRS) can be applied to measure pharmacodynamic effects of central nervous system (CNS)-active drugs. The serotonin precursor 5-hydroxytryptophan (5-HTP), administered together with carbidopa and granisetron to improve kinetics and reduce adverse effects, acutely enhances central serotonergic neurotransmission and induces hypothalamus-pituitary-adrenal-(HPA) axis activation. We studied the hypothalamic levels of glutamate/glutamine (Glx), choline (Chol), N-acetyl-aspartate (NAA) and creatine using 7-Tesla (7T) MRS, and adrenocorticotrophic hormone (ACTH) and cortisol in peripheral blood, after the administration of the 5-HTP function test in healthy volunteers.

METHODS A randomized, double blind, placebo-controlled, two-way cross-over study was performed in 12 healthy males with a 7 day wash-out period. After administration of the oral 5-HTP function test, ACTH and cortisol were measured over four hours and MRS scans at 7T were performed every 30 minutes over three hours measuring Glx: Creatine, Chol: Creatine and NAA: Creatine ratios.

RESULTS In the hypothalamus, the administration of 5-HTP had no effect on the average Glx, Chol or NAA levels over 180 minutes but induced a significant decrease of Glx at 60 minutes on post-hoc analysis. 5-HTP induced significant ACTH release reaching an E_{MAX} of 60.2 ng/l at 80 minutes followed by cortisol with an E_{MAX} of 246.4 ng/ml at 110 minutes.

CONCLUSIONS The reduction in hypothalamic Glx-levels after serotonergic stimulation is compatible with activation of excitatory neurons in this region, which is expected to cause depletion of local glutamate stores. The hypothalamic MRS-response reached its maximum prior to subsequent increases of ACTH and cortisol, which supports the functional relevance of hypothalamic Glx-depletion for activation of the HPA-axis. This exploratory study shows that MRS is capable of detecting neuronal activation following functional stimulation of a targeted brain area.

Introduction

Pharmacological function tests are used to quantify the functionality of central neurotransmitter and neuropeptide systems in health and disease, and following treatment. The serotonergic system can be pharmacologically stimulated with serotonin precursors, selective agonists or re-uptake inhibitors. A test consisting of orally administered (p.o.) L-5-hydroxytryptophan (5-HTP), carbidopa and granisetron acutely increases synaptic availability of serotonin (or 5-hydroxytryptamine, 5-HT) (Gijssman *et al.*, 2002; Smarius *et al.*, 2008). Carbidopa prevents peripheral conversion of 5-HTP to 5-HT (which would preclude brain penetration), while granisetron limits serotonergic side-effects (such as gastro-intestinal stimulation and vomiting) without influencing the neuroendocrine response or 5-HTP pharmacokinetics (PK) (Jacobs *et al.*, 2008). Thus, in the presence of carbidopa and granisetron peripherally, the direct serotonin precursor 5-HTP is decarboxylized centrally into 5-HT. The conversion of 5-HTP to 5-HT is believed to occur in the dorsal raphe nuclei of the brainstem and its associated cortical, subcortical and limbic projections (Dinan, 1996; Leonard, 2005; Sotty *et al.*, 2009). We have previously demonstrated reproducible, concentration-dependent pharmacodynamic effects with acceptable variability associated with this serotonergic function test in healthy volunteers. Also, 5-HTP plasma concentrations demonstrated dose-dependence with a limited PK variability (Smarius *et al.*, 2008). Taken together, these features argue for a reliable pharmacological research tool in examining the central 5-HT system.

Enhanced serotonergic neurotransmission ensuing from the decarboxylation of 5-HTP into 5-HT potently activates the hypothalamus-pituitary-adrenal axis (HPA). The stimulation of 5HT_{2A} or 5HT_{2C} receptors in the paraventricular nucleus (PVN) of the hypothalamus is believed to release corticotrophin releasing hormone (CRH) (Gartside and Cowen, 1990). Subsequently, CRH stimulates adrenocorticotrophic hormone (ACTH) release from the anterior pituitary which in turn induces cortisol release from the adrenal cortices. Ultimately, cortisol exerts negative feedback on CRH and ACTH release via the mineralocorticoid (MR) and glucocorticoid (GR) receptors (de Kloet *et al.*, 2005), leading to a suppression and ultimate cessation of the 5-HTP induced neuroendocrine response.

ACTH and cortisol therefore are the peripheral neuroendocrine reflections of increased central 5-HT neurotransmission.

Neuroendocrine hormone responses in plasma are merely indirect measures of increased neurotransmission, influenced by both central and peripheral amplification, the negative feedback mechanism and the peripheral clearance of the hormones. More direct measurements of drug-induced neuronal activity in the brain could lead to a better understanding of the CNS processes induced by the stimulation of neurotransmitter systems. Neuroimaging techniques of pharmacological CNS-processes such as functional magnetic resonance imaging (fMRI) is often used to demonstrate regional shifts in blood oxygen level dependent (BOLD) signals. For instance, the specific 5-HT_{2C} agonist meta-chlorophenylpiperazine (mCPP) increased the BOLD signal in human brain areas that are particularly rich in 5-HT_{2C} receptors, such as the PVN, hippocampus, caudate, pallidum, amygdala and anterior cingulate (Anderson *et al.*, 2002) which was prevented by the 5-HT_{2C} receptor antagonist SB242084 in rats (Stark *et al.*, 2008). However, BOLD-MRI is not quantitative and the signal tends to drift with time. Consequently, the BOLD-effects of a drug can only be reliably pursued for no more than 30 or 60 minutes, which for most oral drugs is unsuitable. Also, the BOLD-MRI signal is sensitive to nonspecific confounding factors such as (drug-induced) vascular reactivity and shows substantial intersession variance in cross-over designs (Anderson *et al.*, 2008; Stark *et al.*, 2006). These factors limit the application of fMRI in the quantification of drug-induced neuronal activity. Alternatively, *in vivo* proton magnetic resonance spectroscopy (MRS) could be a candidate neuroimaging technique to directly quantify drug-induced neurotransmission. MRS of the brain determines concentrations of protons from tissue chemicals other than water, such as glutamate-glutamine (which cannot be distinguished reliably, hence combined as (Glx)), N-acetyl-aspartate (NAA), myo-inositol, lactate, choline (Chol) and creatine (Creat). In this context, NAA and Glx are generally regarded as surrogate MRS markers for neuronal activity, whereas Chol is rather considered a metabolic marker of membrane density and integrity (Soares and Law, 2009; Wise and Tracey, 2006). To semiquantify changes in the levels of these metabolites, creatine is frequently used as an internal reference, since this is related to neuronal membrane turnover

and is also expected to remain stable after acute administration of (non-proapoptotic) CNS active drugs (Mason and Krystal, 2006). MRS is typically applied in neurodegenerative disease and malignant brain tumours, exhibiting relatively specific abnormalities in NAA (Soares and Law, 2009; De Stefano *et al.*, 2007). Recently, a study using MRS found Glx-increases in the occipital lobe after 7 to 10 days of treatment with the specific serotonin reuptake inhibitor (SSRI) citalopram, while this was not the case for the noradrenaline reuptake inhibitor (NRI) (Taylor *et al.*, 2008). These findings indicate that MRS is not only able to demonstrate and quantify changes in neuronal biochemistry in structural brain disorders but potentially also during (prolonged) pharmacological treatment.

We studied whether neuronal changes can also be measured after acute pharmacological stimulation with a CNS-active drug using MRS. For this, a 5-HTP function test was used that causes a robust serotonergic stimulation of the hypothalamus of healthy male volunteers. This was an exploratory study, since neither the extent and variability nor the time course of hypothalamic MRS-changes could be predicted in advance.

Methods

Study design

A randomized, double blind, placebo controlled, two-way crossover trial with administration of 5-HTP combined with carbidopa and granisetron was performed as described previously. The treatments were administered with a wash-out period of at least 7 days. The study protocol was approved by the Medical Ethics Committee of Leiden University Medical Centre (LUMC) and performed according to legal and regulatory requirements.

Drug administration

The function test consisted of, the administration of carbidopa 100 mg (at t=-180 minutes) and granisetron 2 mg (at t=-60 minutes), followed by a single oral dose of 5-HTP 200mg (at t=0 minutes) and carbidopa 50mg (at t=180 minutes). For all three active drugs, matching double-dummies were administered as placebo (Jacobs *et al.*, 2008; Gijsman *et al.*, 2002; Smarius *et al.*, 2008).

Subjects

Twelve healthy, male volunteers participated in the study. Volunteers without a personal or a first-degree family history of a significant psychiatric disorder according to DSM-IV and who have never used MDMA, methamphetamines or ecstasy were included in the study. Volunteers who used more than 4 units alcohol and/or xanthines on average per day; smoked more than 5 cigarettes per day; used any drugs of abuse or substance within two weeks before the first dosing; had a cardiac pacemaker, piercings or other metal objects attached to the body and suffering from claustrophobia were excluded from study participation. No xanthine or tryptophan containing foods or beverages, tobacco or alcohol were allowed during study days. Concomitant medication other than paracetamol was not permitted during the study period.

5-HTP and carbidopa

5-HTP and carbidopa were obtained from BUFA b.v. (Uitgeest, The Netherlands). Granisetron was obtained from the Department of Clinical Pharmacy of the LUMC. All medication and matching placebo were prepared by the Department of Clinical Pharmacy of the LUMC.

Study days

Volunteers arrived at the Centre for Human Drug Research (CHDR) at 22h00 on the evening preceding each study day. After waking up the next morning, a low tryptophane breakfast was supplied. One intravenous cannula for blood sampling was inserted into the antecubital vein of one arm for blood sampling. At $t=-180$ minutes volunteers received carbidopa 100 mg or placebo at CHDR after which volunteers were transported to the 7T-MRI-unit by taxi, accompanied by a research physician. Granisetron or placebo was administered at $t=-60$ minutes and 5-HTP or placebo subsequently at $t=0$ minutes. Six MRS scans were performed at 30 min before and 30, 60, 90, 120 and 180 minutes following 5-HTP administration. A research physician was present throughout the entire study day. Carbidopa 50 mg was given at $t=180$ minutes. Volunteers were

supplied with a standard meal at $t=420$ minutes. Volunteers were transported back to CHDR after all scanning sessions were performed, and subsequently went home after dinner.

Biochemical measurements

1.2 ml venous blood was collected in prechilled EDTA collection tubes for the determination of plasma ACTH on -10 and 1, 10, 25, 45, 55, 75, 80, 85, 105, 110, 150, 170, 240 and 300 minutes relative to 5-HTP administration. Samples were immediately placed on ice, processed within 30 minutes and stored at -80°C . ACTH was analyzed within six weeks using the Immulite 2500 Analyzer Assay (EURO/DCP, United Kingdom) at the Laboratory for Clinical Chemistry, LUMC, Leiden, the Netherlands. 3 ml venous blood was collected in non-additive tubes for the determination of serum cortisol on -10 and 1, 10, 25, 45, 75, 110, 170, 240 and 300 minutes relative to 5-HTP administration. Samples were allowed to coagulate for 30 minutes at room temperature, centrifuged within 1 hour of collection and serum stored at -20°C until analysis. Serum cortisol was analyzed using the ECLIA assay method on Modular Analytics E170 (Roche, Switzerland) at the Laboratory for Clinical Chemistry, LUMC, Leiden, the Netherlands. The 5-HTP assay was performed at Laboratory for Clinical Chemistry, LUMC, Leiden, the Netherlands according to methods described previously (Gijsman *et al.*, 2002).

MRS acquisition

MRS scans were performed in a Philips Achieva 7.0 Tesla whole body MRI scanner (Philips Healthcare, Best, The Netherlands) installed at the LUMC based C.J. Gorter Center for High-Field MRI. Each individual scanning session had a duration of approximately 10 minutes: first, a T1-weighted MRI scan was performed (with a duration of approximately 3 minutes) followed by MRS (with a duration of approximately 7 minutes). MRS of the hypothalamus was performed using single voxel stimulated echo acquisition mode (STEAM) with the following parameters; repetition time (TR): 2000ms, echo time (TE): 19ms, mixing time (TM): 20ms, 128 averages, 2048 time domain points, and 4000Hz bandwidth. Typical voxel size dimensions were 13 x 12 x 10 mm, in left-right (LR),

anterior-posterior (AP) and feet-head (FH) direction respectively. A typical example of MRS voxel positioning is shown in *figure 1*. For optimization of B₀ field homogeneity, 2nd order pencil beam shimming was applied and 6 saturation slabs were positioned on either side of the voxel to suppress signal from surrounding tissue. After zero-filling to 4,096 data points, exponential multiplication of 2 Hz, Fourier transformation, and linear baseline correction was performed. The integrated area under the curve of N-acetylaspartate (NAA) (referenced at 2.0 parts per million), Glx, Chol and Creat were determined using integration software routines, which were provided by the manufacturer. Creat was used as internal reference for Chol, Glx and NAA. A typical example of the MRS spectra is shown in *figure 2*.

Side-effects

Adverse events were registered from spontaneous reports and hourly inquiries.

Statistical analysis

Since the responses and variability of the MRS measurements were unknown, power estimates could not be based on the expected MRS responses. Based on previous experiments with the 5-HTP-function test (Gijsman *et al.*, 2002; Jacobs *et al.*, 2008; Smarius *et al.*, 2008), a sample size of six was considered large enough to show statistically significant ACTH responses. To confirm that the power calculation was adequate, a blinded interim analysis was executed after dosing of the first six subjects. For the final analysis pharmacodynamic data were logarithmically (LOG) transformed except for the MRS parameters. Within the repeated measures mixed model ANCOVA design, estimated means (least squared means; LSMS) of the neuroendocrine parameters (ACTH, cortisol) were calculated over four hours and LSMS of the MRS ratios (Chol: Creat, Glx: Creat, NAA: Creat) were calculated over 180 minutes using treatment, time, study day and treatment by time as fixed factors, subject, subject by treatment and subject by time as random factors and average prevalue as covariate. The difference from placebo was estimated percentually for ACTH and cortisol (due to

LOG-transformation) and that for the MRS ratios in terms of absolute changes. All estimated differences were presented with 95% confidence intervals.

Pharmacokinetic (PK) analysis

The mean C_{MAX} , T_{MAX} , terminal half life and $AUC_{0-\infty}$ for 5-HTP were calculated with noncompartmental analysis using WinNonLin Professional for windows V5.0 (Pharsight Corporation, 800 West El Camino Real, Suite 200, Mountain View, CA 94040).

Results

Demographic data and subject disposition

The study recruited 12 volunteers who were included after having provided informed consent (mean age 27 years; range 20 - 38 years). Incomplete data sets had occurred during 5-HTP study days due to vomiting (2 study occasions), flawed MRS baseline scans (2 study occasions), technical MRI failure (1 study occasion) and withdrawal of informed consent (1 study occasion). Consequently, neuroendocrine data for 10 subjects and MRS data for 8 subjects were available for 5-HTP analysis, while the data of 12 subjects were available for placebo analysis.

Adverse events

The most common side-effect during placebo treatment was headache (2/12 occasions). Side-effects associated with 5-HTP treatment were 5-HT related and included mild nausea (10/11 occasions), vomiting (5/11 occasions), abdominal discomfort (3/11 occasions) dizziness (3/11 occasions) and headache (3/11 occasions).

Neuroendocrine effects

The mean ACTH and cortisol responses differed significantly from placebo for 5-HTP (Table 1). ACTH reached a maximum mean concentration (E_{MAX}) of 60.2 ng/l at 80 minutes (Figure 4) and cortisol an E_{MAX} of 246.4 ng/ml at 110 minutes (Figure 5) after administering 5-HTP.

MRS effects

Overall, the mean NAA: creat, Chol: creat and Glx: creat ratios did not differ significantly from placebo over the period of 0 to 180 minutes (Table 2). Based on the time profiles, a post hoc statistical analysis was performed of the effects at the average peak time. This secondary analysis showed a significant decline of Glx: creat, one hour after administration of 5-HTP (Table 2 and Figure 3). This Glx: creat trough at 60 minutes preceded the E_{MAX} of ACTH at 80 minutes and E_{MAX} of cortisol at 110 minutes (Figure 6). The other MRS ratios either did not show a clear peak, or this did not differ significantly from the placebo effect at the same time.

5-HTP PK

PK results are presented as means with standard deviations between brackets: oral 200 mg 5-HTP led to a C_{MAX} of 1867 (392) ng/ml, at 176 (82) minutes after its administration (Figure 6). Terminal half life ($T_{1/2}$) was 265.2 (91.1) minutes and $AUC_{0-\infty}$ was 848135 (313093) min*ng/ml. Since 5-HTP was not sampled sufficiently until the end of its concentration-time profile curve, the terminal half-life was not accurately determinable.

Discussion

5-HTP induced a significant hypothalamic glutamate-glutamine (Glx) trough 60 minutes after its administration, while its effects on Glx, Chol and N-acetyl-aspartate (NAA) levels over the entire 180 minutes observation period were probably too short lasted or too small or variable to be detectable in a group of 12 subjects. ACTH reached its E_{MAX} of 60.2 ng/l at 80 minutes followed by cortisol, with its E_{MAX} of 246.4 ng/ml, at 110 minutes after the administration of 5-HTP. The MRS Glx spectrum is constituted by the combined extracellular peaks of glutamate and glutamine since these spectra are inseparable. Glutamate is the most abundant excitatory neurotransmitter in the brain and one of the most important components of cellular energy metabolism (Mason and Krystal, 2006). This would arguably make it the most informative MRS-measurable metabolite associated with (drug-induced excitatory) neurotransmission (Soares and Law, 2009). However,

little research has been done on the effects of serotonergic agents on cerebral Glx levels and it is unclear how 5-HT and Glx are linked mechanistically. In this context, other areas not directly involved in the HPA-axis may be stimulated by the 5-HTP function test, influencing the hypothalamus indirectly. Spectroscopy of a (limbic) control region with lower serotonergic receptor density than the hypothalamus would also have strengthened the evidence for a specific 5-HT mediated hypothalamic effect. However, the time course of the different effects following oral 5-HTP-administration provides support for a functional connection between Glx and the neuroendocrine responses. The timing of the Glx trough, which preceded the E_{MAX} of ACTH by 20 minutes and that of cortisol by 50 minutes, can be reconciled with a serotonin-induced (and probably 5HT_{2A} or 5HT_{2C} mediated) release of CRH from the hypothalamic PVN. Glx showed a significant trough at 60 minutes, ACTH peaked at 80 minutes followed by cortisol at 110 minutes and 5-HTP reached its maximal concentration around 170 minutes (*figure 7*). At the same time, ACTH and cortisol had started to decline from their peaks at 80 minutes and 110 minutes onwards (*figure 7*). Therefore, we can assume that the major part of the 5-HTP-induced hypothalamic activation must have taken place during the 50 to 70 minute period after 5-HTP-administration. From the moment of its release cortisol exerts negative feedback via the mineralocorticoid receptor (MR) and at higher concentrations also via the glucocorticoid receptor (GR) (de Kloet *et al.*, 2005). The decline of ACTH and cortisol in blood is thus a net consequence of both its clearance from the body and negative feedback on the production. In this study, negative feedback would have inhibited hypothalamic 5-HT neurotransmission and consequently also the release of Glx. Thus, the short-lasting effects on Glx levels in the hypothalamus were, at least in part, most probably due to the inhibiting effects of cortisol feedback.

Taylor also looked at the effects of serotonergic treatment on MRS parameters in the brain (Taylor *et al.*, 2008). In this trial, Glx increased in the occipital lobes of healthy subjects treated for 7 to 10 days with 20mg citalopram. These findings are difficult to compare to ours, since there are obvious functional and metabolic differences between the occipital lobe and the hypothalamus. However, there is also a pharmacological difference between acute

administration of a precursor and prolonged treatment with a reuptake inhibitor of serotonin. Serotonergic systems are activated by acute administration of 5-HTP, whereas they are desensitized during chronic treatment with a selective serotonin reuptake inhibitor (SSRI). In this respect, a divergent Glx-response is perhaps not surprising. It is not easy to explain elevations of occipital Glx-levels following medium-term SSRI administration. However, acute Glx-depletion following synaptic stimulation is perhaps more in line with predictions and is also supported by a reduction in Glx after acute cortical excitation with transcranial magnetic stimulation (TMS) (Singh *et al.*, 2009). Following neuronal excitation (in this case by serotonergic stimulation of the hypothalamus), glutamate is released into the synaptic cleft by exocytosis. To interrupt post-synaptic activation and prevent accumulation of toxic glutamate levels, the neurotransmitter is rapidly cleared from the synapse by neuronal and astrocytic reuptake via excitatory amino acid Na⁺-coupled glutamate transporters (EAATs). Astrocytes convert glutamate back into glutamine and release it via the system-N-transporter (SN) into the synaptic cleft for reuptake by neurons (Danbolt, 2001; Iversen *et al.*, 2009). The net effect of this process on local Glx-levels is therefore expected amount to zero, because MRS cannot distinguish glutamate from glutamine, nor intracellular (astrocytic or neuronal) from extracellular (synaptic or interstitial) sources of these metabolites. However, secondary to increased release of extracellular glutamate and glutamine, these compounds are also cleared from the synaptic cleft by diffusion and blood flow (Danbolt, 2001), which could lead to the Glx-reductions that were observed with acute administration of the 5-HTP function test.

It is uncertain whether MRS would be feasible as a neuroimaging technique in the detection of other functional drug-induced changes. 7-Tesla MRS is reliable for volumes of at least 1 to 1.5 cm³. Measurements in a structure with a smaller volume of interest (VOI) can be disturbed by a low signal to noise ratio. Also, imaging multiple brain regions at the same time is associated with its own complexities. We performed spectroscopy of a hypothalamic VOI of ca. 1.5 cm³ surrounding the PVN, while the PVN itself typically comprises a volume of only 15 to 30 mm³. This is only a very small fraction of the scanned VOI, which has undoubtedly limited the sensitivity of the technique to detect PVN-activation. Furthermore,

since we exclusively performed spectroscopy on the hypothalamus, non-specific effects (such as nausea or vomiting) or a more generalized serotonergic effect of 5-HTP on the observed reduction in Glx cannot be excluded. The fact that changes were found nonetheless may indicate that the MRS-changes in the PVN must have been substantial. Detection of this small activation area was also due to the relatively low intersession variability of the much larger surrounding 'dead' volume. The variability of the predose (baseline) Glx: creat ratios was around 20% and the intrasubject variability was 15%. The low baseline variability and the high sensitivity of 7-Tesla MRS despite a fairly large VOI suggest that this technique can be particularly useful for drugs that can be predicted to act on a specific brain region. Such functional anatomical predictions can be made using preclinical *in vivo* techniques like brain tissue microdialysis and registration of local neurophysiological activity.

Creat was used as internal reference for NAA, Chol and Glx. It is possible that the Creat concentrations may have changed during the course of each experimental session. The Glx: creat trough might therefore reflect an increase in Creat concentrations rather than a decrease in Glx concentrations. However, it is impossible to reliably estimate changes in absolute quantitative Creat concentrations because hypothalamic 7T metabolite and water T1 and T2 relaxation values are unknown. Moreover, absolute Creat concentrations are usually calculated based on the unsuppressed water signal as internal reference which may change during the course of each experiment. Metabolite referencing to Creat is therefore not optimal and limits the interpretation of our results. This is however a general problem when using MRS and is not specific for the present trial.

A weakness of this study is that the power for the detection of changes by MRS may be relatively low. Measuring 5-HTP-induced activation of the hypothalamus using MRS at 7T is a novel technique. We based our initial power calculation on 5-HTP's effects on ACTH, and performed a blinded interim assessment to determine if MRS would be just as robust. As expected, the 5-HTP neuroendocrine responses were reliable and comparable to our previous experiences with 5-HTP (Danbolt, 2001; Gijsman *et al.*, 2002; Jacobs *et al.*, 2008; Smarius *et al.*, 2008). However, the MRS-analyses were complicated by technical issues and unforeseen dropouts. Statistical

power was also limited by the short duration of the hypothalamic activation as detected by MRS, which may have been due to the inhibitory effects of cortisol feedback on the hypothalamus.

The potential applications of MRS as novel technique in CNS pharmacology need to be investigated further in well-designed experiments. In this context, careful consideration should be given to the expected CNS effects of the drug under investigation, including the size of the activated area relative to the sampling volume, and the timing and duration of the physiological responses.

THE AUTHORS DECLARE NO CONFLICT OF INTEREST.

Table 1

Neuroendocrine parameters for the period 0 minutes to 240 minutes for the 200mg 5-HTP function test: Estimated means (back transformed Least Square Means) for plasma ACTH (ng/l) and serum cortisol (ng/ml) and estimated difference (%) with 95% confidence interval from placebo for the 200 mg 5-HTP function test.

| Neuroendocrine parameter | Least Square Means | | Estimated difference (%) | |
|--------------------------|--------------------|---------------|--------------------------|----------|
| | placebo n=12 | 5-HTP n=10 | 5-HTP vs placebo | p-value |
| ACTH (ng/L) | 16.9 | 33.4 | +97.8 (56.7, 149.6) | p<0.0001 |
| Serum cortisol (ng/mL) | 95.6 | 159.8 | +67.2 (44.2;93.9) | P<0.0001 |

Table 2

MRS ratios for the period 0 minutes to 180 minutes and peak effects at 60 minutes for the 200mg 5-HTP function test: Estimated means (untransformed Least Square Means) from placebo for Chol: Creat, Glx: Creat and NAA: Creat and estimated difference with 95% confidence interval from placebo for the 200mg 5-HTP function test.

| MRS ratio | | Least Square Means | | Estimated difference | p-value |
|------------------|---------------------------|--------------------|-------|-------------------------|---------|
| | | Placebo | 5-HTP | 5-HTP vs placebo | |
| | | n=12 | n=8 | | |
| Chol:Creat ratio | 0 to 180 minutes | 1.071 | 1.117 | 0.046 (-0.017,0.109) | 0.128 |
| | Peak effect at 60 minutes | 1.159 | 1.064 | 0.096 (0.004, 0.195) | 0.059 |
| Glx:Creat ratio | 0 to 180 minutes | 0.229 | 0.259 | -0.030 (-0.066, 0.006) | 0.090 |
| | Peak effect at 60 minutes | 0.196 | 0.252 | -0.055 (-0.110, -0.001) | 0.045 |
| NAA:Creat ratio | 0 to 180 minutes | 1.041 | 1.057 | 0.016 (-0.049, 0.080) | 0.597 |
| | Peak effect at 60 minutes | 1.036 | 1.057 | 0.021 (-0.076, 0.118) | 0.670 |

Figure 1

Typical example of the MRS planning. The white rectangles represent the borders of the MRS voxel that contains the hypothalamus.

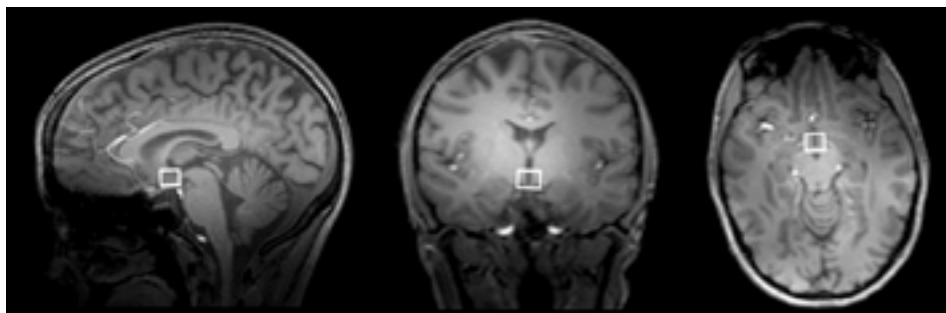


Figure 2

Example of the typical MRS spectra for choline (chol); creatine (creat); glutamate-glutamine (Glx) and N-acetylaspartate (NAA) derived from a volunteer in the present study.

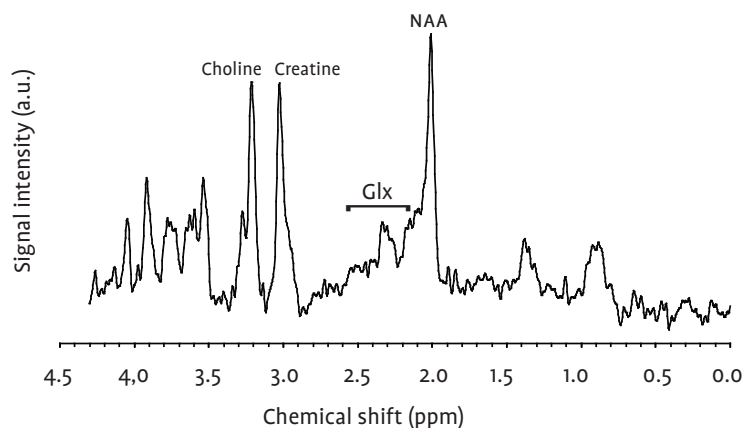


Figure 3

Estimated means with 95% CI error bars for MRS Glx: Creat over the period 0 to 180 minutes, after administration of placebo (n=12) and the 200mg 5-HTP function test (n=8).

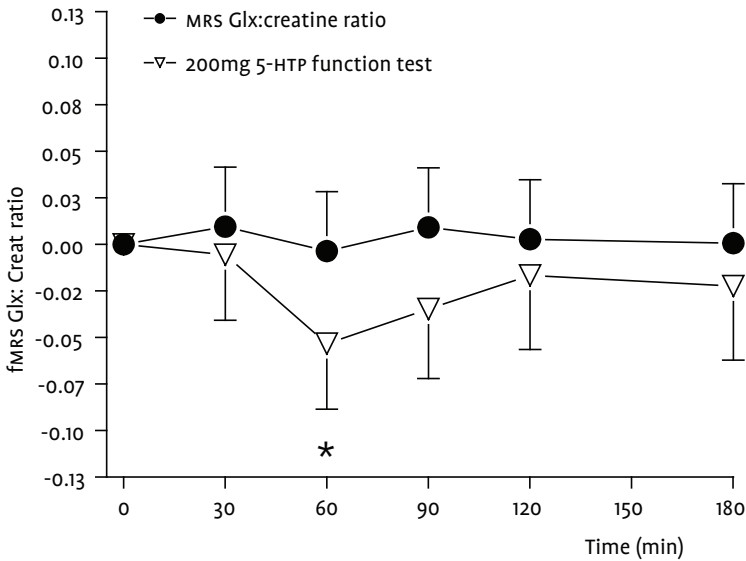


Figure 4

Estimated means (ng/l) with 95% CI error bars for plasma adrenocorticotrophic hormone (ACTH) over the period 0 to 240 minutes, after administration of placebo (n=12) and the 200mg 5-HTP function test (n=8).

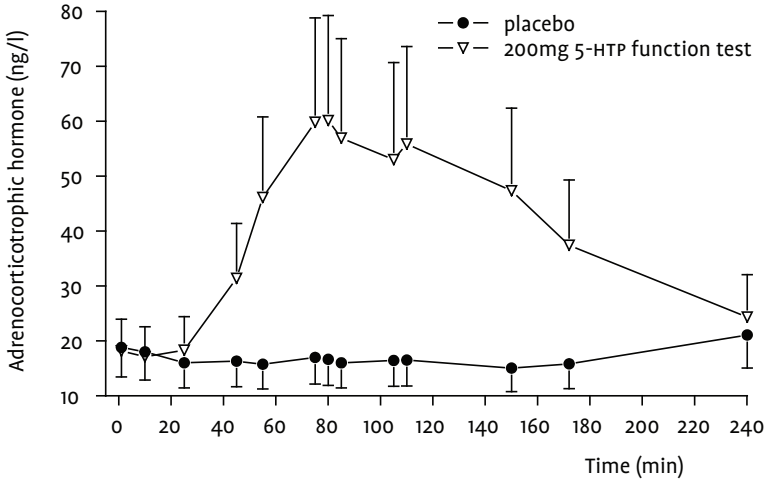


Figure 5

Estimated means (ng/ml) with 95% CI error bars for serum cortisol over the period 0 to 240 minutes, after administration of placebo (n=12) and the 200mg 5-HTP function test (n=8).

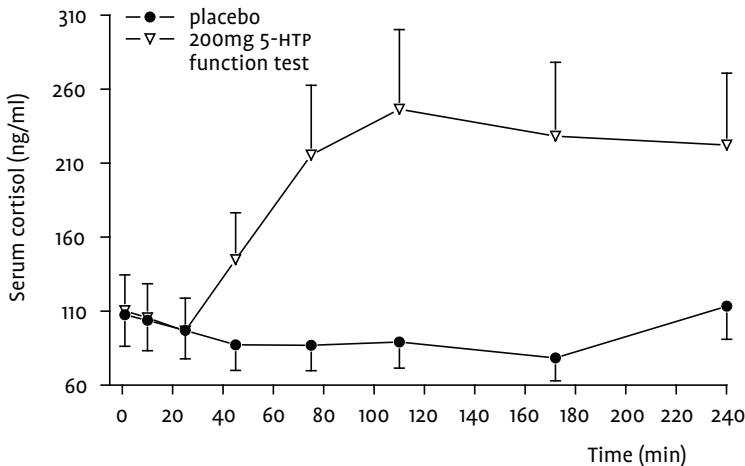


Figure 6

Average time profile with SD error bars of serum 5-HTP (ng/ml) over 300 minutes for the 200mg 5-HTP function test (n=10).

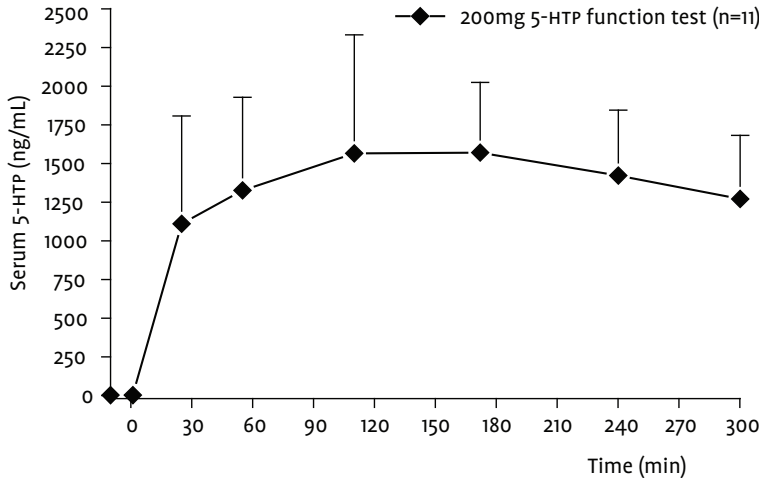
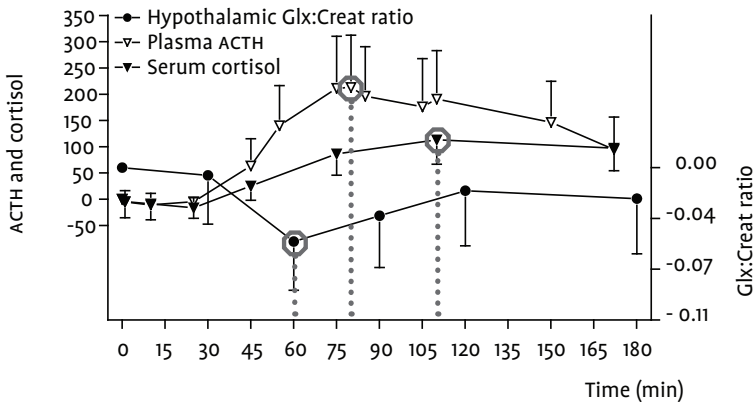


Figure 7

Combined graphical representation of the estimated mean percentual change from placebo (%) with 95% CI error bars for adrenocorticotrophic hormone (ACTH) and cortisol (left Y-axis) and the mean change relative to placebo with error bars for MRS Glx: Creat (right Y-axis), after administration of the 200mg 5-HTP function test. The grey circles and dotted lines represent the respective E_{MAX} for Glx: Creat at 60 minutes, ACTH at 80minutes and cortisol at 110minutes.



REFERENCE LIST

- Anderson I M, Clark L, Elliott R, Kulkarni B, Williams S R, Deakin J F (2002) 5-HT(2C) receptor activation by m-chlorophenylpiperazine detected in humans with fMRI. *Neuroreport* 13: 1547-1551.
- Anderson I M, McKie S, Elliott R, Williams S R, Deakin J F (2008) Assessing human 5-HT function in vivo with pharmacofMRI. *Neuropharmacology* 55: 1029-1037.
- Danbolt N C (2001) Glutamate uptake. *Prog Neurobiol* 65: 1-105.
- de Kloet E R, Joels M, Holsboer F (2005) Stress and the brain: from adaptation to disease. *Nat Rev Neurosci* 6: 463-475.
- De Stefano N, Filippi M, Miller D et al. (2007) Guidelines for using proton MR spectroscopy in multicenter clinical MS studies. *Neurology* 69: 1942-1952.
- Dinan T G (1996) Serotonin and the regulation of hypothalamic-pituitary-adrenal axis function. *Life Sci* 58: 1683-1694.
- Gartside S E, Cowen P J (1990) Mediation of ACTH and prolactin responses to 5-HTP by 5-HT₂ receptors. *Eur J Pharmacol* 179: 103-109.
- Gijsman H J, van Gerven J M A, de Kam M L et al. (2002) Placebo-controlled comparison of three dose-regimens of 5-hydroxytryptophan challenge test in healthy volunteers. *Journal of Clinical Psychopharmacology* 22: 183-189.
- Iversen L L, Iversen S D, Bloom F E, Roth R H (2009) Introduction to Neuropsychopharmacology. 85-103
- Jacobs G, Kamerling I, de K M et al. (2008) Enhanced tolerability of the 5-hydroxytryptophan challenge test combined with granisetron. *J Psychopharmacol*
- Leonard B E (2005) The HPA and immune axes in stress: the involvement of the serotonergic system. *Eur Psychiatry* 20 Suppl 3: S302-S306.
- Mason G F, Krystal J H (2006) MR spectroscopy: its potential role for drug development for the treatment of psychiatric diseases. *NMR Biomed* 19: 690-701.
- Singh S, Mistry S, Jefferson S et al. (2009) A magnetic resonance spectroscopy study of brain glutamate in a model of plasticity in human pharyngeal motor cortex. *Gastroenterology* 136: 417-424.
- Smarius L J, Jacobs G E, Hoeberechts-Lefrandt D H et al. (2008) Pharmacology of rights oral doses of 5-hydroxytryptophan with carbidopa. *J Psychopharmacol*
- Soares D P, Law M (2009) Magnetic resonance spectroscopy of the brain: review of metabolites and clinical applications. *Clin Radiol* 64: 12-21.
- Sotty F, Folgering J H, Brennum L T et al. (2009) Relevance of dorsal raphe nucleus firing in serotonin 5-HT(2C) receptor blockade-induced augmentation of SSRIs effects. *Neuropharmacology* 57: 18-24.
- Stark J A, Davies K E, Williams S R, Luckman S M (2006) Functional magnetic resonance imaging and c-Fos mapping in rats following an anorectic dose of m-chlorophenylpiperazine. *Neuroimage* 31: 1228-1237.
- Stark J A, McKie S, Davies K E, Williams S R, Luckman S M (2008) 5-HT(2C) antagonism blocks blood oxygen level-dependent pharmacological-challenge magnetic resonance imaging signal in rat brain areas related to feeding. *Eur J Neurosci* 27: 457-465.
- Taylor M, Murphy S E, Selvaraj S et al. (2008) Differential effects of citalopram and reboxetine on cortical Glx measured with proton MR spectroscopy. *J Psychopharmacol* 22: 473-476.
- Wise R G, Tracey I (2006) The role of fMRI in drug discovery. *J Magn Reson Imaging* 23: 862-876.

General discussion and conclusions

This thesis focused on the quantification of HPA axis activation in healthy volunteers by applying pharmacological function tests with distinct pharmacodynamic (PD) mechanisms. Its main objective was to contribute to the validation of different function tests for the two most well-characterized HPA axis activation routes. To realize this objective, different experiments were executed in which specific principles that have been proposed to optimize the reliability of pharmacological function tests were observed. hCRH and 5-HTP were applied for the corticotrophinergic (CRH-mediated) route while dDAVP and metoclopramide were used for the vasopressinergic (AVP-mediated) pathway. Additionally, the application of these function tests was expected to contribute to a better understanding of the respective contributions of the corticotrophinergic and vasopressinergic pathways to HPA axis activation in health. Finally, an extra dimension was added to the validation process by applying a neuroimaging technique to measure changes in hypothalamic metabolites during pharmacological stimulation.

What are the major findings of this thesis?

dDAVP is a synthetic analogue of vasopressin which, together with endogenous CRH, functions as a co-activator of pituitary ACTH-release (DeBold et al., 1983; Favrod-Coune et al., 1993; Lamberts et al., 1984). However, it also has cardiovascular, pro-coagulatory and anti-diuretic effects that may cause safety concerns in some patients and could have secondary confounding effects via (sympatho-adrenal-medullary - SAM) stress activation. In chapter 2 the effects of vasopressinergic activation on the HPA-axis were investigated, relative to the potentially confounding autonomic and systemic effects of dDAVP. The neuroendocrine effects of a 10µg dDAVP bolus administered over one minute were similar to those of a 30µg incremental infusion administered over one hour, despite higher dDAVP concentrations after the infusion. In contrast, the cardiovascular- and coagulatory effects of dDAVP were dose-related. This differential effect is probably related to low endogenous CRH levels under physiological conditions and/or maximal v_3 receptor occupancy at doses above 10 µg. These findings indicate that dDAVP administered over one minute at a dose of not more than 10µg is safe and does not cause confounding SAM (stress) effects.

However, the neuroendocrine effects associated with the systemic administration of 10µg dDAVP by itself are so small and variable that its role in future research focusing on (physiological) AVP-mediated HPA axis activation is expected to be limited. Since AVP functions as co-activator of the HPA axis in the presence of CRH, chapter 3 examined whether the concomitant administration of dDAVP and low doses of synthetic CRH - corticorelin or hCRH - could provide a more robust stimulation of the HPA axis in healthy volunteers than with dDAVP alone, and whether such a combination could provide information on the extent of vasopressinergic co-activation relative to major corticotrophinergic activation with hCRH alone. The administration of 100µg hCRH induces robust HPA axis activation while combining 10µg dDAVP with either 10µg or 30µg hCRH boosts vasopressinergic co-activation and causes dose-related ACTH- and CORT release that is larger than with 10µg dDAVP alone, and roughly similar to the neuroendocrine effects of 100µg hCRH alone. Finally, in chapter 3 it was explored whether distinct corticotrophinergic activation and vasopressinergic co-activation of the HPA axis could be examined on a single short laboratory visit by sequentially administering dDAVP and hCRH: the interaction effect of prior 10µg dDAVP administration on corticotrophinergic activation by administering 100µg hCRH two hours later was investigated and was found not to interact in a significant way.

The function tests described in chapters 2 and 3 are associated with problems that may hamper their continued application in HPA axis research. These encompass confounding PD effects (dDAVP and hCRH), safety concerns in some patient population (dDAVP) and the lack of information that (peripheral) pituitary stimulation by dDAVP and hCRH provides about essential more proximal structures such as the hypothalamus, medial prefrontal cortex (MPFC) and limbic system in stress related disorders. For the purpose of stimulating these central systems, two other function tests were examined in Chapters 4, 5 and 6. 5-HTP combined with carbidopa has previously been shown to induce robust activation of the HPA axis by inducing ACTH- and CORT release (Gijssman et al., 2002). The neuroendocrine activation associated with 5-HTP is attributed to actions at hypothalamic 5-HT receptors with subsequent endogenous CRH-release that results in corticotrophinergic HPA axis activation (Gartside and Cowen, 1990). In chapter 4, the dose- and

plasma concentration-effect relationship for orally administered 5-HTP combined with carbidopa was investigated. The combined 5-HTP test was found to be an effective corticotrophinergic function test via the serotonergic route, which exhibits dose-related plasma concentrations and neuroendocrine responsiveness. However, the frequent occurrence of serotonin-associated side-effects such as nausea and vomiting limits its applicability in clinical research. Subsequently, in chapter 5 it was aimed to suppress the serotonergic side-effects associated with the combined 5-HTP/carbidopa function test by the addition of an anti-emetic. The addition of the subtype selective 5-HT₃ receptor antagonist granisetron to the combined 5-HTP/carbidopa function test suppresses nausea and vomiting without influencing the neuroendocrine response or the pharmacokinetic (PK) characteristics of 5-HTP, making it a potentially useful tool to assess centrally-mediated corticotrophinergic HPA axis activation. On the other hand, metoclopramide has been reported to induce vasopressinergic co-activation by stimulating endogenous AVP release (Chiodera et al., 1986; Nomura et al., 1984; Seki et al., 1997; Walsh et al., 2005). The previously reported experiments that have investigated the neuroendocrine effects of metoclopramide were associated with important methodological drawbacks. Additionally, the mechanism by which metoclopramide induces AVP-release remains speculative and the relation of its PD effects to its PK has never been investigated. In the penultimate chapter 6, intravenous administration of the D₂ receptor antagonist metoclopramide was investigated as a centrally acting vasopressinergic function test. Metoclopramide effectively releases ACTH by itself and induces co-activation in the presence of enhanced (5-HTP induced) corticotrophinergic activation: metoclopramide's neuroendocrine effects under physiological and supraphysiological CRH concentrations are compatible with, but not indicative of, endogenous AVP release and subsequent vasopressinergic co-activation of the HPA axis.

The (peripheral) systemic neuroendocrine responses that are induced by function tests of the HPA-axis reflect fluctuations within the (central) pituitary portal system following stimulation. Changes in the concentrations of plasma ACTH and CORT are a net effect of different dynamic processes including central and peripheral amplification, HPA axis negative feedback and the clearance of

neuroendocrine hormones. So far, it has not been possible to quantify each of these different processes sufficiently reliably. More direct measurements of drug-induced neuronal activity in the brain using neuroimaging techniques could lead to a better understanding of the central (pharmacological) CNS processes induced by the stimulation of neurotransmitter systems with function tests, and add one piece to the complex puzzle of HPA-axis regulation. In chapter 7 the effects of the combined 5-HTP/carbidopa/granisetron function test on hypothalamic glutamate/glutamine (Glx), choline, N-acetyl-aspartate (NAA) and creatine levels were studied in healthy volunteers using 7-Tesla (7T) MRS. These responses were subsequently related to the (well-characterized) peripheral ACTH and CORT responses. Following serotonin-mediated corticotrophinergic HPA axis activation with 5-HTP, hypothalamic Glx-levels are reduced and are followed by the release of ACTH from the pituitary and CORT from the adrenal glands. Taken together, this pilot study supports the involvement of hypothalamic Glx-depletion in HPA axis activation and demonstrates that it is possible to quantify serotonin-mediated changes in hypothalamic metabolism using a well-known neuroimaging technique.

What has this thesis provided?

This thesis has shown that the major HPA axis activation routes can be quantified by the application of specific pharmacological stimulation tests. Stimulation can be achieved either directly with peripherally acting agents (hCRH and dDAVP) or indirectly with drugs that have central effects (5-HTP and metoclopramide) (Figure 4). Activation of the corticotrophinergic route is achieved by administering either the CRH analogue hCRH or the direct precursor of serotonin 5-HTP. 100 µg hCRH induces robust ACTH and CORT release by a direct and relatively specific stimulation of the pituitary CRH₁ receptor system. 200mg 5-HTP, combined with carbidopa and granisetron to stabilize the PK and to reduce systemic side-effects of 5-HTP, potentially releases ACTH and CORT in a dose- and concentration dependent manner that is reconcilable with (indirect) 5-HT-induced release of endogenous CRH via the 5-HT_{2A} or 5-HT_{2C} receptors situated in the hypothalamic PVN. Also, 5-HTP-induced HPA axis activation is related to a decrease in

hypothalamic glutamate-glutamine levels in the anatomical region of the PVN. These hypothalamic changes precede the release of ACTH and subsequent CORT and are therefore reconcilable with a depletion of hypothalamic glutamate stores following 5-HT induced CRH release. 100 µg hCRH and 200 mg 5-HTP induce corticotrophinergic HPA axis activation that is associated with similar maximal CORT levels. Importantly, these CORT levels are comparable with the previously maximally attainable CORT levels with 100 µg hCRH (210 ng/ml) (Dinan et al., 1999; Scott et al., 1999) and 0.5mg/kg mCPP (230 ng/ml) (Gijssman et al., 1998) in healthy volunteers. Thus, pharmacological stimulation of the HPA axis in healthy individuals via the corticotrophinergic route seems to be associated with an average CORT ceiling effect in the vicinity of 220 ng/ml. On the other hand, vasopressinergic co-activation can be quantified by applying dDAVP and metoclopramide as function tests. 10 µg dDAVP directly stimulates pituitary V_3 receptors and induces demonstrable neuroendocrine effects that are too small and variable to reliably quantify vasopressinergic co-activation. In fact, the neuroendocrine response induced by 10 µg dDAVP alone is roughly half that of the corticotrophinergic CORT ceiling effect. Previously, dDAVP has been shown to induce dose-dependent vasopressinergic co-activation at 5 µg and 10 µg but not at 15µg. Also, 30 µg dDAVP induces activation that is comparable to that of 10 µg, despite reaching a 1.8 fold higher plasma concentration. This apparently low maximal effect of dDAVP alone is attributed to low endogenous CRH levels under physiological conditions and/or maximal V_3 receptor occupancy at doses above 10µg. The administration of doses of more than 10µg dDAVP will thus not lead to more sizeable vasopressinergic co-activation but is expected to cause non-specific confounding effects that will thwart its validity and applicability even further. Instead, vasopressinergic co-activation can be boosted by exogenously administering (low) doses of hCRH. This thesis showed that combining 10 µg dDAVP with 10 hCRH or 30 µg hCRH both induce a CORT response that is greater than that of 10 µg dDAVP alone, and only marginally smaller than that of the CORT ceiling effect attained with for instance 100 µg hCRH: 10 µg hCRH combined with 10 µg dDAVP induces a CORT response that is 40% greater than 10 µg dDAVP alone, reaching 80% of the CORT ceiling effect, while combining 10 µg dDAVP with 30 µg hCRH induces a

response that is 60% greater than 10 µg dDAVP alone which approaches 90% of the ceiling effect. These findings indicate that vasopressinergic co-activation of the HPA axis is a dose-dependent function of ambient CRH or CRH₁-receptor activation that can be mimicked pharmacologically in healthy volunteers by the concomitant administration of dDAVP and low doses hCRH. The boosted response induced by 30 µg hCRH combined with 10 µg dDAVP approaches the CORT ceiling to such an extent that it makes differentiating vasopressinergic co-activation from corticotrophinergic activation virtually impossible, while 10 µg hCRH combined with 10 µg dDAVP induces a response that distinguishes itself from both vasopressinergic co-activation with 10 µg dDAVP alone and corticotrophinergic activation with 100 µg hCRH alone. The optimal hCRH dose that needs to be combined with 10 µg dDAVP for optimal vasopressinergic co-activation, without obscuring the difference between vasopressinergic co-activation and corticotrophinergic activation, is therefore expected to lie around 10 µg since it is not restricted by flooring- or ceiling effects as in the case of 10 µg dDAVP alone and 100 µg hCRH. However, such a prediction is precarious since we have not examined the full dose-response curve of hCRH (including the effects of 10 µg or 30 µg hCRH alone). PK-PD modeling will have to confirm the optimal combination in future. Alternatively, metoclopramide is believed to induce vasopressinergic co-activation (indirectly) by releasing endogenous AVP from the hypothalamus and/or the pituitary following D₂ receptor stimulation. However, metoclopramide's precise pharmacological mechanism remains unclear and the release of endogenous AVP still needs to be confirmed. At any rate, metoclopramide is a potentially useful tool in vasopressinergic HPA axis research since it has the advantage that it does not need to be combined with hCRH and that it is not associated with potentially confounding (autonomic) effects. Finally, 10 µg dDAVP followed by 100 µg hCRH two hours later do not interact significantly, which allows for a practical function test examining both HPA-activation routes on a single short test occasion.

It is important to realize that the function tests described in this thesis are easily executable under research conditions. They consist of the oral or intravenous administration of registered drugs that is followed by a complete neuroendocrine assessment within 4

hours. Furthermore, the addition of granisetron to 5-HTP suppresses the bothersome 5-HT associated side-effects of 5-HTP, and the adverse effects associated with hCRH and low doses of dDAVP are self-limiting and of little clinical significance. Also, full characterization of the HPA-response provides a dynamic quantification of HPA axis activation: instead of utilizing a few sparse sampling points (as in the case of the CAR), ACTH and CORT are sampled intensively, allowing for a reliable quantitative analysis of neuroendocrine changes as a function of both time and function test drug concentrations. Additionally, the application of 7T spectroscopy provided indications that it is possible to demonstrate changes in neuronal metabolites after the administration of a precursor drug such as of 5-HTP. Taken together, the present function tests are associated with a minimal burden for research subjects, while providing an optimally informative data yield, making them ethically acceptable and practical to administer.

In summary, this thesis has provided a number of pharmacologically well-characterized clinical research tools for the assessment of the major HPA axis activation routes (Table 3). 200 mg 5-HTP combined with carbidopa and granisetron induces safe dose- and concentration dependent activation via the central corticotrophinergic route. Also, this test is well-tolerated and has minimal confounding PD effects and predictable and reproducible PK. 10 µg dDAVP combined with a dose of 10 µg or 30 µg hCRH induces dose-dependent (pituitary) vasopressinergic activation, without the potentially dangerous and confounding effects of higher doses dDAVP. Metoclopramide could be considered as an alternative vasopressinergic function test that probably operates centrally, but replication of its (vasopressinergic) effects and further clarification of its pharmacological mechanism will determine its role as function test in future. hCRH allows for the assessment of direct pituitary corticotrophic stimulation, although the optimal hCRH dose to characterize the full response curve still needs to be established. The sequential administration of dDAVP followed by hCRH presents the possibility to independently assess vasopressinergic as well as the corticotrophinergic aspects of HPA axis activation on a single short study day, without falling prey to interaction effects. Thus, the present tests can be considered suitable for application in clinical HPA axis research in both healthy volunteers and patient groups.

How can these function tests be applied?

The development of novel drugs for the treatment of major depressive disorder (MDD) has stagnated in recent years (Conn and Roth, 2008). Target identification and -validation in the development of novel CNS drugs are generally problematic and undoubtedly contribute to the current impasse (Agid et al., 2007; Pangalos et al., 2007). More importantly, the validity of preclinical (animal) models that are used to predict the efficacy of novel antidepressant drugs (AD's) is limited. These models have been developed and validated after discovery of the antidepressant properties of the tricyclic antidepressant drugs (TCA's), and therefore all rely heavily on the modulation of 5-HT, noradrenaline (NA) and dopamine (DA) circuits (Cryan and Slattery, 2007; Conn and Roth, 2008). Hyperactivity of the HPA axis is one of the most consistent (neuroendocrine) abnormalities associated with (certain subgroups of) MDD and other so-called stress-related psychiatric disorders (Pariante and Lightman, 2008; Schule et al., 2009). Whether such hyperactivity represents a vulnerability trait/risk factor or a pathogenetic factor in the development of stress-related psychopathology, or whether it constitutes a mere epiphenomenon of such pathology remains obscure (Schule et al., 2009). At any rate, the neuropeptides CRH and AVP (in particular) have been implicated in HPA axis hyperactivity in both humans and animals (Dinan et al., 2004; Pariante and Lightman, 2008; Schule et al., 2009). The precise pathophysiological mechanism and the share of depressed patients that display HPA axis hyperactivity still needs to be established (Pariante and Miller, 2001; Pariante and Lightman, 2008). Taken together, the contribution of HPA axis hyperactivity to the pathophysiology of MDD remains elusive and the traditional mono-amine based preclinical animal models are associated with the obvious weakness that they deny the role of neuropeptides in HPA axis hyperactivation. Despite these problems, new compounds that target corticotrophinergic and/or vasopressinergic HPA axis disturbances for the treatment of MDD are currently under development. The hitherto unclear contributions of CRH and AVP in HPA axis hyperfunction and inadequate preclinical models pose a concrete risk for failure of such compounds in future clinical trials. However, the application of pharmacological function tests may guide the development of these

potentially clinically relevant compounds along a rational path, and contribute to further elucidation of the pathophysiology of stress-systems in psychiatric disease.

Pharmacological function tests can be applied as pathophysiological research tools to characterize disturbed HPA axis activation associated with MDD. The nature of aberrant HPA axis activation can be assessed in patient groups by quantifying corticotrophinergic activation (using hCRH alone or 5-HTP combined with carbidopa and granisetron) on the one hand and (enhanced) vasopressinergic co-activation (using dDAVP with or without a low dose hCRH or metoclopramide) on the other hand. Subsequently, the findings in patients can be validated by comparing the HPA axis responses to those of healthy volunteer groups who have received the respective (identical) function tests. Also, part of such validation process can be performed in experimental animals. Such characterization of corticotrophinergic activation and vasopressinergic co-activation in animals, healthy individuals and patients might aid in the future development of a diagnostic and classification system that is based on (endo)pathophysiological characteristics, rather than the currently widely used phenomenological ones. More provocatively, the quantification of HPA axis activation in asymptomatic probands of individuals with MDD or carriers of known genetic predisposing factors such as polymorphisms of 5-HT, CRH and/or V_3 receptors might help identify individuals at risk for developing full-blown MDD timely. Such detailed analyses of complex multifaceted regulatory systems are only possible with well-validated function tests and function parameters.

The development of preclinical models that are able to predict the effect of novel drugs that target the HPA axis deserves priority. The role of pharmacological function tests in this process is still undefined and needs further exploration. For instance, function tests can be applied in animals as pharmacological models for HPA axis hyperactivation instead of the traditional chronic stress models that rely on mechanically induced hyperactivation such as mechanical restraint or repeated electrical stimulation. Once validated, the findings in animals can be integrated with clinical data derived from healthy volunteers and patients. In this sense, pharmacological function tests are natural - but largely underutilized - (bidirectional) translational tools between animals and humans

that can enable researchers to develop (additional) preclinical models that acknowledge the role of disturbed neuropeptide function in depressive disorders, instead of solely relying on the current (reductionist) ones.

Pharmacological function tests can prove informative in “proof-of-pharmacological principle” experiments with novel drugs. For instance, the effects of a v_3 antagonist could be demonstrated using a function test that quantifies vasopressinergic co-activation in both animals and humans. By doing this, the intended mechanism of action can be confirmed, and the dosage regimens can be pharmacologically optimized in animals, healthy volunteers and selected patient groups, before costly clinical trials are launched. Also, instead of examining the effects of drugs under basal conditions, it would be instructive to evaluate their actions in models of pathological states in both humans and animals. In this context MDD-associated HPA axis hyperactivation can be mimicked pharmacologically in healthy individuals using pharmacological function tests. Such an artificially hyperactivated HPA axis is expected to lend itself to pharmacological modulation with compounds that target the CRH_1 and/or v_3 receptors. Artificially induced hyperactivation can also be used to mimic certain (neuroendocrine) aspects of stress-related disorders in healthy volunteers or subjects at risk, and in turn such use as disease models can contribute to the validation of the function test as a predictive tool in drug development. Finally, antidepressant drugs such as the selective serotonin re-uptake inhibitors (SSRI's), the mono-amine oxidase inhibitors (MAOI's) and the TCA's have been shown to modulate HPA axis function. In mice, treatment with different AD's (SSRI's, MAOI's) induces a marked increase in hippocampal MR and GR and decreases CRH mRNA in the PVN (Schule, 2007). Also, a gradual attenuation of HPA axis hyperactivity has been demonstrated with serial application of the DEX/CRH test when treating depressed patients with TCA's and SSRI's (Schule, 2007). Taken together, these findings are indicative of more efficient negative feedback secondary to the upregulation over time of the MR and GR in when treating patients suffering from depression with AD's. Moreover, enhanced negative feedback via the MR/GR is expected to affect the corticotrophinergic and/or vasopressinergic activation routes of the HPA axis. However, changes in the sensitivity of

the CRH₁ and/or V₃ receptor system after treatment with AD's have not been investigated as such.

In this context, function tests can be applied to investigate the effects of widely used, clinically well-validated antidepressant drugs on HPA axis activation. Such information can aid in further clarifying the relationship between the central mono-amine circuits and the hypothalamic neuropeptides in both health and disease.

What still needs to be clarified?

HPA axis function is determined by dynamic interactions between production and clearance, modulation and co-activation, and activation and inhibition, involving different complex regulation systems and negative feedback processes. The studies described in this thesis have focused on the quantification of HPA axis activation and the exploration and partial optimization of pharmacokinetic- and pharmacodynamic properties of pharmacological agents that target different central and peripheral components of the stress system. These studies have not taken the influence of negative feedback on pharmacologically induced HPA axis activation into account. It should be realized that the neuroendocrine responses described in this thesis do not purely reflect activation, but are always the product of concomitant pharmacological stimulation and physiological feedback. A complete physiological model that takes both activation and feedback into account is needed for a more accurate understanding of HPA axis function. However, complete characterization of the major activation centers and feedback loops in both healthy volunteers and different patient groups will be time-consuming, and requires more detailed information about processes that are currently difficult to study in isolation. In this context, physiological PK-PD modeling can be of important value in further characterizing the hitherto incompletely characterized aspects of HPA axis physiology. Moreover, such a model can be useful in predicting the responses of patient populations to the different function tests and the effects of novel drugs targeting HPA neuropeptide systems. In the case of the HPA axis, PK-PD modeling remains a complex and relatively unexplored field. An important reason for this is because HPA axis physiology

is dynamic and that the feedback loops exist on different functional-anatomical levels. Before it would be possible to proceed to model such an intricate system, the different aspects of HPA axis physiology will have to be functionally disentangled. It is not unthinkable that the function tests described in this thesis can be combined with other function tests that temporarily shut off the negative feedback system. For instance, DEX can be administered and followed by 5-HTP to investigate whether the same blunted response as with hCRH can be observed in patients with MDD. With regards to the AVP system, DEX administration can be followed by dDAVP to examine vasopressinergic co-activation in the absence of an intact GR/MR feedback mechanism. For the time being, quantification of the differential activatory and inhibitory aspects of HPA axis physiology should be pursued by applying the different pharmacological function, in an attempt to fill the gaps in the current understanding of HPA axis physiology.

The diurnal rhythm of CORT is an important aspect that has also not specifically deserved attention in this thesis. Healthy humans demonstrate an ultradian pattern of CORT release that forms the basis of the typical diurnal CORT rhythm (Lightman et al., 2008). Additionally, there are robust dynamic interactions between basal CORT pulsatility and normal HPA axis function that determines an individual's ability to respond to (psychological) stress. Whether the same holds true for the neuroendocrine responses following pharmacological stimulation is essentially unclear. In the described experiments, it was attempted to minimize additional variability due to differences in basal CORT levels by administering all function tests between 9 and 11h in the morning and by performing all invasive study procedures (such as insertion of intravenous cannulas) at least 120 minutes before function test administration. Additionally, the basal CORT (and ACTH) levels before administration were included as covariate in the ANCOVA analysis models for the neuroendocrine responses. However, patients suffering from MDD display a disturbed CORT pulsatility and diurnal rhythm. It can therefore not be excluded with certainty that such disturbances will confound the effects of these function tests in patient groups. Abnormal diurnal rhythms will certainly affect the sensitivity and functionality of the different processes involved in the production, release, activation and inhibition

of neuroendocrine hormones. At present however, such data are lacking and this issue will have to be explored in future experiments with patients, using high-frequency sampling methods and deconvolution analyses of hormonal rhythms.

In conclusion, this thesis has shown that the major HPA axis activation routes can be quantified either directly (with peripherally acting hCRH and dDAVP) or indirectly (using centrally acting 5-HTP and metoclopramide) by directed pharmacological stimulation with pharmacological function tests. 5-HTP, hCRH and dDAVP combined with low doses of hCRH are pharmacologically best characterized, making them apt for application in clinical HPA axis research settings in both healthy volunteers and patient groups. On the other hand, metoclopramide's role as vasopressin-ergic pharmacological function test needs to be explored further. Importantly, 10 µg dDAVP followed by 100 µg hCRH two hours later do not interact significantly, which allows for a practical function test examining both HPA-activation routes on a single short clinical laboratory visit. Over the past decades, a wealth of information on HPA axis function in both health and disease has been gathered. The precise physiological mechanisms that underlie both HPA axis activation and negative feedback have been studied extensively, enabling HPA axis researches and pharmacologists alike to further pursue the origins and potential treatment of disturbed HPA axis activation in stress-related psychiatric disorders. In this context, well-characterized pharmacological function tests can be applied as pathophysiological research tools to characterize disturbed HPA axis activation, as translational tools between animal and humans in developing more adequate preclinical models, and in "proof-of-pharmacological principle" experiments with novel drugs that target the different HPA axis activation centers.

Figure 4

Graphic representation of the pharmacological function tests that have been investigated in this thesis: hCRH (direct) and 5-HTP (indirect) for quantification of corticotrophinergic and dDAVP (direct) and metoclopramide (indirect) for the quantification of vasopressinergic activation of the HPA axis (5-HT: 5-hydroxytryptamine/serotonin; 5-HTP: 5-hydroxytryptophane; hCRH: corticotorelin; dDAVP: desmopressin; MCP: metoclopramide; GR: glucocorticoid receptor; ACTH: adreno-corticotrophic hormone).

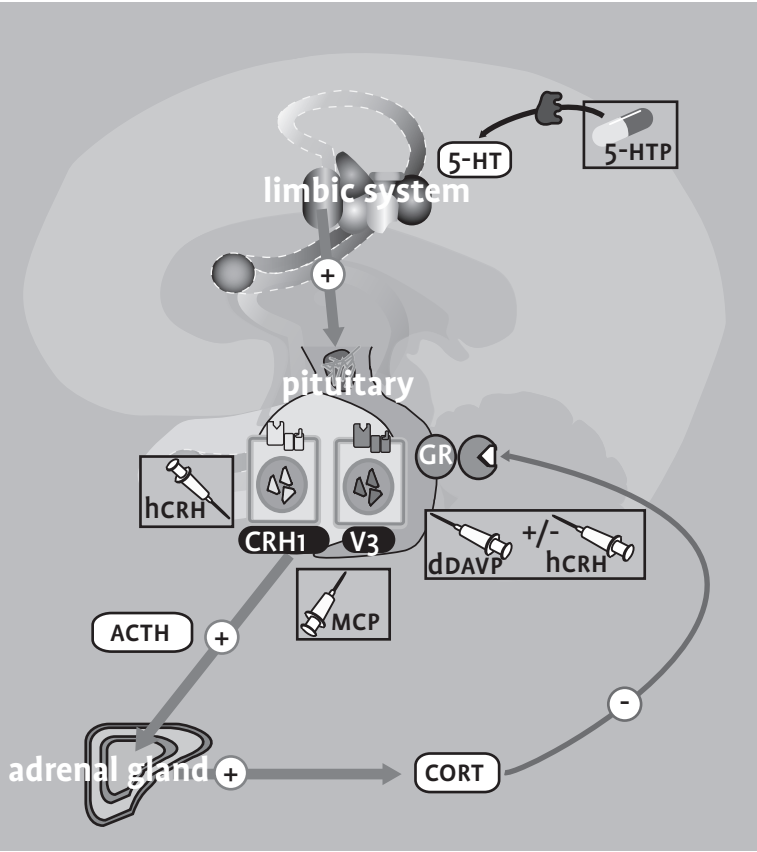


Table 3

Summary of the information provided by this thesis (*in italics*) to the existing corticotrophinergic (hCRH and 5-HTP) and vasopressinergic (dAVP and metoclopramide) function tests to assess HPA axis.

| Characteristic | Corticotrophinergic activation | | Vasopressinergic co-activation | | |
|-------------------------------------|---|--|---|---|--|
| | hCRH | 5-HTP/carbidopa | dAVP | dAVP combined with low doses hCRH | Metoclopramide |
| Pharmacological mechanism | Agonism at the CRH ₁ receptor in the anterior pituitary | Conversion of 5-HTP to 5-HT in the raphe nuclei followed by agonism at 5-HT _{2A} or 5-HT _{2C} receptors in the PVN, leading to depletion of hypothalamic glutamate-glutamine (chapter 7) | Agonism at the V ₃ (V _{1B}) receptor anterior pituitary | CAMP induced synergism of CRH ₁ agonism by dAVP | Unclear, maybe D ₂ antagonism |
| Confounding effects | Blood pressure reduction leading to autonomic nervous system activation | Minimal nausea and vomiting with the addition of granisetron (chapter 5) | Blood pressure reduction leading to autonomic nervous system activation is minimized at $\leq 10 \mu\text{g}$ (chapter 2) | Similar to those with dAVP and hCRH, no additive effect in combination (chapter 3) | Minimal effects (chapter 6) |
| Measurable PD effects | ACTH, CORT, prolactin | ACTH, CORT, prolactin | ACTH, CORT, prolactin | ACTH, CORT, prolactin | ACTH, CORT, prolactin |
| A robust physiological model | Convincingly demonstrated | Dose-concentration dependence for 100mg, 200mg and 300mg (chapter 4) | Convincingly demonstrated for 5 μg and 10 μg ; a ceiling effect exists at $\geq 10 \mu\text{g}$ (chapter 2) | Dose-dependent vasopressinergic co-activation when combining 10 μg dAVP with 10 μg or 30 μg hCRH (chapter 3) | Never demonstrated |

| | | | | | |
|---|--|---|--|---|--|
| Safe/few adverse effects | Few effects, blood pressure reduction is potentially confounding (chapter 3) | Nausea and vomiting, can be suppressed by the addition of granisetron without influencing the neuroendocrine response or 5-HTP PK (chapter 5) | Dose-related cardiovascular, coagulatory effects, minimal effects at $\leq 10 \mu\text{g}$ (chapter 2) | Similar to those with dbAVP and hCRH, no additive effect in combination (chapter 3) | Minimal effects (chapter 6) |
| Wide window between PD – and undesirable effects | CORT ceiling effect around 200ng/mL (chapter 3) | CORT ceiling effect around 200ng/mL with 200mg 5-HTP/carbidopa combined with granisetron with minimal side-effects (chapter 5) | CORT ceiling exists at $\geq 10 \mu\text{g}$ (chapter 2) | 10 μg dbAVP combined with 10 μg hCRH and 30 μg hCRH associated with a CORT ceiling effect respectively approximating and comparable to the CORT ceiling effect with 100 μg hCRH (chapter 3) | Induces co-activation by itself and in the presence of enhanced corticotrophinegic activation with 5-HTP/carbidopa/granisetron (chapter 6) |
| Plausibility | Abnormalities in the CRH system have been associated with MDD | Abnormalities in the 5-HT system are implicated according to the monoamine hypothesis of depression | Abnormalities in the AVP system have been associated with MDD | Abnormalities in both the CRH and AVP systems have been associated with MDD | Unclear |
| Practical | Easy to administer (i.v.) | Administration is complicated by carbidopa and granisetron pretreatment (p.o) | Easy to administer (i.v.) | Easy to administer (i.v.) (chapter 3) | Easy to administer (i.v.); does not need to be combined with low doses hCRH (chapter 6) |
| Ethical | Yes | Yes | Yes | Yes | Yes |

Samenvatting / Summary in Dutch

De hypothalamus-hypofyse-bijnier-as (ook hypothalamus-pituitary-adrenal axis of HPA-as) bestaat uit verschillende endocriene klieren, die elkaar via hormonen stimuleren en ook weer remmen via feedbackmechanismen. Op die manier blijft de activiteit van dit belangrijke stress-systeem binnen de fysiologische perken. De HPA-as is bij een aantal aandoeningen verstoord, onder andere bij bepaalde (ernstige) vormen van depressieve stoornissen en angststoornissen. Om meer inzicht te krijgen in de afwijkingen van het stress-systeem en in de invloed van bestaande en nieuwe medicamenten daarop, zijn goed gevalideerde functietesten nodig, die gedetailleerde informatie geven over elk aspect van de HPA-as. Tot nu toe waren er alleen testen die inzicht gaven in de HPA-as als geheel of in onderdelen daarvan, meestal zonder veel aandacht voor de dynamiek van de verschillende hormonen en hun onderlinge feedforward- en feedbackmechanismen. Dit proefschrift heeft als doel om een aantal farmacologische functietesten van de HPA-as verder te verfijnen. Bij gezonde vrijwilligers werden diverse functietesten met verschillende farmacologische eigenschappen toegepast, die op verschillende manieren de HPA-as beïnvloeden. In elk experiment werd een aantal basisprincipes in acht genomen waarmee de betrouwbaarheid van functietesten kan worden geoptimaliseerd. In hoofdstuk 2 tot 6 werden 5-HTP (dat wordt omgezet in de neurotransmitter serotonine; 5-HT) en hCRH toegepast voor HPA-as activatie via de corticotrofinerge route. dDAVP (een analogon van de neuropeptide vasopressine; AVP) en metoclopramide (dat endogene AVP afgifte zou bevorderen) werden gebruikt voor vasopressinerge co-activatie. We verwachtten dat het toepassen van dit model meer inzicht zou geven in de rol van zowel het corticotrofinerge- als vasopressinerge systeem bij HPA-as activatie. Tenslotte werd een beeldvormende techniek, magnetic resonance spectroscopy (MRS), in hoofdstuk 7 toegepast om veranderingen in hypothalamische metaboliëten gedurende farmacologische stimulatie te meten.

HOOFDSTUK 1: INTRODUCTION

De HPA-as is een functioneel-anatomisch geheel dat bestaat uit verschillende centrale neurotransmitter- en neuropeptide circuits

in de mediaal-prefrontale cortex (MPFC), het limbische systeem en de hypothalamus; en de perifeer geproduceerde stresshormonen afkomstig uit de hypofyse en bijnieren. HPA-as activatie kan worden beschouwd als het neuro-endocriene eindresultaat van de affectregulatie. De hypothalamische productie van corticotrophin releasing hormoon (CRH) leidt tot de afgifte van adrenocorticotrofisch hormoon (ACTH) uit de adenohypofyse en vervolgens tot cortisol (CORT) uit de bijnierschors. De afgifte van ACTH kan via zowel corticotrofinerge activatie als vasopressinerge co-activatie van de HPA-as verlopen. CORT zet een divers spectrum aan gedragsmatige-, cognitieve-, immunologische- en metabole veranderingen in gang en remt tegelijkertijd de neuro-endocriene respons via een complex feedbackmechanisme. In hoofdstuk 1 wordt geconcludeerd dat een overexpressie van de V_3 receptor, verhoogde centrale AVP productie, of een combinatie van deze factoren leidt tot een voortdurend overmatige vasopressinerge co-activatie. Dit mechanisme speelt mogelijk een rol bij een overactieve HPA-as bij verschillende vormen van stress-gerelateerde psychopathologie, waaronder sommige (ernstige) depressieve stoornissen. Hoe verstoorde vasopressinerge co-activatie in relatie staat tot corticotrofinerge activatie of tot het negatieve feedbackmechanisme bij HPA-as pathologie is echter onduidelijk. Het toepassen van betrouwbare klinische instrumenten om de corticotrofinerge- en vasopressinerge aspecten van HPA-as activatie en/of feedback te ontrafelen, zou kunnen bijdragen aan verdere opheldering van deze complexe kluwen. Hiervoor kunnen farmacologische functietesten worden toegepast. Dergelijke functietesten worden vaker gebruikt om inzicht te krijgen in systemen die zich moeilijk lenen voor onderzoek in rust, zoals bijvoorbeeld de orale glucose-tolerantie test en de intracutane allergietesten. Verschillende functietesten die specifiek toegepast worden bij HPA-as onderzoek, worden overzichtelijk besproken. Dit overzicht omvat testen voor HPA-as feedback (de orale DEX test en de DEX/hCRH test) en HPA-as activatie via zowel de corticotrofinerge route (hCRH en 5-HTP) als de vasopressinerge route (dDAVP en metoclopramide). De tekortkomingen van deze testen worden besproken en worden er aan de hand van een aantal basisprincipes voorstellen gedaan om de betrouwbaar-

heid van de betrokken functietesten te vergroten. De verwachting hierbij is enerzijds dat de (eenmaal geoptimaliseerde) functietesten in de toekomst gebruikt kunnen worden om afwijkende HPA-as functie bij verschillende patiëntengroepen te onderzoeken, en dat zij anderzijds toegepast kunnen worden bij het ontwikkelen van nieuwe geneesmiddelen die ingrijpen op de HPA-as.

HOOFDSTUK 2: “DESMOPRESSIN AS A PHARMACOLOGICAL TOOL IN VASOPRESSINERGIC HYPOTHALAMUS–PITUITARY–ADRENAL AXIS MODULATION: NEUROENDOCRINE, CARDIOVASCULAR AND COAGULATORY EFFECTS”

De vasopressinerge HPA-as activatieroute kan worden gestimuleerd door het i.v. geven van het synthetische analogon van AVP, desmopressine (dDAVP). dDAVP is een partiële specifieke AVP receptoragonist, die farmacologisch actief is op zowel de vasopressine 2 – (V_2) als de vasopressine 3 receptor (V_3 of V_{1B}). Stimulatie van V_3 in de adenohipofyse leidt tot activatie van de HPA-as door verhoogde ACTH-synthese. Hierbij induceerden 5µg en 10µg dDAVP i.v. in eerder onderzoek een dosisgerelateerd ACTH- en CORT respons bij gezonde vrijwilligers maar 15µg niet. In dit hoofdstuk worden de effecten van vasopressinerge activatie van de HPA-as met dDAVP onderzocht en gerelateerd aan de potentieel verstoringe autonome en systemische effecten van dDAVP. De neuroendocrine effecten van een 10µg dDAVP zijn vergelijkbaar met die van 30µg, ondanks hogere dDAVP plasmaspiegels bij de hogere dosering. Daarentegen zijn de effecten van dDAVP op het cardiovasculaire systeem en op de stolling dosisafhankelijk. Dit verschil in effect is mogelijk te verklaren vanuit lage endogene CRH spiegels onder basale condities en/of maximale V_3 receptor bezetting bij doseringen hoger dan 10 µg. Deze bevindingen geven aan dat het toedienen van 10µg dDAVP veilig is en geen verstoringe autonome effecten veroorzaakt. De neuroendocrine effecten van 10µg dDAVP zijn echter zo klein en variabel dat de toepasbaarheid daarvan bij AVP-gemedieerd HPA-as onderzoek beperkt zal zijn. Dit probleem kan echter niet worden verholpen door het geven van een hogere dosering, omdat de neuroendocrine effecten van dDAVP boven de 10 µg maar weinig toenemen.

HOOFDSTUK 3: “A PHARMACOLOGICAL TOOL TO ASSESS VASOPRESSINERGIC CO-ACTIVATION OF THE HYPOTHALAMUS–PITUITARY–ADRENAL AXIS MORE INTEGRALLY IN HEALTHY VOLUNTEERS”

CRH en AVP zijn fysiologisch aan elkaar verbonden doordat AVP fungeert als co-activator van de HPA-as in de aanwezigheid van CRH. In hoofdstuk 3 wordt onderzocht of het tegelijk toedienen van dDAVP en lage doseringen synthetisch CRH - corticoreline oftewel hCRH – de HPA-as bij gezonde proefpersonen sterker stimuleert dan dDAVP op zichzelf. Hierbij is de verwachting dat een dergelijke combinatie meer inzicht zou geven in de omvang van vasopressinerge co-activatie (met alleen dDAVP) vergeleken met corticotrofinerge activatie (met alleen hCRH). Het toedienen van 100µg hCRH veroorzaakt robuuste HPA-as activatie, terwijl het combineren van 10µg dDAVP met 10µg of 30µg hCRH tot dosisafhankelijke vasopressinerge co-activatie leidt. De neuro-endocriene respons van beide combinaties is dan ook groter dan met 10µg dDAVP alleen en ongeveer gelijk aan de neuro-endocriene effecten van alleen 100µg hCRH. Tenslotte wordt gekeken of het mogelijk is om corticotrofinerge activatie en vasopressinerge co-activatie onafhankelijk van elkaar te onderzoeken tijdens een kort bezoek aan de kliniek door dDAVP en hCRH achter elkaar toe te dienen. Het blijkt dat dit mogelijk is, omdat er geen sprake is van een interactie-effect op corticotrofinerge activatie met 100µg hCRH wanneer 10µg dDAVP twee uur eerder wordt gegeven.

HOOFDSTUK 4: “PHARMACOLOGY OF RISING ORAL DOSES OF 5-HYDROXYTRYPTOPHAN WITH CARBIDOPA”

Een potentieel bezwaar bij zowel dDAVP als hCRH is dat zij de HPA-as alleen op het niveau van de hypofyse stimuleren en daardoor de rol van de MPFC en het limbische systeem nauwelijks in acht nemen. Het zou daarom wenselijk zijn om de HPA-as met een “proximalere” of “centralere” farmacologische functietest te stimuleren. Er bestaat inmiddels consensus dat de neurotransmitter serotonine (5-HT) een belangrijke betekenis heeft in de affectregulerende circuits, tenminste in het kader van de monoaminerge hypothese van depressie. 5-HT wordt geproduceerd door serotonerge neuronen in de (dorsale) raphé nuclei van de hersenstam en wordt daarvandaan afgegeven in diverse (neurotransmitter)circuits in de hersenen, waaronder het limbische

systeem en hypothalamus. Een functietest die serotonine (5-HT) acuut verhoogt stimuleert daarmee de centrale serotonerge circuits en indirect ook de HPA-as. Orale toediening van 5-hydroxytryptofaan (5-HTP, een stof die direct in serotonine wordt omgezet) in combinatie met carbidopa activeert de HPA-as met krachtige afgifte van ACTH- en CORT tot gevolg. Ofschoon dit model aantrekkelijk is, brengt deze serotonerge test verschillende problemen met zich mee, zoals gebrek aan standaardisering, een variabele kinetiek en een nauw venster tussen neuro-endocriene effecten en bijwerkingen. In hoofdstuk 4 wordt de relatie tussen de dosering/plasmaspiegel en de neuroendocriene effecten bij oraal toegediende 5-HTP gecombineerd met carbidopa onderzocht. Deze gecombineerde 5-HTP test is een effectieve corticotrofinerge functietest via de serotonerge route en laat dosis-afhankelijke plasmaspiegels en neuro-endocriene effecten zien. Het vaak voorkomen van misselijkheid en braken bij hogere doses 5-HTP is echter beperkend in het toepassen van deze test in klinisch HPA-as onderzoek.

HOOFDSTUK 5: “ENHANCED TOLERABILITY OF THE 5-HYDROXYTRYPTOPHANE CHALLENGE TEST COMBINED WITH GRANISETRON”

In hoofdstuk 5 wordt een anti-emeticum toegevoegd aan de gecombineerde 5-HTP/carbidopa functietest. Hiermee wordt gepoogd het venster tussen de gewenste neuro-endocriene effecten en de bijwerkingen te vergroten door het tegengaan van misselijkheid en braken. Het toevoegen van de subtype-specifieke 5-HT₃ receptor antagonist granisetron aan de gecombineerde 5-HTP/carbidopa functietest onderdukt deze hinderlijke bijwerkingen zonder beïnvloeding van de neuro-endocriene respons of de farmacokinetiek (PK) van 5-HTP. Dit verbetert de bruikbaarheid van 5-HTP bij het onderzoeken van centraal-gemedieerde, corticotrofinerge HPA-as activatie bij zowel gezonde vrijwilligers als patiënten; tenminste in combinatie met carbidopa en granisetron.

HOOFDSTUK 6: “METOCLOPRAMIDE AS PHARMACOLOGICAL TOOL TO ASSESS VASOPRESSINERGIC ACTIVATION OF THE HYPOTHALAMUS-PITUITARY-ADRENAL (HPA) AXIS: A STUDY IN HEALTHY VOLUNTEERS”

Eerder onderzoek rapporteerde dat de D₂ receptor antagonist metoclopramide vasopressinerge co-activatie van de HPA-as induceert door de afgifte van endogene AVP te stimuleren. De studies waarin

dit werd onderzocht kennen echter belangrijke (methodologische) bezwaren. Bovendien is het farmacologische mechanisme dat zorgt voor afgifte van AVP speculatief en is de relatie tussen de neuroendocriene effecten en de PK van metoclopramide nooit eerder onderzocht. In hoofdstuk 6 wordt i.v. toegediende metoclopramide onderzocht als een functietest voor centraal- gemedieerde vasopressinerge co-activatie. Metoclopramide (op zichzelf) leidt tot de afgifte van ACTH en CORT en induceert co-activatie tijdens corticotrofinerge activatie door de gecombineerde 5-HTP/carbidopa/granisetron functietest. Deze neuro-endocriene effecten tijdens corticotrofinerge stimulatie suggereren dat metoclopramide de afgifte van endogene AVP versterkt, met vasopressinerge co-activatie van de HPA-as tot gevolg. De afgifte van AVP zelf kon echter niet direct gemeten worden.

HOOFDSTUK 7: “HYPOTHALAMIC GLUTAMATE LEVELS FOLLOWING SEROTONERGIC STIMULATION: A PILOT STUDY USING 7-TESLA MAGNETIC RESONANCE SPECTROSCOPY IN HEALTHY VOLUNTEERS”

De (perifere) neuro-endocriene effecten van de beschreven HPA-as functietesten weerspiegelen veranderingen in centrale neurotransmittercircuits en de hypofyse na farmacologische stimulatie. Het toepassen van beeldvormende technieken om veranderingen op neuronaal niveau te meten, kan daarom inzicht geven in de centrale farmacologische effecten van functietesten. Bovendien kan een dergelijk inzicht additionele informatie over de complexe regelmechanismen van de HPA-as verschaffen. In hoofdstuk 7 worden de effecten van de gecombineerde 5-HTP/carbidopa/granisetron functietest op hypothalamische glutamaat/glutamine (Glx)-, choline-, N-acetyl-aspartaat (NAA)- en creatine (Creat) concentraties bij gezonde vrijwilligers met behulp van 7-Tesla (7T) magnetic resonance spectroscopy (MRS) onderzocht. Vervolgens worden de hypothalamische veranderingen gerelateerd aan de perifere neuro-endocriene effecten (ACTH- en CORT afgifte) van de gecombineerde functietest. Corticotrofinerge activatie van de HPA-as via de serotonerge route (met de gecombineerde 5-HTP functietest) leidt tot een verlaging van de hypothalamische Glx, en wordt gevolgd door afgifte van ACTH uit de hypofyse en CORT uit de bijnieren. Samenvattend ondersteunt deze pilotstudie hypothalamische Glx-depletie bij (serotonerge) HPA-as activatie en toont verder aan dat een beeldvormend

techniek in staat is om 5-HT gemedieerde veranderingen in het hypothalamische metabolisme te meten.

Samenvattend toont dit proefschrift aan dat de corticotrofinerge- en de vasopressinerge activatieroutes van de HPA-as zowel direct (met de perifeer werkende hCRH and dDAVP) als indirect (met de centraal-actieve 5-HTP en metoclopramide) door gerichte farmacologische stimulatie gekwantificeerd kunnen worden. Hierbij kennen 5-HTP, hCRH en dDAVP gecombineerd met een lage dosering hCRH een goede farmacologische karakterisering en een goede verdraagbaarheid. Zij zijn daarom geschikt voor klinisch HPA-as onderzoek bij zowel gezonde vrijwilligers als patiënten. Daarentegen is de rol van metoclopramide als vasopressinerge functietest (vooralsnog) twijfelachtig. Belangrijk is dat 10 µg dDAVP gevolgd door 100 µg hCRH 2 uur later, althans bij gezonde mensen, geen interactie-effect laat zien, wat deze test geschikt maakt voor het onderzoeken van de beide HPA-as activatieroutes tijdens eenzelfde (poli)klinisch bezoek.

Concluderend wordt gesteld dat een systematisch gebruik van goed gevalideerde farmacologische functietesten belangrijke informatie kan verschaffen over de (patho)fysiologie van de HPA-as. Hierbij is er een rol voor dergelijke testen weggelegd als pathofysiologische onderzoeksinstrumenten bij (stress-gerelateerde) psychiatrische aandoeningen, als translationele instrumenten tussen proefdieren en mensen en bij “proof-of-pharmacological principle” experimenten met nieuwe geneesmiddelen die op verschillende plaatsen in de HPA-as ingrijpen.

REFERENCE LIST

- Agid Y, Buzsaki G, Diamond DM, Frackowiak R, Giedd J, Girault JA, Grace A, Lambert JJ, Manji H, Mayberg H, Popoli M, Prochiantz A, Richter-Levin G, Somogyi P, Spedding M, Svenningsson P, Weinberger D (2007) How can drug discovery for psychiatric disorders be improved? *Nat Rev Drug Discov* 6: 189-201.
- Chiodera P, Volpi R, Delsignore R, Marchesi C, Salati G, Camellini L, Rossi G, Coiro V (1986) Different effects of metoclopramide and domperidone on arginine-vasopressin secretion in man. *Br J Clin Pharmacol* 22: 479-482.
- Conn PJ, Roth BL (2008) Opportunities and challenges of psychiatric drug discovery: roles for scientists in academic, industry, and government settings. *Neuropsychopharmacology* 33: 2048-2060.
- Cryan JF, Slattery DA (2007) Animal models of mood disorders: Recent developments. *Curr Opin Psychiatry* 20: 1-7.
- DeBOLD CR, DeCherney GS, Jackson RV, Sheldon WR, Alexander AN, Island DP, Rivier J, Vale W, Orth DN (1983) Effect of synthetic ovine corticotropin-releasing factor: prolonged duration of action and biphasic response of plasma adrenocorticotropin and cortisol. *J Clin Endocrinol Metab* 57: 294-298.
- Dinan TG, Lavelle E, Scott LV, Newell-Price J, Medbak S, Grossman AB (1999) Desmopressin normalizes the blunted adrenocorticotropin response to corticotropin-releasing hormone in melancholic depression: evidence of enhanced vasopressinergic responsivity. *J Clin Endocrinol Metab* 84: 2238-2240.
- Dinan TG, O'Brien S, Lavelle E, Scott LV (2004) Further neuroendocrine evidence of enhanced vasopressin V_3 receptor responses in melancholic depression. *Psychol Med* 34: 169-172.
- Favrod-Coune C, Raux-Demay MC, Proeschel MF, Bertagna X, Girard F, Luton JP (1993) Potentiation of the classic ovine corticotrophin releasing hormone stimulation test by the combined administration of small doses of lysine vasopressin. *Clin Endocrinol (Oxf)* 38: 405-410.
- Gartside SE, Cowen PJ (1990) Mediation of ACTH and prolactin responses to 5-HTP by 5-HT₂ receptors. *Eur J Pharmacol* 179: 103-109.
- Gijsman HJ, van Gerven JM, de Kam ML, Schoemaker RC, Pieters MS, Weemans M, de Rijk R, van der PJ, Cohen AF (2002) Placebo-controlled comparison of three dose-regimens of 5-hydroxytryptophan challenge test in healthy volunteers. *J Clin Psychopharmacol* 22: 183-189.
- Gijsman HJ, van Gerven JM, Tieleman MC, Schoemaker RC, Pieters MS, Ferrari MD, Cohen AF, van Kempen GM (1998) Pharmacokinetic and pharmacodynamic profile of oral and intravenous meta-chlorophenylpiperazine in healthy volunteers. *J Clin Psychopharmacol* 18: 289-295.
- Lamberts SW, Verleun T, Oosterom R, de JF, Hackeng WH (1984) Corticotropin-releasing factor (ovine) and vasopressin exert a synergistic effect on adrenocorticotropin release in man. *J Clin Endocrinol Metab* 58: 298-303.
- Lightman SL, Wiles CC, Atkinson HC, Henley DE, Russell GM, Leendertz JA, McKenna MA, Spiga F, Wood SA, Conway-Campbell BL (2008) The significance of glucocorticoid pulsatility. *Eur J Pharmacol* 583: 255-262.
- Nomura K, Kurimoto F, Demura H, Sakurai H, Nomura T, Zibiki K, Naruse M, Kanai N, Shizume K (1984) Effect of metoclopramide in plasma vasopressin in man. *Clin Endocrinol (Oxf)* 21: 117-121.
- Pangalos MN, Schechter LE, Hurko O (2007) Drug development for CNS disorders: strategies for balancing risk and reducing attrition. *Nat Rev Drug Discov* 6: 521-532.
- Pariente CM, Lightman SL (2008) The HPA axis in major depression: classical theories and new developments. *Trends Neurosci* 31: 464-468.
- Pariente CM, Miller AH (2001) Glucocorticoid receptors in major depression: relevance to pathophysiology and treatment. *Biol Psychiatry* 49: 391-404.
- Schule C (2007) Neuroendocrinological mechanisms of actions of antidepressant drugs. *J Neuroendocrinol* 19: 213-226.
- Schule C, Baghai TC, Eser D, Rupprecht R (2009) Hypothalamic-pituitary-adrenocortical system dysregulation and new treatment strategies in depression. *Expert Rev Neurother* 9: 1005-1019.
- Scott LV, Medbak S, Dinan TG (1999) Desmopressin augments pituitary-adrenal responsivity to corticotropin-releasing hormone in subjects with chronic fatigue syndrome and in healthy volunteers. *Biol Psychiatry* 45: 1447-1454.
- Seki K, Kato T, Sekiya S (1997) Corticotrophin and vasopressin responses to metoclopramide in patients with hypothalamic amenorrhoea. *Clin Endocrinol (Oxf)* 46: 203-207.
- Walsh P, Spelman L, Sharifi N, Thakore JH (2005) Male patients with paranoid schizophrenia have greater ACTH and cortisol secretion in response to metoclopramide-induced AVP release. *Psychoneuroendocrinology* 30: 431-437.

CURRICULUM VITAE

Gabriël Etienne Jacobs was born on 22-05-1977 in Vereeniging, South Africa (SA). He graduated from the Hoërskool Drie Riviere (Vereeniging, SA) in 1995 and went on to study medicine at the University of Pretoria in 1996. After completing his clinical rotations in 2001, he obtained a bachelor degree in medicine and surgery (MBChB). Subsequently, he spent two years working in different medical specialties at the Chris Hani-Baragwanath Hospital (Soweto, Johannesburg, SA) and in community health care at the Ermelo Provincial Hospital (Ermelo, SA). In 2004, he moved to the Netherlands (NL) and commenced his career in psychiatry as resident on the closed unit of the GGZ Drenthe in Emmen (NL). He started his clinical residency in psychiatry at the Leiden University Medical Centre (LUMC)/GGZ Rivierduinen (prof. dr. F.G. Zitman) in 2005. From 2006 to 2009, he interrupted his clinical training to perform the work presented in this thesis at the Centre for Human Drug Research (CHDR) (prof. dr. J.M.A. van Gerven, prof. dr. A.F. Cohen). During the same period he was trained as clinical pharmacologist and received his registration from the Dutch Society for Clinical Pharmacology and Biopharmacy (NVKFB) in 2009. He recommenced his clinical training in 2009 on the closed unit of the GGZ Leiden in Oegstgeest and is currently working on the outpatients department for mood, anxiety and somatoform disorders (SAs) of the GGZ Rivierduinen in Leiden.

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LIST OF PUBLICATIONS

- DESMOPRESSIN AS A PHARMACOLOGICAL TOOL IN VASOPRESSINERGIC HYPOTHALAMUS-PITUITARY-ADRENAL AXIS MODULATION: NEUROENDOCRINE, CARDIOVASCULAR AND COAGULATORY EFFECTS. Jacobs GE, Hulskotte EG, van Gerven JM, Zuurman L, de Kam ML, Elassaiss-Schaap J, Ruigt G, van Pelt J, Peeters BW, Peeters PA, Burggraaf K. *J Psychopharmacol.* 2010 Mar 23. [Epub ahead of print]
- A PHARMACOLOGICAL TOOL TO ASSESS VASOPRESSINERGIC CO-ACTIVATION OF THE HYPOTHALAMUS-PITUITARY-ADRENAL AXIS MORE INTEGRALLY IN HEALTHY VOLUNTEERS. Jacobs GE, van Gerven JM, de Kam ML, Schaap J, Ruigt G, van Pelt J, Peeters BW, Peeters PA, Hulskotte EG. *J Psychopharmacol.* 2010 Apr 1. [Epub ahead of print].
- PHARMACOLOGY OF RISING ORAL DOSES OF 5-HYDROXYTRYPTOPHAN WITH CARBIDOPA. Smarius LJ, Jacobs GE, Hoeberechts-Lefrandt DH, de Kam ML, van der Post JP, de Rijk R, van Pelt J, Schoemaker RC, Zitman FG, van Gerven JM, Gijsman HJ. *J Psychopharmacol.* 2008 Jun;22(4):426-33. Epub 2008 Feb 28.
- ENHANCED TOLERABILITY OF THE 5-HYDROXYTRYPTOPHAN CHALLENGE TEST COMBINED WITH GRANISETRON. Jacobs GE, Kamerling IM, de Kam ML, Derijk RH, van Pelt J, Zitman FG, van Gerven JM. *J Psychopharmacol.* 2010 Jan;24(1):65-72. Epub 2008 Aug 21.
- METOCLOPRAMIDE AS PHARMACOLOGICAL TOOL TO ASSESS VASOPRESSINERGIC CO-ACTIVATION OF THE HYPOTHALAMUS-PITUITARY-ADRENAL (HPA) AXIS: A STUDY IN HEALTHY VOLUNTEERS. Jacobs GE, Hulskotte EG, de Kam ML, Zha G, Jiang J, Hu P, Zhao Q, van Pelt J, Goekoop JG, Zitman FG, van Gerven JM. *Eur Neuropsychopharmacol.* 2010 Jul 22. [Epub ahead of print].
- HYPOTHALAMIC GLUTAMATE LEVELS FOLLOWING SEROTONERGIC STIMULATION: A PILOT STUDY USING 7-TESLA MAGNETIC RESONANCE SPECTROSCOPY IN HEALTHY VOLUNTEERS. Jacobs GE, der Grond J, Teeuwisse WM, Langeveld TJ, van Pelt J, Verhagen JC, de Kam ML, Cohen AF, Zitman FG, van Gerven JM. *Prog Neuropsychopharmacol Biol Psychiatry.* 2010 Apr 16;34(3):486-91. Epub 2010 Feb 4.