Osmomediated natriuresis in humans: the role of vasopressin and tubular calcium sensing

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Abstract
Background. The aim was to investigate the unknown mechanism of osmomediated natriuresis. This is the phenomenon by which hypertonic saline (HS) produces a larger natriuresis than isotonic saline (IS), despite the same sodium content.

Methods. Seven healthy volunteers first received HS and then IS (both 3.85 mmol sodium/kg). To investigate the role of calcium metabolism, four patients received HS, two with an activating mutation (ADH) and two with an inactivating mutation (FHH) of the calcium-sensing receptor (CaSR).

Results. In healthy volunteers, HS produced mild hypernatraemia, a 4-fold rise in vasopressin (to 2.2 ± 0.85 pg/mL) and a 3-fold rise in natriuresis compared with a 1.5-fold rise with IS (∆P = 0.002). This confirmed osmomediated natriuresis. HS caused calciuresis to increase 1.4-fold and then reduce it 1.4-fold, whereas IS failed to increase calciuresis and caused it to fall 3.7-fold (∆P = 0.05). Natriuresis and calciuresis in ADH patients were similar to healthy volunteers receiving HS, whereas a blunted response was seen in FHH patients. Patient vasopressin levels did not exceed 1.3 pg/mL and changes from baseline were variable. In one FHH patient, a 3-fold rise in vasopressin did not prevent the blunted natriuresis and calciuresis. In one ADH patient, natriuresis and calciuresis were similar to healthy volunteers despite a 1.7-fold fall in vasopressin.

Conclusions. Our data suggest that not only vasopressin (possibly via its V1a receptor), but also the CaSR (which is sensitive to high sodium concentrations) may play a role in osmomediated natriuresis. These results shed new light on osmomediated natriuresis and suggest roles for the CaSR beyond calcium regulation.

Keywords: calcium-sensing receptor; hypernatraemia; parathyroid hormone; thick ascending limb

Introduction

The effects of hyperosmolarity on water balance regulation are well characterized and consist of activation of central osmoreceptors, release of arginine vasopressin and ultimately the insertion of aquaporin-2 water channels in the apical plasma membrane of the renal collecting duct to facilitate water reabsorption [1]. Interestingly, hyperosmolality also affects renal sodium excretion. This is illustrated by experiments that demonstrate a greater natriuresis when the same sodium load is given as hypertonic saline compared with isotonic saline [2]. The mechanisms of this phenomenon of ‘osmomediated natriuresis’ are incompletely understood. A previous infusion study for the first time demonstrated this phenomenon in healthy volunteers, showing that hypertonic and isotonic saline produced similar changes in creatinine clearance, haemodynamic parameters and mediators of renal sodium excretion, including renin, angiotensin II, aldosterone and atrial natriuretic peptide (ANP) [2]. As expected, vasopressin only increased in subjects receiving hypertonic saline [2]. In the present study, our objective was to re-investigate the mechanism of osmomediated natriuresis in humans. To do so, we repeated the previous analyses and extended these by studying the renal handling of different solutes, including calcium, magnesium, potassium, urea and phosphate, and the response in parathyroid hormone (PTH), B-type natriuretic peptide (BNP) and catecholamines. Furthermore, the effect of hypertonic saline was studied in two patients with an activating mutation of the calcium-sensing receptor (CaSR), who have autosomal dominant hypocalcaemia (ADH), and in two patients with an inactivating mutation of the CaSR, who have familial hypocalciuric hypercalcaemia (FHH) [3]. This was done to test the hypothesis that the CaSR is involved in osmomediated natriuresis, because recent data have implicated the CaSR both in central osmoregulation [4] and renal tubular ‘salinity sensing’ [5,6].

Subjects and methods

Infusion studies

The experimental protocol was adapted from Andersen et al. [2] and approved by the Ethics Committee of the Erasmus Medical Center (MEC-2005-326). Experiments were performed in seven healthy volunteers (4 females, 3 males, 19–28 years, 50.4–72.6 kg, not taking medication), two sisters with ADH (ADH-1: 45 years, 94.0 kg; ADH-2: 41 years,
Osmomediated natriuresis and calciuresis

88.2 kg, both no medication) and two patients with FHH (FHH-1: father, 57 years, 61.0 kg; FHH-2: daughter, 23 years, 50.4 kg; both no medication). ADH and FHH were confirmed by sequence analysis (ADH: de 2519C>T; FHH: CGA>TGA = R392Sp). Written informed consent was received from all participants. All subjects consented to consume a normal diet for 3 days prior to the experiment, while avoiding salty foods and refraining from alcohol and caffeine. Basal urine sodium and calcium excretion (measured in 24-h urine collected the day prior to the experiment) were 122 ± 23 and 3.7 ± 0.8 mmol in healthy volunteers, 328 and 4.9 mmol in ADH-1, 228 and 4.7 mmol in ADH-2, 104 and 2 mmol in FHH-1 and 74 and 0.5 mmol in FHH-2. Each experiment started at 9 am and was divided into four 90-min periods (baseline, infusion, two post-infusion periods). The subjects emptied their bladders before the experiment. Two catheters were placed in opposite antecubital veins for blood sampling and infusion of saline, respectively. After the baseline period, a sodium load of 3.85 mmol/kg body weight (wt) was infused over the following 90 min by an infusion pump. The load was infused as either 0.9% saline (25 mL/kg body wt) or as 5% saline (4.5 mL/kg body wt) on separate days. The ADH and FHH patients only received an infusion of hypertonic saline. Blood and urine were collected at the end of each 90-min period; blood was also collected at baseline and halfway through the infusion. Withdrawn and excreted fluids were immediately replaced by tap water plus 45 mL (0.5 mL/min) to compensate insensible water loss. The subjects remained seated in an armchair throughout the experiment and were allowed to stand up only for micturition.

Measurements and calculations

Serum and/or urine concentrations of sodium, calcium, magnesium, potassium, phosphate and albumin were measured by a multianalyser (Hitachi 917; Roche). The fractional excretion (FE) of these solutes was calculated as follows: \( FE = \frac{\text{U}_{\text{solute}} \times C_{\text{creatinine}}}{\text{S}_{\text{Osm}} \times C_{\text{creatinine}}} \times 100 \). Renal sodium excretion was calculated as follows: \( \text{U}_{\text{Na}} \times (\text{urine volume} / 90 \text{ min}) \times 1000 \). Serum and urine concentrations of creatinine were measured by conventional spectrophotometry. Serum and urine osmolality (\( S_{\text{Osm}} \) and \( U_{\text{Osm}} \)) were measured by freezing-point depression (Osmomet, Menarini) and used to calculate free water clearance: \( C_{\text{FWC}} = (\text{urine volume} \times (1 - U_{\text{Osm}} / S_{\text{Osm}})) \). Changes in plasma volume were estimated using changes in total protein concentrations. Blood for hormonal analyses was collected in prechilled polyethylene tubes containing EDTA. The samples were centrifuged at 4°C immediately after sampling, and serum was stored at −80°C and analysed within 6 months. Serum vasopressin and aldosterone were measured with commercially available radioimmunoassay kits (DiaSorin, Stillwater, MN, USA, and Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA, USA, respectively). Enzymatically active renin was determined by the radioimmunoassay as previously described [7]. ANP and BNP were measured with immunoradiometric assay kits (Shionoria, Osaka, Japan). Catecholamines were determined by high performance liquid chromatography with fluorimetric detection, as previously described [8]. PTH was determined by a solid-phase sandwich chemoluminescence immunoassay (IMMULITE intact-PTH, DPC, The Netherlands). Finally, blood pressure was recorded semiautomatically by oscillimetry four times during each 90-min period and is reported as mean arterial pressure.

Statistics

Results were presented as mean ± SEM. Data of the healthy volunteers were subjected to a repeated measures general linear model, which analyses the within and between group effects and generates one P-value for differences in the overall trends. If significant, a post hoc analysis was used to evaluate which time-point(s) were significantly different. Because vasopressin levels were not normally distributed, they were log-transformed prior to analysis. No statistics were performed for the patients because of the small sample size.

Results

Osmomediated natriuresis and vasopressin

In healthy volunteers, hypertonic saline produced mild hypoperthaemia (\( S_{\text{Na}} 146 ± 1 \text{ mmol/L} \)) and hyperosmolality (\( S_{\text{Osm}} 292 ± 3 \text{ mOsm/kg} \)) (Figure 1). This caused a rise in serum vasopressin during the infusion from 0.54 ± 0.12 to 2.02 ± 0.85 pg/mL. The courses in \( S_{\text{Na}}, S_{\text{Osm}} \) and serum vasopressin were significantly different (\( P < 0.05 \) for all) from the healthy volunteers receiving isotonic saline, in whom these parameters remained unchanged. The two ADH patients reached higher peak \( S_{\text{Na}} \) levels (148 ± 2 mmol/L). In the two patients with FHH, the initial \( S_{\text{Na}} \) was lower (136.5 ± 0.5 mmol/L), but hypertonic saline produced a similar rise in \( S_{\text{Na}} \) to 142.5 ± 1.5 mmol/L. In all patients, both the rise in vasopressin (0.67 ± 0.43 to 0.75 ± 0.08 pg/mL in ADH and 0.22 ± 0.03 to 0.51 ± 0.34 pg/mL in FHH) and its peak concentrations (all ≤ 1.3 pg/mL) were lower than in healthy volunteers. In healthy volunteers, free water clearance decreased with hypertonic saline, but increased with isotonic saline. In patients, the fall in free water clearance was less in FHH than in ADH. All groups increased renal sodium excretion during infusion. After infusion, the rise in renal sodium excretion persisted in healthy volunteers receiving hypertonic saline (402 ± 96 to 501 ± 77 \( \mu \text{mol/min} \)), whereas it decreased with isotonic saline (379 ± 53 to 286 ± 34 \( \mu \text{mol/min} \), \( P = 0.002 \)). In the ADH patients, the average renal sodium excretion also increased (515 ± 204 to 585 ± 240 \( \mu \text{mol/min} \)). However, in the FHH patients, the rise in renal sodium excretion was attenuated (329 ± 95 to 344 ± 70 \( \mu \text{mol/min} \)).

Extracellular volume, blood pressure and sodium regulating hormones

In healthy volunteers and patients, hypertonic and isotonic saline caused a similar increase in calculated plasma volume and a decrease in serum aldosterone (Figure 2). In healthy volunteers and in the FHH patients, the mean arterial pressure was unaffected by the infusions. However, in the ADH patients, hypertonic saline increased the mean arterial pressure from 94 ± 5.4 mm Hg to 100 ± 3.2 mm Hg (during infusion) and 98 ± 3.8 mm Hg (after infusion). In healthy volunteers, hypertonic and isotonic saline produced similar small rises in serum norepinephrine and serum BNP. Especially in the ADH patients, these increments were more prominent. In all groups, renal function, serum renin activity and serum ANP showed no differences between groups (renal function increased, response in ANP similar to BNP, serum epinephrine slightly increased after the infusions, data not shown).

Changes in calcium homeostasis

During the infusions, all groups exhibited a fall in serum calcium and serum albumin concentrations (4 g/L in all groups, data not shown) and a rise in serum PTH (Figure 3). In healthy volunteers, hypertonic saline increased \( \text{FE}_{\text{Ca}} \) (from 0.89 ± 0.24% to 1.24 ± 0.23%), whereas it decreased with isotonic saline (1.06 ± 0.32% to 0.79 ± 0.22%). \( \text{FE}_{\text{Ca}} \) decreased after both infusions, but because \( \text{FE}_{\text{Ca}} \) remained at a higher level in healthy volunteers receiving hypertonic saline, the difference in \( \text{FE}_{\text{Ca}} \) level became significant after the first post-infusion period (\( \text{P}=0.05 \)). At the end of the experiment, \( \text{FE}_{\text{Ca}} \) had decreased 1.4-fold with hypertonic
Fig. 1. The effects of hypertonic and/or isotonic saline on serum sodium (A), serum osmolality (B), serum vasopressin (C), free water excretion (D) and renal sodium ($\text{Na}^+$) excretion (E) are shown. The left part shows the response in healthy volunteers. The right part shows the response in four patients with mutations in the calcium-sensing receptor (CaSR), including two patients with familial hypocalciuric hypercalcaemia (FHH) and two patients with autosomal dominant hypocalcaemia (ADH). Statistical analysis was performed comparing the responses in healthy volunteers to isotonic and hypertonic saline. A repeated measures general linear model was used, which generated the reported $P$-value for the differences in the overall trend. If significant, a post hoc analysis was done to determine at which individual time-points the difference was significant ($^*P < 0.05$).
Fig. 2. The effects of hypertonic and/or isotonic saline on calculated plasma volume (A), mean arterial pressure (B), serum aldosterone (C), serum norepinephrine (D) and serum B-type natriuretic peptide (BNP) are shown in healthy volunteers (left) and four patients with mutations in the calcium-sensing receptor (CaSR, right). See the caption to Figure 1 for additional information.
Fig. 3. The effects of hypertonic and isotonic saline on serum calcium (A), parathyroid hormone (B) and the fractional excretions of calcium (C), magnesium (D) and urea (E) are shown in healthy volunteers (left) and four patients with mutations in the calcium-sensing receptor (CaSR, right). See the caption to Figure 1 for additional information.
Relative changes in parameters associated with osmomediated natriuresis

Figure 4 shows the relative changes of those parameters that changed significantly between hypertonic and isotonic saline. They were calculated from baseline until peak values or the time at which significance was achieved (FE_{Ca}). The observations in the four patients are also presented. They show individually different responses in vasopressin, a blunted natriuresis and calciuresis in the FHH patients and a response in ADH patients that is similar to healthy volunteers receiving hypertonic saline.

Discussion

In the present study, we sought to clarify the mechanism of osmomediated natriuresis in humans. To do so, an established infusion protocol was used in which the same sodium load was given to healthy volunteers either as hypertonic saline or as isotonic saline [2]. Osmomediated natriuresis was confirmed, because hyperosmolarity induced by hypertonic saline was followed by a significantly greater renal sodium excretion (Figure 1). This is the second study showing osmomediated natriuresis in humans and, compared with the first study, obtained similar results for the courses in renal sodium excretion, estimated plasma volume, serum osmolality and the serum concentrations of sodium, vasopressin, renin, aldosterone and ANP [2].

Because none of the obvious parameters associated with renal sodium excretion was different, the question remains why hypertonic saline induced a greater natriuresis than isotonic saline. For the first time, we also investigated serum BNP, norepinephrine and epinephrine, but were unable to identify an association between these hormones and osmomediated natriuresis. Therefore, we extended our analyses by evaluating the renal handling of calcium, magnesium, potassium, urea and phosphate, and the responses of PTH during hypertonic and isotonic saline.

The principal finding from these new analyses was that hypertonic saline caused an increase in FE_{Ca} despite a rise in PTH and then remained significantly higher compared with isotonic saline (Figure 3). The rise in serum PTH can be explained by the ‘true’ decrease in serum calcium (~50% was caused by the fall in serum albumin), although volume expansion may also have contributed [9]. The excretion of other solutes was not different between the healthy volunteers receiving hypertonic or isotonic saline, although there
appeared to be a trend towards a higher magnesium and lower urea excretion in subjects receiving hypertonic saline (Figure 3). Interestingly, in the ADH patients, hypertonic saline produced a natriuretic and calciuretic response that equalled or sometimes exceeded that of the healthy volunteers receiving hypertonic saline. In contrast, the FHH patients’ response was blunted and resembled the pattern of the healthy volunteers receiving isotonic saline (Figure 4).

The next obvious question is if these findings can shed any light on the physiological mechanisms of osmomediated natriuresis. We emphasize that this study was not designed to locate the nephron segment in which osmomediated natriuresis occurs. In addition, not all factors influencing renal sodium excretion were directly measured. For example, oxytocin and cGMP are both also stimulated by hypernatraemia and can induce a natriuresis [10,11]. However, based on our data and on previous studies, a number of new scenarios can be postulated.

The first scenario is vasopressin. Until recently, the literature on the effect of vasopressin on renal sodium handling was conflicting, with evidence for vasopressin both stimulating and inhibiting renal sodium excretion. Part of the controversy may be explained by the fact that most studies that showed a stimulation of sodium reabsorption administered the V2 receptor (V2R) selective dDAVP [12–14], whereas studies showing an inhibition of sodium reabsorption used arginine vasopressin [15–27], which stimulates both vasopressin receptors. Indeed, the administration of an antagonist to the V1a receptor (V1aR) [28] or the V1aR and V2R [29] before hypertonic saline loading prevented osmomediated natriuresis. A recent study finally explained the opposite findings by showing a differential and dose-dependent effect of vasopressin [30]. That is, lower vasopressin levels stimulate V2R-mediated sodium reabsorption, whereas higher levels stimulate V1aR-mediated natriuresis and overrule the V2R effects [30]. Experiments using paired tracer microinjections of [3H]inulin and 22Na in rats located vasopressin-induced natriuresis in the thick ascending limb (TAL) [19], where vasopressin is believed to act via V1aR [21] and V2R is absent [31]. Because the TAL is also an important site for renal calcium and magnesium handling, this may explain the combined natriuresis and calciuresis. The fact that the fractional magnesium excretion was not significantly different between groups could be a power problem, or counterregulation in more distal parts (e.g. the distal convoluted tubule). In the healthy volunteers receiving hypertonic saline, both the negative free water clearance and the lower fractional urea excretion are additional proof of the biological actions of vasopressin (vasopressin increases urea permeability [32]). The higher free water excretion in FHH patients indicates less vasopressin action and may therefore explain the blunted natriuresis. The reason that FHH patients secreted less vasopressin could be due to a lower peak osmolality, which was caused by their lower basal serum sodium levels. Low-normal basal serum sodium concentrations in FHH patients were previously reported by Kristiansen et al. [33] and suggest a role for CaSR in central osmoregulation [4].

However, vasopressin alone cannot explain all our findings. Namely, vasopressin secretion was normal in FHH-1 and fell in ADH-2, but these patients still had a blunted and normal natriuresis, respectively (Figure 4). The second possible scenario is therefore that the CaSR plays a role in osmomediated natriuresis. CaSR-mediated natriuresis has been demonstrated in experiments in which the basolateral CaSR in the TAL was stimulated by calcium in vitro in isolated perfused tubules [34], in vivo in microperfused loops of Henle of thyroparathyroidectomized rats [35] and in humans using a graded calcium infusion with a PTH clamp [36]. Besides calcium, hypernatraemia can also have a direct renal tubular effect. Kamm and Levinsky showed that by unilaterally elevating the renal arterial sodium concentration in dogs, hypernatraemia decreased tubular sodium reabsorption independently of volume expansion, which persisted even when filtered sodium in the experimental kidney was reduced below that of the control [37]. The question remains if and how hypernatraemia activates the CaSR. Recent evidence has indicated that a variety of factors other than calcium can influence the activity of the CaSR, including ionic strength [38]. However, hyperosmolarity would be expected to increase ionic strength, which would reduce the CaSR affinity for calcium and render the receptor to be less active at the same serum calcium concentration, producing the opposite effect [5]. Alternatively, other nephron segments that express CaSR apically could be involved, including the proximal tubule or the collecting duct. Nijenhuis et al. showed that volume depletion increases sodium and calcium reabsorption in the proximal tubule [39]—the opposite could be true for volume expansion.

This study has a number of limitations. First, only a small number of patients with CaSR mutations were available. Secondly, we estimated plasma volume by changes in total protein concentration, which does not account for possible vasodilatation. Thirdly, as mentioned, further research will need to elucidate the exact nephron segments involved in osmomediated natriuresis (e.g. by using vasopressin-receptor antagonists or calcimimetics).

In conclusion, we report a new association between osmomediated natriuresis and calciuresis and show that these responses are attenuated in patients with an inactivating mutation of the CaSR. Based on these observations, we hypothesize that osmomediated natriuresis is a central phenomenon (vasopressin release, vasopressin-induced natriuresis) or a renal phenomenon (activation of the CaSR, CaSR-mediated natriuresis). The physiological role of osmomediated natriuresis is unclear, but it could play a role in the defence against hypernatraemia or prevent kidney stone formation during hyperosmolarity [40]. Furthermore, osmomediated natriuresis is an interesting physiological example of the intimate interactions between renal water, sodium and calcium regulation. Our study suggests the CaSR to have roles beyond calcium regulation, including central and renal effects on water and sodium homeostasis. These non-classic CaSR effects are especially important given the increasing use of calcimimetics in clinical practice.

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