Effect of Changes in Sodium Intake on Atrial Natriuretic Factor (ANF) and Peptides Derived from the N Terminus of the ANF Prohormone in the Rat (43392)

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Abstract. **The purpose of the present study was to determine whether variations in salt intake would alter the plasma concentrations of atrial natriuretic factor and the N-terminal atrial natriuretic factor prohormone peptides proANF 1-98 and proANF 31-67. Two groups of rats were placed on different salt intakes for 1 week. The low salt group of rats was fed a diet providing <0.1 m*M* NaCl/day and given deionized water to drink. The normal salt group of rats was fed regular rat chow with deionized water to drink, providing them with approximately 2 m*M* NaCl/day. Plasma atrial natriuretic factor was 204 ± 60 pg/ml (mean ± SE) in normal salt rats and was significantly lower in the low salt group (44 ± 13 pg/ml, $P < 0.01$). ProANF 1-98 was also significantly higher in the normal salt group (635 ± 47 pg/ml) compared with the low salt group (353 ± 33 pg/ml, $P < 0.01$). ProANF 31-67 was 123 ± 21 pg/ml in the normal salt group and 59 ± 12 pg/ml in the low salt group ($P < 0.05$). Plasma renin activity in ng angiotensin I/ml/hr averaged 1.80 ± 0.15 in the normal salt group of rats and was significantly higher in the low salt group of rats (5.66 ± 1.07, $P < 0.05$).**

These results suggest that atrial natriuretic factor and the atrial natriuretic factor prohormones may play a role in the physiological adjustments to low salt intake.

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Atrial natriuretic factor (ANF) is a peptide hormone secreted by the heart in response to atrial stretch (1, 2). ANF appears to play a major role in the natriuretic response to acute volume expansion, since this response can be attenuated by either atrial appendectomy (3, 4) or by infusion of antibodies directed against ANF (5). Evidence that ANF plays an important role in chronic adjustments to increases or decreases in sodium chloride intake is more controversial (6). Some studies in both animals (7) and humans (8) have found increased plasma ANF concentrations in response to elevated sodium chloride intake. However, others have found that increases in sodium intake had no effect on plasma ANF in either the rat (9), dog (10), or human (11).

The 28-amino acid (aa) peptide termed ANF is produced in the heart as part of a larger 126-aa prohormone, i.e., proANF. ANF is the 99–126-aa C terminus of the proANF molecule. Recent evidence suggests that the remaining 1–98-aa portion of proANF is also released by the heart and circulates in plasma (12–15), where it is converted at some unknown site to several smaller peptides with proANF 31-67 from the midportion of the N terminus also circulating (14, 15). ProANF 31-67 has vasodilator and natriuretic properties that appear to be similar to ANF 99-126 (16, 17). In humans, both proANF 1-98 and proANF 31-67 circulate in plasma in much higher concentrations than ANF and both are markedly elevated in patients with heart failure (15) or hypertension (18).

The purpose of the present study was to determine whether the plasma concentrations of the proANF peptides are altered by chronic changes in sodium intake.

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We were particularly interested in obtaining measurements of proANF 1-98 and proANF 31-67 because there is little data available on the normal plasma levels of these peptides in the rat.

Materials and Methods

Male Sprague-Dawley rats were purchased from Harlan Sprague-Dawley. They were weighed, placed in metabolism cages, and divided into two groups. The low salt group ($n = 9$) was placed on a sodium-deficient diet (ICN Biochemicals, Cleveland, OH) and was given deionized water to drink. The normal salt group of rats ($n = 8$) was fed a standard rat chow, providing approximately 2 mM of sodium per day, and was also given deionized water to drink. Daily urine output and food and water consumption were measured for the next 7 days. The animals were then weighed and decapitated. A small blood sample from each rat was placed in heparinized tubes for plasma electrolyte measurements. The remaining blood from each rat was placed in chilled tubes containing EDTA and centrifuged at 4°C, and the plasma was frozen at −20°C until assayed for hormone concentrations.

Analysis

For measurement of ANF, 0.5 ml of plasma was extracted on octyl C8 minicolumns (Amersham Corp., Arlington Heights, IL). ANF was measured by radioimmunoassay using the method that we have described previously (19, 20). Radioimmunoassays (RIA) to measure the N terminus of the prohormone were devised to amino acids 1-30 and 31-67 of the 126-amino acid prohormone. Sephadex G-50 gel permeation chromatography revealed that the proANF 1-30 RIA recognized a 10,000-mol wt peptide consistent with it measuring the whole N terminus (i.e., amino acids 1-98 of the prohormone), but lacking the C terminus (i.e., ANF) (15). Thus, although the proANF 1-30 RIA was devised to synthetic amino acids 1-30 of the 126-amino acid prohormone, it immunologically recognizes the whole 1-98-amino acid segment that comprises the N terminus of the prohormone. It does not cross-react with ANF. The proANF 31-67 RIA, on the other hand, recognized mainly (96%) a peptide of 3,900–4,000 mol wt from the midportion of the N terminus of the prohormone (actual proANF 31-67 mol wt, 3,878) (15) and shows a 4% cross-reactivity with proANF 1-98 and no detectable cross-reactivity with ANF.

For measurement of the N-terminal prohormone peptides, 200-μl plasma samples were first extracted with 100% ethanol (1/1 dilution), vortexed, and allowed to stand at 4°C for 30 min. Following 30 min at 4°C, the samples were centrifuged at 3000g for 15 min and the supernatants were taken to dryness via controlled nitrogen flow. The samples were then ready for RIA. All determinations were performed in triplicate. The intra-assay coefficients of variation for proANF 1-30 and 31-67 RIA were 4.8% and 5.3%, respectively. The interassay coefficient of variation was 8% for both proANF 1-30 and 31-67 RIA. The lowest detectable concentrations were 40 fmol/tube and 35 fmol/tube for proANF 1-30 and 31-67, respectively. Serial dilution of pooled sample has revealed excellent parallelism of standard and unknown in these assays (15).

Plasma renin activity was measured by combining 100 μl of plasma with 900 μl of plasma from a nephrectomized animal. The samples were dialyzed for 48 hr against a phosphate buffer (pH 5.3) to remove native angiotensin I (AI). Plasma samples were then incubated for 3 hr at 37°C with phenylmelthyl-sulfonyl fluoride to prevent the breakdown of formed AI. The radioimmunoassay for AI has been described previously (21).

Plasma, urine, and food concentrations of sodium were determined by flame photometry (model 943; Instrumentation Laboratories, Lexington, MA). Food electrolyte contents were multiplied by the amount of food consumed per day to estimate sodium intake.

Statistical significance was determined using an analysis of variance for repeated measures for within-group comparisons and an unpaired t test for between-group comparisons. All post hoc tests for the analysis of variance were performed with Fisher's LSD test. In all cases, a P-value of less than 0.05 was considered the criterion for statistical significance.

Results

The initial body weights (Day 1) were 264 ± 27 g (mean ± SE) for the low salt group of rats and 225 ± 34 g for the normal salt group. These values were not statistically different. The final body weight for the low salt group was 292 ± 23 g. This value was not significantly different from the final body weight of the normal salt group (293 ± 25 g). Plasma Na^+ was 144.8 ± 1.9 mmol/liter in the low salt group rats at the end of the 1-week study and tended to be slightly higher in the normal salt group (148.6 ± 1.1 mmol/liter), although this difference was not significant.

Figure 1 illustrates water intake, urine output, and sodium intake and excretion during the 7 days of the study. Water intake was significantly lower in the low salt group compared with the normal salt group during each of the 7 days of the study ($P < 0.05$). Urine output was significantly greater in the normal salt group compared with the low salt group on Days 2–4, as well as Days 6 and 7 ($P < 0.05$). Sodium intake and excretion are shown in the lower panels of Figure 1 using a log scale because of the large differences in these values between the groups. Sodium intake was much lower in the low salt group compared with the normal salt group ($P < 0.001$). Sodium excretion was significantly lower in the low salt group compared with the normal salt group on Days 2–7 ($P < 0.01$).

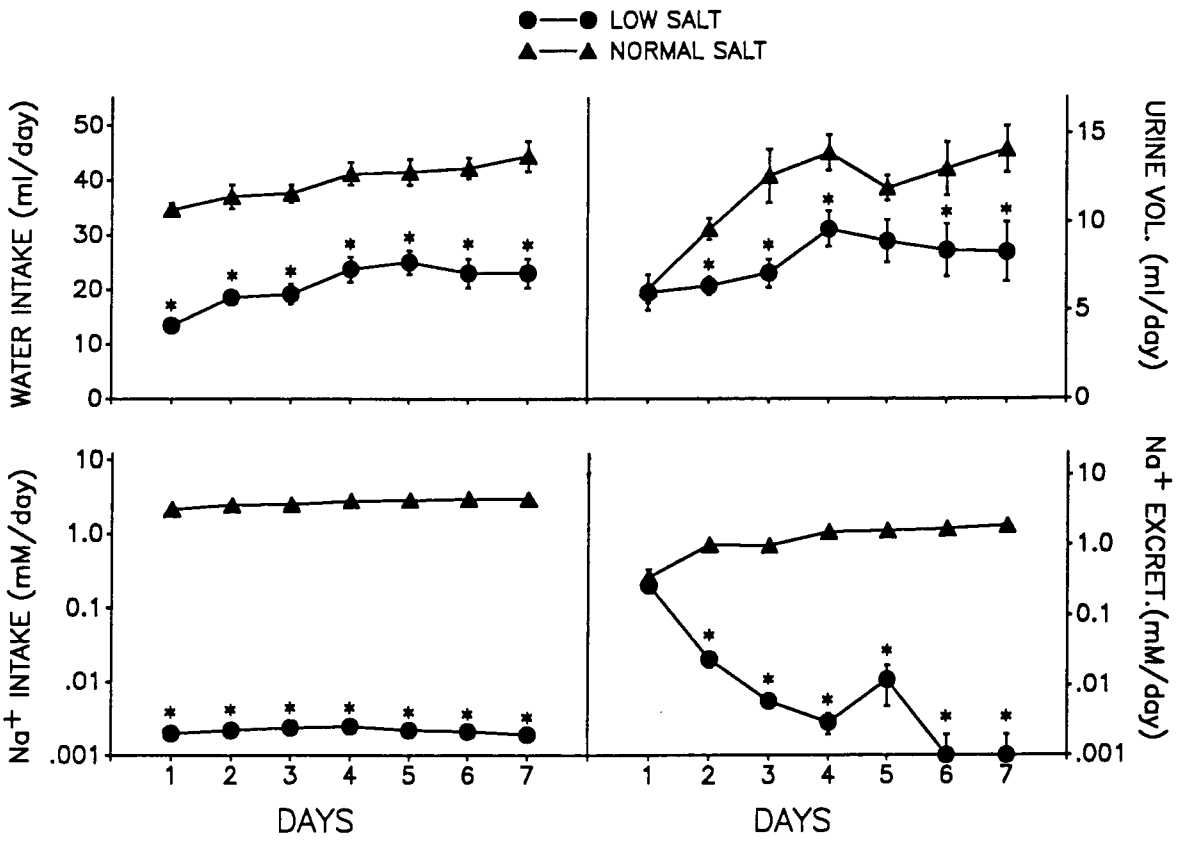

Figure 1. Water intake, urine output, and sodium intake and excretion for the 7-day period of study in two groups of rats maintained on different sodium diets. Values are mean ± SE. Some SE bars are not apparent on these graphs since they are smaller than the symbol size. Asterisk indicates a statistically significant difference from the normal salt group (all $P < 0.05$ or less).

In the low salt group, sodium excretion was higher than sodium intake during the first 5 days of the study, but fell to near undetectable levels by Days 6 and 7. Sodium intake and excretion were similar in the normal salt group.

Plasma hormone concentrations are shown in Figure 2. Plasma ANF concentration (Fig. 2A) was significantly lower ($P < 0.05$) in the low salt group (44 ± 13 pg/ml, $n = 9$) compared with the normal salt group (204 ± 60 pg/ml, $n = 8$). The plasma levels of proANF 1-98 (Fig. 2C) were from 3- to 10-fold greater than the plasma ANF levels on both the low and normal salt diets. ProANF 1-98 was significantly less ($P < 0.001$) in the low salt group (353 ± 33 pg/ml, $n = 9$) compared with the normal salt group (635 ± 47 pg/ml, $n = 8$).

Plasma levels of proANF 31-67 (Fig. 2B) were also higher ($P < 0.05$) in the normal salt group (123 ± 21 pg/ml, $n = 4$) compared with the low salt group (59 ± 13 pg/ml, $n = 4$). Plasma concentrations of this hormone could not be determined in every rat due to an insufficient volume of plasma. Plasma renin activity (Fig. 2D) was significantly higher ($P < 0.001$) in rats on the low salt diet (5.66 ± 1.07 ng AI/ml/hr, $n = 9$) compared with rats on the normal salt diet (1.80 ± 0.15 ng AI/ml/hr, $n = 8$).

Discussion

The present study was designed to examine the effects of altered sodium intake on the plasma levels of ANF and the N-terminal ANF prohormone peptides 1-98 and 31-67. We found dramatic differences in the plasma concentrations of these peptides between groups of rats on normal or low salt intake of 1-week duration (Fig. 2). This suggests a possible role for these peptides in the physiological adaptation to changes in salt intake.

There is strong evidence supporting a role for ANF in the natriuretic and diuretic responses to acute sodium chloride excess. Atrial appendectomy in the rat has been shown to attenuate both the rise in ANF (4) and the natriuresis (3, 4) in response to acute volume expansion. This response can also be attenuated by prior administration of monoclonal antibodies directed against ANF (5). The evidence suggesting a role for ANF in chronic adjustments to increased sodium intake is much more controversial. Salazar *et al.* (10) showed that in the dog, high salt intake had no effect on plasma ANF, but increased sodium output. Several studies in the rat have reported either no change or a decrease in plasma ANF with extremely high salt intakes (7, 9, 22, 23). In humans, a number of laboratories have reported

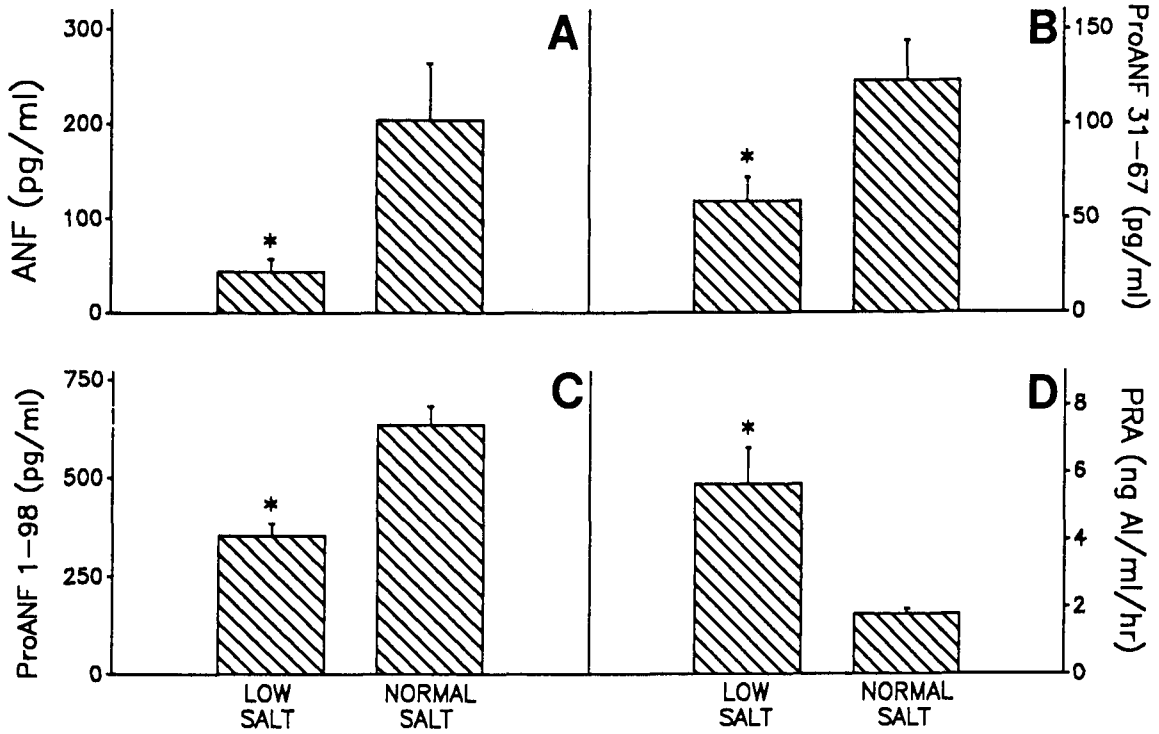

Figure 2. Plasma levels of atrial natriuretic factor, proANF 1-98, and proANF 31-67 and plasma renin activity (PRA) in rats on two different sodium intakes for a duration of 1 week. Values are mean ± SE. Asterisk indicates statistically significant differences from the normal salt group at the 0.05 level or less.

that salt intake and plasma ANF levels are positively correlated (8, 24–27), whereas others have found little effect (11, 28, 29).

Evidence suggests that peptides from the N terminus of the ANF prohormone circulate in humans (12–15, 18). Peptides derived from the N terminus, specifically, proANF 1-30, 31-67, and 79–98, have been found to be natriuretic or kaliuretic (17). In humans, the basal plasma levels of proANF 1-98 and proANF 31-67 are 10- to 20-fold greater than the plasma levels for ANF (15). Also, the plasma levels of these peptides are markedly increased by immersion-induced central hypervolemia (30). We have shown recently that the N-terminal prohormone peptides 1-98 and 31-67 are secreted simultaneously with ANF in response to atrial stretch in the isolated rat atrium (31). In disease states that are characterized by abnormalities of salt and water balance, such as heart failure, the plasma levels of these peptide are markedly increased (15). In fact, patients with mild heart failure (New York Heart, Class I) show significant increases in the plasma levels of proANF 31-67 at a time when plasma ANF (99-126) is not yet significantly increased (15).

Based on the above observations, we have hypothesized that proANF 1-98 and proANF 31-67 would show greater changes than ANF with alterations in sodium intake. We found that the plasma concentrations of ANF and proANF 1-98 and 31-67 were all markedly higher in rats on a normal salt diet of approximately 2 m*M*/day for a duration of 1 week compared with rats on a low salt diet of less than 0.1 m*M*/day (Fig. 2). As in the human (15, 30), the plasma levels of proANF 1-98 in the rat are greater than the ANF levels. In contrast to the human, the plasma concentrations of proANF 31-67 in the rat are similar to the plasma ANF concentrations (Fig. 2). If one compares sodium intake and output for the low salt group (Fig. 1), it is apparent that these rats were in negative sodium balance for the first few days of the study (Days 1–3). This suggests that this group was probably volume depleted, which could account for the lower plasma levels of the atrial peptides in this low salt group compared with the normal salt group.

Therefore, the present data suggest that ANF and peptides derived from the N terminus, i.e., proANF 1-98 and proANF 31-67, may play a role in adjusting renal sodium excretion between low and normal sodium intakes. It is clear that additional studies are needed to determine the contribution of peptides derived from the ANF prohormone in circulatory homeostasis.

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