Natriuresis induced by mild hypernatremia in humans

LARS JUEL ANDERSEN,1,2 JENS LUNDBÆK ANDERSEN,1 BETTINA PUMP,3 AND PETER BIE4
1Department of Medical Physiology, Panum Institute, University of Copenhagen, DK-2200 Copenhagen; 2Department of Aviation Medicine, Rigshospitalet 7805, DK-2200 Copenhagen; 3Department of Clinical Physiology, Herlev Hospital, DK-2730 Herlev; and 4Department of Physiology and Pharmacology, University of Southern Denmark, DK-5000 Odense, Denmark

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IT HAS BEEN RECOGNIZED for decades that renal sodium excretion is modulated by changes in intravascular pressure and body fluid volume (e.g., Refs. 18, 25). In addition, results from a number of animal experiments indicate that stimulation of osmoreceptors located in the brain, the gut, and the liver may increase renal sodium excretion by mechanisms that are not dependent on changes in body fluid volume (7, 8, 12, 20–22, 34–36, 42, 43, 45, 46). In the same animal species, infusion of hypertonic saline has been found to induce a greater natriuresis than that produced by infusion of the same amount of sodium as an isotonic solution, although the former procedure induces the smallest increase in body fluid volume (14–16, 23, 28, 33). This excess natriuresis induced by hypertonic saline infusion may be mediated by stimulation of the above-mentioned sodium-regulating osmoreceptors and could be referred to as concentration-mediated or osmo-stimulated natriuresis.

Although sodium homeostasis may also be under osmoregulatory control in humans, concentration-mediated natriuresis has not been consistently verified in humans. In recent human studies from our laboratory, infusion of hypertonic saline was not associated with excess natriuresis (4–6). However, plasma sodium concentration was increased to rather modest levels, and it could be hypothesized that the absolute increase in plasma sodium concentration is of critical importance so that natriuresis is not mediated until sodium concentration reaches a certain level. The purpose of the present study was to compare the effects of hypertonic vs. isotonic saline loading in subjects that were water deprived for 14 h before the experiments to increase plasma sodium concentration to levels above those obtained in previous experiments.

METHODS

Experiments were performed in six healthy male volunteers. Subjects were 22–29 yr old, weighing 62.6–83.9 kg. All gave informed consent, and the study was approved by the Ethics Committee of Copenhagen (JNR KF 01–104/95).

The subjects were investigated on three different study days, and before all experiments subjects accepted a controlled diet containing 150 mmol NaCl/day for 4 days. During this period, subjects were allowed to drink only tap water. Sodium turnover was assessed by measurements of 24-h urinary sodium excretion. The day before the experiment, subjects consumed their standard evening meal before 1800 and were not allowed any food or fluid intake after this time. The night before the experiment the subject slept at the laboratory. After consumption of a light standardized low-salt breakfast (2 slices of toast, 15 g marmalade, and 100 ml of tap water), two catheters (Venflon) were placed in super-

Address for reprint requests and other correspondence: P. Bie, Dept. of Physiology and Pharmacology, Univ. of Southern Denmark, 21 Winsløwparken, DK-5000 Odense, Denmark (E-mail: pbie@health.sdu.dk).

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ficial cubital veins for blood sampling and infusion of saline, respectively. Between 0800 and 0830, the subject emptied his bladder, and the experiment was initiated. The subject remained seated in an armchair throughout the experiment and was allowed to stand up only for micturition.

Each experiment lasted 6 h and was divided into four 90-min periods. After the baseline period, a sodium load of 3.85 mmol/kg body wt was infused over the following 90 min by an infusion pump. The load was infused as either 0.9% saline (isotonic) or as 5% saline (hypertonic) on separate days. Thus volumes of 25 and 4.5 ml/kg body wt were infused in the isotonic and hypertonic series, respectively. The time control series was without saline infusion but otherwise identical to the infusion series. The three experiments on each subject were separated by at least 8 days.

Urine was collected by voluntary micturition during the last minutes of each 90-min period. The excreted volume was immediately replaced by drinking the same amount of tap water plus 45 ml (0.5 ml/min) as replacement for insensible water loss.

Blood for measurements of hematocrit (Hct), plasma osmolality, onecotic pressure, and plasma concentrations of sodium, protein, albumin, and creatinine was sampled in heparinized tubes in the middle of each period and during the last 10 min of saline infusion. At the same time, blood for hormone analyses was collected in prechilled polyethylene tubes containing aprotonin and EDTA. A total of 150 ml of blood was collected throughout each experiment. Blood samples were replaced by similar amounts of isotonic saline immediately after each sampling. The samples were centrifuged at +4°C immediately after sampling, and plasma for determination of hormone concentrations was stored at −18°C and analyzed within 6 mo.

Hct was determined by centrifugation (Microfuge, Christ). Onecotic pressure was determined by a colloid osmometer (4400 Colloid Osmometer, Wescor). Hemoglobin (Hb), Hct, and plasma concentrations of sodium, potassium, albumin, and protein were measured by a multianalyzer (SMAC3, Bayer). Urine sodium concentrations were measured by flame photometry and osmolality by freezing-point depression (model 3DII, Advanced Instruments). Concentrations of creatinine were measured by conventional spectrophotometry based on the Jaffe reaction.

Arterial systolic and diastolic pressures were recorded semiautomatically by oscillometry four times during each experimental period (Propaq 102, Dacema), and the mean of these values was used to calculate mean arterial pressure (MAP) from the following formula: MAP = diastolic pressure + (1/3) × pulse pressure. Percent change in plasma volume (PV) was calculated from the formula (26)

$$\Delta PV\% = [(Hb_1/Hb_2) \times (100 - Hct_2/100 - Hct_1) - 1] \times 100$$

Plasma concentrations of atrial natriuretic peptide (ANP), ANG II, and arginine vasopressin (AVP) were quantified by RIAIs after extraction on C18 Sep-Pak cartridges (Waters), as recently described (6). Plasma aldosterone concentrations were measured using a commercial kit (COAT-A-COUNT, Diagnostic Products). Plasma renin activity was determined by RIA using the antibody-trapping method of Poulsen and Jørgensen (38) based on conversion of renin substrate to ANG I in the presence of ANG I antibody (no. 3–2008939) at 37°C. After 3 h, addition of cold assay buffer stopped the reaction by cooling and dilution. After incubation with labeled angiotensin (16–20 h, 4°C), separation by incubation (3 h, 4°C) with second antibody (Sac-Cel, IDS, Boldon, UK), and centrifugation, the activity of the sediment was determined. In separate experiments it was found that the present storage procedure, including one freeze-thaw cycle, did not influence the results. The results were quantified by use of the World Health Organization standard (human renin no. 68–356, 0.1 IU per ampoule) obtained from the National Institute for Biological Standards and Control (South Mimms, Hertfordshire, UK).

**Statistics.** Results are presented as means ± SE. Data were subjected to one-way analysis of variance for repeated measures (44). In case of significantly large F values of the ANOVA, all possible differences were evaluated sequentially by Newman-Keuls test. Selected differences between two series, e.g., at the time of maximal deviation, were evaluated using paired Student’s t-test. Level of significance was in all cases 0.05.

**RESULTS**

Plasma sodium concentration increased with hypertonic saline infusion from 142.0 ± 0.6 to 146.2 ± 0.5 mmol/l and decreased slightly but significantly during time control (141.7 ± 0.7 to 140.3 ± 0.6 mmol/l) and isotonic saline loading (142.3 ± 0.6 to 140.3 ± 0.7 mmol/l). Changes in plasma osmolality followed the same pattern (Fig. 1).
Plasma oncotic pressure was identical before saline infusion and decreased in both saline series (Fig. 2). The consistent tendency toward lower values in the isotonic series was evaluated by averaging the observations made during and after the infusion in each subject. After saline loading, the values found in the isotonic series were marginally but significantly smaller than those of the hypertonic series (23.0 ± 0.5 vs. 23.8 ± 0.3 mmHg, P < 0.05). No changes were observed during time control. Similar trends were observed for Hb and plasma concentration of protein and albumin (data not shown). Calculated change in plasma volume increased significantly more during isotonic than during hypertonic saline infusion (15 ± 2 vs. 11 ± 1%, Fig. 2).

Sodium excretion remained stable during time control but increased in both saline series (Fig. 3). The natriuresis after hypertonic saline infusion exceeded that after isotonic saline infusion in the first period after infusion (291 ± 25 vs. 199 ± 24 μmol/min) as well as in the final period of observation (300 ± 23 vs. 224 ± 23 μmol/min).

Urine flow remained unchanged during time control but increased from 0.6 ± 0.1 to 1.4 ± 0.1 ml/min after hypertonic saline infusion and from 0.6 ± 0.1 to 2.6 ± 0.7 ml/min after isotonic saline infusion (Fig. 4). Free water clearance decreased from −1.2 ± 0.1 to −2.3 ±

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**Fig. 2.** A: change in plasma volume. B: plasma oncotic pressure. ○, Time control; ■, isotonic saline infusion; ●, hypertonic saline infusion. *Significantly different from preinfusion value. #Significantly different from corresponding value in hypertonic saline series.

**Fig. 3.** Renal sodium excretion. ○, Time control; ■, isotonic saline infusion; ●, hypertonic saline infusion. *Significantly different from preinfusion values. #Significantly different from corresponding value in isotonic saline series.

**Fig. 4.** A: urine flow. B: free water clearance. ○, Time control; ■, isotonic saline infusion; ●, hypertonic saline infusion. *Significantly different from preinfusion values.
0.1 ml/min after hypertonic saline infusion and remained statistically unchanged in the other series (Fig. 4).

There were no changes in arterial blood pressure in the isotonic series or during time control, but MAP decreased significantly by ∼4 mmHg in the hypertonic series as a result of small decreases in diastolic and systolic arterial pressures (Table 1). Heart rate decreased in all series. Creatinine clearance did not change measurably in any of the series (Table 1).

Plasma hormone concentrations are presented in Fig. 5 and Fig. 6. Isotonic and hypertonic saline infusion induced similar decreases in plasma renin activity, ANG II concentration, and aldosterone concentration. No changes were observed during time control except for aldosterone concentration, which increased during the second and third period of observation, returning to baseline in the final period. Plasma AVP concentration increased from 1.4 ± 0.4 to 3.1 ± 0.5 pg/ml during hypertonic saline infusion and decreased from 1.3 ± 0.4 to 0.6 ± 0.1 pg/ml during isotonic saline infusion. No changes were observed during time control. Plasma concentration of ANP remained unchanged in all series.

**DISCUSSION**

The purpose of the present study was to test the hypothesis that an increase in plasma sodium can induce natriuresis by a mechanism independent of changes in body fluid volume. The data support this hypothesis because hypertonic saline infusion induced an increase in renal sodium excretion that exceeded the natriuresis induced by isotonic saline infusion.

The experiments were performed under well-defined and strictly controlled conditions with regard to sodium status and water balance. Accordingly, baseline sodium concentrations and osmolality were very similar in the different experimental series, at levels to be expected after overnight fasting. During salt loading, isotonic saline infusion caused no further increase in plasma tonicity, whereas hypertonic saline infusion increased plasma sodium concentration and plasma osmolality quite substantially, but still not to a degree that exceeds the physiological levels induced by, for example, moderate to severe dehydration (11, 40). While only the hypertonic saline load induced an os-
natriuretic responses were obtained at different volumetric stimuli, it was argued that part of the natriuresis seen with hypertonic loading was caused by osmостimulation (4, 6). The present results provide a more direct and convincing evidence for a significant role of osmoreceptors in human sodium homeostasis.

The main difference between the present and the previous protocols is the degree of hypernatremia obtained with hypertonic sodium loading. In previous protocols the subjects received an initial water load before the salt loading, and consequently basal as well as postinfusion plasma sodium concentrations were lower than those presented here. The results suggest that the extent to which plasma sodium is elevated is of critical importance for the natriuretic response to osmостimulation. Furthermore, the present application of moderate dehydration and placement of the subject in the seated position is associated with a central blood volume that is reduced compared with the usual conditions of slight overhydration, high sodium intake, and supine position (4, 6). Because osmомедiated natriuresis was more evident under the present circumstances of relative hypovolemia, it seems that the degree of hypernatremia is more important as a stimulus to sodium excretion than any additional increase in central blood volume obtainable by changes in, for example, posture or dietary sodium intake.

Natriuretic mechanisms. The regulation of renal sodium excretion is multifactorial, and the natriuresis after volume loading is generally considered to be mediated by changes in physical as well as neural and endocrine factors. Data from several animal experiments indicate that the natriuretic response to osmостimulation is facilitated by stimulation of specific osmo- or sodium receptors located primarily in the brain (7, 8, 12, 20–22, 34–36, 42, 43, 45, 46) but possibly also in the gut or the liver (for reviews, see Refs. 13, 27, 39). The efferent signal in the natriuresis of central osmостimulation is most likely mediated by a humoral factor because renal denervation does not abolish the natriuresis of osmостimulation (22), but this factor has not been positively identified. In the present study, we measured several well-known mediators of sodium excretion to clarify the mechanism of the osmомедiated natriuresis.

Both loading procedures consistently suppressed the renin-angiotensin-aldosterone axis, suggesting that at least a part of the natriuretic response to isotonic as well as hypertonic sodium loading was mediated by changes in this system. Previous findings confirm the notion that ANG II is one of the main controllers of renal sodium excretion (1, 4–6, 41). However, none of the components in the renin-angiotensin-aldosterone system was reduced more during hypertonic than during isotonic saline infusion. On the contrary, isotonic loading tended to suppress these parameters more than hypertonic loading although this tendency did not reach statistical significance. From these results, it seems quite unlikely that the renin system was responsible for the concentration-mediated part of the natriuresis.

Fig. 6. A: plasma concentrations of atrial natriuretic peptide (ANP). B: plasma concentration of arginine vasopressin (AVP). ○, Time control; ■, isotonic saline infusion; ●, hypertonic saline infusion. *Significantly different from preinfusion values.

According to the classical concept of sodium balance being maintained primarily by volumetric control, the largest natriuresis should have been observed in the isotonic saline series. However, hypertonic saline loading induced a sizable osmotic stimulus at the expense of a smaller volumetric stimulus.

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Plasma ANP remained unchanged in all series, and it is very unlikely that the changes in sodium excretion were induced by ANP. This is in agreement with our previous findings in seated as well as supine subjects, where similar doses of sodium increased renal sodium excretion several times without any change in the plasma concentration of ANP (4-6).

During isotonic saline infusion, AVP secretion was probably suppressed by increments in central blood volume, which have been found to be a very potent inhibitor of AVP secretion, at least in dogs (2). In addition, plasma sodium decreased by just a few millimoles per liter, adding to the suppression of AVP secretion. During hypertonic saline loading, the osmotic stimulus dominated over the volumetric stimuli as expected, and plasma AVP increased significantly. It is unlikely that baroreceptor unloading accounted for the increased AVP release because there was no change in pulse pressure, probably no unloading of cardiopulmonary receptors, and only a 4-mmHg decrease in MAP (24). The effect of AVP on sodium excretion is controversial. In several animal species such as dog, rat, and sheep, there are results to indicate that AVP can promote natriuresis (9, 32). However, this has to our knowledge never been confirmed in humans. In fact, infusion of AVP in doses that increase plasma concentration by only a few picograms per milliliter actually inhibits renal sodium excretion in healthy humans (3). It thus seems unlikely that the present increase in AVP observed during hypertonic saline infusion is responsible for the excess natriuresis observed in this series. However, control experiments during which isotonic expansion is accompanied by infusion of suitable amounts of vasopressin to induce identical increments in plasma AVP have not been performed.

It has been suggested that the concentrating ability of the kidneys is a major limiting factor in the excretion of a saline load (17). However, this did not seem to be of critical importance during the present circumstances because sodium excretion in the hypertonic series indeed exceeded that of the isotonic series, despite significantly lower free water clearances. On the other hand, it may explain why the urine flow increased during hypertonic saline loading despite significant increases in plasma AVP concentration.

Hemodilution has been found to be a potent natriuretic factor (19, 29-31), and this probably contributed to the natriuresis observed in both infusion series. However, because the hemodilution seemed most pronounced during isotonic saline loading, this cannot be the mechanism of excess natriuresis after hypertonic saline loading. Arterial blood pressure decreased slightly after hypertonic saline loading and remained unchanged in the other series. It is, therefore, not possible that sodium excretion increased because of pressure natriuresis. In addition, there were no measurable changes in creatinine clearance. Still it must be considered that the increase in plasma sodium concentration most likely increased the filtered load of sodium and that this could be responsible for at least part of the natriuretic response to hypertonic saline infusion. On the other hand, we have previously shown that an opposite but quantitatively similar decrease in filtered load of sodium induced by oral water loading has no antinatriuretic effects at all (5).

**Summary.** The findings demonstrate for the first time in humans that the natriuresis after hypertonic saline is larger than that of isotonic saline. Our data indicate that this excess natriuresis is induced by the increase in sodium concentration and not by changes in body fluid volume or pressure. The combination of overnight water deprivation, normal sodium intake, and the placement of the subject in the seated position provided an experimental situation characterized by higher plasma osmolality and lower central blood volume compared with the conditions of our previous designs, in which hypertonic saline infusion failed to induce excess natriuresis (4-6). Thus the level to which plasma sodium concentration is increased seems to be of critical importance to the mechanism of osmoregulated sodium excretion. The effector mechanism behind the excess sodium excretion cannot be positively identified from the present data, but it is probably not mediated by changes in arterial blood pressure, by oncocotic pressure, by suppression of the renin-angiotensin-aldosterone system, or by increases in ANP or vasopressin. Finally, the results confirm the previous finding (6, 37) that the seated position is a very stable experimental position with regard to hemodynamic, humoral, and renal excretory functions.

**Perspectives**

The maintenance of a proper sodium balance is mediated by a complex interplay of multiple neuroendocrine and physical factors. Changes in body fluid volume may still be considered a very important stimulus to renal sodium excretion, but the present data provide strong evidence that changes in concentration of the body fluids are equally important in the maintenance of a normal sodium balance.

Disturbances in sodium handling are part of the pathophysiological changes in clinically important conditions such as congestive heart failure, edema formation, and some forms of arterial hypertension. A precise understanding of the mechanisms involved in normal regulation of renal sodium excretion may help clarify the pathophysiology behind these clinical conditions. It is suggested that, in future clinical investigations of, for example, salt-sensitive hypertension, a possible dysfunction of osmoregulated sodium excretion should also be taken into consideration.

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