Effects of Oral Salt Load on Arginine-Vasopressin Secretion in Normal Subjects*

LETIZIA SPINELLI, M.D., PAOLO GOLINO, M.D., FEDERICO PISCIONE, M.D., MASSIMO CHIARIELLO, M.D., AMELIA FOCACCIO, M.D., GIUSEPPE AMBROSIO, M.D., and MARIO CONDARELLI, M.D.

Department of Internal Medicine, Division of Cardiology, 2nd School of Medicine, University of Naples, Naples, Italy

ABSTRACT

Arginine-vasopressin (AVP) plays an important role in regulating water balance in humans. Its secretion is under control of several mechanisms, some of which are not completely understood. The purpose of the present study was to evaluate the effects of an acute oral salt load on AVP secretion in normal subjects. Six normal volunteers received 350 mEq of NaCl per os. Pulmonary capillary wedge pressure and right atrial pressure, plasma AVP, plasma sodium and potassium concentration, plasma osmolality, hematocrit, urinary sodium and potassium excretion, and urinary flow were measured at baseline and every 30 minutes for two hours after the salt load. Hemodynamics as well as urinary sodium and potassium excretion did not change over the study. Ninety minutes after the salt load, plasma AVP increased from the basal value of 6.0 ± 0.9 pg per ml to 10.1 ± 1.2 pg per ml (mean ± SE, p < 0.005) and a significant reduction in diuresis of about 50% was observed. However, plasma osmolality and plasma sodium concentration increased significantly only 120 min after the salt load, from the initial value of 277.7 ± 2.2 mOsm per kg and 145.3 ± 1.4 mEq per l (mean ± SE) to 284.8 ± 2.5 mOsm per kg and 148.7 ± 1.5 mEq per l, respectively (p < 0.01). Ninety minutes after the salt load, no correlation was found between plasma osmolality and plasma AVP concentration, indicating that AVP secretion was independent of changes in systemic blood osmolality. This phenomenon suggests that the observed increase in AVP concentration at 90 minutes was not due to a stimulation of the hypothalamic osmoreceptors since it was not accompanied by any detectable change both in plasma osmolality and plasma sodium concentration. These data suggest that osmoreceptors located in areas other than the hypothalamus (probably the portal vascular bed) may play an important role in regulating AVP secretion in response to osmotic stimuli of dietary origin.

* Address reprint requests to: Paolo Golino, M.D. Via Rocco Iemma 8, 80131 Napoli, Italy.
Introduction

A number of physiologic mechanisms are involved in maintaining the balance between intake and excretion of water and salts. Among these mechanisms, several hormones are known to play an important role in regulating body fluid homeostasis in man.

Arginine-vasopressin (AVP) is a nonapeptide whose main biological action is to conserve water and to concentrate urine. This action of AVP contributes in maintaining constant the osmolality and volume of body fluids. Under normal conditions, AVP release is primarily regulated by osmoreceptors located in the hypothalamus. It has been demonstrated that the infusion in normal subjects of hypertonic saline solutions at a constant rate leads to a release of AVP proportional to the increase in plasma osmolality. It is also well known that the reduction in plasma volume stimulates the release of AVP by reducing the tonic inhibitory impulses from the left atrium to the hypothalamus. It is believed that this action is the result of the effect of hypovolemia on stretch receptors located in the left atrium and perhaps in the pulmonary veins, as well as of the activation of carotid and aortic baroreceptors in response to hypotension.

However, it is not completely defined how osmotic stimuli of dietary origin affect AVP secretion. In a recent review, Bie suggested that the hypothesis of hypothalamic osmoreceptors could not account for all of the results obtained in studies of the relationship between plasma osmolality and AVP release. In anesthetized dogs, receptors located outside the head were reported to play an important role in the osmotic control of plasma AVP concentration, and there is some evidence of hepatic or portal osmoreceptors in rats and dogs. Other investigators, however, could not confirm their existence.

The goal of the present study was to test the hypothesis of whether or not an oral hyperosmotic stimulus would affect AVP release in man before any change in systemic plasma osmolality, thus suggesting the presence of osmoreceptors located in the portal vascular bed. Accordingly, plasma AVP concentration, plasma osmolality, and plasma sodium concentration were monitored after an acute oral salt load. Furthermore, proper consideration was given to the changes in sodium and potassium excretion as well as right atrial and pulmonary capillary wedge pressure because interference from receptors controlling systemic sodium concentration or from stretch receptors could complicate interpretation of the results.

Methods

Six normal volunteers (ages 31 to 42) entered the study. All of them gave consent after being informed of the procedure. The present study was approved by the local Ethical Committee. The patients were not taking any drugs. Five days before the study began a diet was observed with a standard content of sodium (150 mEq per day). No food or water were allowed starting from the night before the experiment. The study was conducted in a quiet room with the patients lying in the supine position.

A Swan-Ganz catheter was inserted percutaneously through the right femoral vein (using 10 ml of a two percent lidocaine solution to induce local anesthesia) and advanced into the pulmonary artery under fluoroscopic control. A urinary catheter of appropriate size was inserted via the uretra into the urinary bladder. After 60 minutes of rest, right atrial pressure and pulmonary capillary wedge pressure (Statham P23 dB transducer) and blood and urine samples were obtained to assess plasma AVP, Na⁺, and K⁺ concentration, diuresis, and uri-
nary Na\(^+\) and K\(^+\) excretion. These measurements were repeated 15 minutes later, and the two values were averaged and considered as baseline.

The patients were then given an oral salt load (350 mEq of NaCl divided in seven tablets of 50 mEq each) with a small amount of water (30 to 50 ml). Haemodynamics were recorded at 30-min intervals over two hours. At the same time intervals, 12 ml of blood were withdrawn to measure AVP, Na\(^+\), and K\(^+\) concentration. In addition, all the urine in the bladder was drained through the catheter, and the quantity was carefully measured; the urine was later used to assay its Na\(^+\) and K\(^+\) content.

**ASSAYS**

Blood samples were collected into plastic tubes containing 0.1 ml of a six percent sodium ethylenediamine tetra-acetic acid (EDTA) solution and immediately placed into ice bath. At the end of the experiment, blood samples were centrifuged and plasma and urine stored frozen at -70\(^\circ\)C.

Plasma AVP concentration was assayed in triplicate by a commercially available radioimmunoassay (RIA) kit.* The AVP was extracted from plasma samples by protein precipitation with absolute ethanol. Air-dryed extracts were then reconstituted with one ml of phosphate buffer for assay. \(^{125}\)I-arginine vasopressin was used as a tracer, and synthetic arginine vasopressin was used for the calibration curve. Anti-AVP serum was obtained from rabbits sensitized against AVP. After seven days of incubation at 4\(^\circ\)C, with the tracer added after two days, bound AVP was separated by the addition of goat gamma globulin anti-rabbit gamma globulin, and centrifugation. Bound and free fraction were counted† with a Beckman Gamma 500 gamma counter. The lower limit of sensitivity in this assay was 0.63 pg per ml. The intraassay and interassay coefficients of variation were 6.9 percent and 10.4 percent, respectively. The antiserum exhibited less than 0.01 percent cross-reactivity with oxytocin, angiotensin I, and angiotensin II, or bradykinin between concentrations of 0 to 1000 pg per ml; however, a 42 percent cross-reactivity was observed with lysine vasopressin.

Urinary and plasma concentrations of Na\(^+\) and K\(^+\) were determined by flame photometry.‡ Plasma osmolality was measured by freezing point depression with a Fiske osmometer.

**STATISTICS**

Data are expressed as mean ± standard error of the mean. Statistical comparisons were made using the one-way analysis of variance with a design for repeated measurements.

**RESULTS**

The oral salt load was well tolerated by the patients; none of them had gastric pain, nausea, or thirst during the observation period. Pulmonary capillary wedge pressure (reflecting left atrial pressure) and mean right atrial pressure did not change during the experiment (table I).

Blood hematocrit decreased significantly 120 minutes after the salt load from the baseline of 42 ± 3 percent to 37 ± 3 percent (p < 0.01, table I).

Plasma K\(^+\) concentration did not change 30, 60, and 90 minutes after the salt load, but it decreased significantly from the initial value of 4.9 ± 0.1 to 3.9 ± 0.1 mEq per l at the end of the experiment, p < 0.001 (figure 1). No signifi-

* Sorin Biomedica.
† Beckman, Gamma 500 gamma counter.
‡ Beckman, model E2 A.
Hemodynamics and Blood Hematocrit After Administration of 350 mEq of NaCl per os

<table>
<thead>
<tr>
<th>Base</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wedge capillary pressure (mmHg)</td>
<td>8 ± 1</td>
<td>7 ± 1</td>
<td>8 ± 1</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>Mean right atrial pressure (mmHg)</td>
<td>3 ± 1</td>
<td>4 ± 1</td>
<td>3 ± 1</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>42 ± 3</td>
<td>42 ± 3</td>
<td>43 ± 4</td>
<td>42 ± 2</td>
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</table>

* p < 0.01
Data expressed as mean ± standard error

Significant changes in plasma Na+ concentration were observed 30, 60, and 90 minutes after the oral salt load as compared to the baseline of 145.3 ± 1.4 mEq per l (figure 1). Only 120 minutes after the salt load, plasma Na+ concentration increased significantly, up to 148.7 ± 1.5 mEq per l, p < 0.01. This represents an increase of about two percent as compared to the initial value. Similarly, plasma osmolality showed no significant differences at 30, 60, and 90 minutes after the salt load respect to the basal value of 277.7 ± 2.2 mOsm per kg, whereas a significant increase in plasma osmolality of about 2.5 percent (284.8 ± 2.5 mOsm per kg) was observed at 120 minutes after the salt load (p < 0.01, figure 2). Interestingly, a significant increase in plasma AVP concentration occurred before any detectable changes in plasma osmolality; in fact, 90 minutes after the oral salt load, when plasma osmolality was not different from the initial value, plasma AVP increased about 60 percent, from 6.0 ± 0.9 to 10.1 ± 1.2 pg per ml (p < 0.001, figure 2).

A close correlation was found between plasma AVP and plasma osmolality before, 30, and 60 minutes after the salt load (r = 0.94, p < 0.01). At 90 minutes, however, plasma AVP increased significantly without a simultaneous increase in plasma osmolality, and the correlation between the two parameters was not significant (r = 0.71, p = NS).

In figure 3, plasma AVP and osmolality are plotted at 30, 60, and 90 minutes for each patient. It is evident that, at 90 minutes, virtually no changes in plasma osmolality occurred, whereas a sharp increase in plasma AVP concentration was observed in all patients.

Plasma AVP further increased at 120 minutes, up to 10.9 ± 1.3 pg per ml, (p < 0.001, figure 2). The increase in AVP concentration at 90 and 120 minutes after the salt load was accompanied by a significant reduction in urine excretion of about 50 percent (table II). The consequent haemodilution may explain the significant decrease in plasma K+ concentration and blood hematocrit observed at the end of the study.

![Figure 1](image1.png)

**Figure 1.** Time-course of plasma sodium and potassium concentration after administration of an oral salt load (350 mEq of NaCl) in normal human subjects (n = 6). Values are expressed as mean ± standard error of the mean. *p < 0.01 and **p < 0.001.
Sodium and potassium excretion remained unchanged throughout the study (table II).

Discussion

The goal of the present study was to test the hypothesis that AVP release in man may also be regulated by osmoreceptors located in areas other than the hypothalamus, namely, the hepatic-portal vascular bed.

The possibility that the portal vascular bed may contribute in regulating water balance was first suggested by Haberich in 1968. This investigator showed that intraportal infusion of hypotonic solutions led to an earlier and higher increase in diuresis as compared to infusion into the inferior vena cava. Subsequently, other investigators have described evidence for peripheral osmoreceptors located in the portal area. The evidence for these receptors is largely based upon experiments in which intraportal infusions of hypotonic or hypertonic solutions were linked to changes in urine flow, plasma AVP concentration, or neural activity of the hypothalamoneurohypophyseal tract. However, it should be pointed out that the osmotic stimuli used in the previously mentioned studies are somehow "unphysiologic". In fact, changes in the composition and osmolality of the portal blood normally represent the net result of intestinal absorption and secretion of fluids and solutes. Portal infusions merely serve as a way of imitating these processes under more precisely defined conditions. Under these circumstances, the actual physiologic contribution of the entero-hepatic circulation per se may be questioned. Furthermore, very little is known about this issue in humans. With that in mind, the hyperosmotic stimulus used in the present study, i.e., 350 mEq of NaCl per os, is to be considered a more physiologic one, since it was given...
TABLE II

Urine Flow, Na\(^+\) and K\(^+\) Urinary Excretion After Administration of 350 mEq of NaCl per os

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Base</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine flow (ml/min)</td>
<td>1.30 ± 0.34</td>
<td>1.47 ± 0.48</td>
<td>1.15 ± 0.37</td>
<td>0.60 ± 0.12</td>
<td>0.60 ± 0.11*</td>
</tr>
<tr>
<td>Na(^+) Urinary excretion (mEq/min)</td>
<td>0.15 ± 0.03</td>
<td>0.14 ± 0.05</td>
<td>0.13 ± 0.04</td>
<td>0.16 ± 0.04</td>
<td>0.14 ± 0.05</td>
</tr>
<tr>
<td>K(^+) Urinary excretion (mEq/min)</td>
<td>0.06 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.03 ± 0.01</td>
</tr>
</tbody>
</table>

*\(p < 0.005\)
Data expressed as mean ± standard error

per os and, therefore, takes into account the intestinal absorption and secretion of fluids and solutes.

In discussing the data of the present study, proper consideration should be given to two findings. First, plasma AVP concentration sharply increased at 90 minutes after the salt load; this increase occurred in all the subjects when no detectable changes both in systemic plasma osmolality and Na\(^+\) concentration were observed. It is important to emphasize that, as expected,\(^9\) a close correlation was found between plasma AVP and osmolality before, 30, and 60 minutes after the salt load, whereas these parameters were not correlated at 90 minutes, indicating that the increase in AVP concentration at this time was independent of changes in plasma osmolality. Furthermore, the increase in AVP concentration at 90 minutes was most likely independent of volume expansion of the intravascular fluid, since no changes in pulmonary capillary wedge pressure and right atrial pressure and hematocrit were observed at this time of the study.

Second, systemic blood osmolality remained unaltered for a relatively long period of time. In fact, in the present study, plasma osmolality increased only 120 minutes after the salt load. These data indicate that the entero-hepatic circulation plays an important role in buffering abrupt changes in systemic blood osmolality as a consequence of dietary intake of fluids and solutes.

A limitation of the present study is represented by the fact that, owing to its intrinsic difficulties, portal osmolality was not measured. Consequently, direct evidence is not available that portal osmolality increased earlier than systemic osmolality after the salt load. However, it has been demonstrated in dogs that a solute load related to food intake produced a larger and earlier increase in portal blood osmolality than that observed in the systemic venous blood.\(^4\) It is, therefore, very likely that portal osmolality increased in a similar fashion in our patients.

Another potential problem of this study is that the salt load could have stimulated the release of gastrointestinal hormones which could have interfered with the AVP assay. Although data are not available regarding the cross-reactivity of gastrointestinal hormones with the anti-AVP serum, the fact that urine flow decreased by 50 percent at 90 min strongly suggests that the substance measured in plasma at that time period was actually AVP.

The importance of osmotic pressure in regulating AVP release has been recognized for many years, and several studies have been conducted to evaluate the changes in AVP secretion after various osmotic stimuli. It is well known that the secretion of vasopressin is controlled by
a very sensitive mechanism that is able to detect and respond to changes in plasma osmolality as little as one percent. From pioneering studies of Verney and other evidence, it has been concluded that the osmoreceptors are located in the anterior part of the brain, presumably in the hypothalamus. The AVP secretion is also influenced by changes in blood volume, and this interacts with blood osmolality in regulating plasma AVP concentration.

Evidence has accumulated over the past decade indicating that signals which modify AVP release may arise not only in the hypothalamic receptors, as first proposed by Verney, but also in an area located somewhere within the portal vascular bed. It has, in fact, been suggested that the hypothesis of hypothalamic osmoreceptors could not entirely explain the results obtained in studies on the relationship between plasma osmolality and AVP release. Haberich demonstrated in rats that the diuretic effects of hypotonic fluid loading were more pronounced if the fluids were infused into the portal vein compared to systemic vein infusion. In anesthetized dogs, receptors located outside the head were reported to play an important role in the osmotic control of plasma AVP concentration. Recently, portal osmoreceptors have been demonstrated in rats and conscious dogs. In particular, the infusion of hypertonic saline into the portal vein of conscious dogs was associated with a prompt increase in plasma AVP concentration in absence of changes in systemic blood osmolality. The maximal increase was observed five minutes after starting the infusion; subsequently, AVP concentration decreased rapidly despite continued infusion and was back at the control level after 10 minutes. Infusion of a more concentrated solution produced another transient elevation of plasma AVP concentration. Interestingly, no changes in plasma AVP were observed if the same experiment was repeated after chronic surgical denervation of the liver, thus indicating a reflex nature of the observed phenomenon.

In conclusion, data from the present study suggest the presence of osmoreceptors located in areas other than the hypothalamus (probably the portal vascular bed) and that they can be activated by an oral salt load. The physiological role of such osmoreceptors in responding to osmotic stimuli of dietary origin may be relevant.

References

12. Schneider, E. G., Davis, J. O., Robb, C. A.,


