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The role of α-adrenoceptors in the regulation of renal tubular sodium reabsorption and renin secretion in the rabbit

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1 A study was undertaken in the anaesthetized rabbit to classify the α-adrenoceptor subtypes responsible for increasing renal tubular sodium reabsorption and renin secretion. Intrarenal administration of noradrenaline, at doses which did not change renal blood flow or glomerular filtration rate, significantly decreased urine flow, absolute and fractional sodium excretion by between 26% and 29%. These renal responses to noradrenaline were abolished by the selective α_1-adrenoceptor antagonist, prazosin, but not by the selective α_2-adrenoceptor antagonist, idazoxan.

2 Noradrenaline, given intrarenally, increased renin secretion between two and three fold and this response was not modified by either prazosin or idazoxan.

3 Intrarenal administration of the selective α-adrenoceptor agonists, phenylephrine and methoxamine, at dose rates which did not change renal haemodynamics, significantly reduced urine flow, absolute and fractional sodium excretion by 15% to 33%, but at doses which reduced blood flow and filtration rate, by between 11% and 26%, urine flow, absolute and fractional sodium excretion decreased between 42% and 49%.

4 Infusion of guanabenz (selective α_2-adrenoceptor agonist), at doses with no renal haemodynamic action, increased urine flow, absolute and fractional sodium excretion by 11% to 15%, while at doses which decreased blood flow by 7%, these other variables did not change.

5 Administration of UK 14304 (selective α_2-adrenoceptor agonist) reduced blood flow and filtration rate by 3% and 9% respectively but had no other measurable action. At higher doses, which decreased blood flow by 14% and filtration rate by 24%, urine flow, absolute and fractional sodium excretion fell by between 27% and 50%.

6 Renin secretion was significantly increased by the high doses of phenylephrine and UK 14304 but not by the low doses of these drugs.

7 These studies show that adrenergic stimulation of renal tubular sodium reabsorption involves the activation of α_1- but not α_2-adrenoceptors. Further, adrenergic activation of the juxtaglomerular cells to release renin does not appear to involve either α_1- or α_2-adrenoceptors.

Introduction

There is now convincing documentation that activity within the renal nerves, at levels which have no influence on renal haemodynamics, can increase both sodium reabsorption and renin secretion (DiBona, 1982). The nerve-mediated increase in sodium reabsorption involves activation of α-adrenoceptors (Zambraski *et al.*, 1976; DiBona & Johns, 1980) which appear to initiate the response in the cells of the proximal tubule (Bello-Reuss *et al.*, 1976) and the ascending limb of the loop of Henlé (DiBona & Sawin, 1982). The neurally induced increase in renin secretion involves activation of β-adrenoceptors which are now recognized as being located on the juxtaglomerular granular cells (Keeton & Campbell, 1980) and have been classified as β_1-adrenoceptors (Johns, 1981; Osborn *et al.*, 1981). These adrenergically mediated renal functional responses have also been described following noradrenaline administration directly into the renal artery of dogs at low doses which did not change renal haemodynamics (Barger *et al.*, 1959; Pearson & Williams, 1968; Winer *et al.*, 1971; Gill & Casper, 1972).

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The pharmacological characterization of the adrenoceptor involved in the control of renal tubular sodium excretion has been the subject of increasing attention but has resulted in conflicting views. Radioligand binding studies applied to kidney membranes indicate the presence of α_1- and α_2-adrenoceptors in the rat renal cortex (U'Prichard & Snyder, 1979; McPherson & Summers, 1981; 1982a) and almost exclusively α_2-adrenoceptors in the guinea-pig kidney (McPherson & Summers, 1982b). Such an analysis gives no indication of the function of these α-adrenoceptors and the question therefore arises as to which subtype(s) of α-adrenoceptor are involved in the increased reabsorption of sodium induced by the adrenergic nervous system. Functional data from this laboratory using the rabbit (Hesse & Johns, 1984a), and from Osborn *et al.* (1983) using the dog, have shown that the increase in sodium reabsorption caused by activation of the renal nerves at low rates is mediated solely by α_1-adrenoceptors. However, whether blood borne noradrenaline could induce increases in sodium reabsorption by activation of both α_1- and/or α_2-adrenoceptors, which may be more accessible to circulating noradrenaline, is not known but might go some way to explaining the differing conclusions arising from radioligand and functional studies.

There is also great controversy about the role of α-adrenoceptors in the control of renin secretion as both stimulatory (Osborn *et al.*, 1982; Blair, 1983; Olson *et al.*, 1983) and inhibitory (Desaulles *et al.*, 1975; Pettinger *et al.*, 1976) effects have been reported with various α-adrenoceptor agonists. However, part of the reason for these differences could reside in the different degree of adrenergic activation used and whether renal haemodynamics were altered or not.

In the present investigation we have examined the ability of selective α_1- or α_2-adrenoceptor blockade to inhibit noradrenaline induced antinatriuresis and increases in renin secretion. The study has been extended to compare the effects of administration of selective α_1- and α_2-adrenoceptor agonists at several dose levels on renal sodium excretion and renin secretion in the anaesthetized rabbit.

Methods

Surgical procedures

Experiments were carried out on male Carolina rabbits (2.0–3.5 kg) anaesthetized with sodium pentobarbitone (120–200 μmol kg^{-1}). Catheters were inserted into the carotid artery for blood pressure measurements (Statham P23Db transducer) and arterial blood sampling, and into the jugular vein for infusion of 150 mM NaCl (saline) at 60 ml h^{-1}, inulin and supplemental doses of anaesthetic. Heart rate was measured with a tachograph (Grass Model 7PA) triggered by the arterial pulse wave. Recordings were made on a Grass Model 7 polygraph.

The left kidney was exposed retroperitoneally through a flank incision, the renal nerves dissected out, sectioned and a non-cannulating electromagnetic flow probe (Carolina Medical Electronics Inc.) placed around the renal artery for renal blood flow measurements (Carolina Medical Electronics Inc. Flowmeter Model FM 501). A cannula was placed in a small lumbar branch of the renal artery to allow the administration of saline or α-adrenoceptor drugs directly into the renal artery at a rate of 0.02 ml min^{-1}. In experiments in which renin secretory rates were studied a cannula was also placed in a lumbar tributary of the renal vein for the collection of renal venous blood. The left ureter was cannulated for urine collections.

At the end of the surgery a priming dose of inulin in saline (40 mg kg^{-1} inulin in a volume of 1 ml kg^{-1}) was administered followed by an infusion of inulin in saline (4 mg ml^{-1}) to maintain a plasma inulin concentration of 20–40 mg 100 ml^{-1}. The first blood samples were taken not earlier than 1 h after the inulin priming dose.

Experimental protocol

Two experiments, each consisting of five clearance periods of 15 min duration, were carried out in each animal. Urine was collected for two control periods then an α-adrenoceptor agonist infusion into the renal artery was begun and 10–15 min later the third urine sample was collected. Two recovery samples were started 10–15 min after the agonist infusion had been changed back to saline. These 10–15 min periods were left in order for the agonist to attain its peak effect and for the kidney to recover from the effect of the drug.

Analyses

Arterial blood samples were taken at the beginning and the end of each clearance period for plasma inulin and sodium determinations. Plasma and urinary sodium were determined by flame photometry and inulin by colourimetry (Bojesen, 1951). Glomerular filtration rate was calculated as the clearance of inulin from plasma. Haematocrit was determined by a microhaematocrit method. Renal vein and arterial blood for renin assay were collected immediately before, during the last minute of α-adrenoceptor agonist infusion and at the end of the recovery periods into ice chilled tubes containing sufficient sodium-EDTA to achieve a final concentration of 2.7 nmol EDTA ml^{-1} blood. Plasma renin activity was deter-

mined by radioimmunoassay of angiotensin I generated during incubation at 37°C (Haber *et al.*, 1969) using procedures previously described (Hesse, 1984). Renin secretion rate was calculated as the product of the renal venous-arterial plasma renin activity difference and renal plasma flow. This was normalised for body weight and expressed as units $min^{-1} kg^{-1}$, where 1 unit represents 1 ng angiotensin I generated in 1 ml of plasma during 1 h of incubation. Renin secretion rate was measured in the noradrenaline, phenylephrine and UK 14304 (5-bromo-6-[2-imadozolin-2-ylamio]-quinoxaline) infusion experiments only.

Drugs

All drugs, with the exception of prazosin, were dissolved in saline immediately before their use and protected from light to minimize oxidation. Differing dilutions were made such that different dosages could be given at the constant rate of infusion of 0.02 ml min^{-1}. Stock solutions of prazosin were made up with 0.01 M lactic acid and diluted as necessary with distilled water.

Experimental series

Two series of experiments were carried out. In the first series paired experiments were performed in which noradrenaline was administered intrarenal arterially before and following an intrarenal arterial infusion of either the selective α_1-adrenoceptor antagonist prazosin (a bolus of 60 nmol kg^{-1} over 3 min + 7.5 nmol $kg^{-1} min^{-1}$) or the selective α_2-adrenoceptor antagonist idazoxan (a bolus of 500 nmol kg^{-1} over 3 min + 15 nmol $kg^{-1} min^{-1}$). Noradrenaline was administered at a dose-rate (ranging from 50–300 pmol $kg^{-1} min^{-1}$) which was adjusted in each animal to just below the threshold for causing changes in renal blood flow; the same dose-rate was infused in both experiments in each animal. The second experiment was begun some 30–40 min after the beginning of the antagonist administration and the antagonist was infused for the duration of the second experiment. The degree and specificity of α-adrenoceptor blockade was tested by measuring the renal vasoconstrictor responses to intrarenal arterial injections of 5 nmol phenylephrine and 30 nmol clonidine immediately before and 10–15 min after the start of each α-adrenoceptor antagonist infusion. Any effects of the antagonists on baseline renal function were evaluated by comparing the mean of the last two clearances of the first experiment with those of the first two clearances of the second experiment. In the second series of experiments four different α-agonists were infused, the selective α_1-agonists phenylephrine and methoxamine, and the selective α_2-agonists guanabenz and UK 14304. Each group of animals was tested with only one agonist at two different dose levels, one which caused no change or one which caused a 5–20% reduction in renal blood flow, and these doses were given in random order.

Statistical analysis

All values were expressed as mean ± s.e.mean. Paired and unpaired Student's *t* test were used to analyse paired and unpaired data, respectively (Snedecor, 1948). Differences were considered significant at $P < 0.05$. The changes in renal function produced by the α-agonists were calculated as a percentage of the mean of the values obtained in the two control and two recovery periods.

Table 1 Effects of intrarenal arterial administration of α-adrenoceptor antagonists on the systemic and renal responses to noradrenaline (NA) in the rabbit

	Saline (n = 14)		*Prazosin (n = 7)*		*Idazoxan (n = 7)*	
	Control	*NA*	*Control*	*NA*	*Control*	*NA*
MBP (mmHg)	99.1 ± 1.6	97.5 ± 3.1	78.0 ± 1.9	77.8 ± 2.5	92.6 ± 3.4	93.1 ± 4.9
HR (beats min^{-1})	300 ± 7	300 ± 7	279 ± 6	283 ± 6	302 ± 8	300 ± 14
RBF (ml $min^{-1} kg^{-1}$)	20.82 ± 0.57	20.09 ± 0.72	20.36 ± 0.66	19.26 ± 0.78	15.41 ± 0.38	14.92 ± 0.63
GFR (ml $min^{-1} kg^{-1}$)	3.50 ± 0.30	3.36 ± 0.28	4.27 ± 0.31	4.06 ± 0.61	2.91 ± 0.13	2.81 ± 0.16
UV (μl $min^{-1} kg^{-1}$)	247 ± 26	176 ± 32‡	67 ± 24	69 ± 28	163 ± 24	114 ± 17‡
$U_{Na}V$ (μmol $min^{-1} kg^{-1}$)	29.03 ± 2.43	20.50 ± 2.68‡	10.31 ± 2.84	10.47 ± 3.44	18.45 ± 1.78	13.73 ± 2.23‡
FE_{Na} (%)	7.93 ± 0.80	5.88 ± 0.69‡	3.12 ± 1.10	3.24 ± 1.42	4.86 ± 0.37	3.55 ± 0.35†

MBP, mean blood pressure; HR, heart rate; RBF, renal blood flow; GFR, glomerular filtration rate; UV, urine flow rate; $U_{Na}V$, sodium excretion rate; FE_{Na}, fractional sodium excretion.* $P < 0.05$; † $P < 0.02$; ‡ $P < 0.01$; ‡‡ $P < 0.001$. *n* = no. of animals.

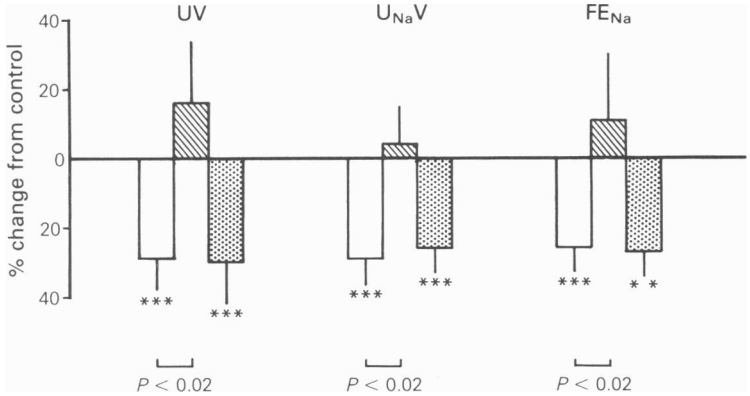

Figure 1 Effect of a 15 min period of intrarenal noradrenaline infusion, at a dose rate which did not change renal haemodynamics, alone (open columns), or in the presence of a continuous infusion of either prazosin (hatched columns) or idazoxan (striped columns), on urine flow rate (Uv), absolute ($U_{Na}V$) and fractional (FE_{Na}) sodium excretions. There were 7 animals in each treatment group. $^{**}P<0.02$; $^{***}P<0.001$.

Results

Effect of renal α-adrenoceptor blockade on noradrenaline induced changes in sodium excretion and renin secretion

The responses to intrarenal noradrenaline infusion in the absence (first experiment) and in the presence (second experiment) of the α-adrenoceptor antagonists are shown in Table 1. The values for the first experiment are the mean for all animals as the magnitude of the renal responses were similar whether the animals were subsequently infused with prazosin or idazoxan. Administration of noradrenaline at dose-rates which did not change renal blood flow had no effect on mean blood pressure, heart rate and glomerular filtration rate in either the first or second experiments. However, noradrenaline significantly decreased urine flow, absolute and fractional sodium excretion by 29% ($P<0.01$), 29% ($P<0.01$) and 26% ($P<0.01$), respectively, during the first experiment.

The intrarenal administration of prazosin (α_1-adrenoceptor antagonist) significantly blocked 80–90% of the phenylephrine-induced renal vasoconstriction while that of clonidine was not significantly affected.

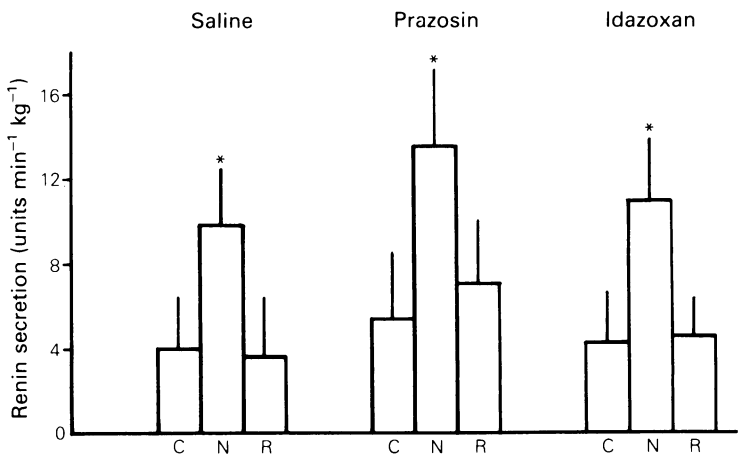

Figure 2 Shows the effect of a 15 min period of intrarenal noradrenaline administration, at dose rates which had no effect on renal haemodynamics, on renin secretion before and in the presence of a continuous infusion of either prazosin or idazoxan. C = control level; N = level during noradrenaline infusion; R = recovery level. There were 7 animals in each group. $^{*}P<0.05$.

Prazosin significantly decreased mean blood pressure ($P<0.01$) without any effect on heart rate and also decreased renal blood flow ($P<0.05$), urine flow ($P<0.01$), absolute ($P<0.01$) and fractional sodium excretion ($P<0.001$) but glomerular filtration rate was not significantly affected. Noradrenaline infusion in the presence of prazosin did not significantly affect urine flow, absolute and fractional sodium excretion and these responses were significantly ($P<0.02$) different from those observed in the absence of prazosin (see Figure 1).

Idazoxan (α_2-antagonist) blocked greater than 90% of the clonidine-induced renal vasoconstriction but was without effect on that induced by phenylephrine. Administration of idazoxan did not change mean blood pressure, heart rate or glomerular filtration rate but significantly decreased renal blood flow ($P<0.02$), urine flow ($P<0.01$) and sodium output ($P<0.01$). In the presence of idazoxan the renal responses to noradrenaline infusion were not modified, and urine flow, absolute and fractional sodium excretion were significantly decreased by 30% ($P<0.01$), 26% ($P<0.01$) and 27% ($P<0.02$), respectively (Figure 1).

The effect of noradrenaline infusion on renin secretion is shown in Figure 2. Noradrenaline infusion into the kidney significantly increased renin secretion by 5.9 ± 2.3 units $min^{-1} kg^{-1}$ ($P<0.05$). In the presence of prazosin, noradrenaline infusion increased renin secretion by 7.3 ± 2.9 units $min^{-1} kg^{-1}$ ($P<0.05$) and in the presence of idazoxan, renin secretion increased by 4.6 ± 1.7 units $min^{-1} kg^{-1}$ ($P<0.05$). None of these responses were significantly different from each other.

Effects of intrarenal infusion of selective α-adrenoceptor agonists on sodium excretion and renin secretion

Infusion of phenylephrine (α_1-adrenoceptor agonist) at both dose-rates had no significant effect on either mean blood pressure or heart rate (Table 2). Low dose rates of phenylephrine (ranging from 0.3–3.0 nmol $kg^{-1} min^{-1}$) were without effect on renal blood flow and glomerular filtration rate but significantly decreased urine flow, absolute and fractional sodium excretion by 15% ($P<0.02$), 23% ($P<0.01$) and 20% ($P<0.01$), respectively. The high dose-rate of phenylephrine (ranging from 0.75–10.0 nmol $kg^{-1} min^{-1}$) significantly decreased renal blood flow and glomerular filtration rate by 11% ($P<0.01$) and 15% ($P<0.02$), respectively, and this was associated with a decrease in urine flow, absolute and fractional sodium excretion of 43% ($P<0.01$), 49% ($P<0.01$) and 42% ($P<0.01$), respectively. These renal responses to the high dose of phenylephrine were significantly greater than those obtained with the low dose of agonist (renal blood flow, $P<0.01$; glomerular filtration rate, $P<0.05$; urine flow, $P<0.02$; absolute sodium excretion, $P<0.02$; fractional sodium excretion $P<0.05$). Renin secretion rate was not significantly affected by the low dose-rates of phenylephrine infusion; however, the high dose-rate significantly increased renin secretion by 2.7 ± 1.0 units $min^{-1} kg^{-1}$ ($P<0.05$) which was significantly ($P<0.05$) different from that observed during the low doses of phenylephrine.

Low doses of methoxamine (α_1-adrenoceptor agonist), ranging from 0.08–0.8 nmol $kg^{-1} min^{-1}$, which were without effect on mean blood pressure, heart

Table 2 Effect of intrarenal arterial administration of phenylephrine (Phe), at low (0.3 to 3.0 nmol $kg^{-1} min^{-1}$) and high (0.75–10.0 nmol $kg^{-1} min^{-1}$) dose-rates, on systemic and renal function

	Low dose			*High dose*		
	Control	*Phe*	*Recovery*	*Control*	*Phe*	*Recovery*
MBP (mmHg)	95.0 ± 4.8	95.7 ± 5.1	99.1 ± 5.6	90.8 ± 8.1	92.6 ± 9.3	88.6 ± 7.3
HR (beats min^{-1})	280 ± 6	274 ± 5	270 ± 5	280 ± 9	281 ± 9	270 ± 9
RBF (ml $min^{-1} kg^{-1}$)	16.6 ± 1.0	16.7 ± 1.1	17.03 ± 1.1	16.6 ± 1.0	14.0 ± 1.0‡	15.6 ± 1.1
GFR (ml $min^{-1} kg^{-1}$)	2.90 ± 0.29	2.77 ± 0.33	2.63 ± 0.26	2.90 ± 0.30	2.50 ± 0.40†	2.90 ± 0.30
UV (μl $min^{-1} kg^{-1}$)	186 ± 40	174 ± 32†	204 ± 36	212 ± 40	120 ± 28‡	206 ± 40
$U_{Na}V$ (μmol $min^{-1} kg^{-1}$)	19.7 ± 5.0	16.4 ± 3.4‡	23.8 ± 5.5	19.3 ± 3.1	11.0 ± 2.6‡	18.4 ± 3.3
FE_{Na} (%)	4.5 ± 1.2	3.9 ± 0.6‡	5.7 ± 1.1	5.2 ± 2.1	3.4 ± 0.9‡	5.2 ± 1.2
RSR (units min^{-1})	2.4 ± 1.5	3.1 ± 1.1	3.2 ± 1.7	3.8 ± 1.1	6.9 ± 1.5*	4.6 ± 1.7

MBP, mean blood pressure; HR, heart rate; RBF, renal blood flow; GFR, glomerular filtration rate; UV, urine flow rate; $U_{Na}V$, absolute sodium excretion; FE_{Na}, fractional sodium excretion; RSR, renin secretion rate. *$P<0.05$; †$P<0.02$; ‡$P<0.01$; ‡‡$P<0.001$. Number of animals = 7.

Table 3 Effect of intrarenal administration of methoxamine (Meth), at low (0.08–0.8 nmol $kg^{-1} min^{-1}$) and high (2.8–10.0 nmol $kg^{-1} min^{-1}$) dose rates, on systemic and renal function

	Control	*Low dose Meth*	*Recovery*	*Control*	*High dose Meth*	*Recovery*
MBP (mmHg)	95.1 ± 2.0	95.8 ± 2.4	93.0 ± 1.4	95.3 ± 1.8	100.5 ± 1.6‡	96.8 ± 0.6
HR (beats min^{-1})	266 ± 5	261 ± 6	256 ± 7	307 ± 9	297 ± 1*	305 ± 7
RBF (ml $min^{-1} kg^{-1}$)	16.0 ± 2.3	16.1 ± 2.1	16.2 ± 2.0	17.6 ± 0.8	14.7 ± 1.2‡	16.8 ± 0.9
GFR (ml $min^{-1} kg^{-1}$)	2.0 ± 0.2	2.1 ± 0.2	2.2 ± 0.2	2.8 ± 0.2	1.9 ± 0.1‡	2.6 ± 0.1
UV (μl $min^{-1} kg^{-1}$)	213 ± 34	155 ± 35‡	235 ± 30	267 ± 29	59 ± 10‡‡	308 ± 32
$U_{Na}V$ (μmol $min^{-1} kg^{-1}$)	21.3 ± 3.2	15.5 ± 3.5†	22.1 ± 3.0	25.9 ± 2.7	6.2 ± 0.3‡‡	27.4 ± 1.9
FE_{Na} (%)	10.5 ± 1.8	7.2 ± 1.6†	9.8 ± 1.5	9.7 ± 0.8	3.2 ± 0.1‡‡	9.8 ± 1.2

Abbreviations and significance symbols as for Table 1. Number of animals = 5

rate, renal blood flow and glomerular filtration rate, significantly decreased urine flow, absolute and fractional sodium excretion by 33% ($P<0.01$), 30% ($P<0.02$) and 28% ($P<0.02$), respectively (Table 3). The high dose rates of methoxamine (ranging from 2.8–10.0 nmol $kg^{-1} min^{-1}$) significantly increased mean blood pressure ($P<0.01$) and decreased heart rate ($P<0.05$). At this dose methoxamine significantly decreased renal blood flow and glomerular filtration rate by 15% ($P<0.01$) and 26% ($P<0.01$), respectively, and also decreased urine flow, absolute and fractional sodium excretion by 79% ($P<0.001$), 76% ($P<0.001$) and 65% ($P<0.001$), respectively. The magnitude of these responses were significantly ($P<0.001$ for all variables) greater than those observed during the low dose of methoxamine.

Guanabenz (α_2-adrenoceptor agonist) was infused at two dose-rates, ranging from 0.03–1.03 at the low dose and ranging from 1.4–10.3 nmol $kg^{-1} min^{-1}$ at the high dose-rates, which resulted in no change and a significant ($P<0.001$) 7% fall in renal blood flow, respectively, while neither dose level significantly affected glomerular filtration rate, blood pressure or heart rate (Table 4). At the low dose-rates guanabenz significantly increased urine flow, absolute and fractional sodium excretion by 16% ($P<0.05$), 18% ($P<0.05$) and 13% ($P<0.05$), respectively. However, the high dose-rate of guanabenz was without effect on urine flow, absolute and fractional sodium excretion. The magnitude of the changes in renal blood flow, glomerular filtration rate, absolute and fractional sodium excretions were significantly ($P<0.001$, $P<0.05$, $P<0.02$ and $P<0.05$, respectively) different from those produced by the low dose of guanabenz.

The changes in systemic cardiovascular and renal function produced by UK 14304 (α_2-adrenoceptor agonist) are shown in Table 5. Low dose rates of UK 14304 (ranging from 0.03–0.6 nmol $kg^{-1} min^{-1}$) did not affect mean blood pressure nor heart rate but significantly decreased renal blood flow by 3% ($P<0.02$) and glomerular filtration rate by 9% ($P<0.05$). However, there were no significant changes in urine flow, absolute and fractional sodium excretion and renin secretion. High dose rates of UK 14304 (ranging from 0.5–1.5 nmol $kg^{-1} min^{-1}$) significantly decreased mean blood pressure ($P<0.05$) and heart rate ($P<0.01$) and also decreased renal blood flow by 14% ($P<0.001$), glomerular filtration rate by 24% ($P<0.001$), and urine flow, absolute and

Table 4 Effect of intrarenal administration of guanabenz (Guan), at low (0.03–1.03 nmol $kg^{-1} min^{-1}$) and high (1.4–10.3 nmol $kg^{-1} min^{-1}$) dose rates, on systemic and renal function

	Control	*Low dose Guan*	*Recovery*	*Control*	*High dose Guan*	*Recovery*
MBP (mmHg)	90.1 ± 5.1	85.9 ± 4.5	83.2 ± 4.3	98.9 ± 3.1	95.6 ± 3.0	97.2 ± 2.8
HR (beats min^{-1})	285 ± 7	283 ± 7	280 ± 6	300 ± 6	287 ± 6	290 ± 6
RBF (ml $min^{-1} kg^{-1}$)	15.7 ± 1.2	15.4 ± 1.6	14.9 ± 1.1	15.6 ± 0.8	14.0 ± 0.9‡‡	14.8 ± 0.9
GFR (ml $min^{-1} kg^{-1}$)	2.5 ± 0.2	2.8 ± 0.2	2.7 ± 0.1	2.5 ± 0.1	2.4 ± 0.2	2.4 ± 0.2
UV (μl $min^{-1} kg^{-1}$)	90 ± 20	108 ± 18*	94 ± 11	87 ± 11	77 ± 10	83 ± 13
$U_{Na}V$ (μmol $min^{-1} kg^{-1}$)	15.2 ± 5.0	16.1 ± 4.1*	12.9 ± 3.0	11.3 ± 1.6	9.8 ± 1.4	9.5 ± 1.4
FE_{Na} (%)	3.5 ± 0.5	3.6 ± 0.4*	3.1 ± 0.5	3.3 ± 0.3	3.1 ± 0.3	3.2 ± 0.5

Abbreviations and symbols of significance as for Table 1. Number of animals = 5

Table 5 Effect of intrarenal administration of UK14304 at low (0.03–0.06 nmol $kg^{-1} min^{-1}$) and high (0.5–1.5 nmol $kg^{-1} min^{-1}$) dose rates, on systemic and renal function

	Control	*Low dose UK14304*	*Recovery*	*Control*	*High dose UK14304*	*Recovery*
MBP (mmHg)	96.9 ± 2.6	94.9 ± 2.3	94.8 ± 2.4	96.7 ± 3.3	89.3 ± 3.4*	95.9 ± 2.9
HR (beats min^{-1})	299 ± 4	292 ± 4	291 ± 2	288 ± 8	263 ± 7‡	279 ± 7
RBF (ml $min^{-1} kg^{-1}$)	18.3 ± 1.1	17.7 ± 1.1†	18.0 ± 1.2	17.1 ± 1.3	14.4 ± 1.2‡‡	16.5 ± 1.2
GFR (ml $min^{-1} kg^{-1}$)	3.0 ± 0.2	2.7 ± 0.2*	2.9 ± 0.2	2.6 ± 0.3	2.2 ± 0.2‡‡	3.0 ± 0.2
UV (μl $min^{-1} kg^{-1}$)	145 ± 43	153 ± 35	136 ± 26	189 ± 36	100 ± 21‡‡	204 ± 48
$U_{Na}V$ (μmol $min^{-1} kg^{-1}$)	18.8 ± 3.1	18.6 ± 1.8	17.4 ± 2.8	20.8 ± 4.4	12.8 ± 3.0‡	28.3 ± 6.5
FE_{Na} (%)	4.7 ± 0.9	5.2 ± 0.8	4.3 ± 0.6	5.3 ± 0.8	3.9 ± 0.7†	5.9 ± 1.2
RSR (units min^{-1})	3.1 ± 1.9	4.5 ± 1.6	5.4 ± 1.7	3.6 ± 1.9	6.4 ± 2.7*	2.7 ± 1.6

Abbreviations and symbols of significance as for Table 1. Number of animals = 7

fractional sodium excretion by 50% ($P < 0.001$), 44% ($P < 0.01$) and 27% ($P < 0.02$), respectively (Table 5). These changes in renal function were significantly different from those recorded during the low rates of UK 14304 infusion ($P < 0.001$, $P < 0.05$, $P < 0.01$, $P < 0.02$, and $P < 0.05$, respectively for renal blood flow, glomerular filtration rate, urine volume, absolute and fractional sodium excretions). Renin secretion rate was significantly ($P < 0.05$) increased by 3.6 ± 1.2 units $min^{-1} kg^{-1}$ which was a response significantly different from that observed during the low dose of UK 14304.

A comparison of the effect on renal sodium excretion of the low dose rates of infusion of α-adrenoceptor agonists is shown in Figure 3. This shows that renal sodium excretion was decreased by the α_1-adrenoceptor agonists phenylephrine and methoxamine and increased by the α_2-adrenoceptor agonists guanabenz and UK 14304.

Discussion

It was the aim of this investigation to undertake a functional investigation of the subtype(s) of α-adrenoceptors mediating the renal tubular action of circulating noradrenaline on sodium excretion and the role, if any, played by α_1- or α_2-adrenoceptors on renin secretion by the juxtaglomerular cells. The experiments were undertaken in kidneys which had been denervated and drugs were infused into the renal artery in order to minimise any systemic or reflex effects of the compounds.

The findings demonstrated that in the rabbit infusion of noradrenaline into the kidney, at dose-rates which did not influence renal haemodynamics, caused an antinatriuresis and this has been taken to represent an increase in tubular sodium reabsorption. In studies using low level renal nerve stimulation, the adrenergic action on sodium reabsorption takes place at both the proximal tubule (Pelayo *et al.*, 1983) and the ascending

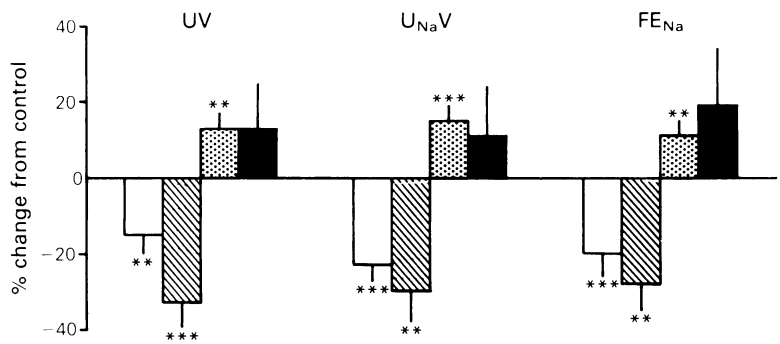

Figure 3 Effect of 15 min periods of intrarenal infusion of phenylephrine (open columns), methoxamine (hatched columns), guanabenz (stippled columns) and UK 14304 (solid columns), all at dose rates which did not change renal haemodynamics, on urine flow (UV), absolute ($U_{Na}V$) and fractional (FE_{Na}) sodium excretions. There were 7 animals in each group except for the methoxamine and guanabenz treated animals in which 5 animals were used. *$P < 0.05$; **$P < 0.02$; ***$P < 0.001$.

limb of the loop of Henlé (DiBona & Sawin, 1982). Administration of the selective α_1-adrenoceptor antagonist, prazosin (Cambridge *et al.*, 1977; Doxey *et al.*, 1977), itself caused a reduction in the output of urine and sodium. However, this phenomenon has been previously reported in the rabbit (Hesse & Johns, 1984a) and was suggested to be a consequence of both the fall in blood pressure, caused by the prazosin entering the systemic circulation, as well as an action of the lactic acid vehicle for prazosin which itself was shown to cause a large fall in basal levels of sodium output (Hesse & Johns, 1984a). Nevertheless, during prazosin infusion the noradrenaline-induced antinatriuresis was completely abolished.

Intrarenal administration of the selective α_2-adrenoceptor antagonist, idazoxan (Doxey *et al.*, 1983), also caused reductions in the excretion rates of urine and sodium, the reasons for which were not apparent. In this case noradrenaline infusion still caused a marked antinatriuresis during functional α_2-adrenoceptor blockade which was comparable to that obtained in the absence of idazoxan. These observations clearly indicate that the α-adrenoceptors mediating the tubular sodium reabsorption are of the α_1-subtype because the response was inhibited by α_1-adrenoceptor blockade but not when the α_2-adrenoceptors were blocked. These results support the findings obtained with renal nerve stimulation experiments in the rabbit (Hesse & Johns, 1984a) and dog (Osborn *et al.*, 1983) which showed that the antinatriuresis of low level renal nerve stimulation was blocked by α_1- but not by α_2-adrenoceptor antagonists. Taken together, all these reports show that whether noradrenaline is released endogenously or administered exogenously there is an increase in tubular sodium reabsorption which is mediated by α_1-adrenoceptors.

In order to provide further evidence for the subtypes of renal tubular α-adrenoceptors mediating sodium reabsorption and to overcome any possible limitations arising from the use of the antagonists, experiments were performed in which the effects of intrarenal administration of a range of selective α-adrenoceptor agonists on renal sodium reabsorption were determined. The data showed that the selective α_1-adrenoceptor agonists, phenylephrine and methoxamine (Starke *et al.*, 1975), at dose-rates which had no effect on renal haemodynamics, significantly increased sodium reabsorption. Similar results have been obtained in the dog, using methoxamine, which increased sodium reabsorption without altering renal haemodynamics (Osborn *et al.*, 1982). In marked contrast, low doses of the selective α_2-adrenoceptor agonists, guanabenz (McGrath, 1982) caused a decrease in sodium reabsorption while UK 14304 (Cambridge, 1981) did not change sodium output. The recent reports of Strandhoy *et al.* (1982) and Strandhoy *et al.* (1983) have shown that guanabenz has an action to inhibit both the release of antidiuretic hormone as well as inhibiting its action at the level of the renal tubule both of which are mediated via α_2-adrenoceptors. It is possible that the changes in sodium and water reabsorption recorded in the present study with guanabenz could be the result of this inhibition of the action of antidiuretic hormone together with some contribution of guanabenz action resulting from its spilling over into the systemic circulation. In contrast, the other α_2-adrenoceptor agonist used in this study, UK 14304, had no effect on sodium or water output but at these dose levels it did reduce glomerular filtration rate which itself could have over-ridden any diuretic or natriuretic activity of the drug.

In the present studies the increased sodium reabsorption caused by the blood-borne agonists noradrenaline, phenylephrine and methoxamine occurred in the absence of changes in systemic haemodynamics, in denervated kidneys and without changes in renal haemodynamics which clearly indicates that these compounds are acting directly on the renal tubules. These results add weight to the contention that renal tubular α_1-adrenoceptors on the proximal tubules and the ascending limb of the loop of Henle can be stimulated by blood borne α_1-adrenoceptor agonists. Therefore an important conclusion from these experiments is that circulating catecholamines have the potential to influence salt and water reabsorption by acting directly on the renal tubular sodium reabsorptive processes.

High dose-rates of both α_1- and α_2-adrenoceptor agonists decreased sodium excretion to a greater degree than the low doses of these drugs but at this level there were substantial reductions in renal blood flow and glomerular filtration rate. Similar haemodynamic and functional responses have been reported for the dog in which methoxamine and phenylephrine caused dose-dependent decreases in renal haemodynamics and sodium output (Osborn *et al.*, 1982; Olson *et al.*, 1983; Blair, 1983). The possibility exists that the changes in renal haemodynamics contributed to and accentuated these falls in sodium excretion. In the case of UK 14304, a further contributory factor to the decreased sodium excretion observed during the high rates of administration was the decreased blood pressure and heart rate. These systemic effects of the α_2-adrenoceptor agonists were probably secondary to central and peripheral sympathetic nervous system α_2-adrenoceptor stimulation with a resultant decrease in sympathetic nerve outflow (Schmitt, 1977; Kobinger, 1978). Another possibility was that the decrease in sodium excretion seen with the high dose-rates of the α_2-adrenoceptor agonists could have been due to nonselective (α_1-) effects at these dose-rates.

There is considerable controversy about the in-

volvement of α-adrenoceptors in the control of renin secretion. Some authors have presented evidence to show that α-adrenoceptors directly inhibit renin secretion (Capponni & Vallotton, 1976; Desaulles *et al.*, 1975; Pettinger *et al.*, 1976). On the other hand, others have observed that α-adrenoceptors stimulated renin release in the absence of changes in renal haemodynamics (Blair, 1983) or in association with marked decrease in renal haemodynamics (Osborn *et al.*, 1982; Olson *et al.*, 1983). The present results showed that when low doses of noradrenaline were administered into the renal artery, renin secretion was increased in the absence of changes in renal haemodynamics which was not affected by either α_1- or α_2-adrenoceptor blockade and indicated that noradrenaline stimulated the juxtaglomerular granular cells to increase renin secretion most probably by the mediation of β-adrenoceptors (Keeton & Campbell, 1980). This conclusion is consistent with the findings observed in nerve stimulation studies which showed that the increase in renin secretion produced by renal nerve stimulation in the rabbit (Hesse, 1984) and the dog (Osborn *et al.*, 1982; 1983) was not affected by renal α_1- or α_2-adrenoceptor blockade. Further support for this view was provided by the converse experiments of the present study in which α-adrenoceptor agonists were administered directly into the kidney and showed that low doses of selective α-adrenoceptor agonists which were without effect on renal haemodynamics but decreased (α_1-), or did not change (α_2-), sodium excretion had no effect on renin secretion. Taken together, the evidence strongly indicates that α-adrenoceptors do not mediate the effect of circulating or nerve released noradrenaline on renin secretion.

When high doses of the α-agonists were administered there was an increase in renin secretion which was associated with substantial decreases in renal blood flow, glomerular filtration rate and sodium excretion. Similar results have been obtained with high doses of methoxamine, phenylephrine or noradrenaline in the presence of propranolol, which decreased renal blood flow by 15–50% (Osborn *et al.*, 1982; Olson *et al.*, 1983; Blair, 1983) and high frequency nerve stimulation, which decreased renal blood flow by 20–50% (Kopp *et al.*, 1981). These observations are in keeping with the view that α-adrenoceptors do not directly stimulate renin secretion by the granular cells of the juxtaglomerular apparatus but rather stimulate secretion indirectly through α-adrenoceptor mediated renal vasoconstriction and/or decreased sodium excretion. The present data show that the involvement of the renal baroreceptor and macula densa mechanisms in α-adrenoceptor stimulation of renin secretion occur when changes of more than 15% in renal blood flow and more than 30% in sodium excretion take place (Hesse 1984) and this would suggest that renal α-adrenoceptors are not important in the control of renin secretion under most physiological conditions.

In conclusion, the results of the present study provide further evidence to indicate that renal tubular α_1-adrenoceptors mediate the antinatriuresis of renal arterial administration of noradrenaline and thereby demonstrate that circulating catecholamines can directly modulate sodium handling by the kidney. They further indicate that α-adrenoceptors, whether α_1- or α_2-, do not directly mediate the noradrenaline-induced renin secretion but may do so indirectly through the renal baroreceptor and/or macula densa mechanisms.

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