CLINICAL STUDY

Effect of exogenous glucocorticoid on osmotically stimulated antidiuretic hormone secretion and on water reabsorption in man

Volker Bähr¹, Norma Franzen¹, Wolfgang Oelkers², Andreas F H Pfeiffer¹ and Sven Diederich^{1,2}

¹Department of Endocrinology, Diabetes and Nutrition, Charité-Universitätsmedizin Berlin, Campus Benjamin Franklin, Hindenburgdamm 30, 12200 Berlin, Germany and ²Endokrinologikum Berlin, Centre for Endocrine and Metabolic Diseases, Berlin, Germany

(Correspondence should be addressed to V Bähr; Email: volker.baehr@charite.de)

Abstract

Objective: Glucocorticoids exert tonic suppression of antidiuretic hormone (ADH) secretion. Hypocortisolism in secondary adrenocortical insufficiency can result in a clinical picture similar to the syndrome of inappropriate ADH secretion. On the other hand, *in vitro* and *in vivo* results provide evidence for ADH suppression in states of hypercortisolism. To test the hypothesis that ADH suppression is of relevance during glucocorticoid therapy, we investigated the influence of prednisolone on the osmotic stimulation of ADH.

Design and methods: Seven healthy men were subjected to water deprivation tests with the measurement of plasma ADH (pADH) and osmolality (posmol) before and after glucocorticoid treatment (5 days 30 mg prednisolone per day).

Results: Before glucocorticoid treatment, the volunteers showed a normal test with an adequate increase of pADH (basal 0.54 ± 0.2 to 1.9 ± 0.72 pg/ml (mean $\pm$ s.d.)) in relation to posmol (basal 283.3 ± 8.5 to 293.7 ± 6 mosmol/kg). After prednisolone intake, pADH was attenuated (<0.4 pg/ml) in spite of an increase of posmol from 289.3 ± 3.6 to 297.0 ± 5.5 mosmol/kg. However, urine osmolar concentration increased normally during water deprivation after prednisolone. Urinary cAMP excretion increased during water deprivation without glucocorticoid treatment from 3.56 ± 0.55 to 6.07 ± 0.76 μ mol/l, reflecting the increased pADH levels. The rise in cAMP excretion was completely blunted by prednisolone treatment.

Conclusions: We speculate that there may be an ADH-independent stimulation of the formation or function of aquaporin-2 channels by prednisolone and/or a direct osmotic stimulation of water reabsorption independent of ADH and glucocorticoid control.

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Introduction

The concentration of cortisol is controlled on multiple levels: The major stimulus for its synthesis in the adrenal is adrenocorticotrophic hormone (ACTH). Synthesis and secretion of this stimulus are controlled by corticotropin-releasing hormone (CRH) and antidiuretic hormone (ADH), also called arginine vasopressin. CRH and ADH are synthesized by neuroendocrine neurons in the medial parvocellular (mp) part of the paraventricular nucleus of the hypothalamus and released by terminals of the neuroendocrine neurons in the median eminence into the hypophyseal portal vasculature. In the anterior lobe of the pituitary gland, CRH and ADH stimulate synthesis and secretion of ACTH. In this traditional scheme, glucocorticoids, as the end-organ hormones, act as a negative feedback signal to inhibit the activity of the control elements, the corticotropes in the pituitary, and the neuroendocrine

neurons in the hypothalamus. ADH derived from the magnocellular neurosecretory cells of supraoptic and paraventricular nuclei is transferred to the posterior pituitary gland where it is secreted in response to osmolar and non-osmolar stimuli. ADH in the peripheral circulation regulates the water permeability of renal collecting ducts via aquaporin-2 channels. A decrease in plasma osmolality centrally inhibits ADH secretion. Thus, ADH is involved in two more or less independent feedback loops.

On the other hand, there is evidence that these two control loops influence one another: Osmotically stimulated endogenous ADH enhances CRH-stimulated ACTH and cortisol secretion in men (1, 2) and corticosterone secretion in rats (3). Some patients with hyponatremia have hypopituitarism with adrenal insufficiency. These patients have an inappropriately high ADH secretion (SIADH) and can be treated very effectively with cortisol replacement (4, 5).

Exogenous cortisol (6) and dexamethasone (7) attenuate osmotically stimulated ADH secretion in dogs. Dogs with hyperadrenocorticism had polyuria and an impairment of osmoregulation of ADH secretion (8). In the present study, we evaluate the effect of an exogenous glucocorticoid on osmotically stimulated ADH secretion and on the antidiuretic action of ADH.

Subjects and methods

Seven male volunteers gave written informed consent to take part in this study, which was approved by the ethical committee of the Charité Berlin. Their age was in the range of 24–37 (average 27.7) years. No volunteer was hypertensive, had diabetes mellitus, renal insufficiency, polyuria, adiposity, hyperlipidemia, osteoporosis, or a history of peptic ulcer.

Fluid deprivation tests were performed between 0800 and 1700 h. There was no food restriction except for soups and food containing much water, like fruits. Every 2 h, blood samples were taken and urine was collected in the same intervals. Ammonium heparinate plasma for the determination of ADH was obtained within 20 min after sampling and stored at -80°C , urine samples at -20°C . After blood and urine sampling at 1600 h, 4 μg V2-receptor-agonist desmopressin acetate (Minirin, Ferring Kiel, Germany) were given intravenously. At 1700 h, the last blood and urine samples were taken (9). Two days after these basal fluid deprivation tests, the volunteers started taking 30 mg prednisolone (Decortin H, Hoechst AG, Frankfurt, Germany) orally at 0800 h for the following 4 days. The fluid deprivation tests were repeated on the first and the fifth days of prednisolone intake.

Plasma and urinary osmolality was measured with an osmometer from Roebeling, Berlin, Germany. Plasma ADH was measured using the method of Morton *et al.* (10). There was no crossreactivity with prednisolone (500 ng/ml, maximum concentration obtainable with 30 mg/day).

Cyclic adenosine monophosphate (cAMP) in urine was determined using a [^3H]-cAMP radioimmunoassay (Amersham International).

Results

The volunteers showed a normal response to fluid deprivation. The rise in plasma osmolality after 8 h thirsting (from 283.3 ± 8.5 to 293.7 ± 6 mosmol/kg (mean $\pm$ s.d.)) was accompanied by an appropriate rise in ADH plasma concentration (0.54 ± 0.2 to 1.9 ± 0.72 pg/ml; Fig. 1). The normal range was defined based on Robertson *et al.* (11) and our own previous investigations (9). The intake of 30 mg prednisolone per day resulted in a suppression of

Figure 1 Plasma antidiuretic hormone (ADH) concentration and plasma osmolality in fluid deprivation tests in men at the fifth day of prednisolone treatment ($\square$) and without glucocorticoid treatment ($\blacksquare$). Normal range and detection limit of ADH indicated by solid and broken lines.

plasma ADH below 0.4 pg/ml (below the sensitivity of the radioimmunoassay). Figure 1 combines the results of the fluid deprivation test at the fifth day of prednisolone intake. Plasma osmolality at the first day of prednisolone intake rose from 289.3 ± 3.6 to 297 ± 5.5 mosmol/kg after 8 h thirsting and at the fifth day of prednisolone from 289 ± 4.4 to 296 ± 4.7 mosmol/kg. There were no differences in blood sugar values before and after treatment with prednisolone.

Prior to the days of prednisolone intake related to the rise in plasma osmolality, there was a rise in urine osmolality from 664.6 ± 222.0 to 1037.3 ± 75.5 mosmol/kg after 8 h thirsting. Although plasma ADH was below 0.4 pg/ml at the first and the fifth day of prednisolone intake, urine osmolality rose after thirsting from 727.4 ± 223.3 to 1061.1 ± 127.0 and from 650.3 ± 278.6 to 1041 ± 182.8 mosmol/kg respectively. Thus, all volunteers were able to concentrate their urine after thirsting at the first and the fifth day of prednisolone intake although plasma ADH was below 0.4 pg/ml (Fig. 2).

Urinary cAMP reflected the dynamic of plasma ADH. Without prednisolone intake, urinary cAMP was stimulated from 3.56 ± 0.55 to 6.07 ± 0.76 $\mu\text{mol/l}$ after thirsting and after i.v. injection of desmopressin acetate to 9.57 ± 1.43 $\mu\text{mol/l}$. At the fifth day of prednisolone intake, thirsting did not induce a rise in urinary cAMP (from 4.6 ± 1.02 to 4.28 ± 0.49) but desmopressin acetate induced a rise to 5.95 ± 0.59 $\mu\text{mol/l}$ ($P < 0.05$).

Figure 2 Urine osmolality and plasma antidiuretic hormone (ADH) concentration in fluid deprivation tests in men at the fifth day of prednisolone treatment ($\square$) and without glucocorticoid treatment ($\blacksquare$). Normal range and detection limit of ADH indicated by solid and broken lines.

Discussion

In this study, prednisolone markedly inhibited osmotically stimulated ADH secretion in healthy subjects. Although it remains to be proven that prednisolone intake does not decrease the half-life of ADH, this may be rather unlikely because, due to the sensitivity of the assay, the half-life would have to be shortened to one-eighth to explain the results. Five days of prednisolone intake did not induce a rise in plasma glucose. Therefore, the osmotic stimuli induced by water deprivation may be comparable. The inhibition of osmotically stimulated ADH secretion by prednisolone may support the concept of glucocorticoid control of ADH secretion into the circulation. Glucocorticoids appear to inhibit ADH secretion physiologically in a tonic way, and thereby prevent ADH hypersecretion and dilutional hyponatremia in stressful situations (4, 5). Cortisol (6) and dexamethasone (7) also inhibit ADH secretion in dogs. Some patients (10–15%) with Cushing's syndrome are polyuric and polydipsic (12, 13). This may be related to suppression of ADH secretion by cortisol, although in our study the suppression of ADH by prednisolone was not accompanied by impaired urine concentrating ability of the kidney. Cortisol and ADH form two overlapping feedback loops. First, ADH synthesized in the parvocellular neurons of the hypothalamus (nuclei paraventricularis) is released into the pituitary portal system at the median eminence. It stimulates corticotropin release in cooperation with CRH. Corticotropin stimulates

cortisol, which feeds back on hypothalamic ADH synthesis and release. Second, ADH synthesized in the magnocellular neurons of the supraoptic and paraventricular nuclei travels along nerve projections into the neurohypophysis, where it is released into the circulation in response to osmotic and non-osmotic (e.g. hypovolemia) stimuli. Circulating ADH acts on the kidney in order to decrease plasma osmolality. While variations in plasma cortisol lead to suppression or de-repression of ADH secretion into the peripheral circulation (6, 7, 12, 13), osmotic stimuli may also influence the feedback loop between ADH, corticotropin and cortisol (1–3).

As far as we are aware, suppression of ADH secretion into the circulation by glucocorticoids without impairment of the renal concentrating ability has not been described previously. In the dog, suppression of plasma ADH by cortisol infusion also impaired the increase in urine osmolality in response to the infusion of hypertonic saline (6). A functional correlate of ADH suppression in our study is the failure of urinary cAMP output to respond to water deprivation. Exogenous desmopressin led to a small increase in urinary cAMP, but the effect of cAMP was blunted in comparison with the studies before prednisolone administration.

There are several lines of evidence that ADH may be the most important regulator of plasma osmolality via aquaporin-2 (AQP2) water channels but not the only one. Brattleboro rats that do not express ADH up-regulate AQP2 in response to osmotic stimulation (14). Dehydration reverses vasopressin antagonist-induced diuresis and AQP2 down-regulation in Wistar rats, although an effective receptor blockade was confirmed by control experiments (15). In men, water loading lowered AQP2 urinary excretion but water loading and additional ibuprofen intake enhanced the secretion of this protein (16).

The mechanism(s) that would explain a vasopressin-independent regulation of AQP2 are not well understood. The adenylate cyclase-coupled vasopressin receptor activates the AQP2 promoter via a dual effect on CRE and AP-1 elements (17, 18). In addition, vasopressin stimulates phosphorylation of AQP2 at Ser²⁵⁶ and its trafficking to the plasma membrane. In primary cultured inner medullary collecting duct cells, angiotensin II stimulates AQP2 expression and plasma membrane insertion probably via stimulation of PKA activity (19). The renal adenylate cyclase activity is also stimulated by β -adrenergic stimuli (20) and prostacyclin (21). The AQP2 promoter is also activated by the tonicity-responsive enhancer binding protein, whose activity is enhanced in collecting duct principal cells by hypertonic challenge (22). The insertion into plasma membrane is not stimulated by hypertonicity (23).

In rat cultured renal papillary collecting tubule cells PKA was stimulated by prostaglandin E₂ (PGE₂) (24). The phosphorylation of AQP2 at Ser²⁵⁶ is not influenced by PGE₂ in rat renal inner medulla cells,

but the translocation of AQP2 to the membrane is inhibited, the ratio of membrane-enriched AQP2 to vesicle-enriched AQP2 in vasopressin-stimulated cells is suppressed to control values by prostaglandin E₂ (25). This is compatible with the above-mentioned stimulation of urinary AQP2 excretion in men induced by PGE₂ synthesis inhibition by ibuprofen (16). This action may be related to the action of prednisolone in this study. Glucocorticoids are also known to inhibit PGE₂ synthesis (26, 27) and it may be possible that they stimulate AQP2 excretion by this mechanism.

In summary, we have shown that supraphysiological glucocorticoid levels totally suppress osmotically stimulated ADH secretion. Therefore, water deprivation tests are highly affected by exogenous glucocorticoids. This should be considered in clinical differential diagnoses of the polyuria–polydipsia syndrome. On the other hand, urinary concentration ability in healthy young men was not influenced in spite of low ADH levels. In order to explain this observation further, *in vitro* and *in vivo* investigations are necessary.

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