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Pharmacological aspects of corticotrophinergic and vasopressinergic function tests for HPA axis activation

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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; Pariante 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; Pariante and Lightman, 2008; Keck, 2006). CRH is primarily produced by the parvocellular neurons of the hypothalamic PVN and acts specifically at the CRH_1 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; Pariante 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 (V_3 or V_{1B}) on the anterior pituitary (Ring, 2005; Ryckmans, 2010). There,

AVP transiently mobilizes calcium into the cytoplasm and subsequently potentiates CRH_1-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 CRH_1 receptors (Nemeroff et al., 1984; Nemeroff et al., 1988). However, recent observations have also implicated AVP in MDD-associated HPA hyperactivity. In MDD, CRH_1 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 V_3 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 (corticorelin - hCRH) alone induces corticotrophinergic activation by directly stimulating the peripheral pituitary CRH_1 receptors. Similarly, direct stimulation of the pituitary V_3 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 vasopressinergic 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 CRH_1 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-suppresion 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 CRH_1 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 CRH_1 receptors with hCRH and V_3 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 V_3 receptors. In this context, the antiemetic D_2 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 raphé 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 D_2- and 5-HT_3 receptor antagonist on the one hand and 5-HT_4 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; Gijsman, 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 (Gijsman

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 shortcoming 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 interinidivudal 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 metclopramide) function tests to assess HPA axis activation and their correspondence to the proposed principles of reliable pharmacological function tests**

	Corticotrophinergic activation		Vasopressinergic co-activation	
Characteristic	**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 $5\text{-}HT_{2A}$ or $5\text{-}HT_{2C}$ 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).

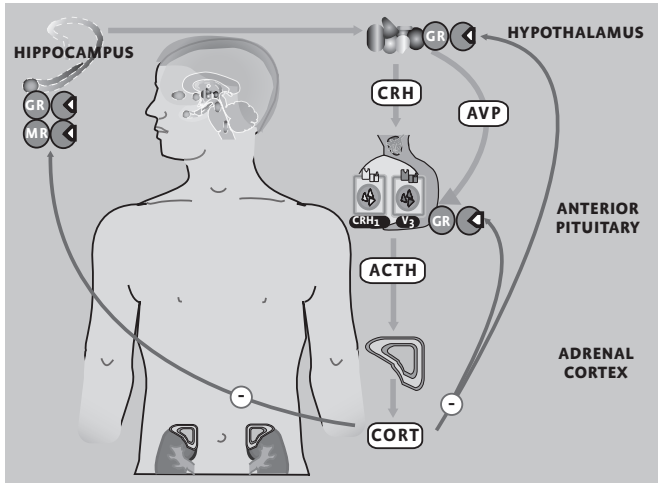


Figure 2 Graphic representation of HPA axis function in severe major depressive disorder (MDD): thick arrows signify hyperactivation and dotted arrows signify decreased inhibition via negative feedback (CRH: corticotrophin-releasing hormone; AVP: vasopressin; ACTH: adrenocorticotrophic hormone; GR: glucocorticoid receptor; MR: mineralocorticoid receptor).

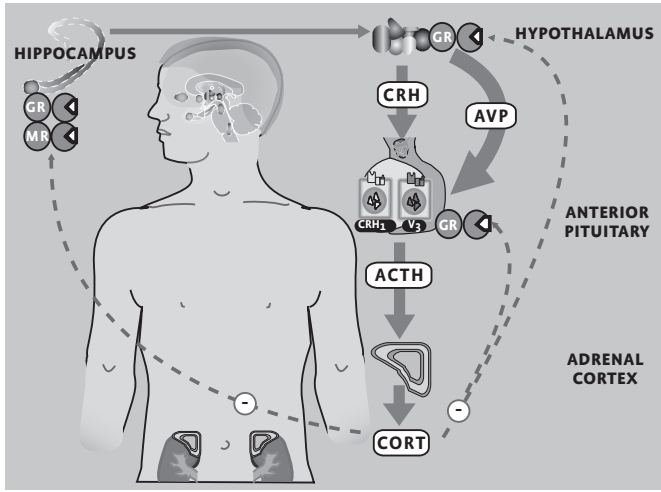
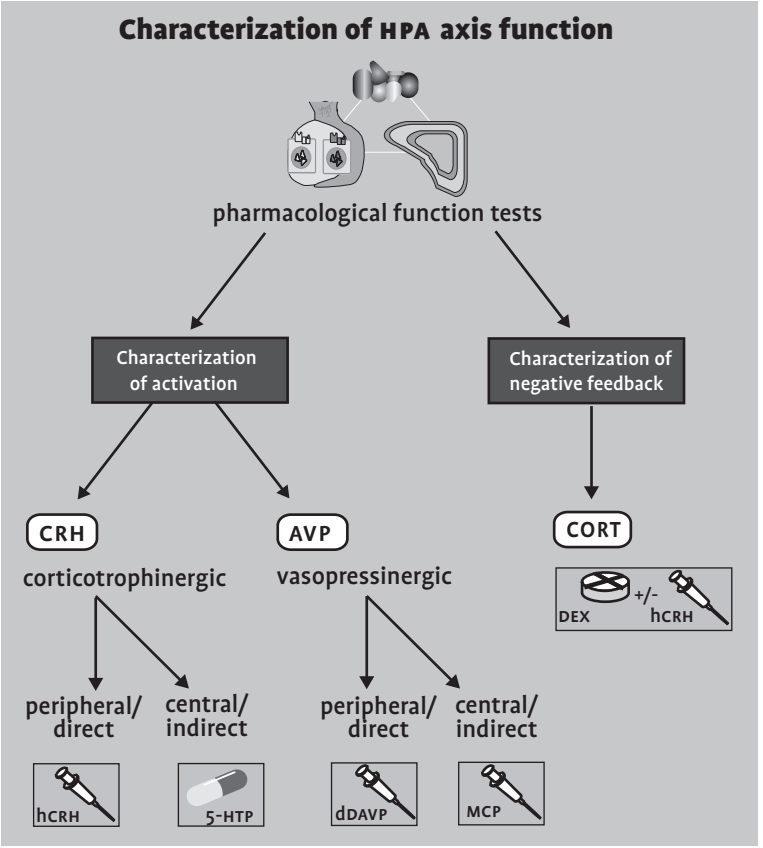


Figure 3

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).



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