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Diuretic and Natriuretic Action of Rat Atrial Natriuretic Peptide (6–33) Administered Intracerebroventricularly in Rats

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ISRAEL, A. AND Y. BARBELLA. *Diuretic and natriuretic action of rat atrial natriuretic peptide (6–33) administered intracerebroventricularly in rats.* BRAIN RES BULL 17(2) 141–144, 1986.—Intracerebroventricular (IVT) administration of rat atrial peptide (6–33) (rANP) to conscious male hydrated or salt-loaded rats, resulted in significant increase in urinary volume. The diuretic effects of rANP occurred during the 3 hr period of urine collection and were most effective during the first hour. Most remarkably, rANP given IVT produced a dose-related increase in urinary sodium excretion at 3 hr. With a high dose of rANP, kaliuresis was significant only at 3 hr. Our results strongly suggest that ANF may play a significant role in central regulation of fluid homeostasis, and that its natriuretic and diuretic effects may be, at least in part, centrally mediated.

Atrial natriuretic peptide Natriuresis Diuresis Intracerebroventricular administration Fluid homeostasis

ATRIAL cardiocytes contain several peptides with natriuretic and diuretic activity [1,10]. Purification and amino acid sequence analysis of several related active peptides have been reported [2]. It has been proposed that some of these peptides play active roles in control of extracellular fluid volume, electrolyte homeostasis and vascular function [1,10]. Atrial natriuretic factor (ANF) is released from the atria in response to an increase in blood volume [8]. The main action of ANF is, in general, to promote reduction of plasma and extracellular fluid volume by eliciting natriuresis and diuresis [3], acting as physiological antagonist of the peripheral renin-angiotensin system [1], and by opposing the action of vasopressin [13], and aldosterone secretion from the adrenal cortex [1,10].

However, in addition to the peripheral effects, certain fluid and electrolyte metabolic effects of ANF could be centrally mediated. In the brain, there is a widespread network of ANF-containing neurons [7,14]. Binding sites for ANF (8–33) [11] and rANP (6–33) [12] were recently localized in specific brain areas, such as the subfornical organ, a circumventricular structure involved with the regulation of blood pressure, fluid metabolism and vasopressin secretion [15]. ANF modulates vasopressin secretion acting at the hypothalamic levels [13]. In addition, there is recent evidence of an inhibitory effect of ANF, administered IVT, on spontaneous or angiotensin-II-stimulated water intake and salt appetite [4,9].

The present study was designed to determine the effects of intracerebroventricular (IVT) administration of rANF (6–33) on urine volume and sodium excretion in both conscious hydrated and salt-loaded rats.

METHOD

Adult male Sprague-Dawley rats (230–290 g) were housed at a constant temperature with light on from 06.00 hr to 18.00 hr and given free access to food and water. In the group of rats where NaCl treatment was studied, a 1% NaCl solution was substituted for the drinking water during the one week period previous to the IVT. A cannula [15] was implanted in the left lateral cerebroventricle, 1 mm caudal to the coronal suture and 1.5 mm lateral to the midsagittal suture, with the aid of a stereotaxic instrument and under pentobarbital anesthesia (40 mg/kg, IP). Acrylic cement was used to secure the cannula to the skull. A minimum of 2 days was allowed for recovery. Single IVT injections were made with a Hamilton syringe fitted with a stop to prevent needle penetration past the cannula tip. Saline solution or rANP (rat atrial peptide, 28 amino acids, Peninsula Laboratories, Inc., Belmont, CA) was given in 5-μl volumes. Ventricular cannula place-

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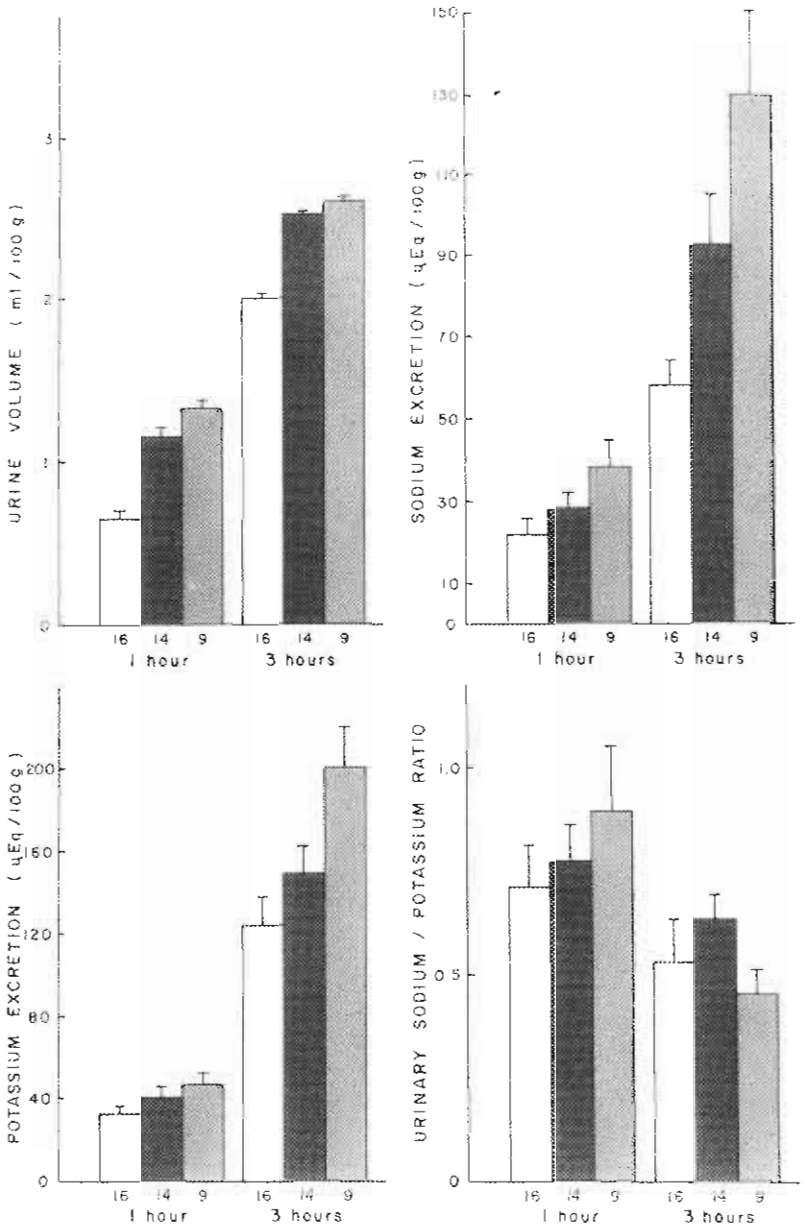

FIG. 1. Effect of a single cerebroventricular injection of rat atrial natriuretic peptide (rANP) (6–33) on urine output, sodium and potassium excretion and Na/K ratios in hydrated rats. Rats recieved an intracerebroventricular injection of saline solution (open column), 0.25 μg/5 μl (dark shaded column) or 1.25 μg/5 μl (light shaded column) of rANP immediately after 20 ml/kg PO water. Food and water were not allowed. Urine was collected after one and three hours. Significant F ratios from one-way analysis of variance were: urinary volume, 1 hr and 3 hr, $F(2,37)=7.6$, $p<0.01$ and 3.28, $p<0.05$ (control<dose-1=dose-2, Newman-Keul's test), sodium excretion, 3 hr, $F(2,37)=7.68$, $p<0.01$ (control<dose-1<dose-2, Newman-Keul's test) and potassium excretion, 3 hr, $F(2,37)=5.46$, $p<0.01$. Number under the bottom of the bars represent animals per group.

ment was confirmed post mortem by examining the distribution of an IVT injection of 5 μl of fast green dye, given before sacrificing the animal. Data were used only if the dye was distributed in the lateral, third and fourth ventricles.

Protocol for rANP Experiment

Rats, with their ventricular cannula, were weighed and placed in individual metabolism cages. At 0900 hr the rats were injected IVT with freshly prepared rANP in saline solution (0.25 μg/5 μl or 1.25 μg/5 μl) or saline (5 μl), followed by 20 ml/kg PO water. Urine was collected at 1 and 3 hr; in addition the bladder was emptied at 3 hr by gentle suprapubic massage. Food and water were not available during the experiment. Urine samples were assayed for sodium

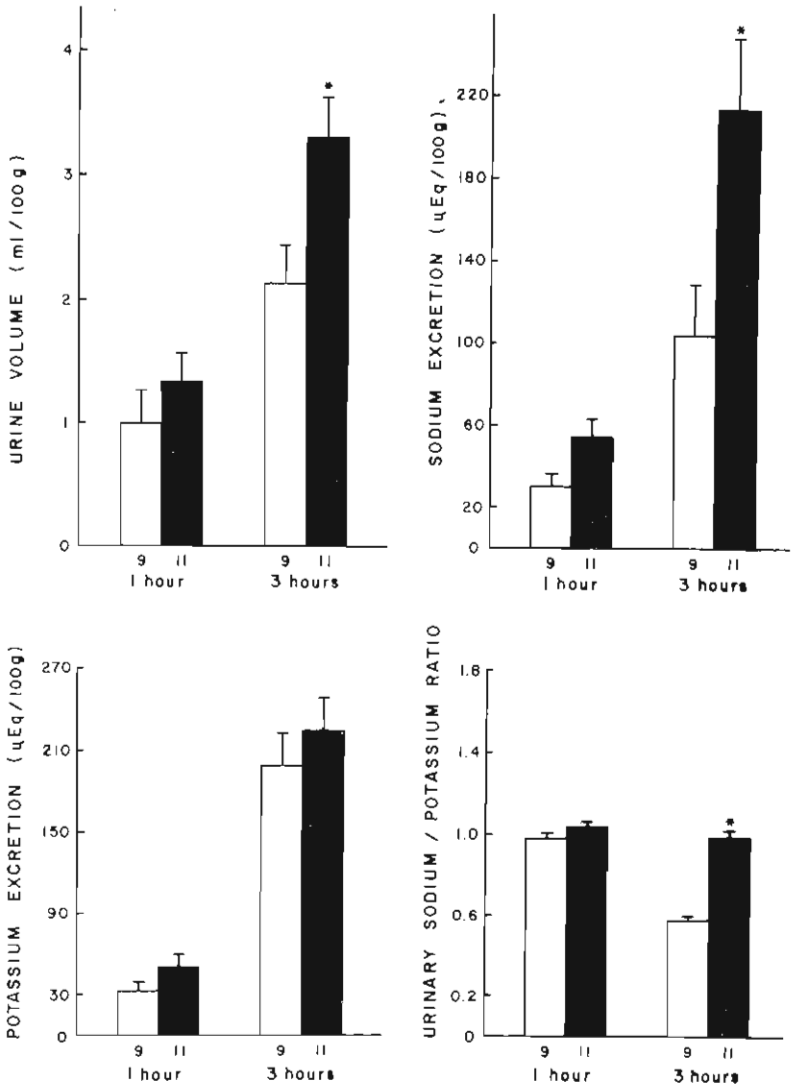

FIG. 2. Urinary output, sodium and potassium excretion and Na/K ratio before and after a single intracerebroventricular injection of rANP (6–33) in salt-loaded rats. Rats recieved an intracerebroventricular injection of saline solution (open column) or 0.25 μg/5 μl of rANP (dark column) immediately after 20 ml/kg PO water. Food and water were not allowed. Urine was collected after one and three hours. Significant differences (Student's t-test) are indicated ($^*p<0.05$). Numbers under the bottom of the bars represent animals per group.

and potassium content by flame photometry.

All the data were presented as mean±S.E.M. Statistical differences between groups were analyzed using the Student's t-test or by one-way analysis of variance (ANOVA) and the Newman-Keul's studentized range statistic.

RESULTS

Effects of IVT rANP in Hydrated Rats

Urine was collected after 0–1 and 1–3 hour periods of single IVT injection of rANP. Most of the effect of rANP on urinary volume appeared to occur within the first hour. The urinary response to IVT rANP are illustrated in Fig. 1. The volume of urine/100 g b.w. increased significantly with both 0.25 μg/5 μl and 1.25 μg/5 μl doses of rANP, at 1 and 3 hr periods of collection, $F(2,37)=7.6$, $p<0.01$, and $F(2,37)=3.28$, $p<0.05$, respectively (one-way ANOVA), but the volumes for the two rANF doses did not differ from one another (control<dose-1=dose-2, Newman-Keul's test).

The increased urinary volume was associated with an enhanced natriuresis and kaliuresis at 3 hr. Urinary sodium excretion (μEq/100 g b.w.) increased significantly at the 3 hr period, $F(2,37)=7.68$, $p<0.01$ (one-way ANOVA) and the increase was dose-related, (control<dose-1<dose-2, Newman-Keul's test). Although there were no significant differences in urinary sodium excretion at the 1 hr period of collection, there was a trend towards an increase. rANP-induced kaliuresis was significant only at 3 hr and with the highest dose of rANP. The urinary Na/K ratio was unaffected by either dose of rANP, in both the 1 and 3 hr periods of collection ($p>0.05$, one-way ANOVA).

Effect of rANP in Salt-Loaded Rats

Urinary sodium excretion in salt-loaded rats increased significantly when compared with normal hydrated rats (29% and 80% at 1 and 3 hr of urine collection, respectively, $p<0.01$). The effects of 0.25 μg/5 μl IVT of rANP in NaCl-loaded rats are illustrated in Fig. 2. rANP induced a significant increase in urinary volume associated with an enhanced natriuresis but not with kaliuresis. The volume of urine/100 g b.w. and the sodium excretion (μEq/100 g b.w.) increased significantly at the 3 hr period of urine collection ($p<0.05$). Evaluation of the urinary Na/K ratio at 3 hr revealed a significant increase ($p<0.05$).

DISCUSSION

In the present study we demonstrate that a single IVT injection of rANP (6–33) induced a significant increase in urine volume, urinary sodium excretion and kaliuresis in conscious rats. ANP (6–33) closely resembles rat ANF (8–33), containing at the N-terminus only two more amino acids (Ser-Leu) than this peptide [1,10].

The observation that central administration of rANP has a significant effect on urine volume and sodium excretion strongly suggests that ANF in the brain may serve as an endogenous defense against hypervolemia and states of sodium excess. The central mechanism could facilitate the reduction of blood volume by increasing the excretion of water and solute, and by reducing the further intake of salt [4] and water [9]. All these central effects, added to the already known peripheral natriuretic and diuretic action of the peptide [1, 3, 10], would form part of the complex regulatory role of ANF in fluid homeostasis.

The mechanisms of the CNS-mediated diuresis may be explained, at least in part, by a suppression of vasopressin secretion. ANF has been reported to decrease vasopressin release through a central mechanism [13]. In addition, ANF may act in opposition to the renin-angiotensin system in the brain [1].

Receptors for ANF have now been described and characterized in the rat brain [11,12]. Binding was concentrated in the subfornical organ and the choroid plexus [12]. The subfornical organ sends projection to the hypothalamus, and specifically to the anteroventral third ventricle (AV3V) region, an area critical for the development and maintenance of experimental hypertension as well as fluid and electrolyte balance [5]. This area contains the largest concentration of ANF-positive neurons [7,14], ANF-receptors [11,12] and a high density of ANG binding sites [6]. Thus, these structures may represent the anatomical link between the peripheral and central ANF system and the site for the antagonistic interaction between ANG and ANF.

In the periphery, ANF directly and selectively reduces basal and ANG-stimulated secretion of aldosterone in the adrenal cortex [1,10]. A possible mechanism for central natriuretic effects of rANF is withdrawal of mineralocorticoid activity in the kidneys, perhaps by a centrally mediated reduction of plasma aldosterone levels.

The fact that ANF seems to act both peripherally and centrally to antagonize the action of ANG suggests that this peptide could have a major and important role in long term regulation of fluid and electrolytes balance.

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