Different Responses of Atrial Natriuretic Peptide Secretion and Its Receptor Density to Salt Intake in Rats

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This study investigated whether high-salt intake influences atrial natriuretic peptide (ANP) system, atrial content, and release rate of ANP as well as receptor density in the kidney were measured in salt intake rats. Male Sprague-Dawley rats received either 0.9% or 2% salt in their drinking water for 10 days. The stretch-induced ANP secretion from isolated perfused nonbeating left atria was accentuated, and the production of cGMP by ANP in renal cortical tissue membranes were pronounced in rats exposed to 0.9% salt for 10 days but not in rats exposed to 2% salt. The levels of ANP receptor density and expression in renal cortex were decreased in 2% salt intake rats but not in 0.9% salt intake rats. No significant differences in atrial and plasma concentrations of ANP and water balance were observed in both salt intakes. Therefore, these results suggest that atrial ANP secretion and its binding sites in the kidney may respond differently to ingested salt concentrations in rats. Exp Biol Med 229:65–71, 2004

Key words: stretch; receptor; kidney; PCR; cGMP; membrane; atrium

Atrial natriuretic peptide (ANP) is a cardiac peptide hormone that is secreted primarily by atrial myocytes in response to atrial wall stretch (1–3). ANP elicits a number of physiological effects including diuresis, natriuresis, vasorelaxation, and inhibitions of the release of vasopressor hormones (4, 5). ANP is an antagonistic hormone to renin-angiotensin-aldosterone system in the regulation of blood pressure. The synthesis of ANP is regulated by developmental, hemodynamic, and hormonal factors (5). Atrial myocytes constitute a major site of synthesis and secretion of ANP, and ventricular myocytes also synthesize ANP during the fetal life (6, 7). After birth, the synthesis of atrial ANP gradually increases, whereas that in the ventricles markedly decreases (6). The ventricular synthesis of ANP, however, is reactivated in pathological conditions associated with cardiac hypertrophy (8–10).

A variety of stimuli such as pressure and volume overloads, dexamethasone, thyroid hormone, and endothelin is known to regulate ANP gene expression (4, 5). Especially, the relationship between ANP system and volume overload induced by salt intake has been extensively studied since the discovery of ANP. Despite several investigations, this relationship is still controversial (11–15). It may be due to many possible factors such as the degree of volume load, route of salt intake, salt concentration, duration of exposure, hydration status before exposure, seasonal variation, or species. They may influence differently transcription and translation levels, release rate of ANP, and its binding to the receptor. A few studies have been done using *in vitro* ANP secretion from rat atria chronically exposed to salt. Recently Wolf and Kurtz (15) suggested that ANP gene expression and secretion are probably not involved in water balance during chronic salt loading. Therefore, the purpose of the present study was to investigate whether high-salt intake influences atrial content of ANP and secretion rate as well as its receptor density in kidney.

Materials and Methods

Animals. Male Sprague-Dawley rats weighing 230–250 g were stabilized for 5 days in metabolic cages, and urine was collected for 3 days as a control period. Rats drank either 0.9% or 2% salt solution or tap water for a given number of days, and urine was collected. Water balance was calculated by subtracting urine volume from water intake. Urinary excretion of Na^+ was measured using flamephotometry.

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Blood Collection and Tissue Preparation. On the day of experiments, rats were sacrificed by decapitation, and blood was collected into a prechilled tube containing aprotinin (200 kallikrein inhibitory units [KIU]/ml), soybean trypsin inhibitor (SBTI, 50 N-a-benzoyl-L-arginine ethyl ester units/ml), phenylmethylsulfonyl fluoride (PMSF, 600 μ*M*/ml), and EDTA (2.7 m*M*/ml). Blood was centrifuged at 10,000 *g* for 15 mins at 4°C, and plasma was kept at –70°C. Plasma ANP was extracted using Sep-Pak C_{18} cartridge (Waters Associates, Milford, MA) as described previously (16, 17). Both atria were separated, weighed, and kept in 2 ml of 0.1 N acetic acid at 4°C. Tissues were boiled for 10 mins, homogenized with Polytron homogenizer, and centrifuged at 10,000 *g* for 15 mins at 4°C. The concentrations of ANP in plasma extracts and supernatants of tissue homogenates were measured by specific radioimmunoassay as described previously (16).

Isolated Perfused Atrial Preparation. An isolated perfused atria was prepared by the method described previously (18). Briefly, the left atrium was rapidly inserted with a Tygon cannula and secured by a ligature. The cannulated atrium was fixed into the organ chamber with water-tight silicone rubber cap and perfused with oxygenated HEPES buffer solution (pH 7.4) at a rate of 0.4 ml/min using a peristatic pump. The composition of HEPES buffer solution was as follows: NaCl 118 m*M*, KCl 4.7 m*M*, $CaCl_2$ 2.5 m*M*, $MgSO_4$ 1.2 m*M*, $NaHCO_3$ 25 m*M*, HEPES 10 m*M*, glucose 10 m*M*, and bovine serum albumin (BSA) 0.1%. The pericardial HEPES buffer solution, which contained [^{3}H]-inulin for the measurement of translocation of extracellular fluid (ECF), was oxygenated by silicone tubing coils located inside the organ chamber. The pericardial space of the organ chamber was sealed and connected with a calibrated microcapillary tube, through which atrial volume was monitored. After stabilization for 30 mins, perfusates were collected two times at 2-min intervals, and then atrial distension was induced for 2 mins by elevating the position of outflow catheter tip to 1 cmH_2O. Atrial contraction was induced by lowering the position of outflow catheter tip to the basal level. Intra-atrial pressure was subsequently increased from 0 to 2, 4, 6, or 10 cm H_2O every 8 mins. A radioimmunoassay (RIA) for ANP (19) in perfusates was done on the day of experiments, and all samples in an experiment were analyzed in a single assay.

Measurement of ECF Translocation. The ECF translocated from atria was measured as described previously (20). Radioactivities of [^{3}H]-inulin in atrial perfusate and pericardial buffer solution were measured with a liquid scintillation counter, and the amounts of ECF translocated through the atrial wall were calculated by total radioactivity in perfusate (counts per minute/minute) divided by radioactivity in pericardial reservoir (counts per minute/milliliter) and atrial wet weight (milligrams).

Activation of Particulate Guanylyl Cyclase (GC) in Renal Tissue Membranes and RIA of cGMP. Particulate GC activity was measured by determination of cGMP generated in protein aliquots of renal tissue membranes according to a method described previously (21, 22). Briefly, renal cortex and medulla were separately homogenized at 4°C in 30 m*M* phosphate buffer (pH 7.2) containing 120 m*M* NaCl and 1 m*M* phenanthrolene by three 30-sec bursts of 27,000 rpm. The homogenates were centrifuged at 8,000 *g* for 10 mins at 4°C, and the supernatants were recentrifuged at 36,000 *g* for 60 mins at 4°C. The membrane pellets were washed three times with 50 m*M* Tris-HCl (pH 7.6) and resuspended in this solution. Five-microgram protein aliquots of the membrane suspension were incubated at 37°C for 15 mins in 50 m*M* Tris-HCl (pH 7.6) containing 1 m*M* isobutyl-1-methylxanthine, 1 m*M* GTP, 0.5 m*M* ATP, 15 m*M* creatine phosphate, 80 μg/ml creatine phosphokinase, and 4 m*M* $MgCl_2$ and various concentrations of ANP. The reaction was stopped by the addition of 375 μl of cold 50 m*M* sodium acetate (pH 5.8) and boiling for 5 mins.

In Vitro Autoradiography. For *in vitro* receptor autoradiography, the kidney was rapidly removed and snap frozen in liquid nitrogen. Transverse plane sections (20 μm) of kidney were cut in a cryostat at –20°C, thaw mounted on gelatin-chrome-alum–coated slides, and then dried in a desiccator overnight at 4°C. Binding conditions of [^{125}I]-ANP to the kidney section were prepared according to a method described elsewhere (23). To measure binding constants, the competitive inhibition of [^{125}I]-ANP binding of the kidney was determined in consecutive sections by coincubating with various concentrations of unlabeled ANP and 250 pM of [^{125}I]-ANP. Subsequently, the sections were rinsed three times with cold distilled water at 4°C and quickly dried under a stream of cold air. Autoradiographic images were generated by exposure of renal sections to Hyperfilm-^{3}H (Amersham International, Little Chalfont, UK) with [^{125}I]-labeled polymer standard strips (Amersham International) at room temperature for 3 days. Autoradiographic images were viewed with a Leica Wild M420 macroscope (Heerbrugg, Switzerland) and captured using a Sony video camera (Tokyo, Japan) with CCD iris and a Hamamatsu AC adaptor (Tokyo, Japan) connected to a Power Macintosh 8100/80AV computer (Uxbridge, UK). The number of ligand-binding sites of different affinities, the apparent K_d, and the maximal binding capacities (B_{max}) on renal glomerulus and medulla were derived separately in each individual using the LIGAND iterative model-fitting computer program.

Semiquantitative Reverse Transcription–Polymerase Chain Reaction (RT-PCR) of Messenger RNAs (mRNAs) for Natriuretic Peptide Receptors (NPRs). Semiquantitative RT-PCR was performed as described previously (23). One microgram of RNA was suspended in 20 μl RT buffer containing 50 m*M* Tris (pH 8.3), 75 m*M* KCl, 3 m*M* $MgCl_2$, 0.125 m*M* each of deoxynucleotide triphosphates (dNTPs), 20 U ribonuclease inhibitor, 5 μ*M* random hexamers, and 200 U SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA). Messenger RNA was reverse transcribed at room temperature for 10

mins and 42°C for 50 mins. The reaction was stopped by heat inactivation for 15 mins at 99°C and then chilled on ice. Complementary DNA products were amplified by PCR with sense and antisense primers.

NPR-A sense	5′-CGCATTGAGTTGACACGA-3′ (8925–8942)
NPR-A antisense	5′-CCCGTCTACCACACAGTT-3′ (9837–9854)
NPR-C sense	5′-GGGCAGTGAGCGAGTGGT-3′ (757–774)
NPR-C antisense	5′-CCCCATCCTTCTTGCTGT-3′ (1140–1157)

Twenty microliters of PCR buffer contained 2 m*M* Tris (pH 8.3), 10 m*M* KCl, 1.5 m*M* $MgCl_2$, 125 μ*M* each of dNTPs, 5 U *Taq* polymerase, and 10 p*M* each of sense and antisense primers. The temperature profile of amplification consisted of 1 min of denaturation at 95°C, 1 min annealing at 65°C, and 2 min extension at 72°C for 40 cycles. PCR products were separated with 2% agarose gels, and bands were visualized by ethidium bromide staining and photographed using Polaroid 665 film (Polaroid, Cambridge, MA). Intensity of the bands was quantified using a densitometer, and the relative amount of mRNAs for NPRs was calculated using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal standard gene.

Statistical Analysis. The results are given as means ± SEM. The statistical significance of the differences was analyzed by repeated-measures analysis of variance (ANOVA) (Fig. 1A–D) or one-way ANOVA (Tables 1 and 2) followed by Bonferroni's multiple-comparison test. Two-way ANOVA analysis for repeated measures was also used. Student's unpaired *t* test was used for analysis of two groups. The critical level of significance was set at $P < 0.05$.

Results

Changes in Water Intake and Renal Functions by Salt Intake. Water intake, urine volume, and urinary excretion of sodium were increased in terms of time during 0.9% salt intake ($n = 15$, all $P < 0.05$). However, responsiveness of water intake to 2% salt intake was variable at the beginning of exposure and then tended to increase after the fifth day ($n = 8$, all $P > 0.05$). Urine volume and urinary excretion of sodium tended to increase without significance. The average of each parameter obtained form individual rat for 6 days after exposure of salt was calculated and shown in Figure 1a–d. With both salt intakes, marked natriuresis and diuresis appeared, as compared with control rats (Fig. 1c and 1d). However, no significant differences in water intake, water balance, urine volume, and urinary excretion of sodium were found between 0.9% and 2% salt intake rats (Fig. 1a–d).

Changes in Atrial and Plasma ANP Concentrations. Atrial and plasma concentrations of ANP did not change significantly at 12 hrs or on the first, third, and sixth day of 0.9% salt intake (Fig. 1E). With 0.9% salt intake for 10 days, atrial and plasma concentrations of ANP also did not change (Table 1). With increasing salt concentration by 2% for 10 days, atrial and plasma concentrations of ANP did not change except the ratio of right atrial ANP concentration to left side (Table 1).

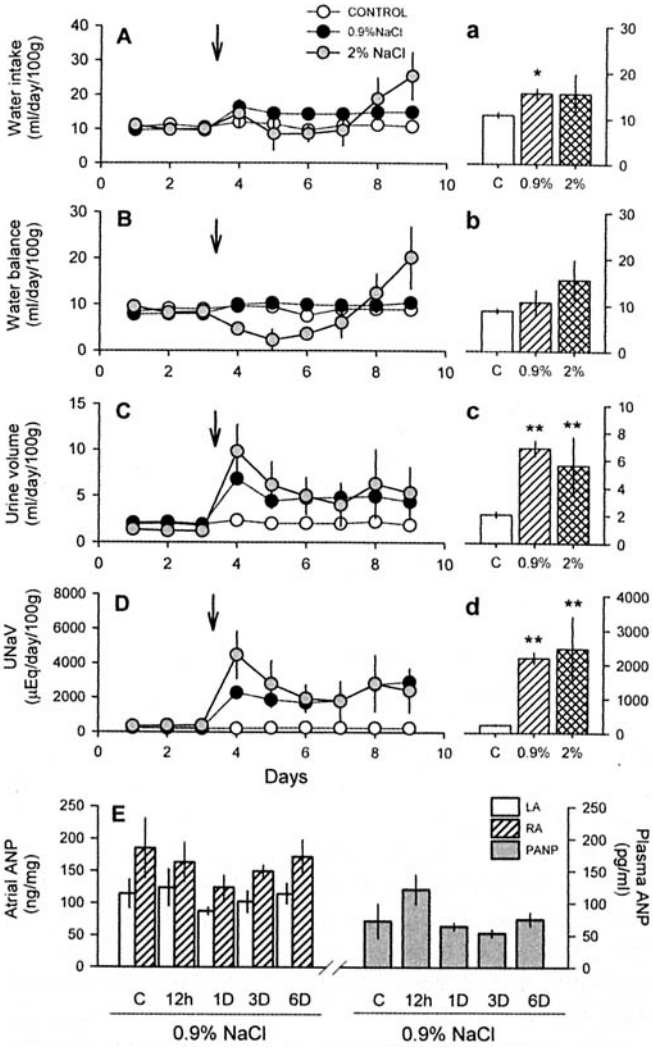

Figure 1. Changes in water intake (A), water balance (B), urine volume (C), urinary excretion of sodium (D), and ANP concentrations in atria and plasma (E) by 0.9% and 2% salt intake. Persistent water retention, diuresis, and natriuresis were observed, but atrial and plasma concentrations of ANP did not change during 0.9% salt intake. The average of each parameter obtained form individual rat for 6 days after exposure of salt was calculated and shown in Figure 1a–d. No significant differences in water intake, water balance, urine volume, and sodium excretion were found between two salt intake groups. Arrow indicates the start of salt intake. UNaV, urinary excretion of sodium; LA, RA, left and right atria, respectively; PANP, plasma ANP concentration; 1D, 3D, 6D, first, third, and sixth days of 0.9% salt intake. *, **, significantly different from control group, $P < 0.05$ and $P < 0.01$, respectively.

Stretch-Induced ANP Secretion from Atria of Salt Intake Rats. Figure 2 shows atrial volume, ANP secretion, and ECF translocation in response to elevations in intra-atrial pressure from left atria of rats receiving 0.9% salt solution (0.9% salt rats, $n = 10$) for 10 days. Changes in

Table 1. Chronic Effects of Salt Intakes for 10 Days on Atrial and Plasma Concentrations of ANP[a]

	CONT		0.9% NaCl		2% NaCl	
	LT	RT	LT	RT	LT	RT
Atrial ANP (ng/mg)	240.37 ± 22.02	295.21 ± 23.53	233.95 ± 23.97	266.72 ± 14.67	222.54 ± 26.6	329.02 ± 14.89
RT/LT atrial ANP (ratio)	1.25 ± 0.07		1.21 ± 0.12		1.54 ± 0.12*	
Plasma ANP (pg/ml)	104.71 ± 20.50		89.40 ± 18.24		53.89 ± 12.21	

[a] Values are the mean ± SE ($n = 9$). CONT, control rats; 0.9% NaCl, 0.9% salt intake rats; 2% NaCl, 2% salt intake rats; LT, RT, left and right atria, respectively.
*, Significantly different from control rats, $P < 0.025$.

atrial volume were proportionate to intra-atrial pressure in both groups and no significant differences in atrial volume were found (Fig. 2B). The basal rate of ANP secretion from atria of 0.9% salt rats was constant and not significantly different from control atria (Fig. 2C). Increases in atrial volume caused proportional increases in ANP secretion, which tended to increase in 0.9% salt rats (Fig. 2C).

We have previously shown that the ANP secretion is regulated by two-step sequential mechanism, the first being the release of ANP from atrial myocytes into the extracellular space, followed by the translocation of ECF with the released ANP into the atrial lumen with atrial contraction (21, 20). Therefore, the translocation of ECF using [^{3}H]-inulin clearance was measured simultaneously with the secretion of ANP. The basal ECF translocation was not significantly different from control atria (Fig. 2D). Increases in atrial volume caused increases in mechanically stimulated ECF translocation in both groups, and no significant differences in ECF translocation between them were found.

To compare the response of ANP secretion to atrial stretch between two groups, changes in ANP secretion and ECF translocation were calculated by subtracting the previous two values from the two peak values at each pressure. Figure 3A–D shows positive relationships among atrial volume, relative changes in ECF translocation, and ANP secretion in response to atrial stretch in 0.9% salt rats and control rats. Increases in atrial pressure caused proportional increases in the atrial volume (Fig. 3A), which closely correlated with mechanically stimulated ECF translocation in both groups (Fig. 3C). Increases in atrial volume caused proportional increases in ANP secretion (Fig. 3B), which closely correlated with mechanically stimulated ECF translocation (Fig. 3C). The positive relationship between ANP secretion and atrial volume, ANP secretion, and ECF translocation shifted leftward and upward by 0.9% salt intake (Fig. 3B and 3D). However, in 2% salt rats, no significant differences in relationships among changes in atrial volume, ANP secretion, and ECF translocation were found (Fig. 3E and 3F, $n = 10$). Therefore, changes in stretch-induced ANP secretion in terms of ECF translocation were markedly increased from 0.328 ± 0.029 to 0.513 ± 0.043 ng/μl ($P < 0.01$) by 0.9% salt intake but not in 2% salt intake (Fig. 4).

Changes in Particulate GC Activity and ANP-Binding Sites in Kidney. To evaluate changes in renal GC-A activity by salt intake for 10 days, the amounts of cGMP production by ANP were measured in tissue membranes of renal cortex and medulla (all $n = 9$). The basal cGMP production in renal cortical membranes was similar among three groups, but that in renal medullary membranes of 2% salt rats was lower than control rats. As shown in Figure 5, ANP caused increases in cGMP production in a dose-dependent manner in renal cortical membranes. The cGMP production by ANP showed accentuation in 0.9% salt rats but not in 2% salt rats, compared with the control rats. In renal medullary membranes, the cGMP production by $10^{-7}M$ ANP was

Table 2. Mean Values of Binding Constants for Regionally Specific Reversible Binding of ^{125}I-ANP in the Kidney of Control and Salt Intake Rats[a]

Sites	Groups	Constants	
		Kd (p*M*)	Bmax (fmol/mm^2)
Glomerulus	Control ($n = 13$)	1.30 ± 0.08	6.43 ± 0.61
	0.9% NaCl ($n = 17$)	1.68 ± 0.27	6.05 ± 0.84
	2% NaCl ($n = 8$)	0.71 ± 0.21**	2.62 ± 0.77***
Medulla	Control	1.78 ± 0.30	1.98 ± 0.40
	0.9% NaCl	2.60 ± 0.07	2.41 ± 0.61
	2% NaCl	3.65 ± 0.88**	2.44 ± 0.54

[a] Values are the mean ± SE. CONT, control rats; 0.9% NaCl, 0.9% salt intake rats; 2% NaCl, 2% salt intake rats.
*, **, ***, Significantly different from control rats, $P < 0.05$, $P < 0.01$, $P < 0.005$, respectively.

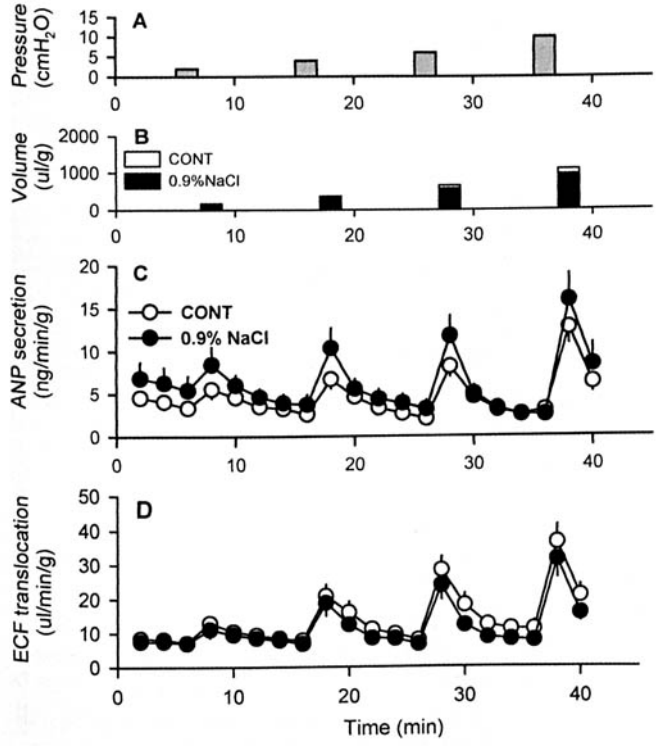

Figure 2. Changes in atrial pressure (A), volume (B), ANP secretion (C), and ECF translocation (D) from left atria of rats exposed to 0.9% NaCl for 10 days. Atrial volume change was increased by the elevation of intra-atrial pressure. A stepwise increase in atrial volume resulted in proportional increases in ANP secretion and concomitant ECF translocation. The stretch-activated ANP secretion tended to increase in 0.9% salt intake rats. Open column and dot indicate control rats (CONT). Closed column and dot indicate 0.9% salt intake rats (0.9% NaCl). Symbols are mean ± SEM.

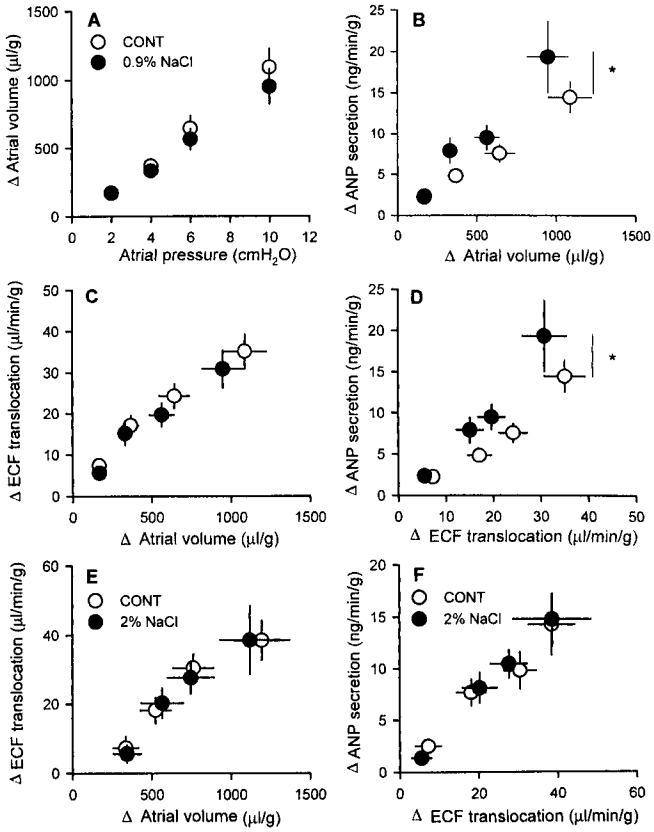

Figure 3. Relationships among atrial pressure, volume, ECF translocation, and ANP secretion in left atria of rats exposed to 0.9% NaCl (A–D) and 2% NaCl (E, F) for 10 days. Close correlations between those parameters were observed. The relationships between ANP secretion and atrial volume (B) and ANP secretion and ECF translocation (D) in 0.9% salt intake rats shifted to the left but not in 2% salt intake rats (F). *, significantly different from control group, $P < 0.05$.

much higher than in renal cortical membranes of control rats (2.91 ± 0.76 vs. 1.17 ± 0.05-fold increase, $P < 0.05$). However, the relative increase in cGMP production by ANP in renal medullary membranes was not modified by intakes of either salt concentration.

To define whether the accentuated response of cGMP production by ANP in 0.9% salt rats is due to changes in ANP receptor population or its affinity, characteristics of [^{125}I]-ANP-binding sites in the kidney were evaluated using *in vitro* autoradiography. Binding sites for [^{125}I]-ANP were distributed in both renal glomeruli and medulla of control rats (Fig. 6A) and completely displaced in the presence of 1μ*M* unlabeled ANP (Fig. 6B). In control rats, the Kd and Bmax values for [^{125}I]-ANP binding sites in renal glomeruli were 1.30 ± 0.08 p*M* and 6.43 ± 0.61 fmol/mm^3 ($n = 13$), respectively, and those in renal medulla were 1.78 ± 0.30 p*M* and 1.98 ± 0.40 fmol/mm^2, respectively (Table 2). No significant differences in Kd and Bmax values of [^{125}I]-ANP binding to the renal glomeruli and medulla of 0.9% salt rats were found ($n = 17$). In 2% salt rats, however, Kd and Bmax values of [^{125}I]-ANP binding to the renal glomeruli were decreased and Kd value [^{125}I]-ANP binding to the renal medulla was increased ($n = 8$).

Changes in mRNA for NPR-A and NPR-C in Kidney. Figure 7A shows a representative agarose gel analysis of RT-PCR products of mRNAs obtained from the renal cortex of control, 0.9%, and 2% salt rats (all $n = 6$). Changes in mRNAs for NPR-A (GC-A) and NPR-C were analyzed using semiquantitative RT-PCR. The amounts of all PCR products were proportionately increased in terms of PCR cycles. Relative amounts of mRNAs for NPRs were obtained using GAPDH mRNA (Fig. 7B). No significant changes in mRNA levels for NPR-A and NPR-C in the renal glomeruli and medulla of salt rats were found except decreased NPR-A mRNA in renal cortex of 2% salt rats.

Discussion

We have demonstrated an accentuation of atrial ANP secretion and ANP-stimulated cGMP production in renal cortical membranes without changes in NPR-A density and expression in 0.9% salt rats. In contrast, in 2% salt rats, the NPR-A density and expression in renal cortex were decreased without changes in ANP secretion and the cGMP production by ANP. These results suggest that atrial ANP secretion as well as its binding sites in the kidney respond differently to ingested salt concentration in rats.

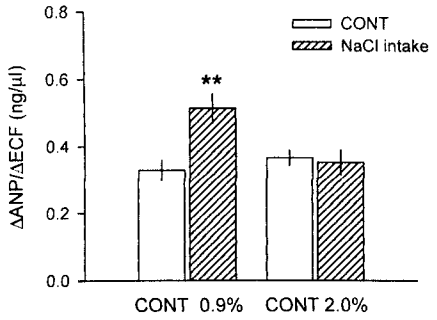

Figure 4. Comparisons of ANP secretion in terms of ECF translocation from left atria of rats exposed to either 0.9% or 2% NaCl. Data were derived from Figure 3. Average of changes in stretch-activated ANP secretion in terms of ECF translocation was accentuated in 0.9% salt intake rats. **, significantly different from control group, $P < 0.01$.

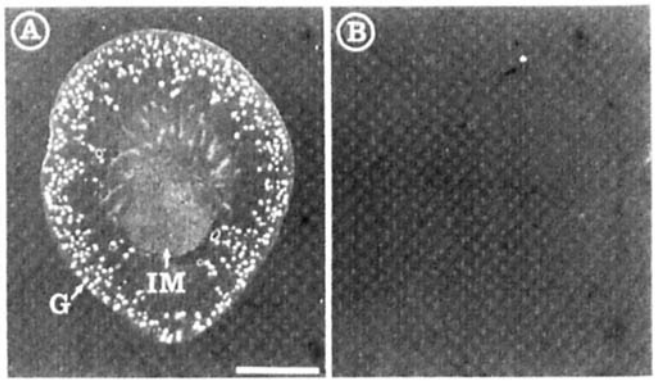

Figure 6. Dark-field photomicrograph of autoradiograms of renal cross-sections from control rats incubated in presence of 250 p*M* ^{125}I-ANP (A) and adjacent sections incubated in 250 p*M* ^{125}I-ANP + 1μ*M* unlabeled ANP (B). IM, inner medulla.

Chronic alterations of dietary salt and water balance have been shown to change ANP level and mRNA in atrial myocytes (11–15). Much attention has been directed toward establishment of the relationship between atrial synthesis and secretion of ANP under various experimental conditions, such as different concentrations of salt (from 0.9% to 8% salt), route of administration, and duration of exposure (from 2 days to 3 weeks).

The present study was performed to investigate the influence of salt intake (0.9% or 2%) on atrial content of ANP, its secretion, and changes in receptor density and expression. The levels of ANP in atrium and plasma were not altered significantly during acute (12 hrs and 1 and 3 days) and chronic exposures (10 days) to 0.9% salt. A 2% salt intake also did not cause any changes in the levels of ANP in atrium and plasma. Our results are consistent with the report showing no change in atrial ANP synthesis (13, 14). However, our results are not consistent with the report showing increased ANP mRNA level and decreased atrial content of ANP in animals given a high-salt diet or volume expansion (11, 12).

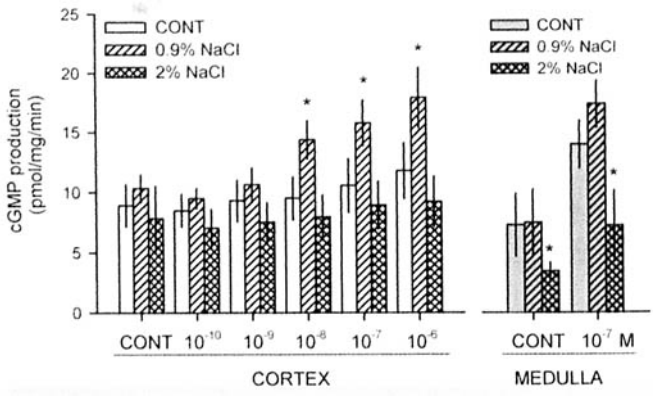

Figure 5. The cGMP production stimulated by ANP in renal tissue membranes of rats exposed to 0.9% or 2% NaCl. ANP caused proportionate increases in cGMP production in renal cortical membranes in a dose-dependent manner, which was accentuated in 0.9% NaCl intake rats but not in 2% NaCl intake rats. The basal cGMP production in renal medullary membranes of 0.9% NaCl intake rats was lower than in control rats. The relative change in cGMP production by ANP in control rats was similar to salt intake rats. *, significantly different from control group, $P < 0.05$.

The decreased atrial content of ANP is explained by a result of increased ANP release to chronic volume load because of a time delay of ANP synthesis. The discrepancy may be due to different levels of water retention caused by salt intakes because hydration level is influenced by many factors, such as environmental temperature, humidity, or ventilation status, despite of the same concentration of salt intake. Another possible explanation is that the responsiveness of ANP secretion to a gradual increase in body fluid (induced by salt intake in the present study) and acute expansion (induced by saline ingestion intravenously or orally) may be different. In the former case, the atrial ANP system may be adapted slowly to changes in body fluid, and other hormonal systems regulating the body fluid may be compensating, making it difficult to detect changes in the atrial ANP system.

In the next experiments, we sought to determine whether the stretch-activated ANP secretion from rat atria of salt intakes is modified despite no changes in atrial ANP content. Surprisingly, stretch-activated ANP secretion in terms of ECF translocation was accentuated in 0.9% salt intake rats but not in 2% salt intake rats. Hence, the secretion of ANP to atrial stretch responds differently in terms of salt concentrations of drinking water. Why did plasma concentration of ANP not increase in 0.9% salt group despite accentuating stretch-activated ANP secretion? One possible factor is the degradation rate of ANP, such as NPR-C density or the metabolic rate of ANP. The relative changes in mRNAs for NPR-A and NPR-C in 0.9% salt intake rats were similar to control rats. We do not know changes in metabolic rate of ANP at present. In these experimental conditions, an increased amount of body fluid (positive water balance) is also an important factor because of the dilution of secreted extra ANP.

Interestingly, the production of cGMP stimulated by ANP in renal cortical tissue membranes was accentuated in the 0.9% salt intake group. Rather, the ANP receptor density and its mRNA in renal glomeruli were decreased in 2% salt

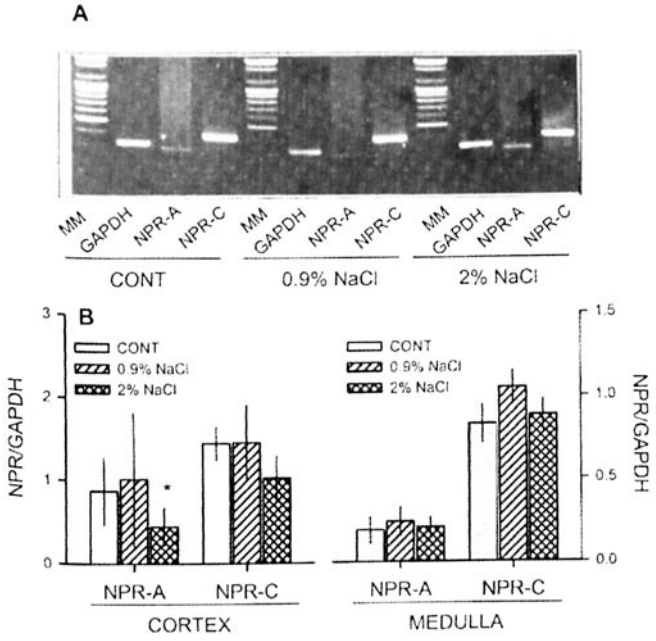

Figure 7. A representative agarose gel analysis of RT-PCR products of mRNAs obtained from the renal cortex of control, 0.9%, and 2% salt intake rats (A). (B) Comparisons of relative changes in mRNAs for NPR-A and NPR-C in terms of GAPDH in renal cortex and medulla of control and salt intake rats. MM, DNA molecular size marker (174 RF DNA, Hae III cut); lane 1, 4, 7, GAPDH; lane 2, 5, 8, NPR-A; lane 3, 6, 9, NPR-C. The mRNA for NPR-A in renal cortex of 2% salt intake rats was lower than in control rats. *, significantly different from control group, $P < 0.05$.

intake rats without a difference in cGMP production by ANP. We do not know the reason for the gap between receptor density (including mRNA level) and cGMP production by ANP. One possible explanation is an increased sensitivity of particulate GC to ANP. Further studies are needed to define the mechanism.

In conclusion, our data suggest that atrial ANP secretion as well as its binding site in the kidney may respond differently to ingested salt concentration. However, considering the fact that natriuresis was observed without an increase in plasma ANP level in salt-administered rats, we also suggest that other hormonal systems may also be involved in the regulation of water balance in high-salt intake rats.

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