Pharmacological aspects of corticotrophinergic and vasopressinergic function tests for HPA axis activation
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Desmopressin as a pharmacological tool in vasopressinergic hypothalamus–pituitary–adrenal (HPA) axis modulation: neuroendocrine, cardiovascular and coagulatory effects

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

Abstract

BACKGROUND   Arginine-vasopressin(AVP) is a physiological co-activator of the hypothalamus-pituitary-adrenal-axis(HPA), together with cortico-trophin-releasing hormone (CRH). A synthetic analogue of AVP, desmopressin(dDAVP) is often used as pharmacological tool to assess co-activation in health and disease. The relation between dDAVP's neuroendocrine, cardiovascular, pro-coagulatory, anti-diuretic and non-specific stress effects has not been studied.

OBJECTIVES   A randomized, double-blind, placebo-controlled, three-way cross-over study was performed in 12 healthy male and female volun-teers(6: 6). dDAVP was administered intravenously as a 10µg bolus (over one minute) or a 30µg incremental infusion (over 60 min-utes). Neuroendocrine, cardiovascular, pro-coagulatory, anti-diuretic effects and adverse events (AE's) were recorded, and auto-nomic nervous system (ANS) activation evaluated.

RESULTS   The incremental infusion reached 1.8 fold higher dDAVP concentra-tions than the bolus. Neuroendocrine effects were similar for the 10µg dDAVP bolus and the 30µg incremental infusion, while cardio-vascular- and coagulatory effects were greater with the 30µg dose. Osmolality and ANS activity remained uninfluenced. AE's corre-sponded to dDAVP's side-effect profile.

CONCLUSIONS   The neuroendocrine effects of a 10µg dDAVP bolus administered over one minute are similar to those of a 30µg incremental infusion administered over one hour, despite higher dDAVP concentrations after the infusion. Cardiovascular and coagulatory effects showed clear dose-related responses. A 10µg dDAVP bolus is considered a safe vasopressinergic function test at which no confounding effects of systemic or autonomic stress were seen.

Introduction

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

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

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

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

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

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

Methods

Study design

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

Main outcome measures

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

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

Drug administration

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

Volunteers

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

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

dDAVP

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

Study days

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

Biochemical measurements

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

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

Vital signs

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

Data analysis

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

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Results

Subject disposition and demographic data

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

Adverse events

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

Pharmacokinetics

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

Neuroendocrine pharmacodynamic effects

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

Pro-coagulant – and anti-diuretic effects

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

Cardiovascular pharmacodynamic effects

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

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

Measures of dDAVP-induced ANS activation

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

Discussion

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

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

attained previously with serotonergic and corticotrophinergic function tests. Meta-chlorophenylpiperazine (mCPP) 0.5 mg/kg administered i.v. lead to a cortisol c_{MAX} 228.4ng/ml (Gijsman et al., 1998), 5-hydroxytryptophan (5-HTP) 300mg administered orally induced a cortisol c_{MAX} of 218.9ng/ml (Smarius et al., 2008) and 100μg human CRH (hCRH) i.v. produced maximal serum cortisol concentrations of 210ng/ml (Dinan et al., 1999; Scott et al., 1999b). This indicates a higher maximum release of ACTH and cortisol following corticotrophinergic stimulation than after vasopressinergic co-activation. This is also illustrated in trials where dDAVP induced a dose-dependent ACTH release for 5μg and 10μg but not for 15μg dDAVP (Scott et al., 1999a) and CRH augmented the vasopressin-induced v_3 effects when administered concomitantly with dDAVP (Favrod-Coune et al., 1993). Such limited vasopressinergic co-activation may be determined by low levels of endogenous CRH, since this study was performed late in the morning and early afternoon, when circadian activity of the HPA-axis is relatively low.

The HPA-axis can also be activated by homeostatic perturbations, like hypotension causing an indirect stress responses via the sympatho-medullary route. dDAVP-induced blood pressure reduction seems to be dependent on v_2 stimulation, causing prostaglandin release from vascular endothelium (Aldasoro et al., 1997; Medina et al., 1999). A reduction in peripheral resistance can activate sympathetic outflow via fibres descending from the vasoconstrictor regions of the medulla oblongata. Concomitantly, direct stimulation of sympathetic outflow fibres terminating on the adrenal cortex can release adrenaline, which in turn might induce ACTH release via AVP neurons (Aldasoro et al., 1997; Scott and Dinan, 2002). On average, 10μg dDAVP reduced diastolic blood pressure (DBP) by 7.2 mmHg, and the incremental 30μg infusion by 8.5 mmHg in the current study. Heart rate showed average compensatory increases of 6.0 bpm after the bolus and 9.7 bpm with the infusion. It could be argued that even these small cardiovascular changes signify an activation of the autonomous nervous system (ANS), inducing ACTH release via alternative routes. However, indirect stress measures (saliva alpha amylase and serum prolactin) that indicate catecholamine-mediated ANS activity via the sympathoadrenal medullary route (Aldasoro et al., 1997; Rohleder et al., 2004) remained unaffected by either dDAVP administration mode.

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

dDAVP displayed a differential concentration-effect relationship for the neuroendocrine parameters on the one hand and cardiovascular and coagulatory parameters on the other. dDAVP's V_2-mediated effects (HR and VWF) were concentration dependent, which was not the case for its V_3-mediated effects (ACTH and cortisol release). The maximal plasma concentrations (c_{MAX}) attained with 30μg dDAVP were nearly twice (1.8-fold) as large as those reached with 10μg dDAVP. This difference was reflected by the concentration-dependence of the cardiovascular and coagulatory effects, but not of the neuroendocrine responses, which were similar for the two doses.

The results suggest that 10μg dDAVP is a safe pharmacological function test in healthy volunteers and patients, at least if some potential restrictions are considered. Neither 10μg dDAVP nor 30μg dDAVP affected serum osmolality with fluid restriction, but dDAVP can cause a reduced osmolality if liberal water consumption is allowed. dDAVP clearly induced von Willebrand factor (VWF) release, but this is unlikely to produce coagulatory complications. The intravenous administration of dDAVP stimulates the release of VWF and factor VIII from the vascular endothelium via V_2 receptors (Lethagen, 1994). At the same time, dDAVP releases tissue plasminogen activator (tPA), which mitigates the prothrombotic effects of VWF (Burggraaf et al., 1994). VWF itself is degraded by the VWF cleaving protease ADAMTS13. Also, a twice higher dose of dDAVP compared to the present trial (0,3μg/kg vs. 0,13μg/kg) lead to (maximal) VWF release (Lethagen, 1994) without the occurrence of coagulation–related AE's in healthy volunteers. The prothrombotic effects of 10μg dDAVP thus appear to be self-limiting, at least in healthy volunteers. Susceptible patients however may be at an increased risk of thrombo-embolic events following dDAVP-induced VWF-elevations, although this is only suggested by rare case reports of patients who developed an acute myocardial infarction after administration of dDAVP for their hemophilia or Von Willebrand's disease (Follenius et al., 1982). Therefore, it is probably sensible to avoid higher doses of dDAVP, and to exclude patients with multiple cardiovascular risk factors or a (past) history of

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

In conclusion, administering 10μg dDAVP intravenously over one minute is an effective pharmacological tool to induce vasopressinergic HPA axis activation in healthy volunteers. Under low corticotrophinergic conditions, this function test produces a clear but limited co-activation of pituitary ACTH-release via the V_3. A 10μg dDAVP bolus does not cause much systemic effects, and avoids non-specific stress responses. The effects on serum osmolality and coagulation are limited, although the safety in specific risk populations remains to be established. Higher doses of dDAVP are not expected to produce much more informative vasopressinergic co-activation, and could induce adverse and confounding V_2-mediated effects.

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Table 1

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

PD parameter	Back transformed Least Square Means (LSM)				Treatment P-value	Estimated difference with 95% Confidence interval (%)		
	Placebo (n=12)		10µg dDAVP bolus infusion (n=11)	30µg dDAVP incremental infusion (n=12)		Bolus infusion vs placebo	Incremental infusion vs placebo	Bolus vs incremental
	50 – 180 min	0 –180 min	50 – 180 min	0 – 180 min		50 – 180 min	0 – 180 min	
Plasma ACTH (ng/L)	9.62	9.73	12.13	12.73	0.011	+26.1 (5.8, 50.3) p=0.013	+30.9 (11.1, 54.2) p=0.003	+13.0 (-5.0, 34.4) p=0.155
Serum cortisol (ng/mL)	117.91	125.46	140.21	147.62	0.036	+18.9 (4.1, 35.8) p=0.014	+17.7 (3.5, 33.8) p=0.016	+2.9 (-9.9, 17.5) p=0.66
Serum prolactin (ng/mL)	4.33	4.54	4.59	3.85	0.001	+6.1 (-502, 18.8) p=0.281	-15.4 (-23.5, -6.4) p=0.03	-19.0 (-27.5, -9.5) p=0.001
Saliva alpha-amylase (U/mL)	110.71	107.56	125.77	119.81	0.689	+13.6 (-13.9, 50.0) p=0.350	+11.4 (-14.5, 45.2) p=0.404	+7.9 (-18.0, 50.0) p=0.568

Table 2 Cardiovascular, coagulatory and anti-diuretic parameters for the period 50 min to 3 hours for the bolus infusion and over 3 hours for the incremental infusion: Estimated difference (%) with 95% confidence interval from placebo for 10µg dDAVP bolus infusion and 30µg dDAVP incremental infusion and the estimated difference (%) with 95% confidence interval between 10µg dDAVP bolus infusion and 30µg dDAVP incremental infusion for systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), von Willebrand factor (VWF) and serum osmolality (mOsmol/kg)

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

Figure 1   Average concentration time profile of plasma adrenocorticotrophic hormone (ACTH) with SD error bars (closed circle: Placebo; open circle: Incremental infusion; open square: Bolus infusion)
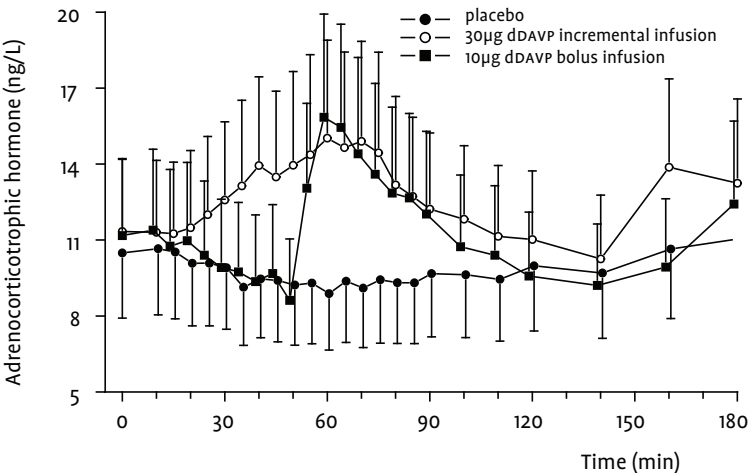

Figure 2   Average concentration time profile of serum cortisol with SD error bars (closed circle: Placebo; open circle: Incremental infusion; open square: Bolus infusion)
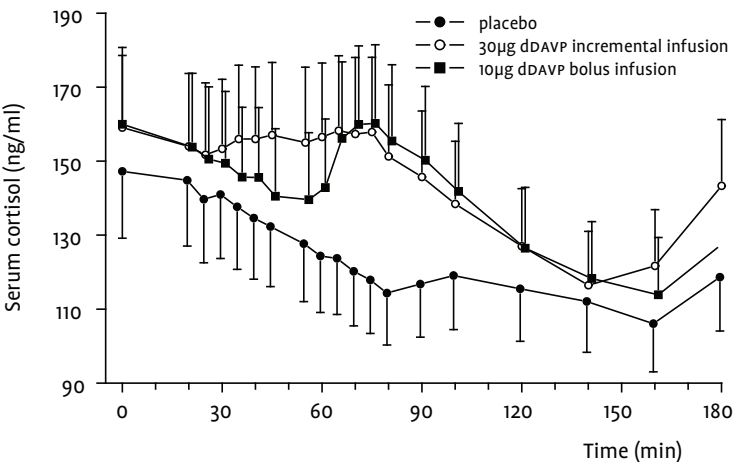
Figure 3   Average concentration time profile of diastolic blood pressure (DBP) with SD error bars (closed circle: Placebo; open circle: Incremental infusion; open square: Bolus infusion)
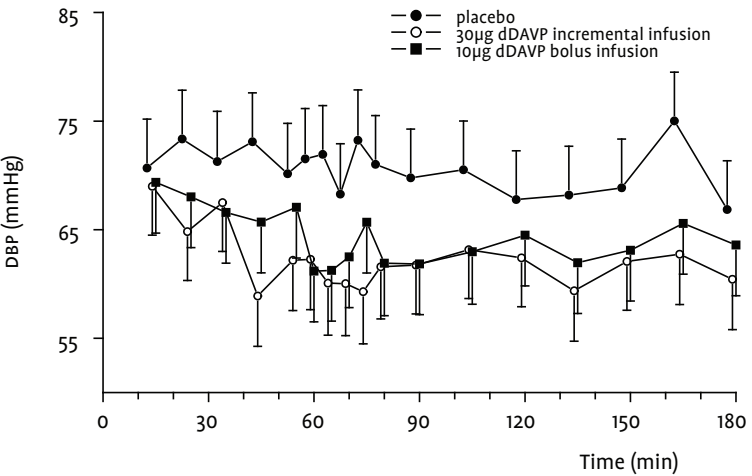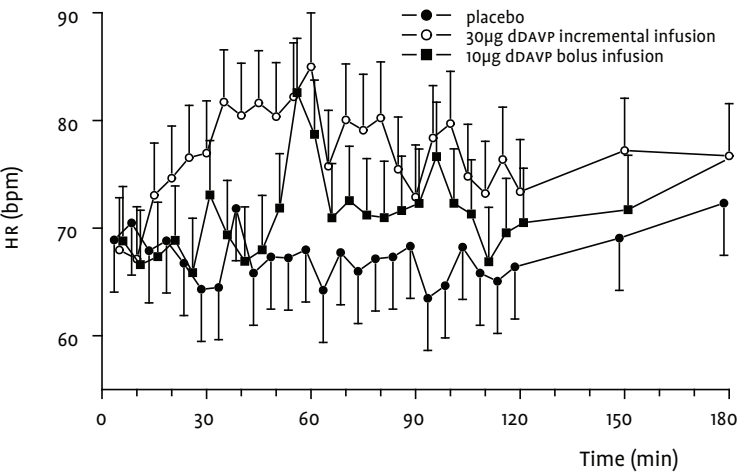

Figure 4   Average concentration time profile of heart rate (HR) with SD error bars (closed circle: Placebo; open circle: Incremental infusion; open square: Bolus infusion)

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