Kidney International, Vol. 42 (1992), pp. 433–441

Prazosin attenuates the natriuretic response to atrial natriuretic factor in man

CHIM C. LANG, ANNA-MARIA J. CHOY, DAVID J.K. BALFOUR, and ALLAN D. STRUTHERS

Department of Clinical Pharmacology, Ninewells Hospital and Medical School, Dundee, Scotland, United Kingdom

Prazosin attenuates the natriuretic response to atrial natriuretic factor in man. The effect of alpha-1-adrenoceptor blockade with 0.25 mg oral prazosin on the renal response to atrial natriuretic factor (ANF) 5 pmol/kg/min was examined in eight healthy male volunteers undergoing maximal water diuresis. ANF on its own decreased mean arterial blood pressure ($P < 0.05$) without altering heart rate or increasing plasma norepinephrine. ANF increased urinary sodium excretion by 130% ($P < 0.01$) from baseline value with accompanying 18% decrease ($P < 0.05$) in PAH clearance (ERPF) without changing inulin clearance (GFR). When compared to placebo infusion, ANF infusion caused a significant increase in fractional excretion lithium (FE_{Li}), a marker of proximal tubular function. Fractional distal delivery of sodium, another marker of proximal tubular outflow as determined by free water clearance, was also increased during ANF infusion. As expected, ANF decreased distal nephron fractional sodium reabsorption as evaluated by both the "lithium method" and by the conventional "solute-free water method." Prazosin on its own had no effect on blood pressure, renal function or hormonal parameters. When given in combination with ANF, prazosin blunted the natriuretic effect of ANF from 130% to 35% ($P < 0.01$). However, prazosin pretreatment did not influence the ANF-induced fall in blood pressure or ERPF nor the ANF-induced suppression of plasma aldosterone. We have therefore found evidence to support the hypothesis that at basal levels of sympathetic tone, the natriuretic effect of ANF in man is dependent on an intact sympathetic nervous system, since sympathetic blockade by prazosin blunts its sodium excretory effects.

Much research activity has gone into examining the interaction between atrial natriuretic factor (ANF) and the renin-angiotensin-aldosterone system [1]. Less attention has been directed towards studying the interaction between ANF and the renal sympathetic nervous system. This is despite the fact that the renal sympathetic nervous system is known to exert a major influence on renal sodium excretion by acting at nearly all sites within the nephron [2]. Recently, low frequency renal nerve stimulation has been shown to blunt the natriuretic response to ANF in anesthetized rats [3], although other similar experiments have produced conflicting results [4, 5]. An alternative proposition supported by experimental data is that ANF functions as a neuromodulator, and that part of the mechanism of action of ANF is to inhibit endogenous renal sympathetic nerve activity [6–8].

Received for publication August 27, 1991
and in revised form February 28, 1992
Accepted for publication March 2, 1992

To date, most previous studies examining the interaction between ANF and the renal sympathetic nervous system have been in experimental animal models of renal nerve stimulation or denervation which does not distinguish the level (pre- or post-synaptic) or adrenoceptor subtype involved in ANF-induced natriuresis [3–5]. In this regard, post-synaptic renal alpha-1-adrenoceptors appear to have the major functional influence in the kidney although, numerically, alpha-2-adrenoceptors are more abundant in the kidney [9]. The aim of this study was to examine the effect of selective alpha-1-adrenoceptor blockade by prazosin on the renal response to ANF in man. Prazosin is a selective alpha-1-adrenoceptor blocker which does not interfere with reflexes mediated via pre-synaptic alpha-2-adrenoceptors [10]. A low dose prazosin was used to avoid any effect on systemic blood pressure since this by itself would alter sodium excretion.

Methods

Eight male volunteers aged 19 to 28 years (mean 23.8 years) were studied. Normal health was established by medical history, physical examination, biochemical and hematological screening, electrocardiogram and urinalysis. None were taking any regular medication. All subjects gave written informed consent to the study protocol, which had been approved by the hospital committee on medical ethics.

Volunteers had a dietary history to ensure they were salt replete and this was confirmed by pre-study timed urine collections. Each volunteer was asked to adhere to his normal diet for the duration of the study and to maintain a similar pattern of meals for the 24 to 48 hours prior to each investigation day. No medication was permitted for the duration of the study.

Subjects were studied on four occasions, with at least one week between each investigation day. All had fasted from 2200 hours the evening before and had abstained from alcohol over the previous 36 hours. Cigarette smoking and caffeine-containing drinks were prohibited on the morning of the study. Each volunteer had taken 500 mg of lithium carbonate 10 hours before the start of the study. On attending the clinical laboratory at 0800 hours, subjects were seated and an intravenous cannula was placed in each antecubital fossa for subsequent infusion and blood sampling. At 0810 hours, priming doses of 50 mg/kg inulin (Inutest, Laevosan Gessellschaft m.b.H., Linz,

Austria) and 10 mg/kg para-aminohippurate (PAH) (Aminohippurate Sodium, Merck Sharpe & Dohme, Westpoint, Pennsylvania, USA) were injected intravenously, followed by continuous infusion of both drugs at doses calculated to achieve steady-state plasma concentrations of 200 mg/liter and 25 mg/liter, respectively.

At 0805 hours, a 20 ml/kg oral water load was administered over a 15 minute period. Every 20 minutes thereafter, subjects stood to void until the end of the study. The volume of urine passed was measured and an aliquot stored for later analysis. On each occasion an equal volume of water was given to drink. In this way, a stable diuresis was established over a period of approximately 1.5 hours at 1000 hours. The last 20 minute collection period of this stabilization period was taken as baseline. At 1000 hours, each subject received orally 0.25 mg of prazosin (Hypovase, Pfizer Ltd., Sandwich, Kent, UK) or placebo tablet. After two further 20-minute collection periods at 1040 hours, an infusion of either placebo (5% D-glucose) or ANF 5 pmol/kg/min [atrial natriuretic factor (99-126), Shire Pharmaceuticals Ltd, Andover, Hampshire, UK] was commenced and continued for 60 minutes. The order of the infusions was random and administered in a single blinded fashion. The study terminated at 1220 hours so that there were two further urine collections after withdrawal of the infusion. Heart rate (HR) was displayed continuously on an ECG oscilloscope (adult monitor 78351A, Hewlett Packard, Boblingen, Germany) and the blood pressure was measured every five minutes with a semi-automatic sphygmomanometer (Dinamap Vital Signs Monitor 1846, Critikon, Tampa, Florida, USA).

Urine samples were obtained at the end of all clearance periods. Venous blood was collected at the midpoint of each collection period into plain glass tubes (5 ml, no preservative) for measurement of electrolytes, including lithium and osmolality, and into chilled tubes (10 ml, lithium heparin) for measurement of plasma PAH and inulin. Venous blood was also collected at the midpoint of the baseline clearance period and the last clearance period during the ANF or placebo infusion for measurement of plasma norepinephrine (2 ml, lithium heparin), plasma angiotensin II (10 ml, 0.05 M o-phenanthroline, 2 g of neomycin/liter, 0.125 M EDTA disodium salt) and plasma aldosterone (2 ml, lithium heparin). All samples were placed on ice (except electrolytes which were allowed to clot), centrifuged at 4°C and the plasma/serum separated off and stored at −20°C (electrolytes, ANF, aldosterone, PAH, inulin) or −70°C (norepinephrine) until assayed.

Analytical and statistical methods

All samples were measured together in one assay run, for any given variable. Plasma norepinephrine was assayed by the double isotope radio-enzymatic method [11]. The intra-assay coefficient of variation (INCV) for this method in our laboratory was 8.0% and the interassay coefficient of variation (ITCV) was 11.1%. Angiotensin II was measured, after plasma extraction, by a radioimmunoassay kit (Immunodiagnostics, Washington, Tyne and Wear, UK). The INCV and ITCV of this assay were 2.2% and 12.1%, respectively. Plasma aldosterone was also measured by a commercially available radioimmunoassay kit (Serono Diagnostics Ltd, Woking, Surrey, UK). The INCV and ITCV for this method were 6.4% and 14%, respectively. Plasma ANF was measured by RIA (Amersham International Ltd, Amersham, Buckinghamshire, UK) after plasma extraction by the method of Richards et al [12]. The INCV and ITCV for this method were 4.9% and 7.8%, respectively. Serum and urinary sodium, and lithium were measured with an indirect reading ion specific electrode (Beckmann System E2A, High Wycombe, Buckinghamshire, UK). Plasma and urinary osmolalities were measured by the freezing point depression method (Osmomat 030, Gonotec, Berlin, Germany). Urine and plasma inulin was assayed spectrophotometrically (Pye Unicam SP9, Phillips, Cambridge, UK) using resorcinol as the color reagent. The INCV and ITCV for this assay in our laboratory was 1.1% and 2.9% for plasma and 1.0% and 3.6% for urine, respectively. Urine and plasma PAH was also assayed spectrophotometrically (Cobas Bio, Roche, Basle, Switzerland) using 4-dimethyl amino benzyldehyde as the color reagent. The INCV and ITCV were 2.1% and 2.0% for plasma, and 1.7% and 2.3% for urine.

The following indices were calculated according to standard criteria. Clearance was calculated as UV/P, where U = urinary concentration, V = urinary flow rate and P = plasma concentration. Clearances of inulin (C_{In}) and PAH (C_{PAH}) were employed to estimate glomerular filtration rate (GFR) and effective renal plasma flow (ERPF), respectively. Fractional excretion (fractional clearance; FE) was calculated as clearance divided by C_{In}.

Segmental tubular sodium handling was estimated from lithium clearance [13]. Fractional excretion of lithium (FE_{Li}), was used as an estimate of the fraction of glomerular filtrate not reabsorbed by the proximal tubule and delivered to the distal nephron. Reabsorption of sodium in the distal nephron was calculated from $C_{Li} - C_{Na}$. Finally, fractional reabsorption of distally delivered sodium was estimated from $(C_{Li} - C_{Na})/C_{Li}$.

As an alternative method for estimating segmental sodium handling, we used the C_{H_2O} and C_{Na} as follows: $(C_{Na} + C_{H_2O})/GFR$ was used as an index of filtered sodium to the diluting segment; and $C_{H_2O}/(C_{H_2O} + C_{Na})$ was used as an index of fractional reabsorption of sodium delivered to the diluting segment [14].

Statistical evaluation

For each parameter, the baseline value was taken to be the last clearance period before the stabilization phase. All values were expressed as means ± standard error. Comparison of treatment groups (placebo, ANF alone, prazosin alone and the combination of prazosin and ANF) on hemodynamic and renal function parameter was assessed by repeated measures analysis of variance (MANOVA, SPSS-plus). Hormonal responses were compared with baseline values using the paired Student's *t*-test following Bonferroni correction. A *P* value of less than 5% was considered statistically significant.

Results

Pre-study screening showed that all subjects were salt replete (24 hr sodium excretion was 168 ± 23 mmol). There were no significant differences in any of the baseline hemodynamic, neuroendocrine and renal function parameters before administration of the prazosin or placebo tablet (Tables 1 and 2).

Table 1. Baseline hemodynamic and renal function parameters on all four study days ($N = 8$)

Study day	P + P	PRZ + P	P + ANF	PRZ + ANF
GFR *ml/min*	101 ± 6	98 ± 6	102 ± 5	100 ± 6
ERPF *ml/min*	580 ± 22	631 ± 22	637 ± 18	616 ± 28
$U_{Na}V$ *umol/min*	161 ± 18	167 ± 15	171 ± 10	175 ± 22
FE_{Li} %	27.8 ± 1.1	30.3 ± 2.1	26.1 ± 2.0	27.5 ± 1.8
$\frac{(C_{Li} - C_{Na})}{C_{Li}}$ %	95.7 ± 0.5	95.9 ± 0.5	95.1 ± 0.2	95.1 ± 0.3
$\frac{(C_{Na} + C_{H_2O})}{GFR}$ %	14.1 ± 1.8	15.6 ± 1.6	12.9 ± 1.1	15.5 ± 1.9
$\frac{(C_{H_2O})}{C_{H_2O} + C_{Na}}$ %	91.4 ± 0.9	91.7 ± 0.6	90.3 ± 0.5	90.7 ± 0.9
Mean arterial pressure *mm Hg*	90 ± 2	92 ± 2	91 ± 2	90 ± 3
Heart rate mm^{-1}	65 ± 2	63 ± 2	62 ± 2	66 ± 2

Values are mean ± SE. Abbreviations are: GRF, glomerular filtration rate; ERPF, effective renal plasma flow; $U_{Na}V$, urinary sodium excretion; FE_{Li}, fractional excretion of lithium; $(C_{Li} - C_{Na})/C_{Li}$, fractional reabsorption of distally delivered sodium; $(C_{H_2O} + C_{Na})/GFR$, sodium delivery to the diluting segment; $C_{H_2O}/(C_{H_2O} + C_{Na})$, fractional reabsorption of sodium delivered to the diluting segment; P, placebo tablet or infusion of 5% D-glucose; PRZ, prazosin 0.25 mg; ANF, ANF 5 pmol/kg/min for 60 min. Samples were taken at −10 min before oral doses of P or PRZ. ANF or P infusion was commenced at +40 min after oral doses of P or PRZ. None of the parameters was statistically different between study days.

Effects of ANF and prazosin on blood pressure and heart rate

Figure 1 shows the effect of ANF infusion on HR and mean arterial BP. ANF infusion decreased mean arterial pressure ($P < 0.05$ vs. placebo by MANOVA) without having any effect on HR. Figure 1 also confirmed that 0.25 mg prazosin administered orally is a non-depressor dose and that it did not influence the hemodynamic response to ANF.

Effects of ANF and prazosin on ERPF, GFR and $U_{Na}V$

During ANF infusion, ERPF decreased by 18% from baseline value (from 637 ± 18 ml/min to 520 ± 37 ml/min, $P < 0.5$ vs. placebo; Fig. 2). Prazosin on its own did not influence ERPF nor did it influence the ERPF response to ANF (a decrease of 19% from baseline, from 616 ± 28 ml/min to 496 ± 31 ml/min, $P < 0.05$ vs. placebo). GFR did not change on any of the study days (Fig. 2).

Urinary sodium excretion increased by approximately 130% from baseline value (from 171 ± 10 μmol/min to 392 ± 48 μmol/min, $P < 0.01$ vs. placebo) during ANF infusion. Prazosin pretreatment blunted this response to a maximal increase of only 35% from baseline value (from 175 ± 22 μmol/min, to 237 ± 31 μmol/min, $P < 0.01$ vs. placebo + ANF study day).

Effects of ANF and prazosin on segmental tubular sodium handling

Segmental tubular sodium handling was estimated from data derived from lithium clearance. ANF alone increased FE_{Li} by 38% from baseline value (from 26.1 ± 2.0% to 36.0 ± 4.2%, $P < 0.05$ vs. placebo). On the prazosin + ANF study day, there was blunting of the ANF induced rise in FE_{Li} with only a maximal rise of 15% from baseline value (from 27.5 ± 1.8% to 30.5 ± 2.0%). Fractional reabsorption of distally delivered sodium $[(C_{Li} - C_{Na})/C_{Li}]$ was decreased by ANF (from 95.1 ± 0.2% to 91.1 ± 0.1%, $P < 0.01$ vs. baseline) and this decrease was also blunted by prazosin pretreatment ($P < 0.05$ vs. placebo + ANF study day; Figs. 3 and 4).

Segmental tubular sodium handling was also estimated by use of the alternative "solute-free water method" (Figs. 3 and 4). These results concur with those obtained with the "lithium method".

Effects of ANF and prazosin on neuroendocrine parameters

ANF infusion caused comparable and significant increases in circulating ANF on the placebo + ANF and prazosin + ANF study days (Table 2). Prazosin on its own did not cause any hormonal changes. ANF infusion caused a significant fall in plasma aldosterone levels and prazosin pre-treatment did not influence these changes (Table 2). There were no significant changes in plasma angiotensin II on all four study days. Despite the significant fall in mean arterial pressure during ANF infusion, plasma norepinephrine remained unchanged. Plasma norepinephrine was also unaltered on the prazosin + ANF study day.

Discussion

This study has two main findings. Firstly, we have shown that a pharmacological dose of ANF in man was associated with a marked natriuresis and a fall in blood pressure which was not accompanied by a reflex tachycardia nor an augmentation of plasma norepinephrine. The lack of a reflex increase in heart rate and plasma norepinephrine despite a fall in blood pressure lends support to the notion that ANF may have a modulatory effect on the sympathetic nervous system. Secondly, our study showed that alpha-1-adrenoceptor blockade by a non-depressor dose of prazosin markedly blunts the renal response to ANF in man. Our results would suggest that the natriuretic effect of ANF in resting man is partly dependent on some intrinsic sympathetic tone since sympathetic blockade blunts its sodium excretory effects. This hypothesis is, however, dependent upon the assumption that the dose of prazosin used in this study results in blockade of the renal sympathetic nervous system. It could be argued that prazosin may have caused a fall in blood pressure and therefore secondarily activate rather than block the renal sympathetic nervous system. It could also be argued that ANF may decrease cardiac preload and consequently lower atrial filling pressures which would then increase renal sympathetic nerve activity. However, the dose of prazosin chosen in this study was such that blood pressure was not affected by prazosin, nor was there evidence of any changes in atrial filling pressures since plasma ANF was not affected by prazosin treatment. While we did not measure urinary norepinephrine, both heart rate and plasma norepinephrine were unchanged by prazosin, which suggests that prazosin did not secondarily activate the sympathetic nervous system. Our results are consistent with recent reports [6–8] that part of ANF's action is to function as a neuromodulator of sympathetic nervous function, and that in the kidney ANF may act by decreasing renal sympathetic nerve activity, which then facilitates the diuretic and natriuretic effects of ANF.

There is now cumulative evidence to suggest that ANF may behave as a neuromodulator of autonomic nervous function [reviewed in 15]. Ackermann et al [16] found that intravenous

Table 2. Neuroendocrine parameters on all four study days ($N = 8$)

Study day	P + P	PRZ + P	P + ANF	PRZ + ANP
ANF *pmol/liter*				
Baseline (−10 min)	6.1 ± 0.6	6.5 ± 0.8	7.0 ± 1.2	6.5 ± 1.8
End of infusion (+90 min)	5.8 ± 0.8	6.6 ± 1.2	67.2 ± 12.7[a]	70.9 ± 9.9[a]
Angiotensin II *pmol/liter*				
Baseline (−10 min)	32.1 ± 3.7	33.7 ± 5.2	38.2 ± 9.3	36.2 ± 3.1
End of infusion (+90 min)	34.2 ± 3.5	34.4 ± 4.8	35.6 ± 8.0	33.5 ± 3.8
Aldosterone *pmol/liter*				
Baseline (−10 min)	426 ± 17	416 ± 14	429 ± 13	421 ± 20
End of infusion +90 min	432 ± 13	443 ± 27	288 ± 12[a]	275 ± 14[a]
Norepinephrine *nmol/liter*				
Baseline (−10 min)	2.93 ± 0.41	3.13 ± 0.38	2.77 ± 0.39	3.16 ± 0.37
End of infusion (+90 min)	3.15 ± 0.49	3.26 ± 0.35	2.90 ± 0.43	3.45 ± 0.64

Results are mean ± SE. Abbreviations are: P, placebo tablet or infusion of 5% D-glucose; PRZ, prazosin 0.25 mg; ANF, ANF 5 pmol/kg/min for 60 min. Samples were taken at −10 min (before oral doses of P or PRZ at 0 min) and at +90 min (10 min before the end of P or ANF infusion). The P or ANF infusion was commenced at +40 min.

[a] $P < 0.01$ vs. baseline by paired Student's t-test

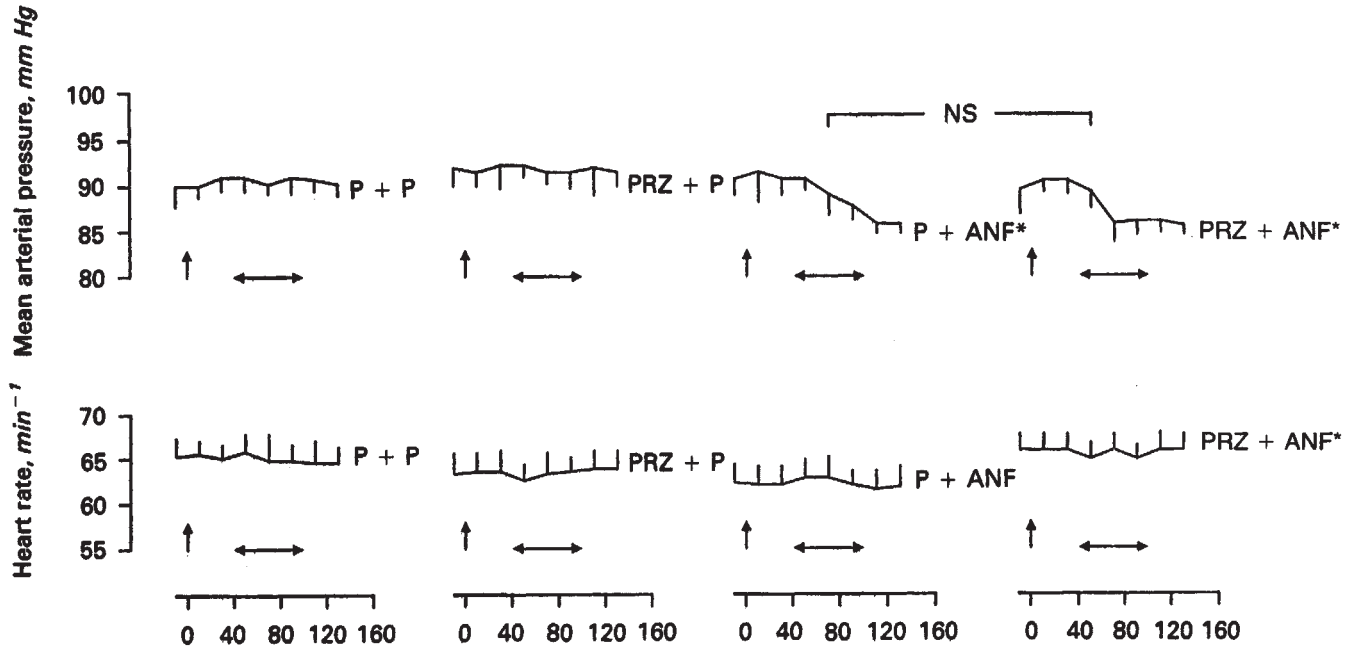

Fig. 1. *Heart rate, min^{-1} (HR) and mean arterial pressure (MAP), mm Hg on all 4 study days* (N = 8). Abbreviations are: P, placebo tablet or 5% D-glucose, ANF, atrial natriuretic factor (99-126) 5 pmol/kg/min; and PRZ, prazosin 0.25 orally. Horizontal and vertical arrows at bottom of figure indicate timing of P/ANF infusion and P/PRZ dosing, respectively. *$P < 0.05$ vs P by MANOVA. MAP decreased significantly ($P < 0.05$ versus P) on both P + ANF and PRZ + ANF study days. MAP responses did not differ between P + ANF and PRZ + ANF study days. HR did not differ between all 4 study days.

administration of atrial extracts to rats caused a reduction in arterial blood pressure, which was accompanied by a decrease in heart rate rather than by the expected baroreceptor-mediated reflex tachycardia. Thoren et al [6] went on to show that atriopeptins (II and III) decreased renal nerve activity in sino aortic-denervated rats and that this effect was eliminated by vagotomy but not by atropine, which suggests that the effects of vagotomy were related to interruption of vagal afferents but not vagal efferents. Schultz et al [7] have recently confirmed this when they showed that blockade of afferent vagal C fibers abolishes the inhibition of renal sympathetic nerve activity produced by ANF. These authors suggested that ANF stimulated vagal afferent endings to produce an inhibitory effect on sympathetic outflow. These observations, in conjunction with our findings, would support a potential role for ANF in modulating autonomic nervous function, a role that could serve to amplify or facilitate the peripheral hormonal action of ANF on vascular and renal structures.

Several theories on the mechanism of action of this neuromodulatory effect of ANF have been postulated, including an effect on the baroreceptor reflex mechanism as well as an effect on peripheral noradrenergic neurotransmission [8]. Takeshita et al [17] have found that ANF 30 μg/kg/min attenuated the reflex increase in forearm vascular resistance during cardiopulmonary baroreceptor activation by lower body negative pressure in man, an observation which has since been confirmed by others

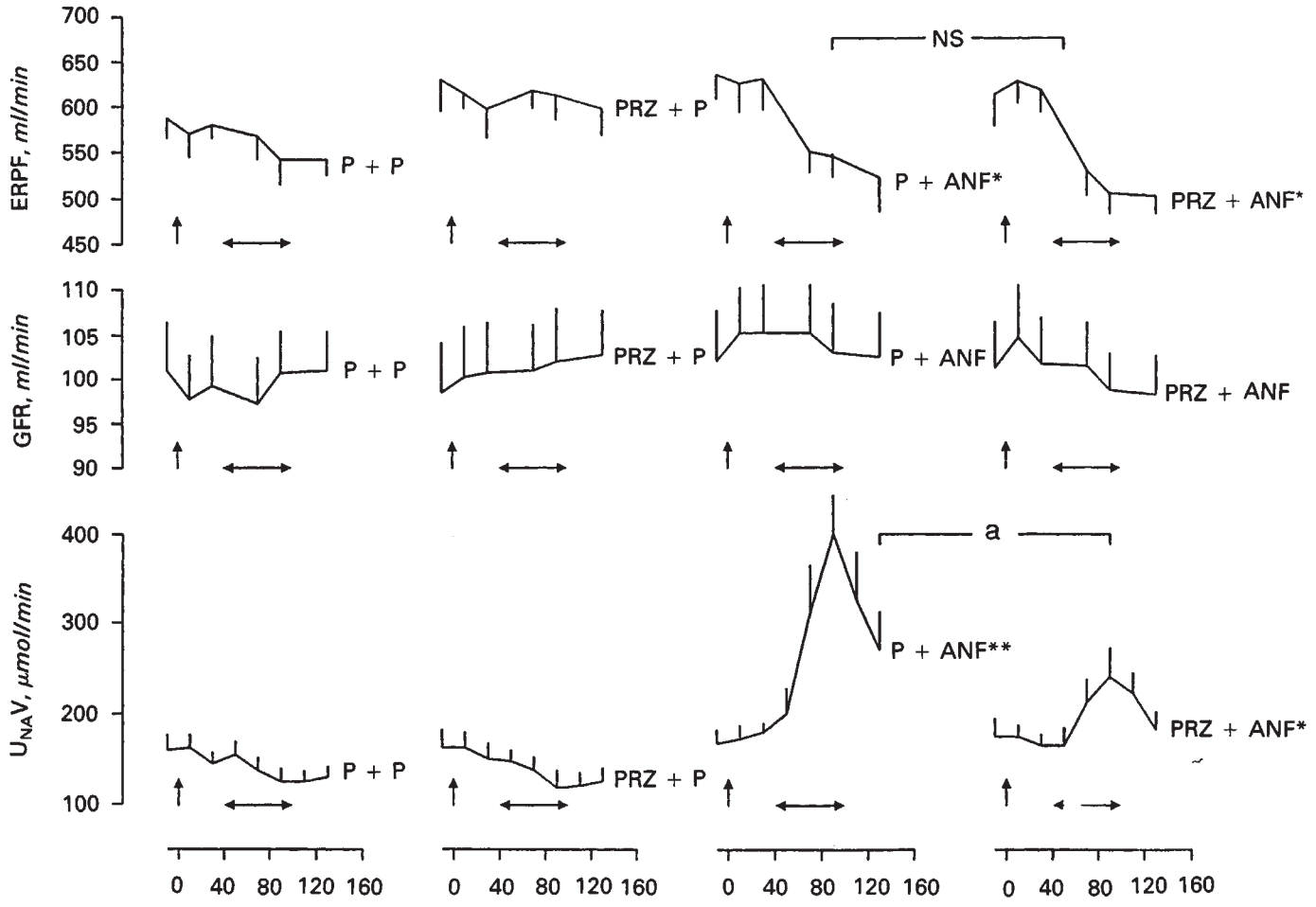

Fig. 2. *Effective renal plasma flow, ml/min (ERPF), glomerular filtration rate, ml/min (GFR) and absolute urinary sodium excretion, μmol/min ($U_{Na}V$) on all 4 study days* (N = 8). Abbreviations are: P, placebo tablet or 5% D-glucose; ANF, atrial natriuretic factor (99-126) 5 pmol/kg/min; and PRZ, prazosin 0.25 mg orally. Horizontal and vertical arrows at bottom of figure indicate timing of P/ANF infusion and P/PRZ dosing, respectively. $^*P < 0.05$, $^{**}P < 0.01$ vs. P by MANOVA. a denotes statistical significance ($P < 0.05$ by MANOVA) of the difference between ANF + P study day and ANF + PRZ study day.

[18, 19]. The mechanism(s) of ANF's influences on baroreceptor reflexes are unknown, although it has previously been suggested that ANF may act centrally to suppress sympathetic nervous activity [20, 21]. However, there is animal evidence to suggest that ANF may also act by inhibiting peripheral noradrenergic neurotransmission. Sasaki et al [22] have shown that ANF-induced reduction in blood pressure was decreased by sympathectomy, which suggests that sympathetic nerves might be involved in the hypotensive action of ANF. Studies by Koepke et al [23, 24] showed that graded doses of synthetic ANF infused into conscious normal rats produced dose-dependent decreases in renal sympathetic nerve activity in association with diuresis, natriuresis and hypotension. More recently Holtz, Sommer and Bassenge [25] have also reported a suppression of epinephrine release rate during ANF infusion in conscious dogs and an absence of reflex tachycardia or augmentation of norepinephrine release in response to ANF-induced hypotension. Likewise, in this study, we found that ANF-induced hypotension was not associated by reflex sympathetic nervous activation in man as indicated by the lack of change in heart rate or plasma norepinephrine. Furthermore, we have also shown that prazosin blunted the natriuretic (although not the hypotensive) effect of ANF in man. This would suggest that the natriuretic effect of ANF in man is dependent on an intact renal sympathetic nervous system. It could be argued that in this study, alpha-1-adrenoceptors blockade by prazosin removed the sympathetic neuromodulator component of ANF's renal action, thereby blunting its natriuretic effect.

However, not all previous data appear to agree with ours, although on closer inspection, it turns out that the level of sympathetic nervous system activity is an important determinant. In fact, an activated level of renal sympathetic nervous system activity appears to limit the renal effects of ANF [3] whereas in basal conditions, as we have found here, an intact intrinsic sympathetic nervous system is required to maintain ANF's natriuretic effects. Indeed, we have previously found that in a similar study to this one, exogenously infused norepinephrine (to mimic an activated sympathetic nervous system) was found to blunt the natriuretic effect of ANF in normal man [26]. In other states with an activated sympathetic nervous system, such as nephrotic or cirrhotic rats, renal denervation

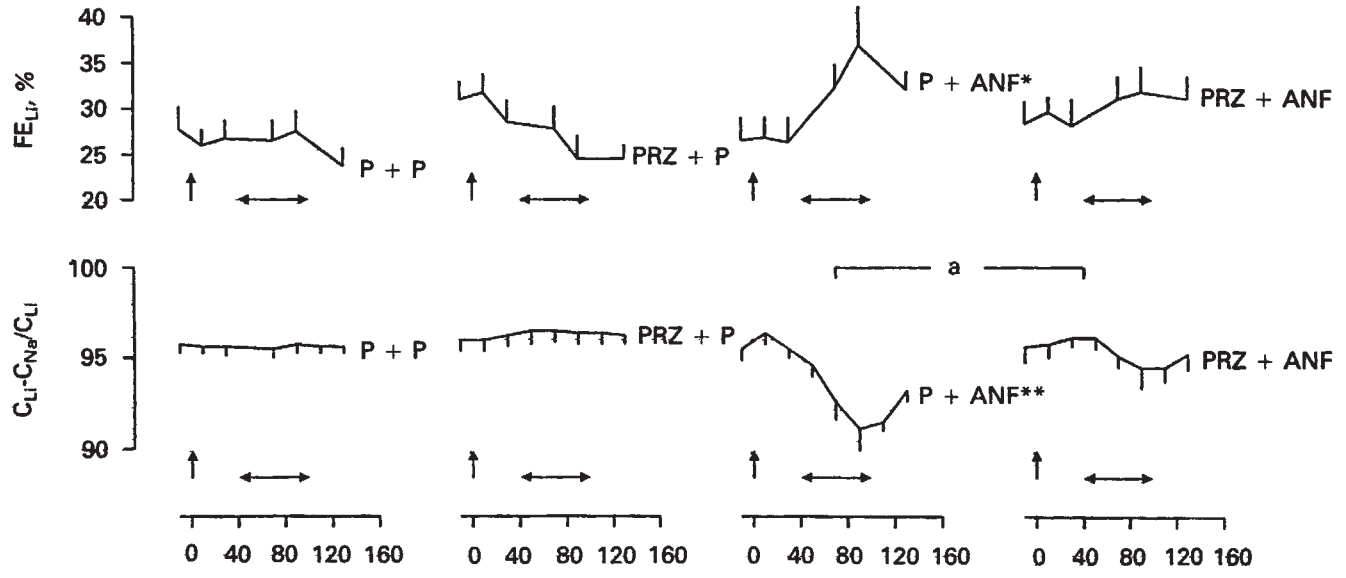

Fig. 3. *Segmental tubular sodium handling as determined by the "lithium clearance" technique on all four study days* (N = 8). Abbreviations are: Fe_{Li}, fractional excretion of lithium which estimates the fractional delivery of glomerular filtrate to distal nephron; $(C_{Li} - C_{Na})/C_{Li}$, fractional reabsorption of distally delivered sodium; P, placebo tablet or 5% D-glucose; ANF, atrial natriuretic factor (99-126) 5 pmol/kg/min; and PRZ, prazosin 0.25 ng orally. Horizontal and vertical arrows at bottom of figure indicate timing of P/ANF infusion and P/PRZ dosing, respectively. *$P < 0.05$ and **$P < 0.01$ vs. P by MANOVA. a denotes statistical significance ($P < 0.05$ by MANOVA) of the difference between ANF + P study day and ANF + PRZ study.

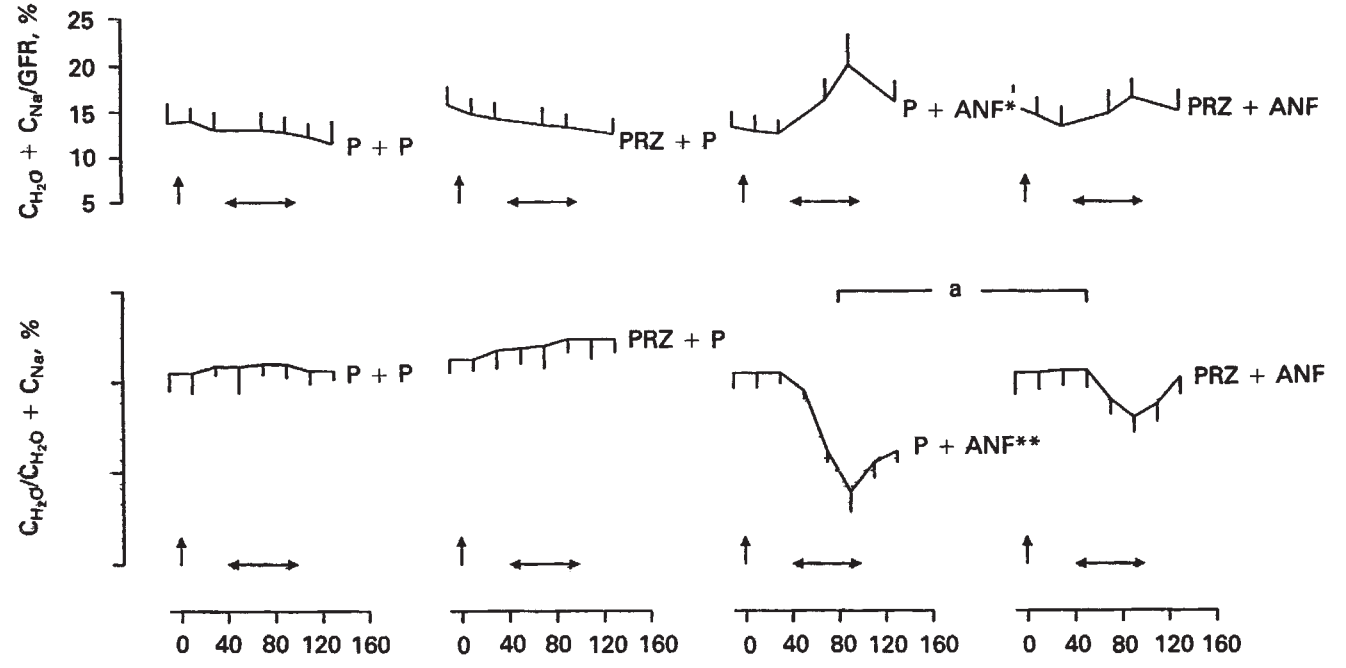

Fig. 4. *Segmented tubular sodium handling as determined by the solute-free water method on all four study days* (N = 8). Abbreviations are: $(C_{H_2O} + C_{Na})$/GFR, fractional sodium delivery to the diluting segment; $C_{H_2O}/(C_{H_2O} + C_{Na})$, fractional reabsorption of sodium delivered to the diluting segment; P, placebo tablet or 5% D-glucose; ANF, atrial natriuretic factor (99-126) 5 pmol/kg/min; and PRZ, prazosin 0.25 mg orally. Horizontal and vertical arrows at bottom of figure indicate timing of ANF infusion and PRZ dosing, respectively. *$P < 0.05$ and **$P < 0.01$ versus P by MANOVA. a denotes statistical significance ($P < 0.05$ by MANOVA) of the difference between ANF + P study day and ANF + PRZ study day.

was found to restore the natriuretic response to ANF [23, 24]. Along similar lines, clonidine, an alpha-2-adrenoceptor agonist which decreases presynaptic norepinephrine release has been shown to restore the originally blunted renal response to ANF in congestive heart failure rats [27].

The reason for conflicting results in the literature could,

however, be due to other factors. We were careful in this experiment to use a dose of prazosin which did not alter systemic blood pressure since this by itself would alter sodium excretion. Some of the previous conflicting results may have been related to changes in systemic blood pressure and hence renal perfusion pressure. Another variable in these experiments relates to renal alpha-2- or beta-adrenoceptors. In our experiment we used prazosin and therefore produced only selective postsynaptic alpha-1-adrenoceptors blockade, whereas other experimental techniques, particularly renal denervation, would have effectively inhibited alpha-2- and/or beta-adrenoceptors. Renal alpha-1-adrenoceptors appear to have the major functional influence in the kidney, although numerically alpha-2-adrenoceptors are more abundant [9].

The site of this interaction between the renal sympathetic nervous system and ANF is unclear. The renal sympathetic nervous system is known to affect the excretion of sodium by acting at various levels of nephron function [2]. Our results do not support an interplay at the glomerulovascular level, since we did not observe any changes in GFR and prazosin did not alter the ANF-induced fall in ERPF. This ANF-induced fall in ERPF, as measured by clearance technique, has generally been reported in man [28–30], although the underlying mechanism(s) for this is unclear. It may be due to a direct efferent arteriolar constriction or perhaps to subtle changes in mesangial cell interstitial pressure which will compress postglomerular blood vessels [reviewed in 31]. While prazosin did not alter ANF's effects on ERPF, it did interfere with the tubular effects of ANF as determined by both lithium clearance and "solute-free water" clearance techniques. It is unclear whether this interaction at the tubular level is via a direct tubular interaction or indirectly via changes in intrarenal hemodynamics, including alteration in medullo-papillary hemodynamics. In this study, we found that a pharmacological dose of ANF increases FE_{Li}, thus suggesting that whole kidney proximal tubular fractional reabsorption of sodium is inhibited by ANF. We also found a significant increase in fractional distal delivery, an independent marker of proximal outflow based on the generation of free water. The finding of a similar change in these two separate markers of proximal tubular outflow, measured in parallel, suggests that we have observed a genuine effect. Although similar observations have been reported in both experimental animals and man [32–36], others have found a lack of proximal tubular outflow [37–39]. Since ANF receptors have not been identified in the proximal tubules [40], it has been suggested the mechanism of these proximal tubular effects of ANF if demonstrated is probably an indirect one [36]. Possible indirect mechanisms include the inhibition of angiotensin II by ANF or an alteration in intrarenal hemodynamics as well as pertubation of peritubular Starling's forces by ANF. In this study, prazosin pretreatment blunted these proximal tubular effects of ANF in man without altering plasma angiotensin II. This influence of prazosin of proximal tubular function (although only apparent during ANF infusion in this study) is in keeping with the presence of proximal tubular adrenergic receptors and demonstrates that synaptically released norepinephrine increases proximal tubular sodium reabsorption [2, 9]. Since prazosin did not alter plasma norepinephrine nor angiotensin II, it is therefore possible that prazosin might have caused undetected changes in intrarenal hemodynamics thereby off-setting ANF's indirect actions on the proximal tubules. Obviously any evidence for this action in man must remain speculative and cannot be directly inferred from this study.

We also found that ANF inhibited fractional distal sodium reabsorption as measured by the "solute free water" method and the "lithium clearance" technique. This is in agreement with a large body of animal evidence which has suggested a major portion of action of ANF may be due to a distal nephron effect especially within the collecting duct system [32, 33, 41]. Prazosin was shown in this study to markedly blunt the distal tubular effects of ANF as determined by clearance techniques. However, it is worth noting that both the lithium clearance and "solute-free water" clearance techniques have their limitations. Use of the lithium clearance technique gives useful data on the tubular sodium handling in man [13], but its interpretation may sometimes be difficult. For instance, recent evidence suggests that lithium handling may also occur beyond the proximal tubule, within the loop of Henle [31, 42, 43]. As such, one must take this into account when interpreting lithium clearance data. This is especially relevant in this study since reabsorption at the loop of Henle may be affected by both renal sympathetic nerves [44] and also by ANF [45, 46]. While the loop of Henle may be a possible site of interaction between prazosin and ANF, one cannot exclude an interaction at both the proximal and distal tubules (distal tubule proper and collecting ducts) where alpha-adrenoceptors are present [2, 9], and where ANF has been shown to have an effect [31–34]. Given the limitations of clearance techniques, it is difficult to state the exact location of the interaction between prazosin and ANF, although the evidence in this study suggests that it is at the tubular level. Clearly further studies are required to examine the exact location of this interaction between ANF and the sympathetic nervous system in the kidney.

In this study, ANF treatment decreased plasma levels of aldosterone. This inhibitory effect of ANF on aldosterone secretion has been previously described in man, and may result partly from a decrease in plasma renin activity and/or a direct action on aldosterone secretion in the adrenal gland [reviewed in 1]. In this study, there was no significant change in plasma angiotensin II, although it is likely that the our measurement of plasma angiotensin II does not adequately reflect the small changes in renin secretion rate. Prazosin treatment alone was not accompanied by any changes in plasma levels of angiotensin II and aldosterone, nor did it affect the ANF-induced fall in plasma aldosterone.

In summary our results have shown that a pharmacological dose of ANF in man was associated with a marked natriuresis, and a fall in blood pressure which was not accompanied by a reflex activation of the sympathetic nervous system. We have also shown that a non-depressor dose of prazosin caused a significant reduction in ANF's effects on tubular sodium excretion in man without any alteration by prazosin of ANF's effects on systemic blood pressure, renal blood flow and the renin-angiotensin-aldosterone system. We have, therefore, found evidence to support the hypothesis that at basal levels of sympathetic tone, the natriuretic effect of ANF is dependent on some intrinsic sympathetic tone, in particular, renal alpha-1-adrenoceptor activity at the renal tubules.

Acknowledgments

This study was supported by a grant from the British Heart Foundation (BHF Gt. No. 88/5). We are grateful to Miss F. Zaccarini for typing the manuscript.

Reprint requests to Dr. C.C. Lang, Department of Clinical Pharmacology, Ninewells Hospital and Medical School, Dundee DD1 9SY, Scotland, United Kingdom.

References

1. Lang CC, Struthers, AD: Effects of atrial natriuretic factor on the renin-angiotensin-aldosterone system, Chapter 6, in *Frontiers in Pharmacology & Therapeutics: Atrial Natriuretic Factor*, edited by Struthers AD, Oxford, Blackwell Scientific Publications Ltd, 1990, pp 115–140
2. DiBona G: The functions of the renal nerves. *Rev Physiol Biochem Pharmacol* 94:75–181, 1982
3. Morgan DA, Peuler JD, Koepke JP, Mark AL, DiBona GF: Renal sympathetic nerves attenuate the natriuretic effects of atrial peptide. *J Lab Clin Med* 114:538–544, 1989
4. Krayacich J, Kline RL, Macchi A, Calaresu FR: Renal responses to atriopeptide II are not dependent on renal nerves. *Am J Physiol* 251:R187–R191, 1986
5. Pettersson A, Hedner J, Hedner T: Relationship between renal sympathetic activity and diuretic effects of atrial natriuretic peptide (ANP) in the rat. *Acta Physiol Scand* 135:323–333, 1989
6. Thoren P, Mark AL, Morgan DA, O'Neil TP, Needleman P, Brody MJ: Activation of vagal depressor reflexes by atriopeptins inhibits renal sympathetic nerve activity. *Am J Physiol* 252:H1252–H1259, 1987
7. Schultz HD, Gardner DG, De Schepper CG, Coleridge HM, Coleridge JCG: Vagal C-fiber blockade abolishes sympathetic inhibition by atrial natriuretic factor. *Am J Physiol* 245:F322–F328, 1988
8. Kuchel O, Debinski W, Racz K, Cusson JR, Larochelle P, Canton M, Genest J: An emerging relationship between peripheral sympathetic nervous activity and atrial natriuretic factor. *Life Sci* 40:1545–1551, 1987
9. DiBona GF: Neural control of renal function: Role of renal alpha adrenoceptors. *J Cardiovasc Pharmacol* 7 (Suppl 8):S18–S23, 1985
10. Stanaszek WF, Kellerman D, Brodgen RN, Romankiewicz J: Prazosin update. A review of its pharmacological properties and therapeutic uses in hypertension and congestive heart failure. *Drugs* 25:339–384, 1983
11. Brown MJ, Jenner DA: Novel double isotope technique for enzymatic assay for catecholamines permitting high precision, sensitivity and plasma sample capacity. *Clin Sci* 61:591–598, 1981
12. Richards AM, Tonolo G, McIntyre GD, Leckie B, Robertson JS: Radioimmunoassay for plasma alpha-human atrial natriuretic peptide: A comparison of direct and pre-extracted methods. *J Hypertens* 5:227–236, 1987
13. Thomsen K: Lithium clearance: A new method for determining proximal and distal tubular reabsorption of sodium and water. *Nephron* 37:217–223, 1984
14. Kaojarern S, Chennavasin P, Anderson S, Brater DC: Nephron site of effect of nonsteroidal anti-inflammatory drugs on solute excretion in humans. *Am J Physiol* 244:F134–F139, 1983
15. Lang CC, Struthers AD: Interactions between atrial natriuretic factor and the autonomic nervous system. *Clin Autonom Res* 1:329–336, 1991
16. Ackermann U, Irizawa TG, Milojevic S, Sonnenberg H: Cardiovascular effects of atrial extracts in anesthetized rats. *Can J Physiol Pharmacol* 62:819–826, 1984
17. Takeshita A, Imaizumi T, Nakamura N, Higashi H, Sasaki T, Nakamura M, Kangawa K, Matsuo H: Attenuation of reflex forearm vasoconstriction by alpha-human atrial natriuretic peptide in man. *Circ Res* 61:555–559, 1987
18. Imam K, Maddens M, Mohanty PK, Felicetta JV, Sowers JR: Atrial natriuretic peptide attenuates the reflex sympathetic responses to lower body negative pressure. *Am J Med Sci* 289:1–7, 1989
19. Kubo SH, Rector TS, Heifetz SM, Sato H, Cohn JN: Atrial natriuretic factor attenuates sympathetic reflexes during lower body negative pressure in normal man. *J Cardiovasc Pharmacol* 16:881–889, 1990
20. Ermirio R, Ruggeri P, Cogo C, Molinari C, Calaresu F: Neuronal and cardiovascular responses to ANF microinjected into the solitary nucleus. *Am J Physiol* 256:R577–R582, 1989
21. Schultz HD, Steele MK, Gardner DG: Central administration of atrial peptide decreases sympathetic outflow in rats. *Am J Physiol* 258:R1250–R1256, 1990
22. Sasaki A, Kida O, Kawgawa K, Matsuo H, Tanaka K: Improvement of sympathetic nerves in cardiosuppressive effects of α-human atrial natriuretic polypeptide (α-hANP) in anaesthetized rats. *Eur J Pharmacol* 120:345–349, 1986
23. Koepke JP, DiBona GF: Blunted natriuresis to atrial natriuretic peptide in chronic sodium-retaining disorders. *Am J Physiol* 2TZ: F865–F871, 1987
24. Koepke JP, Jones S, DiBona GF: Renal nerves mediate blunted natriuresis to atrial natriuretic peptide in cirrhotic rats). *Am J Physiol* 252:R1019–R1023, 1987
25. Holtz J, Sommer O, Bassenge E: Inhibition of sympatho-adrenal activity by atrial natriuretic factor in dogs. *Hypertension* 9:350–354, 1987
26. McMurray J, Struthers AD: Effects of angiotensin II and atrial natriuretic peptide alone and in combination on urinary water and electrolyte excretion in man. *Clin Sci* 74:419–425, 1988
27. Feng Q, Hedner T, Hedner J, Pettersson A: Blunted renal response to atrial natriuretic peptide in congestive heart failure rats is reversed by α_2-adrenergic agonist clonidine. *J Cardiovasc Pharmacol* 16:776–782, 1990
28. Cottier C, Matter L, Weidmann P, Shaw S, Gnadinger MP: Renal response to low dose infusion of atrial natriuretic peptide in normal man. *Kidney Int* 34 (Suppl 25):S72–S78, 1988
29. Janssen WMT, De Zeeuw D, van der Hem GK, de Jong PE: Atrial natriuretid peptide-induced decreases in renal blood flow in man: Implications for the natriuretic mechanism. *Clin Sci* 77:55–60, 1989
30. Solomon LR, Atherton JC, Bobinski H, Hillier V, Green R: Effect of low dose infusion of atrial natriuretic peptide on renal function in man. *Clin Sci* 75:403–410, 1988
31. Green R: Renal actions of atrial natriuretic factor, Chapter 5, in *Frontiers in Pharmacology & Therapeutics: Atrial Natriuretic Factor*, edited by Struthers AD, Oxford, Blackwell Scientific Publications Ltd, 1990, pp 89–113
32. Burnett JM, Granger J, Opgenorth T: Effects of synthetic atrial natriuretic factor on renal function and renal renin release. *Am J Physiol* 247:F863–F866, 1984
33. Burnett J, Opgenorth T, Granger J: The renal action of atrial natriuretic peptide during control of glomerular filtration. *Kidney Int* 30:16–19, 1986
34. Garvin JL: Inhibition of JV by ANF in rat proximal straight tubules requires angiotensin. *Am J Physiol* 257:F907–F911, 1989
35. Harris PJ, Thomas D, Morgan TO: Atrial natriuretic peptide inhibits angiotensin-stimulated proximal tubular sodium and water transport. *Nature* 326:697–698, 1987
36. McMurray J, Seidelin PH, Struthers AD: Evidence for a proximal and distal nephron action of atrial natriuretic factor in man. *Nephron* 51:39–43, 1989
37. Sonnenberg H, Cupples W, de Bold A, Veress A: Intra-renal localisation of the natriuretic effects of cardiac atrial extract. *Can J Physiol Pharmacol* 60:1149–1152, 1982
38. Zeidel M, Brenner B: Actions of atrial natriuretic peptides on the kidney. *Semin Nephrol* 7:91–97, 1984
39. Huang C, Lewicki J, Johnson L, Cogan M: Renal mechanisms of action of rat atrial natriuretic factor. *J Clin Invest* 75:769–773, 1985
40. Kosecki C, Hayashi Y, Torikai S: Localization of binding sites for rat atrial natriuretic polypeptide in the rat kidney. *Am J Physiol* 250:F210–F216, 1986
41. Fried TA, Osgood RW, Stein JH: Tubular sites of action of atrial natriuretic peptide in the rat. *Am J Physiol* 255:F313–F316, 1988
42. Gaillard CA, Koomans HA, Rabelink AJ, Dorhout-Mees EJ:

Effects of indomethacin on renal response to atrial natriuretic peptide. *Am J Physiol* 253:F868–F873, 1987
43. KOOMANS HA, BOER WH, DORHOUT-MEES EJ: Evaluation of lithium clearance as a marker of proximal tubule sodium handling. *Kidney Int* 36:2–12, 1989
44. DIBONA GF, SAWIN LL: Effect of renal nerve stimulation on NaCl and H_2O transport in Henle's loop of the rat. *Am J Physiol* 243:F576–F580, 1982
45. ROY DR: Effect of synthetic ANP on renal and loop of Henle function in the young rat. *Am J Physiol* 201:F220–F225, 1986
46. SCHNERMANN J, BRIGGS JP: Renal effects of atrial natriuretic peptides. *Klin Wochenschr* 65 (Suppl VIII):92–96, 1987