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α_1-Adrenoceptor subtypes mediating antinatriuresis in Wistar and stroke-prone spontaneously hypertensive rats

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Abstract

This study examined the α_1-adrenoceptor subtypes involved in mediating adrenergically induced Na^+ reabsorption in the kidney of pentobarbitone anaesthetised Wistar and stroke-prone spontaneously hypertensive rats (SHRSP). Close renal-arterial phenylephrine (50–100 $\mu g\ kg^{-1}\ h^{-1}$) administration in Wistars, with regulated renal perfusion pressure, caused small reductions in renal haemodynamics but large reductions, of 35%, 64% and 57% (all $P < 0.05$), in urine volume, absolute and fractional Na^+ excretions. The magnitude of these excretory responses to phenylephrine were similar in the presence of the α_{1B}-adrenoceptor alkylating agent, chloroethylclonidine (10 $\mu g\ kg^{-1}\ h^{-1}$), but were blocked during administration of the α_{1A}-adrenoceptor antagonist, 5-methylurapidil (10 $\mu g\ kg^{-1}\ h^{-1}$). Phenylephrine infusion in the stroke-prone spontaneously hypertensive rats caused changes in renal haemodynamics and fluid excretion of comparable magnitude to that achieved in Wistars which was blocked by 5-methylurapidil but not chloroethylclonidine. These observations suggest that in Wistar and stroke-prone spontaneously hypertensive rats the adrenergically induced Na^+ reabsorptive responses are mediated by α_{1A}-adrenoceptors.

Keywords: Na^+ excretion; α_1-Adrenoceptor subtype; Hypertension; Kidney function

1. Introduction

The kidney receives a dense innervation of sympathetic nerves which penetrates both the vascular and tubular components (Barajas et al., 1984) and represents a major way by which the homeostatic control of extracellular fluid volume may be regulated (Kopp and DiBona, 1992). At the vascular level, activation of the sympathetic nerves leads to a reduction in renal haemodynamics as a consequence of arteriolar constriction (Hesse and Johns, 1984) while at the juxtaglomerular cells they cause the release of renin (Johns, 1981). There is now a large body of histochemical and physiological evidence which shows that the renal nerves also act directly on the epithelial cells of the proximal tubules (Bello-Reuss et al., 1977) and the thick ascending limb of the loop of Henle (DiBona and Sawin, 1982) to increase the rate of Na^+ reabsorption.

There have been a number of studies to determine the type of α-adrenoceptors that are involved in mediating the actions of the renal nerves at the tubular epithelial cells. Functional studies have indicated that activation of α_1-adrenoceptors stimulate tubular Na^+ reabsorption in the dog (Osborn et al., 1983), rabbit (Hesse and Johns, 1985) and the rat (Johns and Manitius, 1986) whereas α_2-adrenoceptor activation is without direct effect. There is now a growing body of evidence that in the spontaneously hypertensive rat there is a raised sympathetic nerve activity, including that to the kidneys. Previous studies have shown that the α_2-adrenoceptor density within the cortex is elevated in genetic hypertension (Graham et al., 1982; Pettinger et al., 1982) whereas that of α_1-adrenoceptors is unaltered and the question arises as to whether this might modify the functionality of the major subtype mediating adrenergically induced tubular reabsorption of Na^+ in the genetic models of hypertension, the spontaneously hypertensive and the stroke-prone spontaneously hypertensive rats (SHRSP).

More recently selective α_1-adrenoceptor agonists and antagonists have become available which can be

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used to discriminate between subtypes of α_1-adrenoceptors and both molecular biological and functional evidence has shown α_{1A}- and α_{1B}-adrenoceptor subtypes to be present in many tissues (Perez et al., 1991; Schwin et al., 1990). Recent investigations to analyse the subtype involved in adrenergic control at the level of the renal vasculature of the normotensive Wistar rat (Munavvar and Johns, 1991) and the stroke-prone spontaneously hypertensive rat (Munavvar and Johns, 1992) have found that the α_{1A}-adrenoceptors play a prominent role in mediating the actions of the renal nerves in the control of the vascular smooth muscle and hence renal haemodynamics.

The aim of this study was to provide information on the α_1-adrenoceptor subtype(s) involved in modulating Na^+ reabsorption at the level of the renal tubules in the normotensive state and a second objective was to determine whether in the genetically hypertensive rat in which sympathetic drive is elevated there might be a change in α_1-adrenoceptor subtypes. This was performed by infusing phenylephrine, an α_1-adrenoceptor agonist, close arterially into the kidney at a dose that had minimal haemodynamic actions and determining whether the antinatriuresis and antidiuresis was dependent on α_{1A}- or α_{1B}-adrenoceptors by utilising selective blocking drugs, 5-methylurapidil, an α_{1A}-adrenoceptor antagonist (Gross et al., 1988) and chloroethylclonidine, an α_{1B}-adrenoceptor alkylating agent (Han et al., 1987a)

2. Materials and methods

All animals were maintained on a 12 h light/dark cycle and at 20–22°C with humidity varying between 50 and 72%. Food (SDS Rat diet, Betchworth, Surrey, UK) and water were supplied ad libitum. Male stroke-prone spontaneously hypertensive rats which had been imported from the National Institutes of Health, USA, and bred in the Biomedical Services Unit, whereas, the normotensive Wistar rats were purchased from Charles River (Kent, UK) and kept in the animal facility for at least 1 week.

2.1. Surgical preparation for renal functional studies

Animals, 290–320 g in weight, were starved overnight and anaesthetised with an intraperitoneal injection of 60 mg kg^{-1} sodium pentobarbitone (May and Baker, UK). The trachea was cannulated to provide a clear airway passage. The left jugular vein was cannulated to allow supplementary injections of anaesthetic (sodium pentobarbitone diluted 1:1 in 150 mM NaCl) to be given as required using bolus doses of 0.05–0.1 ml. The right carotid artery was cannulated for the measurement of systemic arterial blood pressure using a pressure transducer (model 23 Gould Statham Instruments) connected to a polygraph (model 7D Grass Instruments).

The left kidney was exposed via a midline abdominal incision and the abdominal contents were carefully moved to the right. A cannula was inserted into the left iliac artery and was advanced into the abdominal aorta, such that its tip lay at the level of the renal artery to enable the infusion of saline and administration of all drugs to be given close renal arterially. The left renal nerves, passing from the coeliac and aortico-renal ganglia, were identified using a Vickers microscope and sectioned. The left renal artery was cleared of connective tissue so that an electromagnetic flowmeter probe (EP100 series probe connected to a Square-wave Electromagnetic flowmeter, Carolina Medical Electronics Model FM501 King, NC) could be fitted for measurement of renal blood flow. The left ureter was cannulated to enable collection of urine. The aorta was carefully exposed above both renal arteries and a suture taken around the aorta. This thread was carefully passed through a short length of polythene tubing and clamped firmly in position just touching the aorta. The cut ends of the loop were tied together and attached to a screw device which, when turned, caused the thread to be shortened such that it tightened around the aorta causing constriction with the consequence that renal perfusion pressure could be adjusted. 2 ml of inulin (10 mg ml^{-1}) in saline (150 mM NaCl) was given as a primer via the jugular vein cannula and an infusion of saline containing inulin 10 mg ml^{-1} and sodium pentobarbitone (12.5 mg kg^{-1} h^{-1}) was begun at a rate of 6 ml h^{-1} via the arterial cannula.

2.2. Experimental protocol

Following completion of surgery, the animals were allowed to stabilise for 2 h.

The experiment was divided into two parts, each of which consisted of a series of five 15 min clearance periods. Two clearances were taken before and two after a period during which the α-adrenoceptor agonist, phenylephrine, 50–100 μg kg^{-1} h^{-1} was administered close renal arterially. Renal perfusion pressure was regulated, if necessary, to prevent any changes in systemic blood pressure from influencing kidney function. After the first set of five clearance collections, the experiment was repeated infusing the saline vehicle to act as a time control group, or in the presence of 5-methylurapidil or chloroethylclonidine at a dose of 10 μg kg^{-1} h^{-1}.

Blood samples were taken at the beginning and end of each pair of clearances before and after phenylephrine was given. Arterial blood samples (0.4 ml) were withdrawn from the carotid cannula into a pre-cooled syringe, centrifuged for 2 min (6000 rpm) and

the plasma removed. The remaining packed blood cells were resuspended in an equal volume of saline and reinfused into the animal within 5 min. The clearance period was started 5–10 min after the reinfusion of the blood sample when the cardiovascular variables had settled. The urine produced during each clearance period was measured gravimetrically. Plasma and urine samples were assayed for inulin using the modified method of Bojesen (1952) and the glomerular filtration rate was calculated as the clearance of inulin (Johns and Manitius, 1986). Plasma and urine electrolytes were measured by flame photometry (Corning model 410C, Halstead, Essex, UK).

2.3. Preparation of drugs

All α-adrenoceptor agonists and antagonists were made up in saline with the exception of 5-methylurapidil, 5-methyl-6[[3-[4-(2-methyoxyphenyl)-1-piperazinyl]-propyl]amino]-1,3-dimethyluracil, (Research Biochemicals, USA) which was dissolved in 10 mM lactic acid. Stock solutions of 5-methylurapidil and chloroethylclonidine, 2-[2,6-dichloro-(*N*-β-chloroethyl-*N*-methyl)-4-aminomethyl]phenylimino-2-imidazoline dihydrochloride (Research Biochemicals, USA) had concentrations of 100 μg ml^{-1} and were kept refrigerated and used within 3 days. Dilution of stock solutions was undertaken in saline immediately prior to use. Phenylephrine (Boots, UK) was diluted fresh daily from sealed ampoules containing 10 mg ml^{-1}. The dose of phenylephrine used varied between 50 and 100 μg kg^{-1} h^{-1} and was dependent on the closeness of the cannula tip to the exit of the renal artery from the aorta and was chosen as one which had minimal effects on renal blood flow.

2.4. Statistics

The renal responses to phenylephrine were measured by taking the average value of the two clearances before and two following the administration of phenylephrine and comparing it with the value recorded during the phenylephrine infusion period (experimental value). All data were expressed as means ± S.E.M. Statistical analysis was performed by one way analysis of variance on repeated measures (Superanova statistical package, Abacus, USA) followed by Bonferonni-Dunn all means post-hoc test. Differences between the means were considered significant at the 5% level. The absolute and percentage changes quoted in the text represent the mean value calculated from individual rats.

3. Results

3.1. Normotensive Wistar rats

Phenylephrine infusion (Table 1) caused significant ($P < 0.05$) reversible increases in blood pressure of approximately 12%, in both parts of the experiment while renal perfusion pressure was maintained at an unchanged level. Renal blood flow was reduced by some 15–20% ($P < 0.05$) during both periods of phenylephrine infusion (Table 1) while glomerular filtration was reduced but not significantly. Fig. 1 shows that in these vehicle infused animals phenylephrine administration caused significant (all $P < 0.05$) reductions in urine volume, absolute and fractional Na^+ excretions ranging from 30 to 50%, the magnitude of which were the same in both the first and second parts of the experiment.

Close renal arterial infusion of phenylephrine in the first part of the experiment before chloroethylclonidine administration resulted in a significant ($P < 0.05$) increase in mean arterial pressure, unchanged renal perfusion pressure, small falls in renal blood flow and glomerular filtration rate (Table 2) and significant decreases in urine volume, absolute and fractional Na^+ excretions, of approximately 35, 64 and 57%, respec-

Table 1
Renal haemodynamic responses to phenylephrine in the time control group of the Wistar rats ($n = 6$)

	MAP (mm Hg)	RPP (mm Hg)	RBF (ml kg^{-1} min^{-1})	GFR (ml kg^{-1} min^{-1})
Vehicle				
Control	102 ± 3	102 ± 3	21.3 ± 1.4	3.9 ± 0.3
Phen	112 ± 2 [a]	103 ± 2	13.9 ± 1.5 [a]	3.5 ± 0.4
Recovery	101 ± 3	100 ± 3	19.5 ± 0.8	4.0 ± 0.2
Vehicle				
Control	100 ± 3	101 ± 3	19.5 ± 1.1	3.8 ± 0.2
Phen	113 ± 5 [a]	101 ± 3	14.7 ± 1.2 [a]	2.8 ± 0.4
Recovery	100 ± 3	100 ± 2	18.5 ± 1.2	3.6 ± 0.2

MAP: mean arterial pressure; RPP: renal perfusion pressure; RBF: renal blood flow; GFR: glomerular filtration rate; Control: average values of two clearances before phenylephrine infusion; Phen: values obtained during phenylephrine infusion; Recovery: average values of two clearances after phenylephrine infusion. [a] $P < 0.05$.

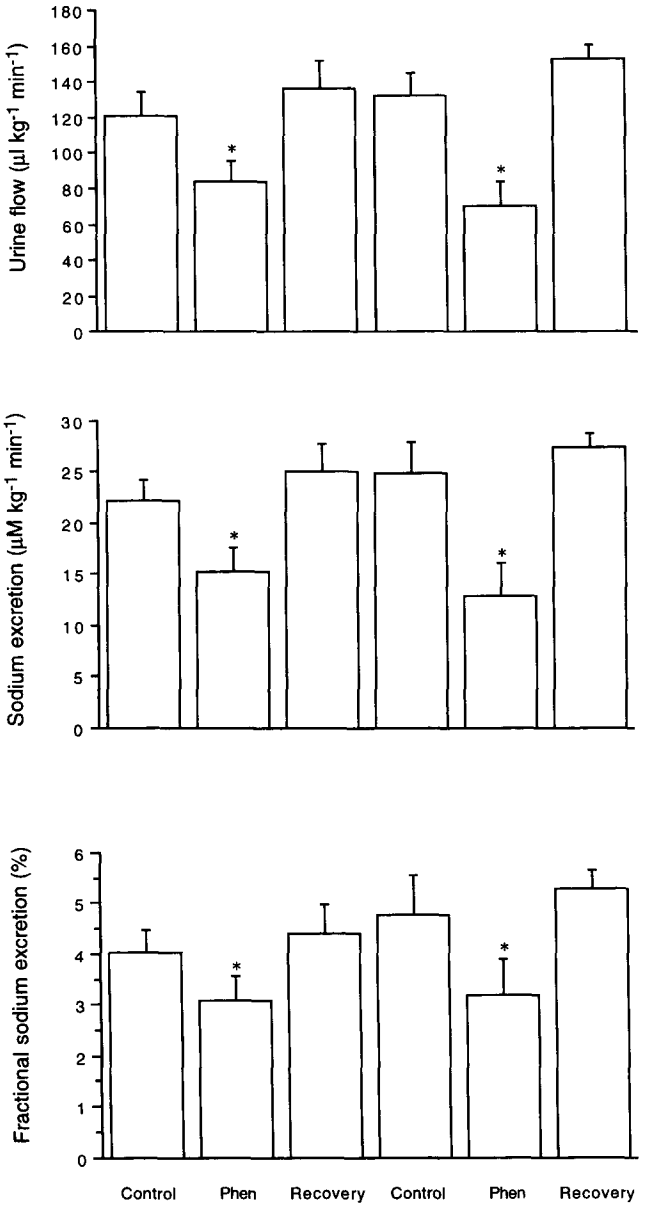

Fig. 1. The effect of two periods of phenylephrine, 50–100 μg kg^{-1} h^{-1} on urine flow (UV), absolute Na^+ ($U_{Na}V$) and fractional Na^+ excretion (FE_{Na}) while saline was infused throughout the experiment in normotensive Wistar rats. * $P < 0.05$ vs. control (ANOVA), $n = 6$.

tively (all $P < 0.05$), during infusion of phenylephrine and all variables returned to levels not significantly different from the control values once the phenylephrine was stopped (Fig. 2).

The administration of chloroethylclonidine had no effect on either blood pressure, renal blood flow and glomerular filtration rate or on any of the renal excretory variables. Phenylephrine infusion in the presence of chloroethylclonidine caused a similar reversible rise in the mean arterial pressure, as observed in its absence, renal perfusion pressure was unchanged and there were no effects on either renal blood flow or glomerular filtration rate (Table 2). Under these conditions, the phenylephrine infusion caused significant reductions in urine volume, absolute and fractional Na^+ excretions of 48, 55 and 63% (all $P < 0.05$, Fig. 2) which returned to control levels once the phenylephrine was stopped. The magnitudes of these phenylephrine induced reductions in Na^+ and water excretions were not significantly different from those ob-

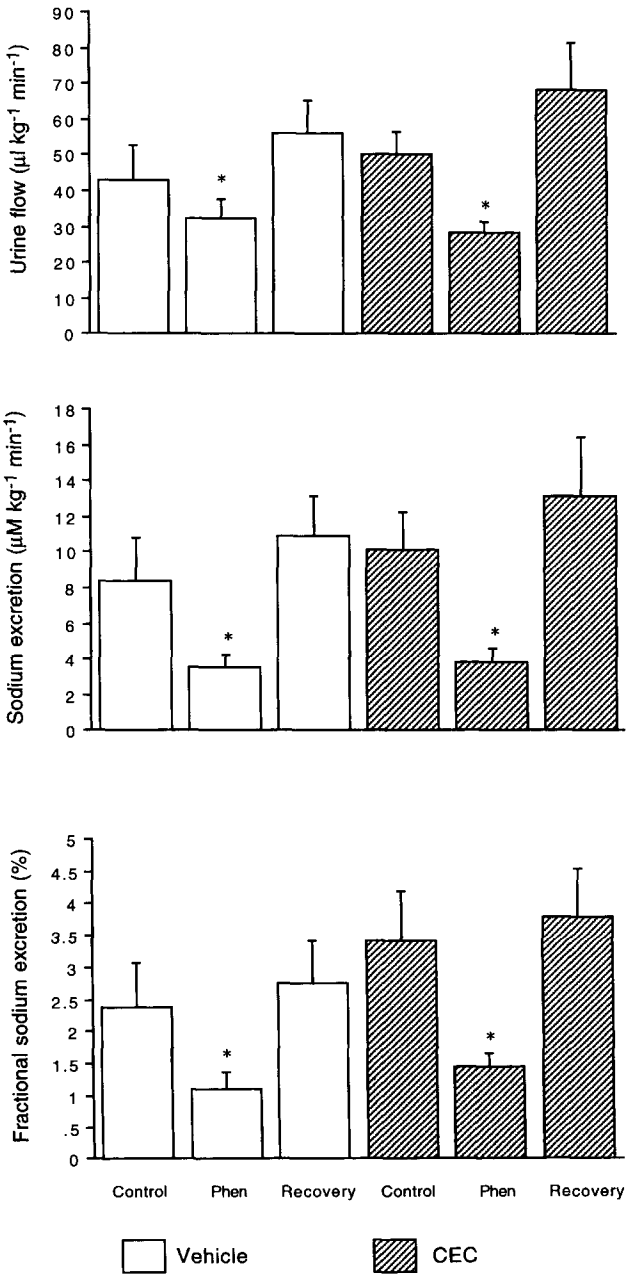

Fig. 2. The effect of phenylephrine, 50–100 μg kg^{-1} h^{-1} on the urine flow (UV), absolute Na^+ excretion ($U_{Na}V$) and fractional Na^+ excretion (FE_{Na}) in the absence and presence of chloroethylclonidine (CEC), 10 μg kg^{-1} h^{-1} in the normotensive Wistar rat. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. control (ANOVA), $n = 5$.

Table 2
Renal haemodynamic responses to phenylephrine in the absence and presence of chloroethylclonidine (CEC) in Wistar rats ($n = 5$)

	MAP (mm Hg)	RPP (mm Hg)	RBF (ml kg^{-1} min^{-1})	GFR (ml kg^{-1} min^{-1})
Vehicle				
Control	105 ± 4	106 ± 5	23.2 ± 0.8	3.1 ± 0.2
Phen	130 ± 5 [a]	106 ± 5	19.4 ± 0.9	2.9 ± 0.3
Recovery	106 ± 7	105 ± 5	20.9 ± 1.6	3.4 ± 0.2
CEC				
Control	103 ± 7	101 ± 6	23.3 ± 1.8	2.6 ± 0.2
Phen	130 ± 5 [a]	101 ± 6	19.8 ± 1.0	2.2 ± 0.2
Recovery	105 ± 6	101 ± 6	20.4 ± 1.8	2.7 ± 0.3

Refer to Table 1 for abbreviations. [a] $P < 0.05$.

tained in the first part of the experiment when chloroethylclonidine was not given.

A similar study was undertaken in a third group of rats, but in which 5-methylurapidil was given during the second part of the study (Table 3, Fig. 3). Infusion of phenylephrine in this group of rats resulted in a significant ($P < 0.05$) increase in mean arterial pressure and while renal perfusion pressure was regulated there was a small reversible reduction in renal blood flow ($P < 0.05$) but no change in glomerular filtration rate (Table 3). There were significant (all $P < 0.05$) reductions in urine volume, absolute and fractional Na^+ excretions during the infusion of phenylephrine of approximately 38, 41 and 37% which returned close to control levels once the infusion ceased (Fig. 3). The magnitudes of these renal excretory responses were very similar to those obtained in the first part of the study in the animals which received chloroethylclonidine.

The infusion of 5-methylurapidil caused significant reductions in both mean arterial pressure and renal perfusion pressure (both $P < 0.01$) of between 12 and 15 mm Hg while neither renal blood flow nor glomerular filtration rate were changed (Table 3). At the same time, there were significant ($P < 0.01$) falls in urine volume, absolute and fractional Na^+ excretions of between 30 and 50% (Fig. 3). Administration of phenylephrine during the 5-methylurapidil caused a significant increase in mean arterial pressure but neither the renal haemodynamic (Table 3) or excretory variables (Fig. 3) were changed. Both the magnitudes and patterns of these responses were very different from those observed in the first part of the study as well as those obtained in both the first and second half of the animals given chloroethylclonidine.

3.2. *Stroke-prone spontaneously hypertensive rats (SHRSP)*

The data for the SHRSP are shown in Tables 4 and 5, and Figs. 4 and 5; the mean arterial blood pressure of these rats was approximately 40–50 mm Hg higher than the Wistar rats ($P < 0.001$), renal blood flows were generally lower ($P < 0.001$), glomerular filtration rates were higher ($P < 0.05$) while water and Na^+ excretions were lower than in the Wistar rats (both $P < 0.05$). In the first group of SHRSP, phenylephrine infusion during the first half of the experiment significantly ($P < 0.01$) increased blood pressure and while renal perfusion pressure remained unaltered, neither renal blood flow nor glomerular filtration rate were changed (Table 4). Under these conditions, the phenylephrine induced reversible reductions in urine volume, absolute and fractional Na^+ excretions of 50, 56 and 46% ($P < 0.05$, $P < 0.01$ and $P < 0.05$, respectively; Fig. 4). Administration of chloroethylclonidine had no effect on control levels of blood pressure or renal haemodynamic and excretory variables (Table 4 and

Table 3
Renal haemodynamic responses to phenylephrine in the absence and presence of 5-methylurapidil (5 Me-U) in Wistar rats ($n = 7$)

	MAP (mm Hg)	RPP (mm Hg)	RBF (ml kg^{-1} min^{-1})	GFR (ml kg^{-1} min^{-1})
Vehicle				
Control	106 ± 4	117 ± 4	22.6 ± 1.5	3.9 ± 0.5
Phen	123 ± 2 [c]	115 ± 4	16.0 ± 0.9 [c]	3.1 ± 0.6
Recovery	115 ± 2	116 ± 4	20.5 ± 1.1	3.6 ± 0.4
5 Me-U				
Control	99 ± 3	107 ± 2	19.5 ± 1.1	3.2 ± 0.3
Phen	109 ± 4 [a]	107 ± 2	17.4 ± 1.1 [b]	2.9 ± 0.4
Recovery	100 ± 3	104 ± 1	19.2 ± 1.0	3.1 ± 0.5

Refer to Table 1 for abbreviations. [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$.

Fig. 4). Infusion of phenylephrine during chloroethylclonidine administration caused a significant ($P < 0.01$) reversible increase in mean arterial pressure while renal perfusion pressure was regulated at an unchanged level but had no effect on either renal blood flow or glomerular filtration rate. Under these conditions, phenylephrine reversibly reduced urine volume, absolute and fractional Na^+ excretions by 52, 71 and 50% ($P < 0.05$, $P < 0.01$ and $P < 0.05$, respectively). The pattern and magnitudes of these responses were very similar to those obtained in the first half of the experiment.

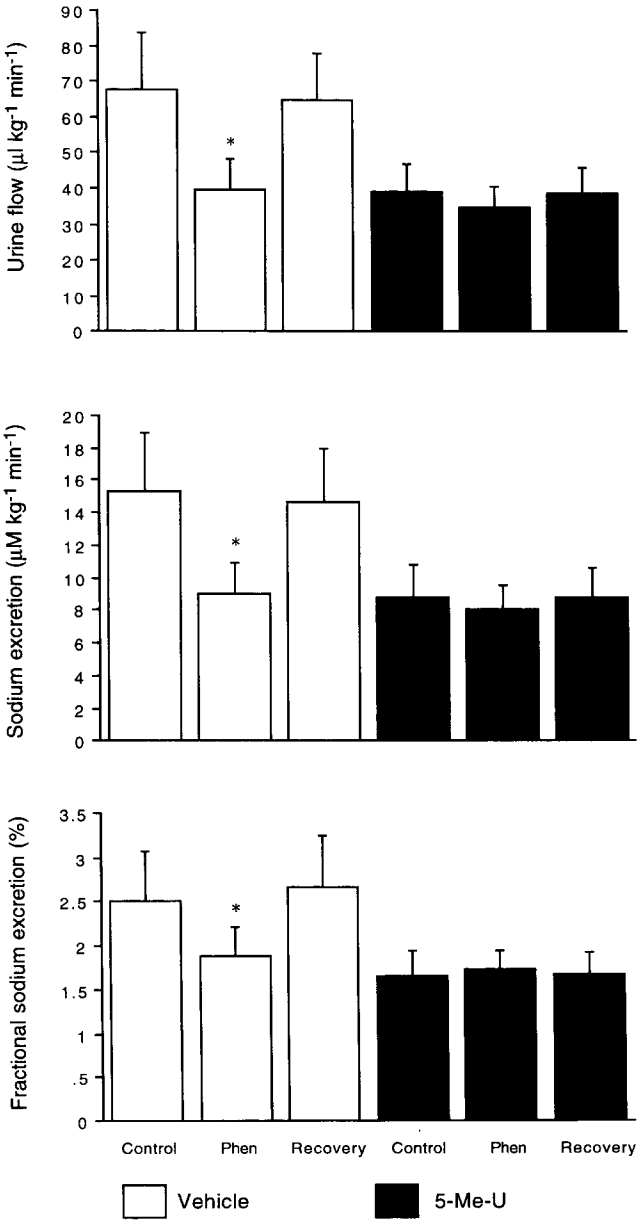

Fig. 3. The effect of phenylephrine, 50–100 $\mu g\ kg^{-1}\ h^{-1}$ on the urine flow (UV), absolute Na^+ excretion ($U_{Na}V$) and fractional Na^+ excretion (FE_{Na}) in the absence and presence of 5-methylurapidil (5 Me-U), 10 $\mu g\ kg^{-1}\ h^{-1}$ in the normotensive Wistar rats. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. control (ANOVA), $n = 5$.

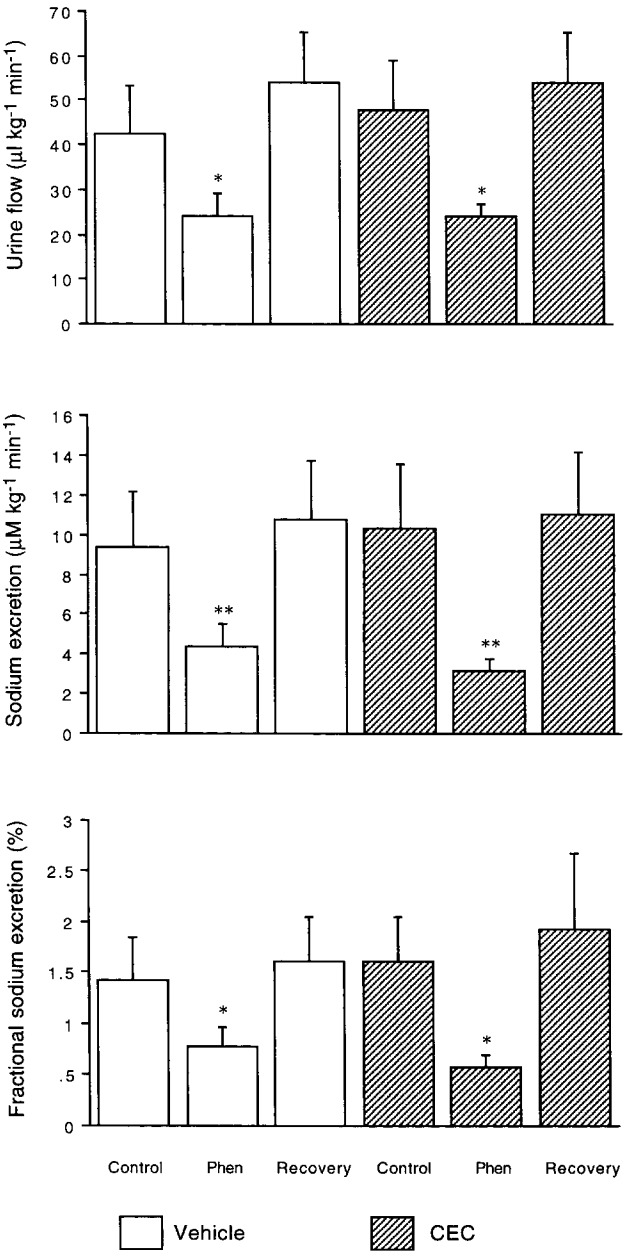

Fig. 4. The effect of phenylephrine, 100 $\mu g\ kg^{-1}\ h^{-1}$ on the urine flow (UV), absolute Na^+ excretion ($U_{Na}V$) and fractional Na^+ excretion (FE_{Na}) in the absence and presence of chloroethylclonidine (CEC), 10 $\mu g\ kg^{-1}\ h^{-1}$ in the SHRSP rats. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. control (ANOVA), $n = 5$

The second group of SHRSP were given the two challenges to phenylephrine. The first challenge significantly ($P < 0.001$) raised mean arterial pressure, had no effect on renal perfusion pressure or renal haemodynamics (Table 5) but reversibly reduced urine flow absolute and fractional Na^+ excretions by 56, 69 and 66%, respectively (all $P < 0.01$). The size and pattern of these responses were comparable to those obtained

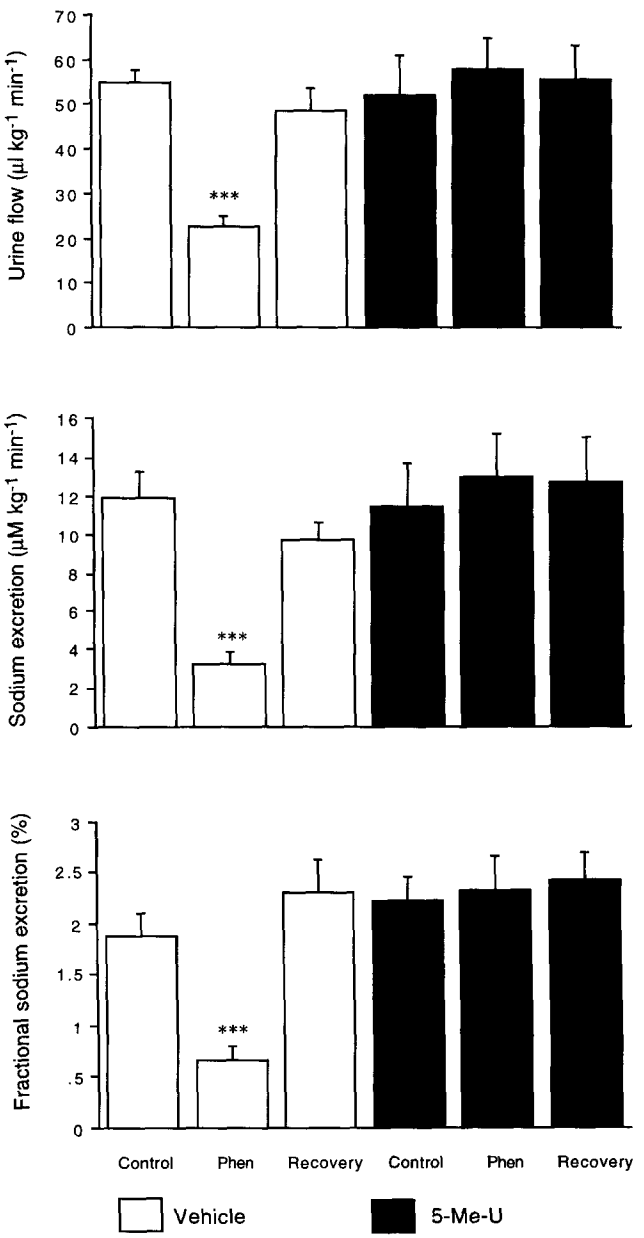

Fig. 5. The effect of phenylephrine, 100 μg kg^{-1} h^{-1} on the urine flow (UV), absolute Na^+ excretion ($U_{Na}V$) and fractional Na^+ excretion (FE_{Na}) in the absence and presence of 5-methylurapidil (5 Me-U), 10 μg kg^{-1} h^{-1} in the SHRSP rats. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. control, (ANOVA), $n = 5$.

in both the first and second parts of the previous hypertensive group of rats. Administration of the 5-methylurapidil caused an approximate 9 mm Hg fall in mean arterial pressure but had no effect on any renal haemodynamic or excretory variable (Table 5 and Fig. 5). Infusion of the phenylephrine resulted in a small reversible rise in mean arterial blood pressure ($P < 0.01$) and reduction in renal blood flow ($P < 0.01$) while neither glomerular filtration rate, urine volume, absolute or fractional Na^+ excretion were changed. These responses were very different from those obtained in the absence of 5-methylurapidil obtained in the first part of the study.

4. Discussion

There is now good evidence that the α_1-adrenoceptors can be classified into α_{1A}- and α_{1B}-adrenoceptors based on their requirement for either the influx of extracellular Ca^{2+} or the release of intracellular stores of Ca^{2+} for activation (Han et al., 1987b). Accordingly, α_{1A}-adrenoceptors exhibit a high sensitivity for the competitive antagonists WB 4101, 5-methylurapidil and (+)-niguldipine while the α_{1B}-adrenoceptors are potently inactivated by chloroethylclonidine which preferentially alkylate this subtype of adrenoceptors (Morrow and Creese, 1986; Han et al., 1987a; Gross et al., 1988; Boer et al., 1989). The approach undertaken in the present investigation was to infuse into the kidney, phenylephrine, which is known to stimulate both α_{1A}- and α_{1B}-adrenoceptors (Han et al., 1987a) before and in the presence of selective α_1-adrenoceptor antagonists to categorise the α_1-adrenoceptor subtypes involved in mediating the tubular antinatriuresis and antidiuresis.

The route of drug administration chosen was close renal arterially in an attempt to have a high local effect within the kidney with minimal systemic spill-over. The dose of phenylephrine required to achieve these end points varied depending on the closeness with which the tip of the cannula lay to the renal artery as it arose from the aorta. Furthermore, it was essential to ensure

Table 4
Renal haemodynamic responses to phenylephrine in the absence and presence of chloroethylclonidine (CEC) in SHRSP ($n = 5$)

	MAP (mm Hg)	RPP (mm Hg)	RBF (ml kg^{-1} min^{-1})	GFR (ml kg^{-1} min^{-1})
Vehicle				
Control	155 ± 4	153 ± 3	13.6 ± 0.7	4.7 ± 0.7
Phen	168 ± 4 [b]	151 ± 4	12.4 ± 0.7	4.0 ± 0.8
Recovery	156 ± 3	151 ± 4	12.4 ± 0.7	4.5 ± 0.5
CEC				
Control	136 ± 3	136 ± 4	13.1 ± 2.5	4.3 ± 0.3
Phen	167 ± 4 [b]	134 ± 5	12.1 ± 1.2 [a]	3.6 ± 0.4 [b]
Recovery	141 ± 4	134 ± 5	13.8 ± 1.4	4.4 ± 0.6

Refer to Table 1 for abbreviations. [a] $P < 0.05$; [b] $P < 0.01$.

Table 5
Renal haemodynamic responses to phenylephrine in the absence and presence of 5-methylurapidil (5 Me-U) in SHRSP ($n = 6$)

	MAP (mm Hg)	RPP (mm Hg)	RBF (ml kg^{-1} min^{-1})	GFR (ml kg^{-1} min^{-1})
Vehicle				
Control	153 ± 6	157 ± 3	15.5 ± 1.0	4.6 ± 0.5
Phen	170 ± 6 [c]	157 ± 3	13.0 ± 1.0	3.7 ± 0.3
Recovery	158 ± 5	157 ± 3	13.1 ± 0.6	3.2 ± 0.4
5 Me-U				
Control	149 ± 3	151 ± 2	13.7 ± 0.5	3.5 ± 0.4
Phen	157 ± 2 [b]	151 ± 2	11.5 ± 0.4 [b]	3.9 ± 0.3
Recovery	150 ± 2	149 ± 2	13.0 ± 0.7	3.6 ± 0.3

Refer to Table 1 for abbreviations. [b] $P < 0.01$; [c] $P < 0.001$.

that renal perfusion pressure did not change when phenylephrine was infused as this would have a major effect on water and Na^+ excretion (Roman and Cowley, 1985). The small increases in blood pressure in the Wistar and SHRSP rats when phenylephrine was infused indicated a spill-over of agonist into the systemic circulation which meant that the kidney was subjected to a high local concentration. Renal blood flow and glomerular filtration rate decreased marginally in both parts of the experiment which potentially could have contributed to the antinatriuresis and antidiuresis due to a reduction in filtered load. However, Hesse and Johns (1984) using the rabbit, showed that a neurally induced reduction in blood flow of up to 15% had only a minor influence on the magnitude of the associated antinatriuresis and antidiuresis. A further point to note is that the larger decrease in renal blood flow than filtration rate would have increased filtration fraction thereby enhancing the drive for fluid reabsorption. However, the importance of this mechanism was probably small as in the presence of 5-methylurapidil the same pattern of renal haemodynamic changes were induced by the phenylephrine but the excretory responses were blocked. Together these observations would suggest that the changes in fluid excretion induced by phenylephrine given at this dose could be taken as being primarily due to a direct action of the agonist present on the tubular epithelial cells.

The first group of rats given saline throughout demonstrated that the responses to phenylephrine were reversible and reproducible over time. Administration of phenylephrine caused significant reductions in both absolute and fractional Na^+ excretion as well as urine volume which was consistent with α_1-adrenoceptors being involved as previously reported by Akpogomeh and Johns (1991). Administration of chloroethylclonidine, at the dose used in the present study, has been used previously to selectively block the α_{1B}-adrenoceptors (Sattar and Johns, 1994) at the level of renal vasculature. At this dose, chloroethylclonidine, did not cause any major changes to the basal levels of urine flow or Na^+ excretion as compared to those obtained during the infusion of vehicle. Furthermore, phenylephrine infusion during blockade with chloroethylclonidine was found to produce a pattern of reversible reductions in water and Na^+ excretion similar in magnitude to those obtained when the agonist was given alone demonstrating that chloroethylclonidine was not able to modify the phenylephrine induced antidiuresis or antinatriuresis. These findings are consistent with the view that α_{1B}-adrenoceptors were probably not involved in the Na^+ reabsorptive processes at the epithelial cells of the tubules.

In the second group of normotensive Wistar rats, the administration of phenylephrine alone caused similar minor changes in renal haemodynamics associated with major reductions in Na^+ and water excretion to a similar degree as obtained in the first group of Wistar rats. The 5-methylurapidil was infused at a dose level which had been used previously to selectively block α_{1A}-adrenoceptors at the renal vasculature (Sattar and Johns, 1994). This dose level of 5-methylurapidil caused a significant fall in blood pressure as had been observed previously in the normotensive Wistar rat studies (Sattar and Johns, 1994). Concomitantly, the infusion of the antagonist resulted in a reduction in the basal levels of Na^+ excretion and urine volume by about 40% and although the reasons for this are unclear, one strong possibility could be a pressure mediated antinatriuresis and antidiuresis as the denervated kidney is particularly sensitive to changes in pressure (Roman and Cowley, 1985). In spite of the new lower stable baseline levels of water and Na^+ output, the antinatriuretic and antidiuretic actions of phenylephrine were abolished. It was unlikely that this lack of effect was due to a time related decrease in responsiveness as in the group of animals given saline throughout the experiment the two phenylephrine challenges gave similar magnitudes of response. This finding supported the contention that the α_{1A}-adrenoceptor subtype was mainly involved in the adrenergically induced antidiuresis and antinatriuresis in the Wistar rat.

Blood pressure in the SHRSP was much higher compared to the normotensive Wistar rats while the

left renal blood flow was lower and glomerular filtration rate tended to be higher in these SHRSP. Interestingly, in spite of the higher pressure in the SHRSP Na^+ excretion was similar to or even lower than in the Wistar rats. Administration of phenylephrine alone resulted in significant reversible falls in absolute and fractional Na^+ excretions and urine volume similar in magnitude to those observed with the normotensive rats. This observation was compatible with the involvement of α_1-adrenoceptors in mediating the Na^+ and water excretion in the SHRSP and was consistent with the observations of Akpogomeh and Johns (1990) in which the adrenergically induced antinatriuresis and antidiuresis were blocked by prazosin, an α_1-adrenoceptor antagonist, but not by an α_2-adrenoceptor antagonist, idazoxan. Administration of chloroethylclonidine caused a 15–20 mm Hg fall in blood pressure although both renal blood flow and glomerular filtration rate were unchanged. Further, chloroethylclonidine did not cause any change in the basal values of the excretory variables which suggested that the blockade of α_{1B}-adrenoceptors did not make an important contribution to the basal levels of Na^+ and water output in the SHRSP. The findings clearly demonstrated that the reductions in the Na^+ and water output when phenylephrine was given in the presence of chloroethylclonidine were very similar in magnitude when it was given alone. This evidence makes it possible to argue that the α_{1B}-adrenoceptors probably did not contribute to any great extent to the antidiuretic and antinatriuretic responses induced by phenylephrine.

The second group of SHRSP rats received 5-methylurapidil which caused a fall in blood pressure (of about 10 mm Hg) but had no meaningful effect on renal haemodynamics or the basal values of Na^+ or water excretion. This situation was in contrast to that obtained in the normotensive Wistar in which the 5-methylurapidil induced large reductions in Na^+ and water excretion. It is not clear why there should be this difference but one possibility is that the kidney of the normotensive Wistar was more sensitive to pressure changes as compared to that of the SHRSP. Phenylephrine infusion into the SHRSP caused reversible decreases in absolute and fractional Na^+ excretion and water output response and these responses were abolished in the presence of 5-methylurapidil. This provided evidence for the argument that α_{1A}-adrenoceptors were responsible in large part for the phenylephrine induced antinatriuresis and antidiuresis in this particular genetic model of hypertension.

The present study examined the α_1-adrenoceptor subtype(s) that were involved in the adrenergically induced antinatriuresis and antidiuresis in the normotensive Wistar and SHRSP. The results obtained suggested the α_{1A}-adrenoceptor played a major role in mediating the adrenergic regulation of tubular Na^+ and water reabsorption. Furthermore, the α_{1A}-adrenoceptor subtype also appeared to be primarily involved in modulating fluid reabsorption in the SHRSP, a genetic model of hypertension.

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