Centrally Infused Atrial Natriuretic Polypeptide Attenuates Exaggerated Salt Appetite in Spontaneously Hypertensive Rats

HIROSHI ITOH, KAZUWA NAKAO, GORO KATSUURA, NARITO MORII, SHOZO SHIONO, MAKOTO SAKAMOTO, AKIRA SUGAWARA, TAKAYUKI YAMADA, YOSHIHIKO SAITO, AKIRA MATSUSHITA, AND HIROO IMURA

We have previously shown that atrial natriuretic polypeptide is present in the brain with the highest concentration in the hypothalamus and septum and that intracerebroventricular injection of atrial natriuretic polypeptide inhibits water drinking induced by centrally injected angiotensin II or 24-hour water deprivation in rats. To study further the role of brain atrial natriuretic polypeptide in the control of water and electrolyte balance, the effect of chronic intracerebroventricular infusion of atrial natriuretic polypeptide on salt appetite in spontaneously hypertensive rats and normotensive Wistar-Kyoto rats was examined with a free-choice, two-bottle preference test. The intracerebroventricular infusion of 100 ng/hour and 500 ng/hour of α-human atrial natriuretic polypeptide preferentially suppressed the intake of 0.30 M NaCl solution and attenuated the elevated preference for the hypertonic saline in spontaneously hypertensive rats while centrally infused α-human atrial natriuretic polypeptide had no significant effects on drinking behavior in Wistar-Kyoto rats. Blood pressure did not change significantly throughout the experiment in either rat strain. It is concluded that the exaggerated salt appetite in spontaneously hypertensive rats is blunted by centrally administered atrial natriuretic polypeptide. Such an effect of atrial natriuretic polypeptide along with its antidiuretic effect suggests that brain atrial natriuretic polypeptide plays a role in water and electrolyte homeostasis and in blood pressure control. (Circulation Research 1986; 59:342-347)

From the 2nd Division, Department of Medicine, Kyoto University School of Medicine, Kyoto (H.I., K.N., N.M., S.S., M.S., A.S., T.Y., Y.S., H.I.), Radiisotope Research Center, Kyoto University, Kyoto (K.N.), and Shionogi Research Laboratories, Shionogi & Co., Ltd., Osaka, Japan (G.K., A.M.).


Address for reprints: Kazuwa Nakao, M.D., Ph.D., 2nd Division, Department of Medicine, Kyoto University School of Medicine, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606, Japan. Received December 30, 1985; accepted June 10, 1986.

of circulating ANP originating from the atrium by showing that the major component of ANP in the brain is of small molecular size and the regulation of ANP levels in the brain differs from that in the atrium.\textsuperscript{14,15} We further confirmed the widespread distribution of ANP using an immunohistochemical method.\textsuperscript{16,17} ANP immunoreactive cell bodies and varicose fibers are most prevalent in the preoptic and anterior hypothalamic areas, corresponding to the anteroventral third ventricular region (AV3V), which is known to be involved in the maintenance of body fluid homeostasis and blood pressure.\textsuperscript{18} In addition, we demonstrated that intracerebroventricular (ICV) administration of ANP suppressed water-drinking induced by angiotensin II or 24-hour water deprivation in rats\textsuperscript{19} and antisera for ANP administered intracerebroventricularly enhanced water intake.\textsuperscript{20} From these findings stemmed the impetus to investigate the effect of centrally administered ANP on salt appetite in SHR in order to clarify the possible role of brain ANP in water and electrolyte handling and blood pressure control as a neuropeptide.

Materials and Methods

Twenty-six male SHR (347 ± 4 g) at the age of 17 weeks and 16 age-matched male normotensive WKY (328 ± 7 g) were used. Animals were housed individually and fed standard rat chow CA-1 (Japan CLEA, Tokyo, Japan) containing 0.50% sodium and 0.84% potassium ad libitum. They were maintained under conditions of 12 hours of light and 12 hours of dark (light on 7:00 AM to 7:00 PM) in a temperature-controlled (25 ± 1°C) room.

---

**EXAGGERATED** preference for salt or stronger "need-free" innate salt appetite in spontaneously hypertensive rats (SHR) compared with Wistar-Kyoto rats (WKY) has been reported by several authors\textsuperscript{1-3} and has attracted attention as a possible factor in the development of hypertension.\textsuperscript{3-5}

de Bold et al discovered that extracts of rat atria possess potent diuretic, natriuretic, and vasorelaxant activities.\textsuperscript{4} This discovery led to the isolation of multiple forms of atrial natriuretic polypeptide (ANP) in both rat and human atria.\textsuperscript{5-6} We developed a radioimmunoassay (RIA) for α-ANP with 28 amino acids\textsuperscript{7} and reported that ANP is released from the heart and circulates in the body as a hormone.\textsuperscript{8} We also demonstrated that ANP is present not only in the atrium but also in the brain,\textsuperscript{12} especially in the hypothalamus and septum, and suggested that brain ANP is independent
Procedure
A test period consisted of 9 consecutive days following a 3-day acclimation period. The rats were offered a choice of 0.30 M NaCl solution and tap water to drink in a free-choice, two-bottle preference test during both the control and ICV infusion periods. Intakes of 0.30 M NaCl solution and water were measured to the nearest 0.1 ml daily at 10:00 AM. Blood pressure was measured every 2 days by the tail cuff method. Values for the first 2 days were averaged and served as a control. On the third day of the test period, the rats were anesthetized with sodium pentobarbital (Nembutal, Pitman-Moore, 50 mg/kg intraperitoneal), and a stainless steel cannula with an outer diameter of 0.3 mm was stereotaxically implanted into the left lateral cerebral ventricle through a guide cannula (0.7 mm o.d.) with the coordinates 6.5 mm anterior to the lambdoidal suture, 1.3 mm lateral to the midline, and 4.5 mm below the skull surface following the procedure previously reported.21 An Alzet osmotic minipump (Alza) was inserted subcutaneously into the interscapular region and connected to the intracranial cannula by P.E. 60 tubing. After the surgery, the animals were returned to their individual cages and the measurement continued for a further 7 days. At the end of the infusion, the pumps were removed and the correct delivery of the solution was confirmed. α-Human ANP (α-hANP) (Protein Research Foundation, Osaka, Japan) dissolved in isotonic saline or isotonic saline as a vehicle control was delivered by the osmotic minipump at the rate of 1 μl/hour for 7 days. SHR were divided into three groups; Group I (n = 7) received isotonic saline as a vehicle, Group II (n = 9) received 100 ng/hour of α-hANP, and Group III (n = 10) received 500 ng/hour of α-hANP. WKY were divided into two groups; Group I (n = 9) received isotonic saline and Group II (n = 7) received 500 ng/hr of α-hANP. Results are expressed as mean ± SE for each group. Intakes of water, 0.30 M NaCl solution, and total fluid (water plus 0.30 M NaCl solution) were expressed per 100 g of body weight and preference for 0.30 M NaCl was expressed in percent as follows: Preference for 0.30 M NaCl = 100 x 0.30 M NaCl solution consumed/total fluid consumed.

Statistical Analysis
Daily intakes of water, 0.30 M NaCl solution, and total fluid and daily preference for 0.30 M NaCl were assessed by a one-way analysis of variance of split-type design for the multigroup repeated measurements study. Cumulative intakes of water, 0.30 M NaCl solution, and total fluid and changes of blood pressure were assessed by Dunnet's test following a one-way analysis of variance for multiple comparisons.22 Differences in mean preference for 0.30 M NaCl between the control and ICV infusion periods were assessed by Student's paired t test.

Results
Drinking Behavior in the Control Period
In the control period, SHR (n = 26) drank 5.5 ± 0.8 ml/100 g b.wt. per day of 0.30 M NaCl solution and 12.0 ± 0.7 ml/100 g b.wt. per day of total fluid, while WKY (n = 16) consumed 0.8 ± 0.3 ml/100 g b.wt. per day of 0.30 M NaCl solution and 10.0 ± 0.5 ml/100 g b.wt. per day of total fluid. Thus, preference for 0.30 M NaCl was 42.3 ± 3.3% in SHR and 6.7 ± 2.9% in WKY. Therefore, SHR consumed a significantly larger volume of 0.30 M NaCl solution and total fluid (p < 0.05, respectively) and showed nearly sevenfold higher preference for 0.30 M NaCl than WKY.

Effects of the ICV Infusion of α-hANP on Fluid Intake
The effects of centrally infused α-hANP on daily intakes of water, 0.30 M NaCl solution, and total fluid and daily preference for 0.30 M NaCl in SHR and WKY are shown in Figure 1. In SHR, in the control period, there were no differences in intakes of water and 0.30 M NaCl solution among the three groups. General suppressive effects of surgery on fluid intake were equally observed in all three groups on the 1st day of the ICV infusion. Thereafter, SHR in Group I (saline) resumed drinking water and 0.30 M NaCl solution comparable to those in the control period. In contrast, SHR in both Group II (α-hANP, 100 ng/hour) and Group III (α-hANP, 500 ng/hour) showed the tendency of suppressed intakes of 0.30 M NaCl solution and total fluid and decreased preference for 0.30 M NaCl throughout the infusion period (0.05 < p < 0.15). The water intake in those two groups during the infusion did not differ from that of saline-infused SHR or from those in the control period.

Cumulative intakes of water, 0.30 M NaCl solution, and total fluid in SHR and WKY are shown in Figure 2 and Table 1. The suppressive effect of centrally administered α-hANP on the intake of the hypertonic saline was evident. Compared to Group I, a significant reduction of the intake of 0.30 M NaCl solution was observed first on the 5th day in Group II (p < 0.05) and on the 4th day in Group III (p < 0.05), and this trend persisted until the end of the infusion. Concomitantly, a significant decrease of the intake of total fluid was observed as early as on the 2nd day in Group II (p < 0.05) and on the 3rd day in Group III (p < 0.05). At the end of the 7-day infusion, the cumulative intakes of 0.30 M NaCl solution and total fluid in both Group II and Group III of SHR were significantly different from those of SHR in Group I (p < 0.05, for both Group II and Group III), while there was no significant difference in water intake among the three groups (Table 1). This attenuated salt intake during the infusion was reflected by a reduction of salt preference as shown in Table 2. Mean preference for 0.30 M NaCl during the infusion in Groups II and III of SHR was significantly lower than the corresponding value during the control period (p < 0.05, p < 0.025, respectively), whereas saline-infused rats showed no significant change in salt preference.

In contrast to SHR, WKY showed no differences in intake of water or 0.30 M NaCl solution between before and during the infusion or between Groups I and II.
Effects of the ICV Infusion of α-hANP on Blood Pressure

In the pre-infusion period, there were no differences in blood pressure among the three groups of SHR (Group I, 187 ± 2 mm Hg; Group II, 186 ± 2 mm Hg; Group III, 188 ± 3 mm Hg) or between the two groups of WKY (Group I, 145 ± 2 mm Hg; Group II, 148 ± 2 mm Hg). No significant change was observed in blood pressure on the 7th day of α-hANP infusion compared to that in the pre-infusion period in either SHR (Group I, -3.7 ± 7.1 mm Hg; Group II, +3.1 ± 4.6 mm Hg; Group III, +0.2 ± 7.0 mm Hg) or WKY (Group I, +1.6 ± 2.4 mm Hg; Group II, -4.9 ± 3.5 mm Hg).

Discussion

The present study demonstrated that chronic ICV administration of α-hANP (100 ng/hour and 500 ng/hour) suppresses the exaggerated intakes of hypertonic saline and total fluid and attenuates elevated salt preference in SHR. The findings that there are no changes of water intake in SHR and no influence of α-hANP administration on the drinking behavior in WKY rule out the possible nonspecific inhibitory effects on fluid intake behavior induced by the peptide.

In this study, α-hANP of 100 ng/hour and a fivefold higher dose (500 ng/hour) had approximately the same magnitude of inhibitory effect on salt appetite. In our preliminary experiment, 20 ng/hour of α-hANP did not exhibit a sufficient inhibitory effect. Although no dose-response relation was observed, doses around 100 ng/hour are considered to be required in the reduction of salt appetite in SHR.

On the 7th day of the infusion, the intake of 0.30 M NaCl solution of SHR in Group I appeared to be suppressed. During the experiment, SHR exhibited higher preference for 0.30 M NaCl than WKY, but there existed day-to-day variation in the intake of 0.30 M NaCl solution of SHR. This variation of salt intake of SHR has been observed not only by us but also by others. Catalanotto et al reported that preference of SHR for 0.30 M NaCl ranged from 45 to 62%, and DiNicolantonio et al showed that the intake of 0.9% saline varied from about 18 to 26 ml/day per 100 g b wt. in the ICV infusion. So, low intake of 0.30 M NaCl solution of SHR in Group I on the 7th day of the infusion might be accounted for by this variation. Furthermore, we could not observe such a sudden decline of the intake of hypertonic saline in another series of experiments.

It is possible that centrally administered ANP leaked...
into the peripheral circulation and lowered blood pressure, which, in turn, reduced exaggerated diuresis and natriuresis in SHR and attenuated sodium intake, assuming that the increased sodium intake in SHR is the consequence of exaggerated natriuresis. This seems unlikely, however, because the reduction of salt appetite did not accompany appreciable changes of blood pressure in this study, whereas intravenous administration of 100 ng/hour of atrial natriuretic factor, the same dose we employed, is known to normalize blood pressure in SHR without significant changes of diuresis and natriuresis.

The mechanism responsible for the manifestation of salt appetite is not fully understood. Preference for salt in rats was reported to be elicited by a variety of experimental interventions; adrenectomy, salivarectomy, ICV administration of angiotensin II, treatments with angiotensin-converting enzyme inhibitors, relatively high doses of deoxycorticosterone acetate, diuretics, and so forth. In the enhancement of salt preference,
peripherally, the salivary sodium concentration, which modulates the gustatory input to the central nervous system, and plasma aldosterone level, and centrally, the brain renin–angiotensin system (RAS) may be involved.

There are several lines of evidence suggesting an overactivity of brain RAS in SHR, including hypertensive effects of ICV administration of an angiotensin II receptor antagonist, salarasin, or angiotensin-converting enzyme inhibitors, and increased immunoreactive angiotensin II containing neurons in the hypothalamus. The enhanced salt preference of SHR was reported to be suppressed by ICV infusion of an angiotensin II-converting enzyme inhibitor, captoril. These findings suggest the role of enhanced brain RAS in SHR in manifesting the development of hypertension and the exaggerated salt appetite. Recently, we have found elevated levels of ANP in the hypothalamus and septom in SHR compared with WKY, and this difference was observed as early as 4 weeks of age — before the development of hypertension. Thus, brain ANP might hold the key to elucidating the mechanism of the altered salt appetite in SHR.

During the preparation of this paper, Fitts et al reported the inhibitory effect of atriopeptin II on saline intake in sodium-depleted rats. In their experiment, salt appetite was elicited in sodium-deficient state by the administration of furosemide and a low sodium diet. Craving for salt in such state vanishes after sodium repletion. We have examined innate “need-free” salt appetite in SHR to investigate the role of ANP in sodium intake and blood pressure control. While the effect of atriopeptin II in their experiment was transient and no effect on normally hydrated rats was observed, we could observe a sustained effect of α-hANP to suppress salt appetite in SHR over days. Our results of the selective attenuation of salt appetite in SHR by chronic infusion of α-hANP suggest an antagonistic relation between ANP and angiotensin II in the brain, like the peripheral opposing effects of ANP to angiotensin II on aldosterone secretion and vasoconstriction. Previous works of the suppressive effect of ICV injection of α-hANP on water intake induced by angiotensin II or water deprivation also support this hypothesis.

The relation between salt appetite and hypertension is widely recognized but poorly understood. There is some evidence indicating that development of hypertension in SHR is retarded by a low sodium diet and accelerated by a high sodium diet and saline. In humans reduction of sodium intake is widely recommended for the management of hypertensives, and some investigators have postulated that the salt recognition thresholds of hypertensives are higher compared to those of normotensives. However, no significant relation between salt intake and blood pressure was found. Our results showed that the reduction of sodium intake was not coupled with the change of blood pressure. But the observation period was rather short in the present experiment, and our results do not exclude the possible contribution of salt appetite to the development of hypertension in SHR. The role of brain ANP in blood pressure control is now under investigation in our laboratory.

It is too soon to draw any conclusion on the physiological role of brain ANP from our present study. Further studies are necessary to determine whether or not endogenous brain ANP does actually modulate salt appetite.

Acknowledgments
We thank Mrs. Hiroko Tabata and Miss Kazuko Horii for the secretarial assistance.

References
9. Kangawa K, Matsuo H: Purification and complete amino acid

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatment group</th>
<th>No. of rats</th>
<th>Control period</th>
<th>ICV infusion period</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR</td>
<td>Group I (saline)</td>
<td>7</td>
<td>42.7 ± 6.7</td>
<td>37.6 ± 3.6</td>
</tr>
<tr>
<td></td>
<td>Group II (α-hANP 100 ng/hour)</td>
<td>9</td>
<td>39.9 ± 5.7</td>
<td>27.9 ± 2.7†</td>
</tr>
<tr>
<td></td>
<td>Group III (α-hANP 500 ng/hour)</td>
<td>10</td>
<td>44.3 ± 5.7</td>
<td>29.9 ± 3.6‡</td>
</tr>
<tr>
<td>WKY</td>
<td>Group I (saline)</td>
<td>9</td>
<td>9.3 ± 4.8</td>
<td>6.5 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>Group II (α-hANP 500 ng/hour)</td>
<td>7</td>
<td>3.4 ± 1.9</td>
<td>6.9 ± 2.6</td>
</tr>
</tbody>
</table>

*The ratio of 0.30 M NaCl solution intake to total fluid (water plus 0.30 M NaCl solution) intake. Values are the means ± SE.
†‡Significantly different from control period: †p < 0.05, ‡p < 0.025.
Salt Appetite and Atrial Natriuretic Polypeptide

Itoh et al

sequence of α-human atrial natriuretic polypeptide (α-hANP).

Biochem Biophys Res Commun 1984;118:131–139


Key Words • atrial natriuretic polypeptide • salt appetite • spontaneously hypertensive rats • water and electrolyte • blood pressure
Centrally infused atrial natriuretic polypeptide attenuates exaggerated salt appetite in spontaneously hypertensive rats.

H Itoh, K Nakao, G Katsuura, N Morii, S Shiono, M Sakamoto, A Sugawara, T Yamada, Y Saito and A Matsushita

_Circ Res._ 1986;59:342-347
doi: 10.1161/01.RES.59.3.342

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1986 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/59/3/342