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The interaction between atrial natriuretic peptides and angiotensin II in controlling sodium and water excretion in the rat

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1 The present study was designed to determine how the natriuretic and diuretic actions of atrial natriuretic peptides were modulated by circulating angiotensin II.

2 In sodium pentobarbitone-anaesthetized rats, administration of bolus doses of atriopeptin III (1000 ng kg^{-1}) had no effect on blood pressure, renal blood flow, or glomerular filtration rate but caused reversible increases (all $P < 0.001$) in urine flow, of 53.9 ± 14.4 $\mu l\,kg^{-1}\,min^{-1}$, absolute sodium excretion, of 13.4 ± 2.9 $\mu mol\,kg^{-1}\,min^{-1}$ and fractional sodium excretion of 3.26 ± 0.74%. Similar effects were seen following a second dose of the atriopeptin III.

3 Following blockade of the renin-angiotensin system with captopril (900 $\mu g\,kg^{-1}\,h^{-1}$), control levels of blood pressure and haemodynamics were unchanged but there were significant (all $P < 0.001$) increases in urine flow, from 39.96 ± 5.05 to 88.70 ± 8.41 $\mu l\,kg^{-1}\,min^{-1}$, absolute sodium excretion, from 8.35 ± 1.08 to 21.62 ± 1.62 $\mu mol\,kg^{-1}\,min^{-1}$ and fractional sodium excretion, from 3.82 ± 0.23 to 5.34 ± 0.32%. Under these conditions, atriopeptin III-induced increases in urine flow (110.2 ± 8.7 versus 43.9 ± 6.2 $\mu l\,kg^{-1}\,min^{-1}$) absolute (24.0 ± 1.8 versus 9.3 ± 1.2 $\mu mol\,kg^{-1}\,min^{-1}$) and fractional ($5.16 \pm 0.24$ versus 2.08 ± 0.33%) sodium excretions were significantly ($P < 0.001$) greater.

4 In another group of rats given captopril, angiotensin II at 10 ng $kg^{-1}\,min^{-1}$ was also infused; this had no effect on blood pressure or renal haemodynamics, but partially restored basal levels of sodium and water excretion to those obtained before captopril. Atriopeptin III reversibly increased urine flow and absolute sodium excretion to the same degree as that obtained without captopril, but fractional sodium excretion was significantly larger than that obtained in the absence of captopril. In rats infused with angiotensin II at 15 ng $kg^{-1}\,min^{-1}$ together with the captopril the basal levels of fluid output were unchanged, while the magnitudes of the urine flow and sodium excretory responses to atriopeptin III were identical to those obtained before captopril and angiotensin II.

5 In animals subjected to two weeks of a low-sodium diet, atriopeptin III reversibly increased urine flow, absolute and fractional sodium excretions by between 53% and 74%; these responses were significantly ($P < 0.001$) smaller than those obtained in sodium replete rats. Administration of atriopeptin III, to low sodium diet rats given captopril, induced excretory responses which were significantly larger than those obtained in the absence of captopril.

6 The findings of this investigation demonstrate that in acute situations, angiotensin II exerts an important modulatory influence on the natriuretic potency of the atrial peptides by attenuating their action on the kidney. Long-term activation of the renin-angiotensin system depresses the renal excretory responses to atrial natriuretic peptides but suppression of angiotensin II production only partially restores the responsiveness of the kidney.

Keywords: atrial natriuretic peptides; angiotensin II; sodium excretion; renal function

Introduction

The atrial natriuretic peptides are released from the atria and act on the kidney to promote sodium and water excretion (Needleman *et al.*, 1989). The mechanisms underlying this action are unclear at present but a number of possibilities exist. An early finding was that atrial natriuretic peptides inhibited aldosterone release (Brenner *et al.*, 1990) but this would not account for the prompt and transient renal excretory responses. A further observation was that administration of high doses of atrial natriuretic peptides increased both renal blood flow and glomerular filtration rate, and one consequence would be increased filtered load which would tend to increase fluid output. Nevertheless, at low doses of atrial natriuretic peptides which have no renal haemodynamic action, there is still an impressive natriuretic and diuretic response (Brenner *et al.*, 1990) and this is now accepted as being due to a direct action of the peptides on tubular reabsorptive processes.

There is a high density of receptors for atrial natriuretic peptides at the glomerulus where they may be involved in the regulation of hydraulic conductivity via mesangial cell relaxation, whereas the evidence for receptors at the proximal tubules which may suppress tubular reabsorption is poor (Brenner *et al.*, 1990). By contrast, high affinity binding sites have been shown to exist in the inner medulla (Healy & Fanestil, 1986; Koseki *et al.*, 1986) and micropuncture studies have demonstrated that the atrial natriuretic peptides suppress fluid reabsorption along the cortical, medullary and papillary sections of the collecting tubules (Sonnenberg *et al.*, 1986). A consequence of such an action would be to diminish water retention and increase sodium excretion.

Recently, it has become apparent that one component of the tubular action of atrial natriuretic peptides could be due to its suppression of the action of angiotensin II at the proximal tubule where it normally stimulates sodium reabsorption (Harris & Skinner, 1990). Conversely, evidence is emerging that angiotensin II itself can inhibit the natriuretic action of atrial natriuretic peptides. Recently, Salazar *et al.* (1987) and Showalter *et al.* (1988) found that intrarenal infusion of angiotensin II in dogs blunted the natriuretic response to atrial natriuretic peptides when renal haemodynamics were

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Table 1 Responses in blood pressure and renal haemodynamic and excretory function to bolus doses of atriopeptin III (APIII, 1000 ng kg^{-1}) in sodium replete animals

		Control	*APIII 1*	*Control 2*	*APIII 2*
MAP (mmHg)	Group 1	116 ± 3	115 ± 4	115 ± 3	112 ± 3
	Group 2	113 ± 2	111 ± 2	112 ± 2	109 ± 3
	Group 3	118 ± 2	117 ± 3	116 ± 2	116 ± 2
	Group 4	114 ± 2	113 ± 2	113 ± 3	111 ± 3
RBF (ml kg^{-1} min^{-1})	Group 1	14.09 ± 0.84	14.32 ± 0.82	13.70 ± 0.16	13.88 ± 0.77
	Group 2	12.76 ± 1.38	12.38 ± 1.27	14.89 ± 1.80	14.89 ± 1.71
	Group 3	13.70 ± 0.83	14.39 ± 0.95	13.80 ± 0.72	13.89 ± 0.77
	Group 4	11.93 ± 1.27	11.81 ± 1.29	11.86 ± 1.39	11.42 ± 1.43
GFR (ml kg^{-1} min^{-1})	Group 1	2.81 ± 0.17	2.81 ± 0.22	2.70 ± 0.16	2.74 ± 0.19
	Group 2	2.53 ± 0.06	2.76 ± 0.08	2.52 ± 0.09	2.76 ± 0.14
	Group 3	2.40 ± 0.10	2.59 ± 0.13	2.17 ± 0.14	2.37 ± 0.09
	Group 4	2.67 ± 0.15	2.77 ± 0.11	2.48 ± 0.22	2.30 ± 0.27
UV (μl kg^{-1} min^{-1})	Group 1	52.55 ± 5.05	106.47 ± 11.69**	61.43 ± 9.58	121.06 ± 16.94**
	Group 2	39.96 ± 5.04	83.89 ± 10.88**	88.70 ± 8.41	198.86 ± 14.02**
	Group 3	67.90 ± 6.63	133.91 ± 14.85**	80.48 ± 5.45	148.97 ± 15.23**
	Group 4	55.94 ± 8.94	103.30 ± 15.97*	61.95 ± 7.89	98.79 ± 9.31*
$U_{Na}V$ (μmol kg^{-1} min^{-1})	Group 1	11.95 ± 1.05	25.35 ± 2.47**	15.00 ± 2.70	30.07 ± 4.32**
	Group 2	8.35 ± 1.08	17.67 ± 2.27**	21.62 ± 1.62	45.62 ± 2.28**
	Group 3	12.68 ± 1.20	24.12 ± 2.71**	16.06 ± 1.29	27.35 ± 1.47**
	Group 4	10.78 ± 1.94	20.36 ± 3.29*	13.41 ± 1.86	21.61 ± 2.54*
FE_{Na} (%)	Group 1	3.05 ± 0.40	6.31 ± 0.80**	3.43 ± 0.41	6.44 ± 0.72**
	Group 2	2.21 ± 0.29	4.29 ± 0.60**	5.65 ± 0.52	10.81 ± 0.57**
	Group 3	3.82 ± 0.23	6.79 ± 0.88**	5.34 ± 0.32	8.37 ± 0.60**
	Group 4	2.80 ± 0.47	5.02 ± 0.79*	3.77 ± 0.63	5.77 ± 0.88*

Group 1, normal saline infusion ($n = 6$); Group 2, captopril 900 μg kg^{-1} h^{-1} infused in the second part of the experiment ($n = 7$); Group 3, as Group 2, but angiotensin II 10 ng kg^{-1} min^{-1} infused with captopril ($n = 6$); Group 4, as Group 3, but angiotensin II at 15 ng kg^{-1} min^{-1} ($n = 6$). MAP, mean arterial pressure; RBF, renal blood flow; GFR, glomerular filtration rate; UV, urine flow, $U_{Na}V$, urinary sodium excretion, FE_{Na}, fractional sodium excretion. Control values 1 and 2 (first and second part of the experiment) represent the average values of the two clearances before and following atriopeptin III. APIII 1 and 2 represent the values achieved during atriopeptin III administration. n = number of animals.
Significantly different from control, *$P < 0.01$; **$P < 0.001$.

unchanged. Conversely, Bie *et al.* (1990) reported that blockade of angiotensin II production in the dog enhanced the action of ANP on renal fluid excretion, although in this study canrenoate was also used to block aldosterone activity.

The aim of this study was to determine whether the natriuretic response to the atrial natriuretic peptides is influenced by the circulating level of angiotensin II. This was done by comparing the increase in sodium excretion caused by atrial natriuretic peptides before and following the blockade of angiotensin II production with captopril. Further, the natriuretic effectiveness of atrial natriuretic peptides was determined when physiological levels of angiotensin II were restored by exogenous intravenous infusion, or elevated chronically subsequent to two weeks of a low sodium diet.

Methods

The experiments were performed on male Wistar rats (mean body weight 350 g) which had been fasted overnight. The animals were anaesthetized with pentobarbitone sodium, 60 mg kg^{-1} body weight i.p., and maintained with a constant infusion of pentobarbitone sodium (12 mg kg^{-1} h^{-1}) which was infused continuously until the end of the experiment. After the anaesthesia was induced, a tracheostomy was performed and catheters were placed in the right carotid artery for the measurement of arterial pressure and obtaining blood samples, and in the left jugular vein for infusions of saline and drugs. The intravenous infusion of isotonic saline (6 ml h^{-1}) was commenced immediately and continued for the duration of the experiment. The left kidney was exposed via a mid-line incision, its ureter cannulated for urine collection and the renal artery was carefully cleared and a flowmeter probe was then placed around the artery. Silver wire electrodes were applied onto the coeliac/aortico-renal ganglia and pulses of 15 V, 10 Hz and 0.2 ms were delivered for 10 s which caused a transient blanching of the kidney. If blanching did not occur, the animal was considered denervated and not included in the study. The arterial catheter was connected to a pressure transducer (Statham P231D) and the renal blood flow was measured with a square wave electromagnetic flowmeter (FM 501, Carolina Medical Instruments). Both arterial pressure and renal blood flow were continuously recorded on a polygraph (Model 7D Grass Instruments, U.S.A.). After surgery was completed a priming dose of 2 ml inulin in saline (1 g 100 ml^{-1}) was given intravenously and isotonic saline infusion was replaced with one containing inulin (1 g 100 ml^{-1}). A 2 h period of equilibration and stabilization was allowed before the clearance studies were performed.

Experimental protocols

Sodium replete animals The animals had free access to standard rat diet (sodium content 0.35%) and tap water. Ten clearance periods of 15 min each, were performed in two series of five clearances. Each series comprised two basal clearance periods followed by one experimental clearance, after which 15 min of recovery was allowed and then two recovery clearances were performed. Thirty min later the second series of clearances were undertaken. Arterial blood samples (300 μl each) were taken before the first and the third and after completion of the third and the fifth clearance periods and the same pattern was used in the second series of clearances. The blood samples were immediately centrifuged and plasma obtained, the red cells were resuspended in an equivalent volume of heparinized saline and reinfused into the animal. A 5 min equilibration period was allowed before the next collection period commenced. Urine was collected in pre-weighed microcentrifuge capped tubes.

Group 1 ($n = 6$) Control. Rat atriopeptin III (APIII, ANF5-28, Cambridge Research Biochemicals Limited, Cambridge, England) was given as a bolus dose of 1000 ng kg^{-1} in 0.3 ml normal saline intravenously 2 min before the experimental clearances.

Group 2 ($n = 7$) Animals were given APIII as in group 1. An infusion of captopril (900 μg kg^{-1} h^{-1}) was started after the

first series of clearances and continued throughout the rest of the experiment. The mean arterial pressure and renal blood-flow responses to angiotensin I 200 ng, given as a bolus dose in 0.2 ml saline, and angiotensin II 100 ng, as a bolus dose in 0.2 ml saline were obtained before the captopril infusion and at the end of the experiment at which time the responses to angiotensin I but not angiotensin II were abolished. The second series of clearances began 30 min after the start of captopril infusion.

Group 3 ($n = 6$) The experimental protocol was the same as that of group 2, but angiotensin II, 10 ng kg^{-1} min^{-1} was infused together with captopril.

Group 4 ($n = 6$) The same protocol was used as in group 3, but angiotensin II was infused at 15 ng kg^{-1} min^{-1} along with the captopril.

Low sodium animals The animals were placed on low sodium diet (Special Diet Services, Essex containing 0.05% sodium chloride) and distilled water two weeks before experiments. Only the first series of five clearances was performed in each animal according to the protocol described above. The animals were divided into two groups.

Low sodium (LS) group ($n = 6$) Animals received APIII in the same dose as sodium replete animals.

Low sodium animals receiving converting enzyme inhibitor (LS + CEI group) ($n = 5$) The infusion of captopril 900 μg kg^{-1} h^{-1} was started 30 min before the end of equilibrium period and continued throughout the experiment. The responses to bolus doses of angiotensin I and II were measured as described above.

Chemical assays

Urinary and plasma sodium concentration was measured by flame photometry (Corning 410C). Plasma and urine samples were deproteinised (Somogyi, 1930) and the inulin content assayed following the method of Bojesen (1952). Plasma inulin levels were taken as the average of the inulin values before and at the end of the paired or single clearance periods and glomerular filtration rate was calculated as inulin clearance.

Statistical analysis

All values represent means ± s.e.mean. The mean values of two basal and two recovery clearance periods were calculated for each series of five clearances and were considered as control values which were compared to the experimental value obtained during the influence of APIII. The statistical analysis was performed with two-way analysis of variance (ANOVA, FASTAT Software). The differences were taken to be significant when $P < 0.05$.

Results

Sodium replete study (Groups 1–4)

Table 1 presents the mean blood pressure and renal haemodynamic and functional variable responses to APIII administration for all experiments. Group 1 animals represent time controls and administration of APIII in the first half of the experiment had no effect on blood pressure, renal blod flow or glomerular filtration rate but significantly (all $P < 0.001$) and reversibly increased urine flow, by 53.9 ± 14.4 μl kg^{-1} min^{-1} absolute and fractional sodium excretions by 13.4 ± 2.9 μmol kg^{-1} min^{-1} and 3.26 ± 0.74%, respectively. In the second part of the study, control values of urine flow, absolute and fractional sodium excretion were slightly, but not significantly, raised and repeating this dose of APIII in the second part of the experiment gave a virtually identical pattern of excretory changes, the magnitudes of which could not be distinguished from those of the first part of the study. A comparison of these responses is shown in Figure 1.

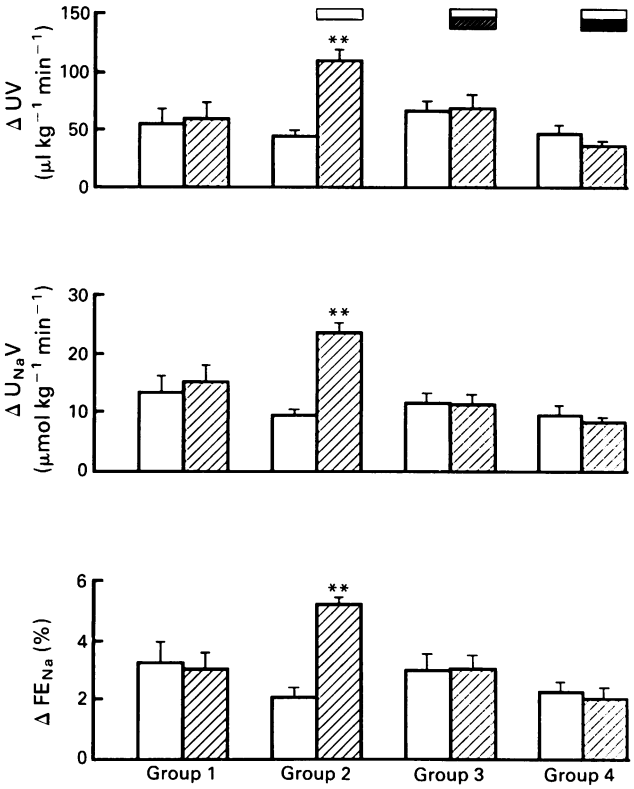

Figure 1 This shows the changes (Δ) in urine flow (UV), absolute ($U_{Na}V$) and fractional sodium excretion (FE_{Na}) from control levels in response to atriopeptin III (1000 ng kg^{-1}). The open histograms represent the responses to the first test with APIII, and the hatched histograms the responses observed following the second dose of APIII. Open horizontal bar: captopril, 900 μg kg^{-1} h^{-1}; hatched bar: angiotensin II, 10 ng kg^{-1} min^{-1}; solid bar: angiotensin II, 15 ng kg^{-1} min^{-1}.
**Significantly different from the first atriopeptin III challenge in that group.

In Group 2 animals (Table 1), the control values of all variables were similar to those of group 1 animals and administration of the first dose of APIII caused comparable reversible increases in urine flow, absolute and fractional sodium excretions (all $P < 0.001$). Infusion of captopril had no effect on blood pressure, renal blood flow or glomerular filtration rate, while control values of urine flow, absolute and fractional sodium excretion were all ($P < 0.001$) greater when compared to the first part of the experiment (Table 1). During the captopril infusion, the reversible increases in urine flow, absolute and fractional sodium excretions induced by APIII were all significantly ($P < 0.001$) larger than those obtained in the first part of the experiment (Figure 1) and those observed in the second part of the group 1 animals.

The animals of group 3 represent those in which exogenous angiotensin II was given together with the captopril (Table 1). The control values for blood pressure, renal haemodynamics excretory variables and excretory responses to APIII were very similar in both group 1 and 2 animals. In the second part of the experiment, angiotensin II was infused at 10 ng kg^{-1} min^{-1} together with the captopril and although this had no effect on the control values of urine flow or absolute sodium excretion, fractional sodium excretion was significantly ($P < 0.02$) raised. The administration of APIII under these conditions reversibly increased urine flow and absolute sodium excretion, the magnitudes of which could not be distinguished from those obtained either in the first half of the experiment or those obtained in group 1 in the second part of the experiment while the response in fractional sodium excretion (Figure 1) was significantly larger ($P < 0.02$).

Table 2 Responses in blood pressure and renal haemodynamic and excretory function to bolus doses of atriopeptin III (APIII) in low sodium animals

		Control	*APIII*
MAP	LS	107 ± 3	106 ± 3
(mmHg)	LS + CEI	105 ± 2	104 ± 2
RBF	LS	13.60 ± 0.92	14.02 ± 1.02
($ml\,kg^{-1}\,min^{-1}$)	LS + CEI	16.22 ± 1.27	16.15 ± 1.36
GFR	LS	2.35 ± 0.20	2.63 ± 0.23
($ml\,kg^{-1}\,min^{-1}$)	LS + CEI	3.77 ± 0.21	3.91 ± 0.26
UV	LS	28.97 ± 3.84	44.53 ± 5.57*
($\mu l\,kg^{-1}\,min^{-1}$)	LS + CEI	60.15 ± 2.39	102.46 ± 15.03*
$U_{Na}V$	LS	3.64 ± 0.74	6.37 ± 0.96**
($\mu mol\,kg^{-1}\,min^{-1}$)	LS + CEI	11.39 ± 0.85	21.09 ± 2.92**
FE_{Na}	LS	1.03 ± 0.16	1.62 ± 0.18**
(%)	LS + CEI	2.09 ± 0.12	3.68 ± 0.36**

LS, low sodium diet for two weeks ($n = 6$); LS + CEI, as LS but receiving captopril $900\,\mu g\,kg^{-1}\,h^{-1}$ i.v. from 30 min before experimental collections were started ($n = 5$). Abbreviations and significance values as in Table 1.

Group 4 animals were comparable to group 3, except that angiotensin was infused at a higher rate, $15\,ng\,kg^{-1}\,min^{-1}$, during the blockade of the renin-angiotensin system (Table 1). The control values of blood pressure and renal haemodynamics and excretory variables for the first part of the experiment were comparable to those obtained in the other groups over this period. There were no blood pressure or renal haemodynamic responses to APIII (Table 1) and the increases in urine flow, absolute and fractional sodium excretions (all $P < 0.001$) were very similar to all the other groups. The bolus dose of APIII during the co-administration of captopril with angiotensin II at $15\,ng\,kg^{-1}\,min^{-1}$ caused reversible increases in urine flow, absolute and fractional sodium excretions (all $P < 0.01$), which were similar in magnitude to those obtained in the first half of the experiment and those of the group 1 animals given the second dose of APIII (see Figure 1).

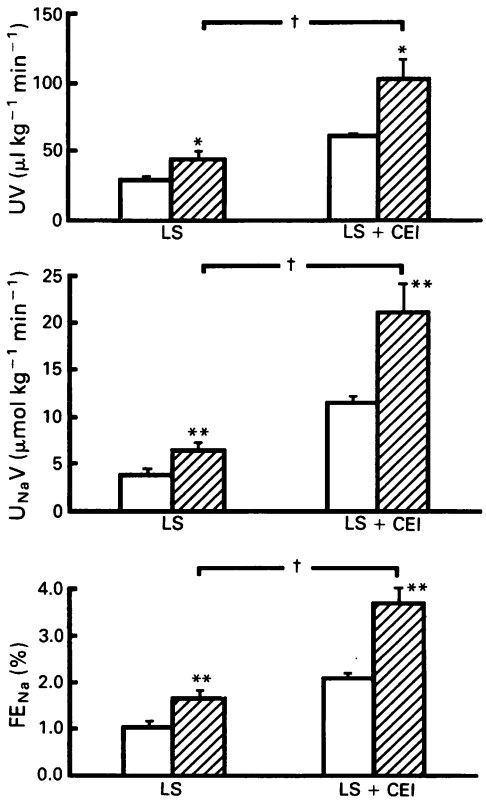

Figure 2 The responses in urine flow (UV), absolute ($U_{Na}V$) and fractional sodium excretion (FE_{Na}) to atriopeptin III (APIII) $1000\,ng\,kg^{-1}$ compared to control (mean of before and following APIII) in animals fed a low sodium diet for two weeks (LS) or also given captopril at $900\,\mu g\,kg^{-1}\,h^{-1}$ (LS + CEI). Open bars represent control values, hatched bars the values obtained after APIII bolus. Significantly different from control: *$P < 0.01$; **$P < 0.001$. Significantly different from values in LS group †$P < 0.001$.

Low sodium study

The control level of blood pressure in the low sodium diet animals (Table 2) was significantly lower than in the sodium replete animals ($P < 0.01$) and although both renal blood flow and glomerular filtration rate were similar to the replete groups, urine flow, absolute and fractional sodium excretions were all significantly ($P < 0.001$) lower. Administration of APIII to the animals fed a low sodium diet had no effect on blood pressure or renal haemodynamics but reversibly and significantly ($P < 0.001$) increased all excretory variables, the magnitudes of which were smaller when compared to the sodium replete animals (all $P < 0.001$).

Infusion of captopril into the animals fed low sodium diet (Table 2) had no effect on blood pressure, which remained lower than sodium replete animals, although renal blood flow was slightly raised. However, in these animals glomerular filtration rate was significantly ($P < 0.001$) greater as was urine flow, absolute and fractional sodium excretions (all $P < 0.001$). When the bolus dose of APIII was given, blood pressure and renal haemodynamics did not change but urine flow, absolute and fractional sodium excretions were significantly and reversibly elevated (all $P < 0.001$) and the responses in absolute and fractional sodium excretion were significantly (both $P < 0.05$) larger than those obtained in the rats subjected to the low sodium diet alone but were comparable to those obtained in the sodium replete animals (Figure 2).

Discussion

This study was undertaken to gain insight into the way in which angiotensin II could modulate the excretory responses of the kidney to atrial natriuretic peptides. We used a synthetic APIII at doses which had been shown in earlier studies to have no or minimal effects on renal haemodynamics (Johns & Rutkowski, 1989; 1990a,b). Under these conditions, the excretory responses obtained would be due primarily to a direct tubular action of the natriuretic peptide. In all animals we ensured that the innervation of the kidney was intact as there is evidence that renal nerve activity can blunt the excretory responses to the atrial natriuretic peptides, at least under some pathological situations (Koepke *et al.*, 1987).

The results from the first group of rats showed that the dose of $1000\,ng\,kg^{-1}$ of APIII caused an approximate doubling of both urine flow and sodium excretion comparable to the

effects reported earlier by ourselves (Johns & Rutkowski, 1989; 1990a,b) and Luft *et al.* (1986) using similar dose levels. These natriuretic and diuretic responses were most probably due to a direct tubular action of the peptide, possibly at some point along the tubule, collecting duct or medullary vasculature site (Harris & Skinner, 1990). It was clear from this time control group of rats that a second dose of ANP produced excretory responses which were similar in magnitude to those obtained to the first dose of peptide showing that the experimental preparation was stable and the results reproducible.

The contribution of angiotensin II, if any, to modulating the effectiveness of the ANP on the tubular responses of the kidney was approached initially by suppressing endogenous production of angiotensin II. Captopril was given in the second part of the study at $900\,\mu g\,kg^{-1}\,h^{-1}$ and at the end of the experiment was sufficient to abolish the vasopressor and renal vasoconstrictor response to a bolus dose of angiotensin I (200 ng) which was taken as good evidence that the renin-angiotensin system had been blocked and that circulating angiotensin II was at a low level. The captopril administration was associated with an increase in baseline levels of urine flow and sodium excretion which was most likely due to the removal of the antinatriuretic and antidiuretic action of angiotensin II (Johns, 1989). Administration of APIII in the presence of captopril led to an enhanced natriuretic and diuretic response compared to that obtained when the renin-angiotensin system was intact. The mechanism underlying this interaction of angiotensin II with APIII is unclear. One possibility could be that removal of the action of angiotensin II at the proximal tubule would result in a greater fluid load being presented to the more distal nephron segments, including the collecting duct, which would allow the APIII to act on a greater proportion of the fluid load, thereby generating a proportionately greater response.

These observations in the anaesthetized rat were similar to those reported by Bie *et al.* (1990) using the anaesthetized dog. They found that in animals given the aldosterone inhibitor, canrenoate and an intra-renal infusion of ANP there was an enhanced natriuresis following administration of a converting enzyme inhibitor. These findings were not supported by two recent papers describing experiments in conscious man (Gaillard *et al.*, 1988; Wamback *et al.*, 1989) in which the atrial natriuretic peptide-induced natriuresis was blunted following converting enzyme inhibition. The reasons for these differences remain to be resolved, and may be dependent on surgical stress activating the renin angiotensin system. However, in both human studies (Gaillard *et al.*, 1988; Wamback *et al.*, 1989) there was a concomitant reduction in blood pressure which would itself have acted to attenuate sodium excretion and possibly limited the response to the peptide.

In an attempt to implicate more clearly an action of angiotensin II, it was infused continuously during blockade of the renin-angiotensin system at a rate which was aimed to raise circulating levels to within the physiological range, but not such as to cause an increase in blood pressure or to reduce renal blood flow. Initially a rate of $10\,ng\,kg^{-1}\,min^{-1}$ angiotensin II was used but under these conditions the basal levels of sodium excretion were still somewhat elevated compared to the period before either captopril or angiotensin II. Nevertheless, the magnitude of the natriuretic and diuretic responses to APIII were suppressed compared to captopril alone and were similar to those obtained when captopril was not given. A second study was undertaken in which angiotensin II was infused at $15\,ng\,kg^{-1}\,min^{-1}$ in the presence of captopril and in this case basal levels of urine flow and sodium excretion were no different from those obtained in the absence of either compound; under these conditions the excretory responses to APIII were suppressed compared to those obtained when captopril was given alone, but were similar to those obtained in response to the first challenge with APIII. These studies complement and strengthen results of the initial study showing that it is indeed angiotensin II which attenuates the ability of the atrial natriuretic peptides to induce a natriuresis and diuresis. This viewpoint is supported by the findings of Salazar *et al.* (1987) and Showalter *et al.* (1988) using dogs in which angiotensin II, either in the presence or absence of an angiotensin convering enzyme inhibitor, appeared to limit the natriuretic effect of the atrial peptides. The exact sites within the kidney where these interactions might occur could not be assessed during the current approach. As proposed above, the action of angiotensin II at the proximal tubule would have reduced the amount of fluid presented to the collecting duct and thereby limited the magnitude of the response to the APIII.

The final study was an attempt to elevate chronically the levels of circulating angiotensin II by subjecting animals to two weeks of a low dietary sodium intake. The results clearly showed that under these conditions the magnitude of the natriuretic and diuretic responses were smaller compared to those obtained in sodium replete rats. Administration of the captopril to the low sodium rats resulted in higher levels of glomerular filtration rate and excretion of water and sodium than in its absence which was consistent with removal of elevated levels of angiotensin II (Johns, 1989). However, it was striking that when the renin-angiotensin system was blocked, although the natriuretic and diuretic responses to the atrial natriuretic peptides were enhanced, they only became comparable in magnitude to those obtained in the sodium replete animals. Indeed, it could be argued that the responses should have been of a similar size to those obtained in the replete rats receiving captopril. The reason for this not occurring is unclear, but may indicate that factors other than angiotensin II are involved under these conditions of chronic stimulation of the renin-angiotensin system. Indeed, Steele & Challoner-Hue (1990), using the isolated, buffer-perfused rat kidney, in which circulating angiotensin II was not present, found that the magnitude of the excretory responses to atrial natriuretic peptides were much less in rats subjected to four weeks of low sodium diet. These results and those of the present study implicate some factor, in addition to angiotensin II as being involved in modulating the renal excretory responses to atrial natriuretic peptide following low dietary sodium intake.

This study has attempted to define the role of angiotensin II in modulating the natriuretic and diuretic responses of the kidney to atrial natriuretic peptide. Blockade of the renin-angiotensin system enhanced, and exogenous infusion of angiotensin II suppressed, the natriuretic action of atriopeptin III. Further, the action of the atrial natriuretic peptide on the renal excretory responses was lower in the animals subjected to low sodium diet and was partially enhanced following converting enzyme inhibition. This study has shown that angiotensin II is an important factor which attenuates the action of atrial natriuretic peptides on the kidney and limits the magnitude of the natriuretic response. Under conditions of chronically elevated angiotensin II other factors appeared to come into play.

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References

BIE, P., WANG, B.C., LEADLEY, R.J. & GOETZ, K.L. (1990). Enhanced atrial natriuretic peptide natriuresis during angiotensin and aldosterone blockade in dogs. *Am. J. Physiol.*, **258**, R1101–R1107.

BOJESEN, E. (1952). A method for the determination of inulin in plasma and urine. *Acta Med. Scand.*, **142** (suppl 266), 275–282.

BRENNER, B.M., BALLERMAN, B.J., GUNNING, M.E. & ZEIDL, M.L. (1990). Diverse biological actions of atrial natriuretic peptides. *Physiol. Rev.*, **70**, 655–699.

GAILLARD, C.A., KOOMANS, H.A. & DARHOUT MEES, E.J. (1988). Enalapril attenuates natriuresis of atrial natriuretic factor in man. *Hypertension*, **11**, 160–165.

HARRIS, P.J. & SKINNER, S.L. (1990). Intra-renal interactions between angiotensin II and atrial natriuretic factor. *Kidney Int.*, **38** (suppl 30), S87–S91.

HEALY, D. & FANESTIL, D.D. (1986). Localisation of atrial natriuretic peptide binding sites within the kidney. *Am. J. Physiol.*, **250**, F573–F578.

JOHNS, E.J. (1989). Role of angiotensin II and the sympathetic nervous system in the control of renal function. *J. Hypertension*, **7**, 695–701.

JOHNS, E.J. & RUTKOWSKI, B. (1989). A comparison of the action of atriopeptin III on renal function in normal and DOCA-salt hypertensive rats. *J. Hypertension*, **7**, 675–682.

JOHNS, E.J. & RUTKOWSKI, B. (1990a). Renal actions of atriopeptin III in genetic and renovascular models of hypertension in the rat. *Eur. J. Pharmacol.*, **185**, 125–135.

JOHNS, E.J. & RUTKOWSKI, B. (1990b). The action of atriopeptin III on renal function in two models of chronic renal failure in the rat. *Br. J. Pharmacol.*, **99**, 317–322.

KOEPKE, J.P., JONES, S. & DiBONA, G.F. (1987). Renal nerves mediate blunted natriuresis to atrial natriuretic peptide in cirrhotic rats. *Am. J. Physiol.*, **252**, R1019–R1023.

KOSEKI, C., HAYASHI, Y., TORIKA, S., FURUYA, M., OHNUMA, N. & IMAI, M. (1986). Localisation of binding sites for α-rat atrial natriuretic polypeptide in rat kidney. *Am. J. Physiol.*, **250**, F210–F216.

LUFT, F.C., LANG, R.E., ARONOFF, G.R., RUSKARAKO, H., TOTH, M., GANTEN, D., STERZEL, R.B. & UNGER, T. (1986). Atriopeptin III kinetics and pharmacodynamics in normal and anephric rats. *J. Pharmacol. Exp. Ther.*, **236**, 416–418.

NEEDLEMAN, P., BLAINE, E.H., GREENWALD, J.E., MICHENER, M.L., SAPER, C.B., STOCKMAN, P.T. & TOLUNAY, H.E. (1989). The biochemical pharmacology of atrial peptides. *Annu. Rev. Pharmacol. Toxicol.*, **29**, 23–54.

SALAZAR, F.J., GRANGER, J.P., FIKSEN-OLSEN, M.J., BENTLEY, M.D. & ROMERO, J.C. (1987). Possible modulatory role of angiotensin II on atrial peptide-induced natriuresis. *Am. J. Physiol.*, **253**, F880–F883.

SHOWALTER, C.J., ZIMMERMAN, R.S., SCHWAB, T.R., EDWARDS, B.S., OPGENORTH, J.J. & BURNETT, J.C. (1988). Renal response to atrial natriuretic factor is modulated by intrarenal angiotensin II. *Am. J. Physiol.*, **254**, R453–R456.

SOMOGYI, M. (1930). A method for the preparation of blood filtrates for the determination of sugar. *J. Biol. Chem.*, **237**, 655–663.

SONNENBERG, H., HONRATH, V., CHONG, C.K. & WILSON, D.R. (1986). Atrial natriuretic factor inhibits sodium transport in medullary collecting duct. *Am. J. Physiol.*, **250**, F963–F966.

STEELE, T.H. & CHALLONER-HUE, L. (1990). Intrarenal angiotension II inhibition influences the actions of atrial natriuretic peptide. *Clin. Sci.*, **78**, 475–480.

WAMBACK, G., SCHITTENHELM, U., STIMPEL, M., BONNER, G. & KAUFMAN, W. (1989). Natriuretic action of ANP is blunted by ACE inhibition in humans. *J. Cardiovasc. Pharmacol.*, **13**, 748–753.

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