Effect of Prolonged High Salt Diet on Atrial Natriuretic Factor in Rats[1] (43087)

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Abstract. **Normotensive Sprague-Dawley rats were given 8% NaCl for 5 weeks. This salt load did not affect their blood pressure nor hematocrit, and plasma atrial natriuretic factor (ANF) showed no change at 3 weeks but decreased after 5 weeks of the experimental period when compared with control rats. The responsiveness of particulate guanylate cyclase and formation of cGMP in ANF target organs suggested an augmented baseline activity of the cGMP system but its relative hyporesponsiveness to exogenous ANF following prolonged salt loading. Decreased plasma ANF levels cannot be explained by its altered production since atrial levels of the peptide were comparable in rats with or without salt loading. Atrial ANF mRNA was unaffected by the salt regimen. This study demonstrates that plasma ANF does not increase during long-term NaCl loading and even decreases after 5 weeks of 8% NaCl. The changes in plasma ANF are associated with changes in the functional state of ANF receptors coupled to particulate guanylate cyclase, but in the opposite direction than expected from lowered plasma ANF. Thus, ANF may not play a significant role in the regulation of sodium excretion in response to prolonged high salt consumption or, if it does, it is not reflected by expected changes in its plasma levels.** [P.S.E.B.M. 1990, Vol 194]

Alterations in sodium handling, especially in response to salt loading, appear to be involved in the pathogenesis of several diseases. Some forms of human essential hypertension may be related to a decreased ability to excrete excess dietary salt (1). Several rat strains genetically predisposed to arterial hypertension when fed a salt-enriched diet have been developed, supporting such a possibility. The best known Dahl salt-sensitive rats manifest severe hypertension on a high NaCl diet, and their survival rapidly decreases after 5 weeks on 8% NaCl ingestion (2).

Atrial natriuretic factor (ANF) (3), a new natriuretic peptide, has become a potential candidate in mediating adaptation to sodium loading and/or restriction as well as in taking part in development of hypertension, since ANF also exerts potent cardiovascular effects (4). There are several reports on ANF responses to short-term salt loading (5–8), including recent studies on ANF reactivity to prolonged NaCl treatment (9, 10). The results of these experiments are not unanimous, and serious doubts have been expressed about the physiologic and/or pathologic significance of ANF in the regulation of sodium excretion (4, 11, 12). In this investigation, we used salt levels (5 weeks on a 8% NaCl diet) which are known to produce severe hypertension in Dahl rats. We expected that under such extreme experimental conditions it would be possible to detect changes, if any, in the production and plasma levels of ANF as well as in the reactivity of functional ANF receptors.

Materials and Methods

Male Sprague-Dawley rats (Charles River, St.-Constant, Quebec, Canada) were used in this study. They arrived at our animal facilities at age 5 weeks. After a 1-week period of acclimatization, they were divided into two groups: the first was kept on normal rat chow (0.8% NaCl content) and tap water *ad libitum*, and the second on an 8% NaCl diet (Ralston Purina, Rich-

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Table I. Biologic Parameters in Weeks after 8% Salt Diet

	First		Third		Fifth	
	Control	NaCl	Control	NaCl	Control	NaCl
Weight (g)	152.0 ± 1.0[a]	147.0 ± 2.0	243.0 ± 5.0	224.0 ± 9.0[b]	322.0 ± 3.0	289.0 ± 4.0[b]
Blood pressure (mm Hg)	94.0 ± 3.0	93.0 ± 3.0	106.0 ± 2.0	103.0 ± 5.0	107.0 ± 3.0	109.0 ± 4.0
Urine volume (ml) (24 hr)	21.2 ± 1.3	75.5 ± 3.0[b]	20.5 ± 0.9	73.5 ± 4.7[b]	20.8 ± 2.3	72.1 ± 6.2[b]
Na^+ excretion (mmol/24 hr)	1.8 ± 0.1	24.4 ± 1.0[b]	2.5 ± 0.1	33.1 ± 1.3[b]	2.0 ± 0.2	24.7 ± 1.9[b]
K^+ excretion (mmol/24 hr)	4.5 ± 0.2	3.6 ± 0.1[b]	7.6 ± 0.4	4.9 ± 0.2[b]	4.8 ± 0.5	4.5 ± 0.3
Creatinine excretion (μmol/24 hr)	119. ± 9	115.0 ± 5	109. ± 3	112. ± 4	126. ± 14	157. ± 9[b]
Hematocrit (% volume)	30.6 ± 0.4	30.9 ± 0.8	41.4 ± 0.4	42.2 ± 1.0	44.4 ± 0.6	44.7 ± 0.4
Plasma renin activity (ng/ml/hr)		—	—	—	3.8 ± 0.7	0.4 ± 0.2[b]
Plasma aldosterone (ng/dl)		—	—	—	7.7 ± 0.9	5.3 ± 0.7[b]

[a] Mean ± SE; $n = 7$–17.
[b] $P < 0.05$ as compared with control.

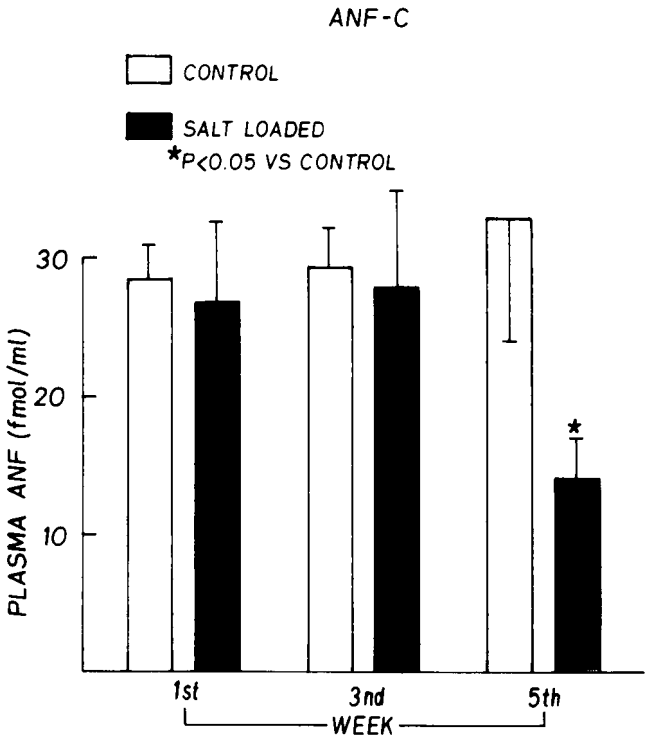

Figure 1. Plasma C-terminal IR-ANF concentrations in salt-loaded and control rats in the first, third, and fifth weeks of high salt intake.

mond, NY) until the end of the experiment at 1, 3, and 5 weeks, respectively. Body weight and blood pressure were measured weekly. To assess urinary excretion, the rats were placed in individual metabolic cages (Nalgen, Canlab) and after 3 days of acclimatization, 24-hr monitoring was performed for 2 days. After 3 and 5 weeks, the food was prepared by dicing in 50 ml of water/cup of food to make it more palatable. The animals were decapitated on days corresponding to the first, third, and fifth weeks of salt feeding. Blood samples were collected in chilled plastic tubes containing EDTA, phenyl methyl sulfonyl fluoride, and pepstatin, and centrifuged at 4000*g* at 4°C for 10 min; plasma was stored at −80°C until assayed. The hearts (divided into left and right atria and ventricles), kidneys, and adrenals were removed and either frozen on dry ice or in liquid nitrogen for mRNA extraction and stored at −80°C, or placed in appropriate buffers before enzymatic or cyclic nucleotide analysis.

Plasma immunoreactive-ANF (IR-ANF) was measured by radioimmunoassay (RIA) after extraction with Sep-Pak cartridges, as described previously (13). Plasma renin activity (14) and plasma aldosterone (15) were evaluated by RIA.

IR-ANF Measurements. Two RIA were performed, one employing an antibody directed against the COOH-terminal portion of pro-ANF (IR(C)ANF), the other using anti-NH_2-terminal antibodies (IR(N)ANF), as reported elsewhere (13, 16). Iodinated ANF was purified in a C_{18} 5-μm Spherisorb column yielding a monoiodinated compound. The cross-reactivity of this antibody to the ANF prohormone was 42.5 ± 2.6% ($n = 6$). The assay for IR(N)ANF detects 100% of pro-ANF (Asn 1-Tyr 126) and N-terminal ANF (Asn 1-Arg 98) and does not cross-react with ANF (Ser 99-Tyr 126) or any other C-terminal portions of the peptide.

ANF mRNA Detection. The specific mRNA species in rat hearts coding for pro-ANF was identified by northern blot hybridization analysis, using a specific rat cDNA probe. A detailed description of this procedure is given elsewhere (17).

Particulate Guanylate Cyclase (GC) activity and cGMP. Particulate GC activity was measured in protein preparations of rat adrenocortical membranes incubated with increasing concentrations of ANF in 20 m*M* triethanolamine buffer (pH 7.5) at 37°C for 30 min, according to assay conditions described earlier (18). cGMP was measured in plasma, urine (19), and kidney glomeruli (20) by RIA following chromatographic extraction.

Catecholamines (CA). CA, with their methylated and/or oxidized metabolites in kidneys and their uri-

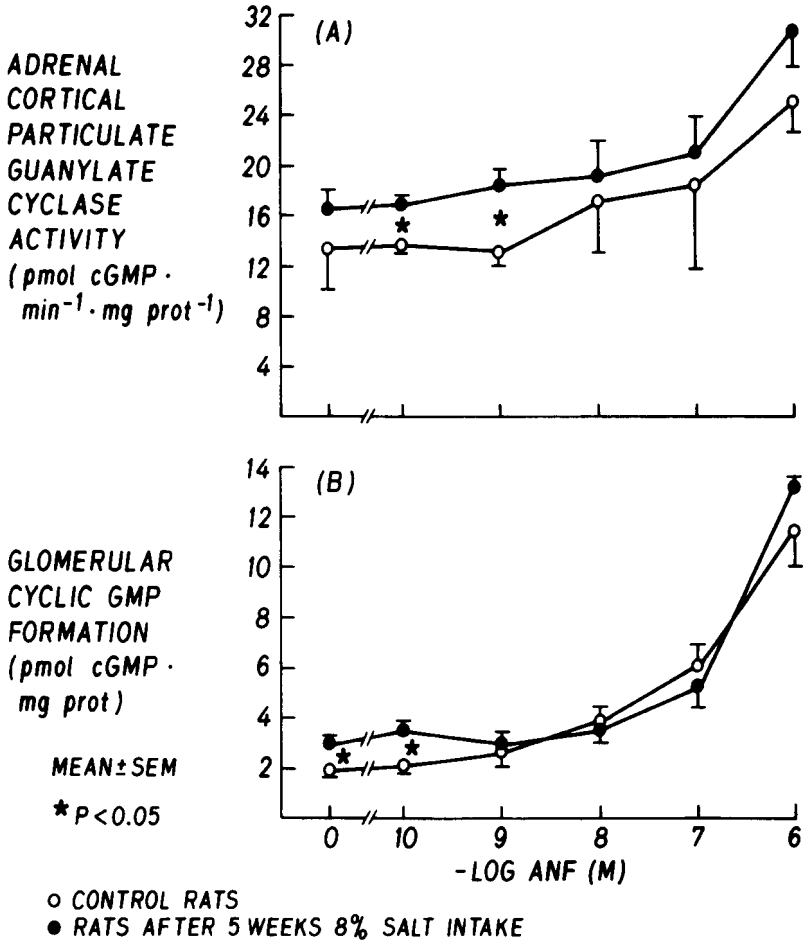

Figure 2. Adrenocortical particulate guanylate cyclase activity (A) and glomerular cGMP formation (B) and their response to ANF *in vitro* in rats on high salt intake and control rats. Points represent averages of three determinations from pooled tissues.

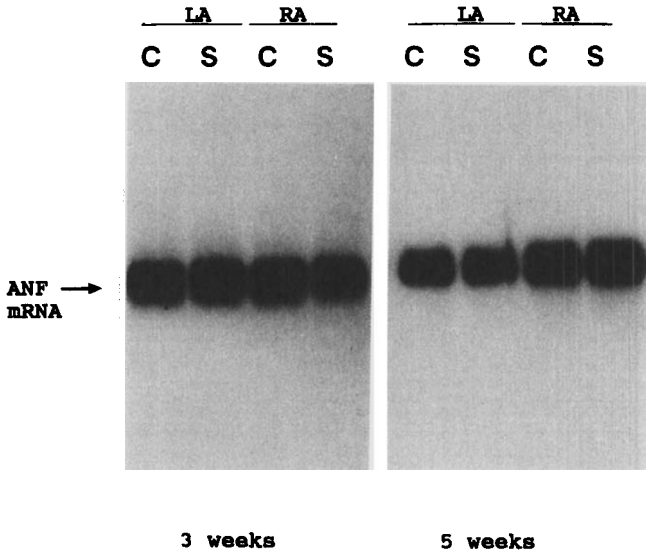

Figure 3. Messenger RNA of ANF in atria (LA left, RA right) following 3 and 5 weeks of high salt intake (C, control; S, salt loading). Each lane contains 1 μg of total RNA.

nary excretion, were measured by a radioenzymatic technique or high-performance liquid chromatography separation with electrochemical detection. Tissue preparation and urine analysis were performed as described previously (21). The urinary excretion of electrolytes and creatinine was determined by standard procedures. The data were analyzed by unpaired Student's *t* test.

Results

Table I summarizes some biologic data on rats subjected to long-term salt loading. The weight of these animals remained comparable to that of the controls in the first week of the experiment, but decreased in the third and fifth weeks of salt treatment as compared with controls. There were no changes in blood pressure. Salt-fed animals had a considerably higher urine output than the controls. Sodium excretion on 8% NaCl exceeded control values at least 10-fold. Potassium excretion, decreased in the first and third weeks of observation, was not different after 5 weeks. Urinary creatinine increased significantly in response to salt loading only after 5 weeks of treatment. Interestingly, hematocrit was maintained equal between both groups. As ex-

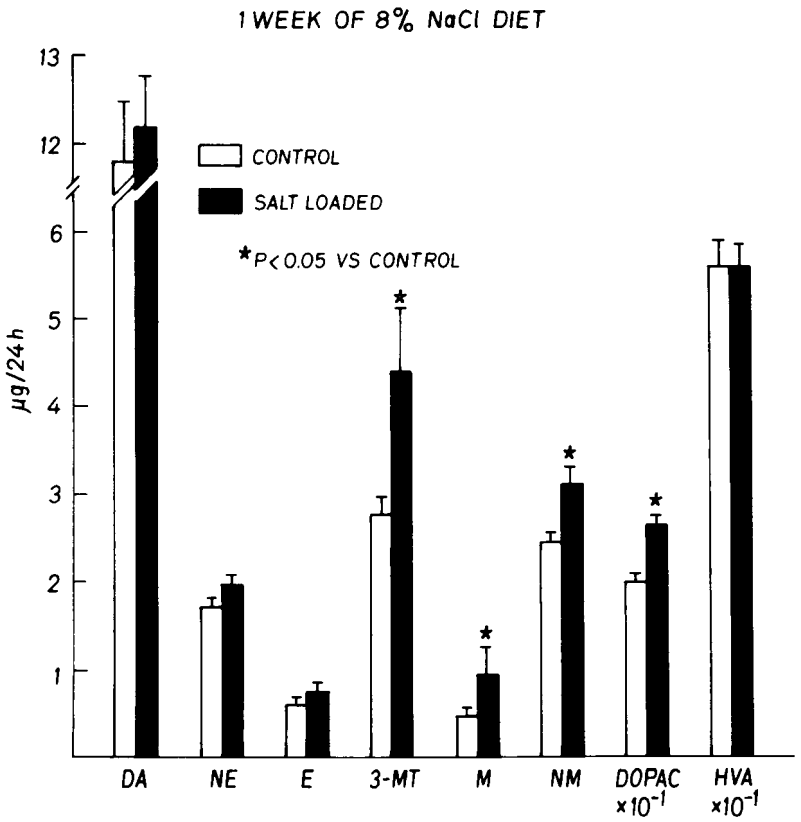

Figure 4. Urinary excretion of catecholamines and their metabolites 1 week following high salt intake. For abbreviations, see text.

pected, plasma renin activity was almost completely suppressed during salt loading and plasma aldosterone was decreased concomitantly.

Plasma ANF values, as measured with the anti-COOH-terminal antibody (Ir(C)ANF), are shown in Figure 1. After 1 and 3 weeks of NaCl loading, Ir(C)ANF concentrations were unchanged when compared with control rats. Surprisingly, 5 weeks of high salt ingestion resulted in about a 50% decrease in plasma IR(C)ANF in comparison to control values. A similar pattern was observed for ANF measured with anti-NH_2 terminal antibody (IR(N)ANF). One- and 3-week periods of salt loading did not cause significant differences. However, as in the case of IR(C)ANF, but not to such extent, IR(N)ANF concentrations were significantly decreased after 5 weeks of treatment (694 ± 42 vs 545 ± 46 fmol/ml, $P < 0.05$).

It is generally accepted that under most conditions, plasma ANF concentrations may parallel plasma cGMP levels (22). Therefore, we measured plasma cGMP and its urinary excretion. Plasma cGMP did not change (5.6 ± 0.9 vs 5.6 ± 0.7 pmol/ml in controls) but daily cGMP excretion was higher after 5 weeks of salt loading (16.2 ± 2.0 vs 10.2 ± 1.1 mmol/cGMP × mmol creatinine^{-1}, $P < 0.05$) than in control rats.

In view of the decreased plasma ANF levels after 5 weeks of salt treatment, it seemed relevant to evaluate ANF effector organ responsiveness in terms of ANF second messenger system activity. Figure 2 illustrates particulate GC activity in the adrenals and cGMP accumulation in kidney glomeruli. The basal glomerular cGMP formation and at low ANF doses the values of both GC and cGMP formations were higher after salt loading than in the controls. The magnitude of the glomerular cGMP formations in response to ANF added to the medium was, however, lower in the high salt treated than in the control group, i.e., 1.8-fold vs 3.3-fold increase ($P < 0.01$) at 10^{-7} ANF and a 4.6-fold vs 6.1-fold increase from basal values at 10^{-6} ANF.

ANF mRNA extracted from hearts of four groups of animals in two separate experiments showed no differences in the left and right atria between salt-loaded and control rats (Fig. 3). The content of IR(C)ANF in left atrium was 94 ± 6 pmol/gland vs 88 ± 4 and in right atrium 116 ± 15 vs 117 ± 11 in control and high salt diet group, respectively.

The urinary excretion of CA and their metabolites is shown in Figure 4. Salt-treated animals excreted more 3-methoxytyramine, metanephrine, normetanephrine, and dihydroxyphenylacetic acid after 1 week of high salt intake. After 3 weeks (Fig. 5), the only difference was in metanephrine excretion and after 5 weeks (not shown), the values were similar in both treated and nontreated rats. The kidney concentrations of dopa-

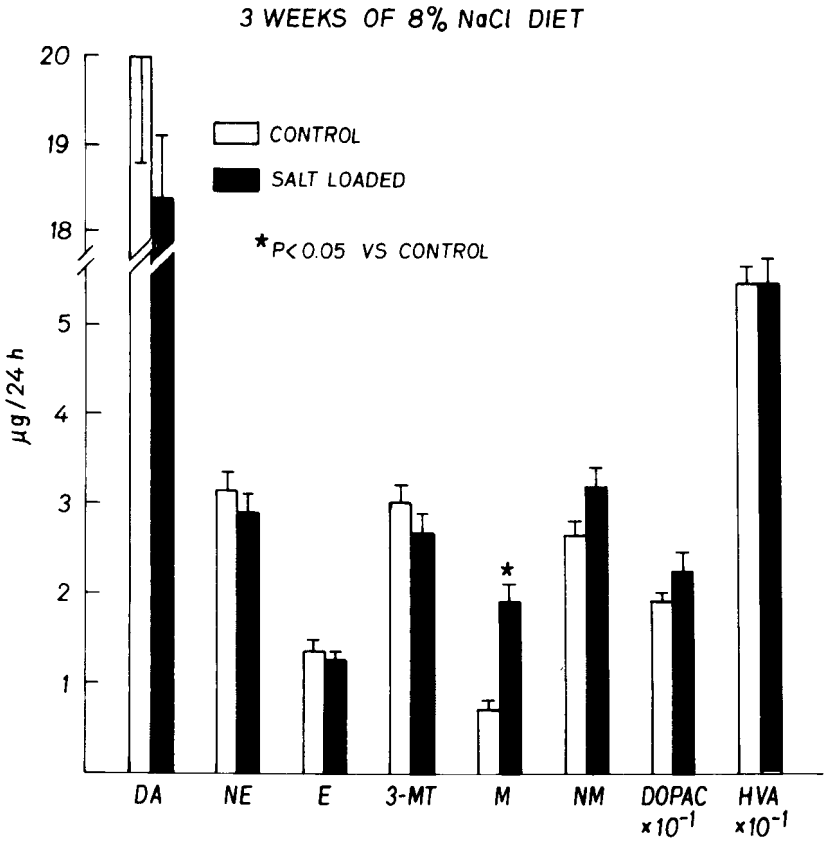

Figure 5. Effect of 8% NaCl on atrial ANF mRNA levels. Urinary excretion of catecholamines and their metabolites after 3 weeks of high salt intake. For abbreviations, see text.

mine (12.7 ± 1.0 ng/g tissue vs 12.4 ± 1.1 in the controls, n = 10–11), norepinephrine (207 ± 13 ng/g tissue vs 224 ± 17 in the controls, n = 10–11), and epinephrine (4.8 ± 0.9 ng/g tissue vs 4.6 ± 0.7 in the controls, n = 10–11) were not affected by salt loading.

Discussion

Our study confirms the efficiency of adaptation to prolonged and massive sodium chloride ingestion in normotensive Sprague-Dawley rats. The excess sodium is appropriately excreted and does not result in increased blood pressure in this strain. There is only slight weight loss which may be attributed to a lower consumption of salty food (2) or greater catabolism as witnessed by higher creatinine excretion. These rats thus behave like Dahl salt-resistant rats (2). The lack of change in hematocrit indicates that the intravascular ratio between plasma volume and formed blood elements remained constant. This implies that either hematopoiesis matched the anticipated increase in volume or that there is no real change in plasma volume despite challenge by the 8% NaCl diet. Our finding of unaltered hematocrit values is in agreement with previous studies (23).

Against this background, none of the circulating forms of IR-ANF (C- and N-terminal) differed quantitatively from that in rats on regular chow after the first and third weeks of salt loading. Five weeks of 8% NaCl feeding caused, however, a significant decrease in both molecular forms of plasma ANF albeit more prominent for IR(C)ANF. This unexpected finding contrasts with studies demonstrating an increase in ANF in animals or humans after an acute sodium chloride load (5, 6, 11). Similarly to our studies, Iwao *et al.* (9) demonstrated that salt ingestion for 2 weeks resulted in no significant changes in circulating C-terminal ANF and even tended to lower the values of this group. In addition, 8 weeks of 8% salt diet in Dahl salt-resistant rats also decreased plasma IR(C)ANF values, although, because of the high variation, this decrease did not achieve statistical significance (10). Moreover, carefully controlled studies in humans have also demonstrated an unresponsiveness of humoral ANF to salt loading, as indicated by measurements of circulating ANF (8). It may be suggested that the time and potency of the stimulus play a determining role in the secretory ANF response. Several observations favor this possibility. For example, rats autoimmunized against ANF exhibit some abnormalities only during acute volume expansion but are not different from their controls in chronic maneuvers affecting cardiovascular regulation (11). Species dependence and target tissue responses may

also play a pivotal role since atrial distension, a maneuver increasing plasma ANF in rats, does evoke a comparable rise in sodium and urine excretion in dogs without stimulation of ANf secretion (12).

Atrial distension is a major stimulus for the release of ANF from the atria (23). Consequently, any change in atrial pressure (e.g., elevated arterial blood pressure or increased central volume) may result in an enhanced ANF release (24). In the present experiment, neither blood pressure nor probably its volume changed. However, there may still be a shift toward the central or peripheral circulation which would require separate measurements.

Plasma ANF does not react to prolonged salt loading for up to 3 weeks and then diminishes after 5 weeks of 8% NaCl feeding. We virtually excluded the possibility of ANF production impairment in accordance with other investigators (9, 10) since mRNA and IR-ANF levels in heart atria did not differ in rats on high or normal salt intake. This, however, does not exclude a decrease in ANF turnover.

We demonstrated that high salt intake does alter, to some extent, the functioning of the second messenger system for ANF, particulate GC activity and cGMP generation (20, 25). Under baseline conditions, the glomerular cGMP production was higher in rats after 5 weeks of salt loading than in their controls. ANF added to the assays, however, evoked a similar (and in glomerular cGMP formation an even smaller) effect in the salt-loaded group. Taking into account the evidently increased urinary cGMP excretion, we may suspect that cGMP system was in some way directly (or mediated by other salt-responsive factors), activated after prolonged salt ingestion in rats. In consequence, the responsiveness of this system to the exogenous ANF was diminished. It is tempting to speculate that this new steady state may occur in a framework of a negative feedback loop and, therefore, the release and plasma level of circulating ANF was negatively affected appropriately to the functional state of the salt-responsive cGMP system.

Recent studies by Maack *et al.* (26) demonstrated that the majority of ANF receptors may represent a buffering system for circulating ANF. These receptors, distinct from those mentioned above on the basis of their molecular weights and pharmacologic properties, would have a great capacity to capture circulating ANF. The results obtained in this study could be well explained by changes in the number and/or affinity of so-called C-ANF (clearance) receptors. Indeed, our recent data indicate alterations in the distribution volume of ANF determined by the uptake of circulating ANF in different organs containing large numbers of C-ANF receptors (27). Thus, this phenomenon could be greatly responsible for the high salt diet-induced decrease in plasma ANF.

In contrast to this unexpected ANF response to prolonged salt-loading, renin-angiotensin-aldosterone and CA follow well-known patterns (23), underscoring the dominant role of these systems in adaptation to prolonged, massive salt loading.

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