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

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CHAPTER 8

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 (Gijsman 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-HT_3 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 D_2 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 CRH_1 receptor system. 200mg 5-HTP, combined with carbidopa and granisetron to stabilize the PK and to reduce systemic side-effects of 5-HTP, potently 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 $5\text{-}HT_{2A}$ or $5\text{-}HT_{2C}$ 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) (Gijsman 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 CRH_1-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 D_2 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 CRH_1 and/or V_3 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 vasopressinergic 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: corticorelin; dDAVP: desmopressin; MCP: metoclopramide; GR: glucocorticoid receptor; ACTH: adrenocorticotrophic hormone).

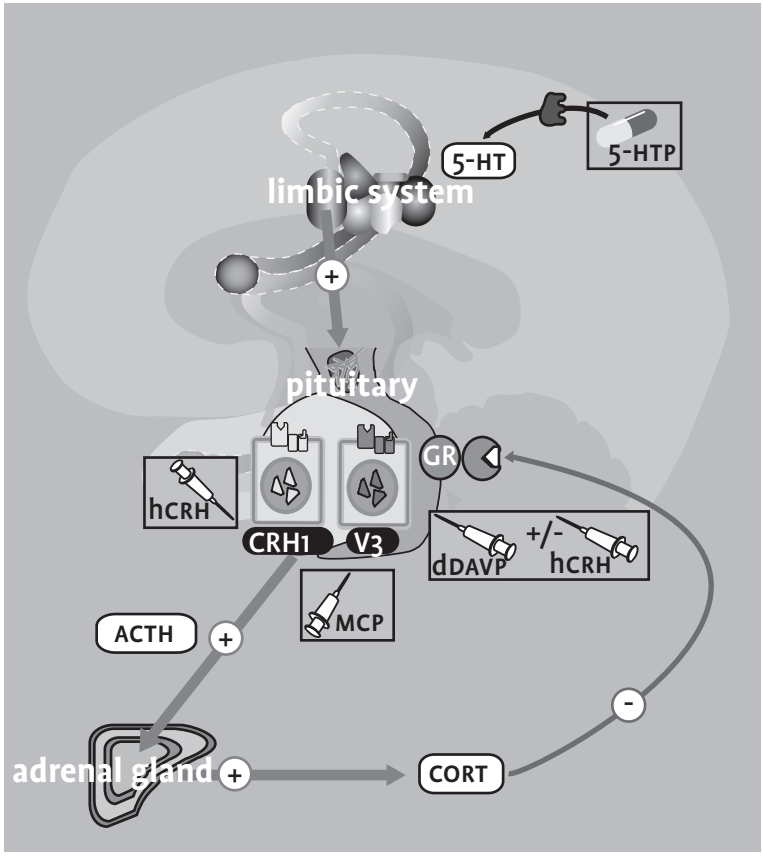

Table 3 Summary of the information provided by this thesis *(in italics)* to the existing corticotrophinergic (hCRH and 5-HTP) and vasopressinergic (dDAVP and metoclopramide) function tests to assess HPA axis.

Characteristic	Corticotrophinergic activation		Vasopressinergic co-activation		
	hCRH	5-HTP/carbidopa	dDAVP	dDAVP 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 raphé nuclei followed by agonism at $5\text{-}HT_{2A}$ or $5\text{-}HT_{2C}$ 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 dDAVP	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 ≤ 10 µg (chapter 2)*	*Similar to those with dDAVP 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 ≥ 10 µg (chapter 2)*	*Dose-dependent vasopressinergic co-activation when combining 10 µg dDAVP 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 suppresses by the addition of granisetron without influencing the neuroendocrine response or 5-HTP PK (chapter 5)*	*Dose-related cardiovascular, coagulatory effects, minimal effects at ≤ 10 µg (chapter 2)*	*Similar to those with dDAVP 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 ≥ 10 µg (chapter 2)*	*10 µg dDAVP 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 corticotrophinergic 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