High Salt Intake-Induced Changes in Atrial Natriuretic Factor Kinetics Are Mediated by Clearance Receptors (43288)

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Abstract. **We have reported a paradoxical plasma atrial natriuretic factor (ANF) decline following prolonged high salt intake that was attributed to an increased tissue uptake of circulating ANF, leading to its augmented distribution volume (V_{ss}) and metabolic clearance rate (MCR) as compared with control rats on a standard diet. To explore this phenomenon further, we evaluated possible chronic salt-loading-induced changes in ANF clearance (C-ANF) receptors, which appear to play a major role in ANF removal from the circulation. We studied changes in plasma [^{125}I]ANF(1–28) and its pharmacokinetics after preoccupation of C-ANF receptors by its specific ligand, C-ANF(4–23), in high-salt-treated rats and their controls. Following C-ANF(4–23) administration, we detected significantly higher circulating [^{125}I]ANF levels throughout the study period (8 min) in high-salt-fed rats compared with the controls (280–470% vs 100–215% increase of basal values, $P < 0.05$). C-ANF(4–23) infusion caused a significantly greater decrease of the metabolic clearance rate and distribution volume of [^{125}I]ANF in high-salt-fed rats than in control animals (74 ± 6% vs 41 ± 6% and 75 ± 4% vs 50 ± 5% of basal values, respectively; $P < 0.05$). These data suggest that a prolonged high salt diet may increase the availability of C-ANF receptors and, through this mechanism, may negatively modulate plasma ANF concentrations. C-ANF receptors may thus fulfill a regulatory function on circulating ANF during prolonged salt loading in rats.** [P.S.E.B.M. 1991, Vol 198]

Atrial natriuretic factor (ANF) is produced and released into the circulation mainly by cardiac atria (1, 2). Through its several properties (natriuretic, diuretic, vasodilatory, neuromodulatory, and aldosterone inhibitory) (3–6), the peptide appears to play a role in the regulation of circulatory homeostasis (7, 8). At least two types of ANF-binding sites have recently been demonstrated (9). The less abundant biological ANF receptors mediate ANF's known biological effects and can be identified by their association with particulate guanylate cyclase (10). Clearance (C-ANF) receptors, the second type, representing a majority of the peptide's binding sites, are believed to fulfill an important function in plasma ANF clearance by regulating circulating ANF levels (11, 12). The possible influence of various experimental or pathological conditions on C-ANF receptor expression remains largely unexplored.

We have shown recently that normotensive Sprague-Dawley rats on a prolonged high salt diet (8% NaCl for 5 weeks) present a paradoxical decrease of plasma ANF (COOH- as well as NH_2-terminal ANF) concentrations, compared with controls fed standard rat chow (0.8% NaCl) (13). This fall in plasma ANF, unaccounted for by reduced atrial ANF production (13) is associated with a high-salt intake-induced elevated tissue uptake of [^{125}I]ANF(1–28) by ANF-binding sites with a resulting increased distribution volume (V_{ss}) and metabolic clearance rate (14). An enhanced availability of ANF-binding sites after salt loading may thus explain the observed decrease of plasma ANF levels. Since we could not distinguish the respective involvement of the different ANF receptors, the present study was performed to evaluate whether C-ANF receptors, mainly responsible for plasma ANF clearance (11, 12), may be

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behind these high-salt intake-induced changes. If this hypothesis is true, administration of C-ANF(4–23), a COOH-terminal ring-deleted specific ligand of C-ANF receptors, will occupy these receptors to a larger extent in salt-loaded rats than in controls. We investigated the *in vivo* changes in circulating [^{125}I]ANF(1–28) levels and their pharmacokinetics in C-ANF(4–23)-pretreated rats on a high salt and standard diet.

Materials and Methods

Peptides. A portion of the C-ANF(4–23), a COOH-terminal ring deleted analog of ANF (12, 15), used in this study was donated by Dr. T. Maack from Cornell University, New York, and the remaining portion was purchased from Peninsula Laboratories (Belmont, CA). Its purification and exact amino acid sequence have been reported elsewhere (15). C-ANF(4–23) has a high affinity for C-ANF, but not for biological ANF receptors (15). [^{125}I]ANF(1–28) was prepared according to the lactoperoxidase technique (16). Monoiodinated ANF was purified in a C_{18} 5-μm Spherisorb column (0.45 × 25.0 cm). ANF(1–28), located at the COOH-terminal of ANF prohormone, is the main circulating form of the peptide in the rat.

Experimental Design. After arrival, male Sprague-Dawley rats (Charles River, St. Constant, Quebec, Canada) were housed in our animal facilities under controlled temperature and humidity for a 1-week acclimatization period and then divided into two groups: (i) controls maintained on standard rat chow (0.8% NaCl; n = 16); and (ii) animals fed a high salt diet (8% NaCl; Ralston Purina, Richmond, NJ) for 5 weeks (n = 16). Both groups had access to drinking water *ad libitum*. On the final day of the experiment, their carotid arteries and jugular veins were cannulated with PE-50 tubing under pentobarbital anesthesia (50 mg/kg body wt) for blood pressure monitoring, blood collection, and atrial peptide administration. All rats were infused with 0.9% NaCl (0.05 ml/min) for 45 min through the left jugular vein. Each of these two (control and salt-treated rats) groups was divided into two subgroups (A and B). Subgroup A (n = 7 in each group) received a bolus injection of [^{125}I]ANF(1–28) (~0.4 μCi/rat) through the right jugular vein in a total volume of 0.3 ml of saline, followed by a 0.15-ml saline flush. The radioactivity remaining in the dead space (syringe and catheter) was subtracted from the injected values. Several arterial blood samples (0.2 ml) were collected during an 8-min period after the iodinated ANF injection (see Results) and were replaced by equal volumes of saline through the jugular vein. After blood centrifugation, 0.1-ml plasma samples were precipitated with 0.5 ml of 10% trichloroacetic acid, as described elsewhere (12). The pellets, corresponding to the intact labeled ANF molecule (12), were counted in a gamma counter. Subgroup B (n = 9 in each group) underwent the same experimental protocol as Subgroup A, except that an intravenous bolus injection of C-ANF(4–23) (10 μg/kg body wt) was followed by a continuous infusion of the same compound (1 μg/min/kg body wt^{-1}), commencing 15 min before the [^{125}I]ANF injection and continuing for 45 min to the end of the experiment (12). Blood pressure was monitored throughout the study period. Rats with a 15 mm Hg or greater decline in mean blood pressure were eliminated from the study.

High Performance Liquid Chromatography. To evaluate further the nature of the measured radioactivity, 2 ml of arterial blood replaced by the same volume of saline were collected between 0.5 and 1 min and between 4.5 and 5 min after the [^{125}I]ANF injection from rats on high salt and standard diets. Plasma (1 ml) was processed on Sep-Pak C_{18} cartridges, as described elsewhere (1). The eluates were evaporated in a Speed-Vac, reconstituted in 0.1 M acetic acid, and loaded on a Vydac C_{18} column (0.46 × 25 cm) of a Varian 5000 high performance liquid chromatography system. A linear gradient from 10% to 50% CH_3CN over 40 min was used and 1-ml fractions were collected and counted for radioactivity in a gamma counter.

To verify the high salt intake, sodium excretion was measured in the urine of controls and salt-treated rats by flame photometry from samples collected for 24 hr.

Pharmacokinetic Analysis. The plasma concentration time curves were fitted to an open two-compartmental model, assuming first-order distribution and elimination using a nonlinear regression curve-fitting program (PC-NONLIN; 17). Initial estimates for the computer program and kinetic parameters not estimated by the NONLIN program were calculated using model independent equations (18). In brief, the area under the curve (AUC) was calculated by the trapezoidal method. The metabolic clearance rate (MCR) was determined by dividing the administered dose with the AUC. The apparent volume of distribution (V_α) was calculated using the equation

$$V_\alpha = \text{dose}/A + B \quad [1]$$

where A and B are intercepts of the distribution and elimination phases. The total volume of distribution (W_β) was calculated using the following equation:

$$V_\beta = \text{dose}/\text{AUC} \times \beta \quad [2]$$

where β is the slope (× 2.303) of the elimination phase of the curve. Distribution volume at steady state was calculated according to the equation

$$V_{ss} = V_\alpha \times [(k_{12} + k_{21})/k_{21}] \quad [3]$$

where k_{12} and k_{21} are distribution rate constants between compartments 1 and 2, and 2 and 1, respectively. The V_{ss} can, in fact, only be predicted by this method,

since steady state conditions were not achieved following a single intravenous dose.

Statistics. Intergroup differences were evaluated by two- or three-way analysis of variance and by unpaired Student's t test. Differences were considered statistically significant at $P < 0.05$. All values are expressed as mean ± SE.

Results

Body weight of rats ranged from 290 g to 350 g, with no intergroup differences. As expected, high salt intake for 5 weeks significantly increased sodium excretion compared with the controls on standard rat chow (25 ± 2 vs 2.3 ± 0.2 mmol/24 hr). Systolic blood pressure was not modified by prolonged salt loading (106 ± 4 mm Hg in controls, 110 ± 7 mm Hg in salt-treated rats).

Figure 1 shows the decay of plasma precipitable radioactivity after [^{125}I]ANF injection in all four groups. High salt intake for 5 weeks (Group 2A) markedly decreased circulating precipitable radioactivity, compared with the controls (Group 1A), through the 8-min study period. As expected, C-ANF(4–23) infusion substantially increased plasma precipitable radioactivity in both the control (Group 1B) and salt-loaded (Group 2B) rats. However, this increase was greater after prolonged salt intake in the salt-loaded rats than in the control rats, with significant differences between both C-ANF(4–23)-treated groups (2B vs 1B). These differences became even more apparent when we compared the percentage of increment of circulating precipitable radioactivity after C-ANF(4–23) infusion (Fig. 2). As depicted, the increase in plasma radioactivity levels after C-ANF(4–23) pretreatment in rats on a high sodium diet was markedly higher at each time point studied, compared with controls on a standard diet.

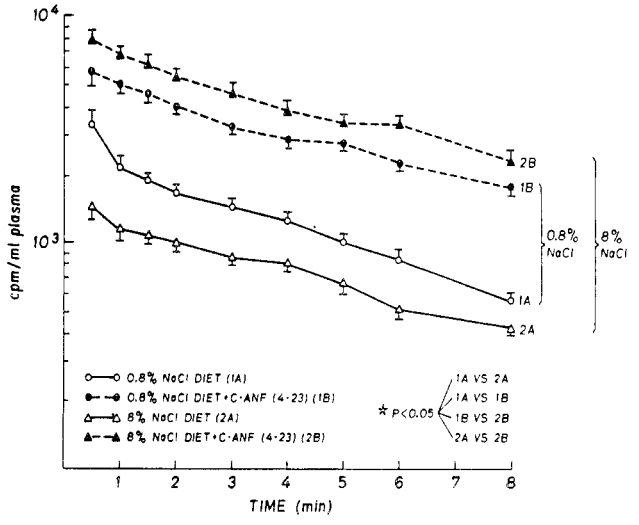

Figure 1. Decay of plasma precipitable radioactivity after [^{125}I]-ANF(1–28) injection and its changes during C-ANF(4–23) infusion in rats on standard and high salt diet.

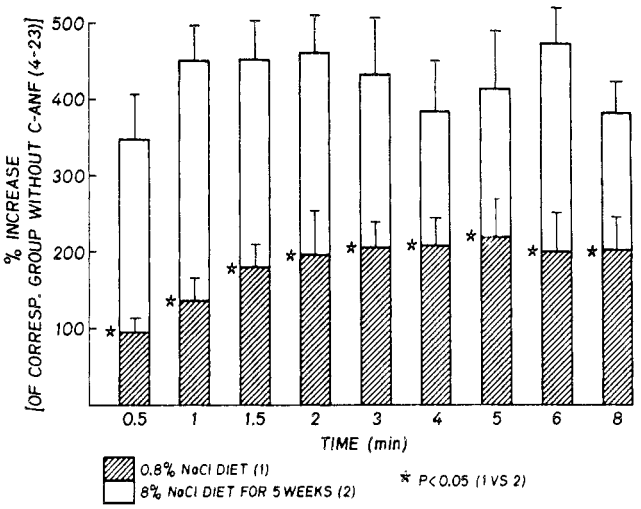

Figure 2. Changes in plasma precipitable radioactivity during C-ANF(4–23) infusion at different time intervals after labeled ANF injection in rats on standard and high salt diet.

The high performance liquid chromatography profile of plasma radioactivity at two different time intervals after [^{125}I]ANF injection in control rats is shown in Figure 3. At the early time interval (0.5–1 min), most (70%) of the total radioactivity appeared between 26 and 30 min, when the [^{125}I]ANF standard was eluted. This proportion was very similar to the proportion of precipitable radioactivity (72%) found in the original sample. Two smaller peaks that eluted at around 11 and 17 min may represent iodinated ANF degradation products, as judged by their further increase in the later time interval (4.5–5 min after [^{125}I]ANF injection). At this interval, the [^{125}I]ANF peak diminished so that it represented only 18% of total radioactivity, which again corresponded to the proportion of precipitable radioactivity (16%) in the original sample. Similar but lower peaks of radioactivity were obtained from precipitable plasma radioactivity after prolonged high salt intake (data not shown). Thus, we considered precipitable plasma radioactivity to be an indicator of the intact labeled ANF molecule.

The pharmacokinetic parameters of [^{125}I]ANF in all studied groups are depicted in Table I. Prolonged salt consumption resulted in significantly higher MCR and distribution volumes (V_α, V_β, and V_{ss}), in comparison to controls on the standard rat diet. C-ANF(4–23) infusion markedly decreased the MCR values and all distribution volumes in both groups. There were no intergroup differences in $t_{½\alpha}$ or $t_{½\beta}$. The percentages of decrease in MCR and distribution volumes after C-ANF(4–23) treatment in salt-loaded and control rats are shown in Figure 4. C-ANF(4–23) administration reduced the MCR, V_α, V_β, and V_{ss} of [^{125}I]ANF to a significantly greater extent in rats on high salt diets than in their controls.

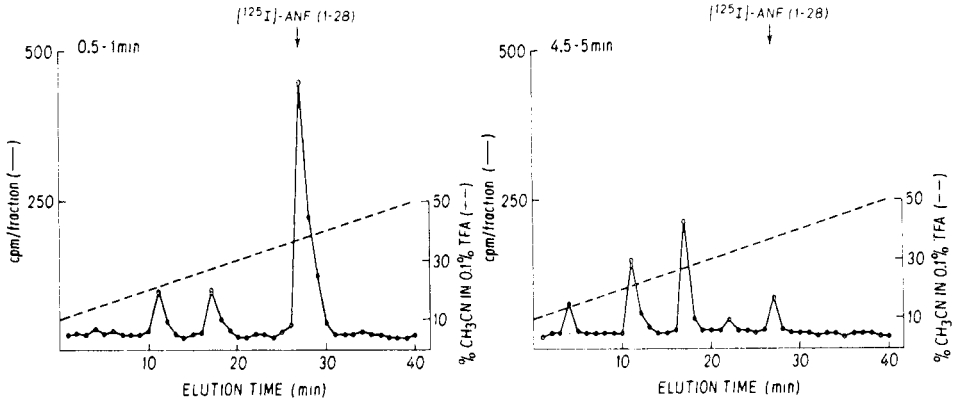

Figure 3. HPLC profile of [^{125}I]ANF(1–28) extracted from rat plasma at different time intervals after labeled ANF injection.

Table I. Changes in Pharmacokinetics of [^{125}I]ANF(1–28) after C-ANF(4–23) Infusion in Rats on Standard and High Salt Diet[a]

Group	MCR (ml/min)	V_α (ml)	V_β (ml)	V_{ss} (ml)	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (min)
1A Controls (n = 7)	32 ± 6	61 ± 6	187 ± 35	174 ± 34	0.12 ± 0.2	3.0 ± 0.3
1B Controls + C-ANF (4–23) (n = 7)	19 ± 2 *vs 1A	44 ± 6	94 ± 11 *vs 1A	92 ± 9 *vs 1A	0.13 ± 0.02	3.6 ± 0.8
2A High salt intake (n = 6)	69 ± 13 *vs 1A, 2B	116 ± 19 *vs 1A, 2B	345 ± 50 *vs 1A, 2B	283 ± 32 *vs 1A, 2B	0.11 ± 0.2	3.4 ± 0.3
2B High salt intake + C-ANF (4–23) (n = 6)	18 ± 4	43 ± 7	74 ± 16	72 ± 10	0.13 ± 0.03	3.2 ± 0.4

[a] Values are means ± SE; * $P < 0.05$. Abbreviations used in this table: MCR, metabolic clearance rate; V_α, apparent volume of distribution; V_β, total volume of distribution in the second phase of disappearance; V_{ss}, volume of distribution at steady state; $t_{1/2\alpha}$ and $_\beta$, plasma half life in the first and second phases of disappearance, respectively.

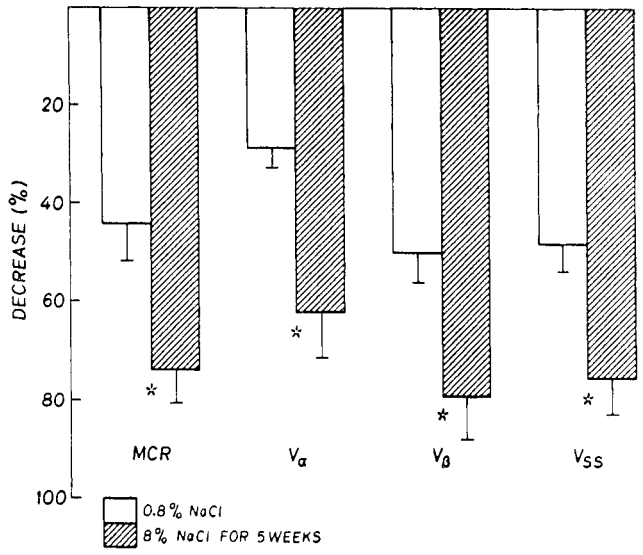

Figure 4. Changes in [^{125}I]ANF(1–28) kinetics after C-ANF(4–23) infusion in rats on standard and high salt diet ($P < 0.05$).

Discussion

A comparison of the proportion of precipitable plasma radioactivity in the original samples with proportional radioactivity co-eluted with the [^{125}I]ANF standard on high performance liquid chromatography suggests that the former represents the intact labeled ANF molecule and not degraded fragments. Since it has been shown that [^{125}I]ANF disappearance is similar to the nonlabeled peptide (12, 19) and iodinated ANF possesses biological activity in bioassays (16, 20), our pharmacokinetic data may also apply to unlabeled ANF.

The present study confirmed our previous observation of increased [^{125}I]ANF disposal resulting from the peptide's higher uptake by its binding sites after prolonged high salt intake in the rat (14). The degree of the MCR increase after prolonged high salt loading in this investigation appears to account for the previously found (13) decrease in rat plasma ANF levels (39 ± 8 vs 98 ± 27 pg/ml in controls) after prolonged high salt intake. Our data suggest that C-ANF receptors may be involved in this phenomenon. Indeed, comparison of the extent of changes in plasma-labeled ANF and its pharmacokinetics after preoccupation of C-ANF receptors by their specific ligand in salt-loaded rats and their controls suggests salt-induced alterations in C-ANF re-

ceptors. However, the parallel decreases of MCR and volume of distribution after C-ANF(4–23) in both groups may explain the lack of changes in $t_{1/2}$. The larger increases of plasma [^{125}I]ANF levels, as well as the decreases in MCR and distribution volumes after C-ANF(4–23) in salt-loaded rats, indicate that this ligand occupied the C-ANF receptors to a markedly greater extent in rats on prolonged high salt intake than in the controls. Therefore, these results are consistent with the notion of increased availability of C-ANF receptors after prolonged high salt consumption. Although a detailed C-ANF receptor binding study *in vitro* could better reveal receptor changes, the phenomenon of switching subtypes of ANF receptors between *in vivo* and *in vitro* conditions (21) may diminish the value of *in vitro* studies, and kinetic *in vivo* studies may better reveal receptor subtype changes.

Since the metabolism of ANF by degradative enzymes plays a role in ANF clearance (19), C-ANF(4–23) could also compete for this pathway of ANF degradation, and/or salt loading could change proteolytic enzyme activity. Both these possible phenomenon may explain our results. However, the former hypothesis was not confirmed in a previous study (12), which showed that C-ANF receptors are the important determinants of MCR and V_{ss} of ANF. Indeed, occupation of C-ANF receptors by their specific ligand in our study nearly resulted in a 50% decrease in the MCR and V_{ss} of [^{125}I]ANF in control rats, which is in agreement with other data (12). The important role of C-ANF receptors in the MCR of ANF also suggests that they may function as a hormonal buffer system, modulating the plasma levels of endogenous ANF (15). In addition, degradation pathways such as those realized by endopeptidase 24.11 (22, 23) may also play an important role in the metabolism of ANF. Of particular interest is the observation that the endopeptidase inhibitor SCH 39370 clearly enhanced the effect of ANP(4–23) on the pharmacokinetics of ANF in the rat (24). This suggests that the plasma ANF concentrations are determined by the interplay of the C-ANF receptor and the neutral endopeptidase system. Thus, until endopeptidase activity is tested, we cannot completely rule out the possibility of high salt-induced changes of the degradation enzyme activity.

One might still argue that the present evidence of C-ANF receptor changes on high salt intake merely represents a secondary phenomenon after the decline of plasma ANF levels. Therefore, a decrease in ANF production could be responsible for the plasma ANF reduction accompanying prolonged high salt diet. However, in view of our findings of unchanged immunoreactive atrial ANF, as well as ANF mRNA with high left ventricle IR-ANF levels (13) and even higher right atrial distention after chronic salt loading (14), this possibility seems to be very unlikely.

Our study provides circumstantial evidence that a prolonged high salt diet induces changes in C-ANF receptors. This phenomenon may explain the decreased plasma ANF levels after prolonged 8% NaCl feeding and may thus constitute a negative factor in the preservation of sodium balance during chronic high salt intake. C-ANF receptors appear to have a regulatory function on circulating ANF during prolonged salt loading in rats. Further studies are needed to enhance our knowledge of C-ANF receptor regulation under different physiological and pathological conditions.

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