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Jacobs, G.E.

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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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G.E. Jacobs MD^{1,3}, E.G.J. Hulskotte PhD², M.L. de Kam MSc¹,
G. Zha MSc⁴, J. Jiang MD⁴, P. Hu MD⁴, Q. Zhao PhD⁴, J. van Pelt PhD⁶,
J.G. Goekoop MD PhD⁵, F.G. Zitman MD PhD³, J.M.A. van Gerven MD PhD¹

¹ Centre for Human Drug Research, Leiden, The Netherlands

² NV Organon, part of Schering-Plough Corporation, Department of Clinical Pharmacology and Kinetics, Oss, the Netherlands

³ Department of Psychiatry, Leiden University Medical Centre, Leiden, The Netherlands

⁴ Clinical Research Center Laboratory, Peking Union Medical College Hospital, Beijing, China

⁵ Rivierduinen Mental Health Care Organisation, Oegstgeest, The Netherlands

⁶ Department of Clinical Chemistry, Leiden University Medical Centre, Leiden, The Netherlands

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 meth-amphetamines, 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 fluoroimmuno-metric 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.

Table 2

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 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	0-130min	60-130min	60-130min
Plasma ACTH (ng/l)	14.0	13.5	19.5	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	52.6 (27.1, 83.2) p<0.0001
Serum cortisol ($\mu\text{g/l}$)	85.3	76.4	125.2	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 ($\mu\text{g/l}$)	4.3	4.2	45.5	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

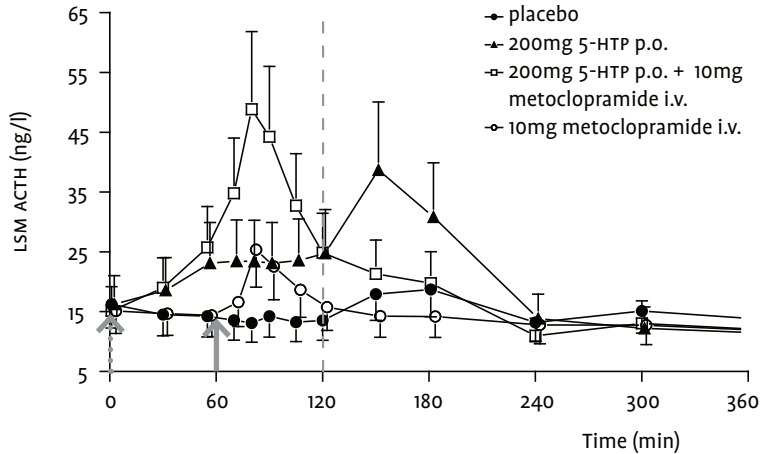
Table 3

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 $AUC_{0-\infty}$ ($\text{min}\cdot\mu\text{g/L}$).

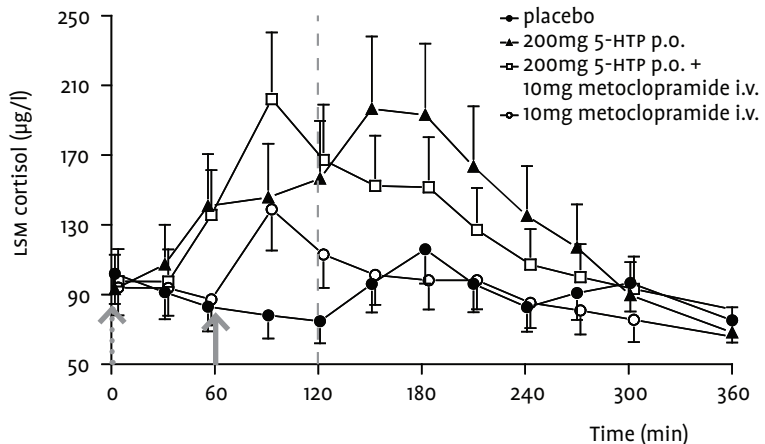
PK parameter; Mean (SD)	Metoclopramide		5-HTP	
	administered i.v.	administered i.v. 60min following 200mg 5-HTP p.o.	administered p.o.	administered p.o. 60min preceding 10mg MCP i.v.
Terminal half life ($t_{1/2}$)	270.0 (55.1)	256.7 (81.6)	205.6 (95.3)	212.5 (141.3)
T_{MAX} (min)	46.2 (64.4)	63.7 (105.2)	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).



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