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α_{1b}-Adrenoceptors mediate renal tubular sodium and water reabsorption in the rat

Abdelhamid M. Elhawary & [1]Catherine C.Y. Pang

Department of Pharmacology & Therapeutics, Faculty of Medicine, The University of British Columbia, Vancouver B.C., Canada, V6T 1Z3

1 It is known that activation of α_1-adrenoceptors causes renal vasoconstriction and increased tubular Na^+ and water reabsorption, with the α_{1a}-subtype mediating the constrictor effect.

2 This study examines which subtype of α_1-adrenoceptors mediates tubular Na^+ and water reabsorption in pentobarbitone-anaesthetized rats. In order to avoid systemic effects, phenylephrine (0.3 to 30 $\mu g\ kg^{-1}$), methoxamine (0.1–10 $\mu g\ kg^{-1}$) and vehicle were infused into the right renal artery (via the suprarenal artery) of three groups of rats. Two other groups of rats were continuously infused with the irreversible selective α_{1b}-adrenoceptor antagonist, chloroethylclonidine (3 $mg\ kg^{-1}\ h^{-1}$) for 1 h, prior to the construction of dose-response curves to phenylephrine or methoxamine. Another group was continuously infused with the irreversible selective α_{1a}-adrenoceptor antagonist, SZL-49 (10 $\mu g\ kg^{-1}\ h^{-1}$) for 1 h, prior to the construction of dose-response curves to phenylephrine. Mean arterial pressure (MAP), heart rate (HR), urine flow, Na^+ and K^+ excretion, and urine osmolality were monitored.

3 Phenylephrine and methoxamine did not affect MAP or HR but dose-dependently and significantly decreased urine flow, urine osmolality as well as Na^+ excretion and, slightly increased K^+ excretion, although this was significant only for phenylephrine.

4 The antidiuretic, antinatriuretic and kaliuretic effects of phenylephrine were abolished by pretreatment with chloroethylclonidine, but were not inhibited by SZL-49. The inhibitory effects of methoxamine on urine flow and Na^+ excretion were also almost totally abolished by chloroethylclonidine.

5 Our results show that α_{1b}-adrenoceptors mediate renal tubular Na^+ and water reabsorption.

Keywords: α_1-Adrenoceptor subtype; chloroethylclonidine; SZL-49; renal tubules; sodium and water reabsorption

Introduction

α_1-Adrenoceptors are known to mediate renal vasoconstriction as well as sodium and water reabsorption induced by the stimulation of renal sympathetic nerves (DiBona, 1985; Jeffries & Pettinger, 1989). The antidiuresis and antinatriuresis mediated by α_1-adrenoceptors can occur in the absence of changes in the glomerular filtration rate of the whole kidney or single nephron, renal blood flow or intrarenal distribution of blood flow, and it is not dependent on intrarenal actions of prostaglandins or angiotensin II (DiBona & Sawin, 1982; Gottschalk *et al.*, 1985). Radioligand binding studies (Schmitz *et al.*, 1981; Snavely & Insel, 1982), autoradiographic analyses (Summers, 1984; Muntz *et al.*, 1985) as well as microdissection studies using radioligand binding techniques (Kusano *et al.*, 1984) have quantified α_1-adrenoceptors in the rat renal membrane. These studies have shown the existence of a high density of α_1-adrenoceptors in the cortex, especially in proximal tubules and ascending thick limb of the loop of Henle, a lower density in the medulla and no apparent binding in the collecting ducts.

Multiple subtypes of α_1-adrenoceptors are known to exist. Separate genes for α_{1a}- (Lomasney *et al.*, 1991), α_{1b}- (Cotecchia *et al.*, 1988; Voigt *et al.*, 1990) and α_{1c}-adrenoceptors (Schwinn *et al.*, 1990) were cloned from different tissues, despite the recent controversy as to whether the cloned 'α_{1a}' is indeed α_{1a}, or rather, a novel α_{1d}-adrenoceptor-subtype (Perez *et al.*, 1992). Pharmacological agents with apparent selectivity for α_{1a}- and α_{1b}-adrenoceptors have been used to identify, functionally and biochemically, these subtypes of receptors. These include the alkylating agent, chloroethylclonidine (CEC) which selectively inactivates α_{1b}-adrenoceptors (Han *et al.*, 1987; Minneman, 1988; Minneman *et al.*, 1988) and SZL-49, an analogue of prazosin, which alkylates CEC-resistant α_1-adrenoceptors, presumably the α_{1a}-subtype (Babich *et al.*, 1987; Kusiak *et al.*, 1989; Piascik *et al.*, 1989).

The rat renal artery has been shown to contain only α_{1a}-adrenoceptors *in vitro* (Han *et al.*, 1990). Elhawary *et al.* (1992) showed that the inactivation of rat renal α_{1a}-adrenoceptors abolished *in vivo* α_1-adrenoceptor-mediated renal vasoconstriction, indicating the inclusive invovlement of α_{1a}-adrenoceptors in renal vasoconstriction. The subtype of α_1-adrenoceptors mediating renal tubular sodium and water reabsorption has not yet been identified. High densities of both α_{1a}- and α_{1b}-adrenoceptors are present in the rat renal membrane (Han *et al.*, 1987). This suggests the possible involvement of both adrenoceptor subtypes in the mediation of renal tubular responses. The aim of this study was to investigate the effect of selective alkylation of α_1-adrenoceptor subtypes on antidiuretic and antinatriuretic actions of α_1-adrenoceptor agonists.

Methods

Male Sprague Dawley rats (300–350 g) were anaesthetized with sodium pentobarbitone (50 $mg\ kg^{-1}$, i.p.). A rectal thermometer connected to an Animal Blanket Control Unit was used to maintain body temperature at 37.5°C. Cannulae (PE50) were inserted into the left femoral artery, for the continuous measurement of mean arterial pressure (MAP) with a Statham pressure transducer (Model P23 DB, Gould Statham, CA), and into the left femoral vein, for continuous infusion of saline (30 $\mu l\ min^{-1}$) throughout the experiment. Heart rate (HR) was derived electronically from the upstroke of the arterial pulse pressure with a tachograph (Grass, model 7P4G). MAP and HR responses were monitored by a Grass polygraph (Grass, model RP57C8). The abdominal cavity was opened through a ventral midline incision. The right suprarenal artery was located and its origin from the renal artery was verified. A tapered PE-10 tube was inserted retrogradely into the suprarenal artery as described by Smits

[1] Author for correspondence.

et al. (1983), and connected to a syringe pump (SAGE 341A) for the infusion of drugs. A PE-50 cannula was inserted into the right ureter for the collection of urine every 10 min in pre-weighed vials. The rats were given 1 h for stabilization after surgery before the study began.

Experimental protocol

Six groups of rats ($n = 7-9$ each, except for groups II and V, where $n = 5$) were used. Groups I, II and III were infused with the vehicle (0.45% NaCl) for 1 h followed by the construction of dose-response curves to phenylephrine (PE, 0.3–30 $\mu g\,kg^{-1}$; 10 min each dose), methoxamine (Methox, 0.1–10 $\mu g\,kg^{-1}$; 10 min each dose) and an equal volume of the vehicle (0.45% NaCl), respectively. In Groups IV and V, chloroethylclonidine (CEC; 3 $mg\,kg^{-1}\,h^{-1}$) was infused into the renal artery for 1 h followed by the infusions of various doses of PE and Methox, respectively. Group VI was given continuous renal arterial infusion of SZL-49 (10 $\mu g\,kg^{-1}\,h^{-1}$) for 1 h followed by PE. The doses of the CEC and SZL-49 selected were those which maximally alkylated and blocked renal vascular responses to stimulation of α_{1b}- and α_{1a}-adrenoceptors, respectively (Elhawary *et al.*, 1992). Urine was collected at the end of each 10 min infusion period. A blood sample was obtained at the end of the stabilization period and after the completion of the study to monitor any changes in the haematocrit, plasma osmolality and levels of sodium (Na^+) and potassium (K^+) during the course of the study. The duration of the studies was approximately 2 h, counting from the end of the stabilization period to the completion of drug infusions. Urine volume was determined gravimetrically. Urine Na^+ and K^+ concentrations were determined by flame photometry (Fisher Scientific IL 143) and urine osmolality was measured by a WESCOR 5500 vapour pressure osmometer.

Materials

SZL-49, {1-(4-amino-6, 7-dimethoxy-2-quinazolnyl)-4- (2-bicyclo [2,2,2] octa-2,5-dienylcarbonyl)-piperazine}, and CEC were purchased from Research Biomedicals, Inc. (Natick, MA, U.S.A.). PE and Methox were obtained from the Sigma Chemical Co (St. Louis, MO, U.S.A.). All drugs were dissolved in 0.45% NaCl solution except SZL-49, which was first solubilized in 80% ethanol and then diluted with 0.45% NaCl solution to make a final concentration of 0.3% ethanol (v/v).

Statistics

All data are expressed as mean ± s.e.mean. The results were analysed by analysis of variance followed by Duncan's multiple range test, with $P < 0.05$ as the level of statistical significance.

Results

The infusion of the vehicle into the renal artery caused minimal and insignificant changes in MAP, HR, urine volume, urine Na^+/K^+ or urine osmolality (Table 1). Intra-renal arterial infusion of PE also caused negligible changes in MAP and HR (Figure 1). PE dose-dependently and signifi-

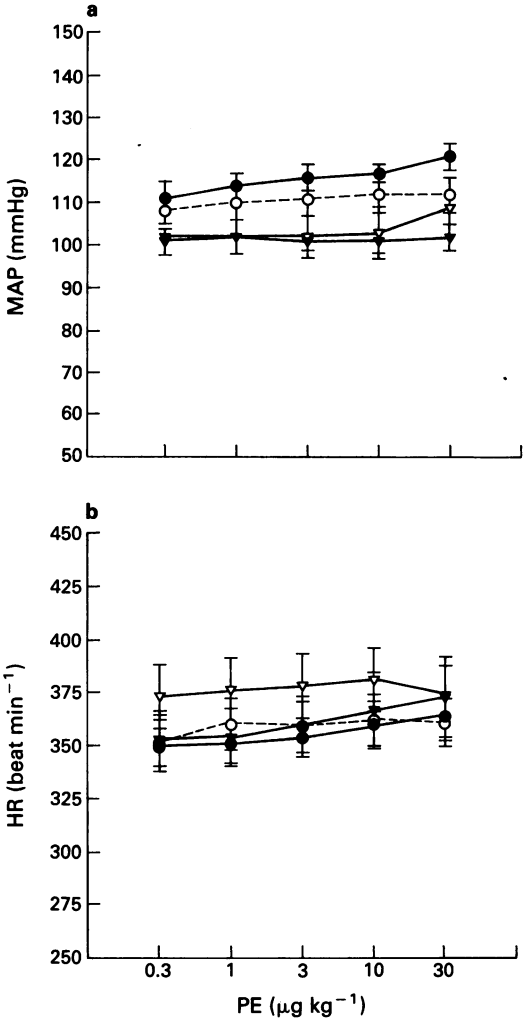

Figure 1 Dose-response effects on mean arterial pressure (MAP, a) and heart rate (HR, b) following renal arterial infusions of vehicle (○, 0.45% NaCl, $n = 8$), phenylephrine (●, PE, $n = 7$), PE after infusion of SZL-49 (▽, 10 $\mu g\,kg^{-1}\,h^{-1}$, $n = 9$), and PE after infusion of CEC (▼, 3 $mg\,kg^{-1}\,h^{-1}$, $n = 7$) in four groups of pentobarbitone-anaesthetized rats. Data are shown as mean ± s.e.mean. For abbreviations, see text.

Table 1 Effect of vehicle (0.45% NaCl) on mean arterial pressure (MAP, mmHg), heart rate (HR, beats min^{-1}), urine flow (UF, $\mu l\,min^{-1}$), Na^+ and K^+ excretions (mEq l^{-1}) and urine osmolality (Osm, mOsm kg^{-1}) during the six sampling periods (C1 to C6) in pentobarbitone-anaesthetized rats

	Time control					
	C1	*C2*	*C3*	*C4*	*C5*	*C6*
MAP	108 ± 3	108 ± 3	110 ± 3	111 ± 4	112 ± 4	112 ± 4
HR	349 ± 13	350 ± 12	351 ± 10	354 ± 9	360 ± 11	364 ± 11
UF	5.1 ± 0.3	4.9 ± 0.3	4.6 ± 0.3	4.8 ± 0.3	4.5 ± 0.4	4.9 ± 0.6
Na	59 ± 5	55 ± 4	56 ± 8	54 ± 7	56 ± 7	62 ± 8
K	163 ± 17	156 ± 20	167 ± 20	171 ± 19	172 ± 18	170 ± 15
Osm	1408 ± 97	1415 ± 109	1447 ± 107	1477 ± 111	1446 ± 112	1460 ± 111

$n = 8$.

cantly reduced urine flow (Figure 2a), urine osmolality (Figure 2b) and Na^+ excretion (Figure 3a) but slightly increased K^+ excretion, which, for the third and fifth PE doses, were significantly different from the corresponding readings in control rats given the vehicle (Figure 3b). Neither 1 h of close arterial infusion of CEC nor of SZL-49 affected baseline values of MAP, HR, urinary flow, urine values of Na^+, K^+ or osmolality, haematocrit or, blood values of Na^+, K^+ or osmolality (Table 2). CEC and SZL-49 also did not affect either MAP or HR response to PE (Figure 1a,b). CEC abolished the effects of PE on urine flow (Figure 2a), urine osmolality (Figure 2b) and K^+ excretion (Figure 3b). In the

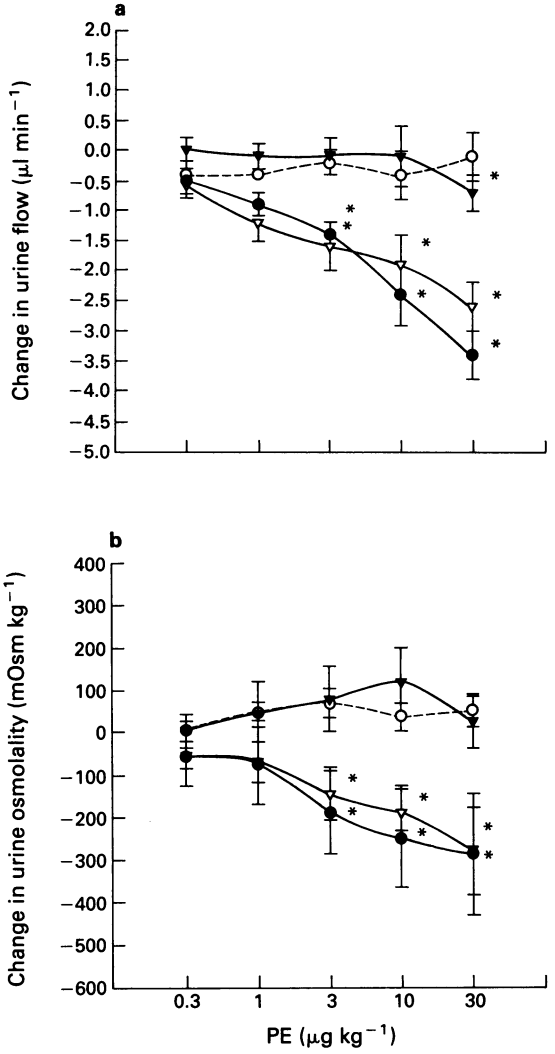

Figure 2 Dose-response effects on urine flow (a) and osmolality (b) following renal arterial infusions of vehicle (○, 0.45% NaCl, $n = 8$), phenylephrine (●, PE, $n = 7$), PE after infusion of SZL-49 (▽, 10 μg kg^{-1} h^{-1}, $n = 9$), and PE after infusion of CEC (▼, 3 mg kg^{-1} h^{-1}, $n = 7$) in four groups of pentobarbitone-anaesthetized rats. Data are shown as mean ± s.e.mean. *Significantly different from values in vehicle-treated rats. For abbreviations, see text.

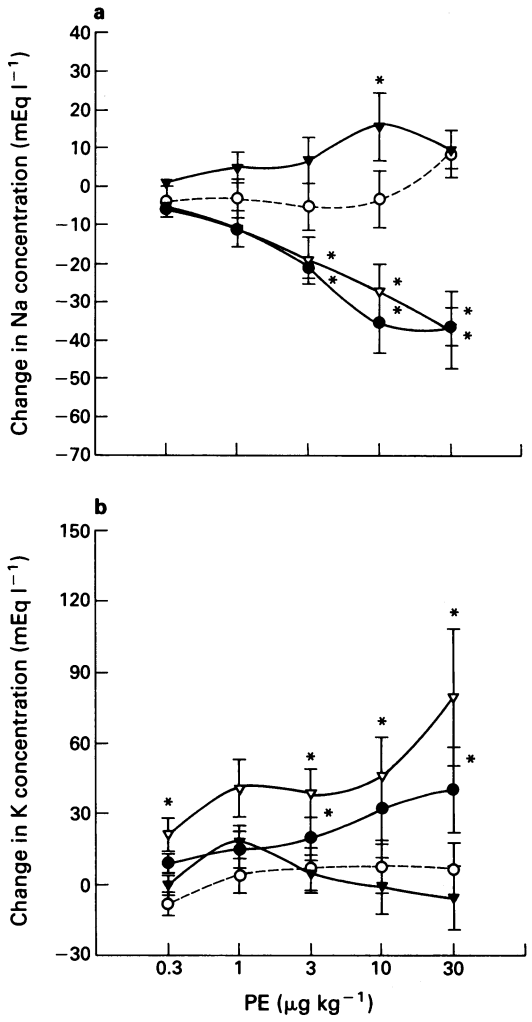

Figure 3 Dose-response effects on urinary excretions of Na^+ (a) and urinary K^+ (b) following renal arterial infusions of vehicle (○, 0.45% NaCl, $n = 8$), phenylephrine (●, PE, $n = 7$), PE after infusion of SZL-49 (▽, 10 μg kg^{-1} h^{-1}, $n = 9$), and PE after infusion of CEC (▼, 3 mg kg^{-1} h^{-1}, $n = 7$) in four groups of pentobarbitone-anaesthetized rats. Data are shown as means ± s.e.mean. *Significantly different from values in vehicle-treated rats. For abbreviations, see text.

Table 2 Baseline values of mean arterial pressure (MAP, mmHg), heart rate (HR, beats min^{-1}), urine flow (UF, μl min^{-1}), urine sodium (U Na, mEq l^{-1}), urine potassium (U K, mEq l^{-1}), urine osmolality (U Osm, mOsm kg^{-1}), haematocrit (HC%), blood sodium (Bl Na, mEq l^{-1}), blood potassium (Bl K, mEq l^{-1}) and blood osmolality (Bl Osm, mOsm kg^{-1}) following 1 h infusion of vehicle (0.45% NaCl), SZL-49 (10 μg kg^{-1} h^{-1}) or CEC (3 mg kg^{-1} h^{-1}) in five groups of pentobarbitone-anaesthetized rats to be infused with phenylephrine (PE) or methoxamine (Methox)

Group	*MAP*	*HR*	*UF*	*U Na*	*U K*	*U Osm*	*HC%*	*Bl Na*	*Bl K*	*Bl Osm*
Vehicle, PE	108 ± 4	347 ± 13	5.3 ± 0.4	62 ± 7	173 ± 19	1507 ± 33	48 ± 2%	137 ± 4	3.3 ± 0.5	295 ± 7
SZL, PE	102 ± 2	372 ± 13	5.3 ± 0.6	74 ± 11	164 ± 23	1537 ± 171	47 ± 2%	136 ± 7	3.4 ± 0.5	298 ± 5
CEC, PE	102 ± 1	348 ± 11	5.3 ± 0.4	50 ± 7	166 ± 16	1370 ± 151	49 ± 1%	138 ± 8	3.6 ± 0.8	297 ± 7
Vehicle, Methox	103 ± 3	380 ± 15	4.7 ± 0.8	53 ± 5	194 ± 14	1695 ± 90	48 ± 1%	137 ± 2	3.4 ± 0.3	297 ± 6
CEC, Methox	108 ± 3	370 ± 12	4.9 ± 0.8	68 ± 13	225 ± 7	1634 ± 180	45 ± 4%	134 ± 6	2.9 ± 0.6	296 ± 8

$n = 7$–9 per group.

Table 3 Dose-response effects of 10 min i.a. infusion of methoxamine on mean arterial pressure (MAP, mmHg), heart rate (HR, beats min^{-1}), urine flow (UF, $\mu l\ min^{-1}$), urine sodium (Na, $mEq\ l^{-1}$), urine potassium (K, $mEq\ l^{-1}$) and urine osmolality (Osm, $mOsm\ kg^{-1}$) in pentobarbitone-anaesthetized rats in the absence ($n = 5$) and presence ($n = 5$) of i.a. infused chloroethylclonidine (CEC, $3\ mg\ kg^{-1}\ h^{-1}$)

	Methoxamine ($\mu g\ kg^{-1}$)						*Methoxamine* ($\mu g\ kg^{-1}$) + *CEC* ($3\ mg\ kg^{-1}\ h^{-1}$)					
	C	0.1	0.3	1	3	10	C	0.1	0.3	1	3	10
MAP	103 ± 3	102 ± 3	102 ± 4	101 ± 5	101 ± 5	103 ± 6	108 ± 3	107 ± 4	109 ± 3	108 ± 4	108 ± 5	109 ± 5
HR	380 ± 15	380 ± 15	351 ± 10	354 ± 9	360 ± 11	364 ± 11	370 ± 12	360 ± 17	364 ± 15	368 ± 15	376 ± 19	382 ± 23
UF	4.7 ± 0.7	4.1 ± 0.7	3.5 ± 0.6*	2.8 ± 0.7*	2.1 ± 0.4*	1.2 ± 0.6*	4.9 ± 0.8	5.8 ± 0.8	5.6 ± 0.8	5.7 ± 0.7	5.8 ± 0.7	6.0 ± 0.9
Na	53 ± 5	45 ± 7	42 ± 6*	29.8 ± 4*	24.8 ± 3*	19 ± 2*	68 ± 13	67 ± 12	64 ± 11	72 ± 16	74 ± 15	75 ± 15
K	194 ± 14	200 ± 12	198 ± 15	219 ± 14	222 ± 16	206 ± 26	225 ± 7	234 ± 6	242 ± 6	234 ± 8	214 ± 21	213 ± 24
Osm	1695 ± 90	1566 ± 97	1490 ± 97	1417 ± 101*	1350 ± 49*	1238 ± 123*	1634 ± 180	1408 ± 97	1415 ± 109	1506 ± 208	1447 ± 247	1440 ± 246

*Indicates significant difference from the baseline value.

presence of CEC, PE slightly increased Na^+ excretion, which was significantly different from the vehicle effect at the fourth dose (Figure 3a). SZL-49, in contrast, did not inhibit the effects of PE on urine osmolality, flow (Figure 2a,b) or Na^+ (Figure 3a) and it slightly but insignificantly potentiated the effect of PE on K^+ excretion, as K^+ excretions in the presence were not different from those in the absence of SZL-49 (Figure 3b). To ascertain that the renal tubular effects of PE were mediated via the activation of α_1-adrenoceptors, Methox was also infused into the renal artery. Like PE, Methox significantly and dose-dependently reduced urine flow, urine osmolality and urine Na^+, slightly but insignificantly increased urine K^+ and did not affect MAP or HR (Table 3). CEC completely inhibited the effects of Methox on urine flow and Na^+ but it incompletely, though significantly, suppressed the effect on urine osmolality (Table 3). Haematocrit and blood values of Na^+, K^+ and osmolality at the end of the experiments were similar to the corresponding values (Table 2) after the stabilization period (results not shown).

Discussion

The results of this study show that renal arterial infusion of PE induced dose-dependent reductions in urine flow and Na^+ excretion. This reduction was accompanied by a decrease in urine osmolality and a slight increase in K^+ excretion. Pretreatment with the irreversible and selective α_{1b}-adrenoceptor antagonist, CEC, abolished the antidiuretic and antinatriuretic effects of PE, suggesting that these responses were mediated via the activation of α_{1b}-adrenoceptors. The infusion of Methox into the renal artery also caused similar dose-dependent decreases in urine flow, osmolality as well as Na^+ and, slight (but insignificant) increases in urine K^+. Since all these changes were inhibited by CEC, it suggests that renal tubular effects of Methox were also mediated via the activation of α_{1b}-adrenoceptors. In contrast, pretreatment with the alkylating agent, SZL-49, did not inhibit the effects of PE on urine volume, osmolality or Na^+. Accordingly, our results indicate that α_{1b}-adrenoceptors are the primary α_1-adrenoceptors involved in the mediation of renal tubular Na^+ and water reabsorption.

In the present study, the renal tubular effects of PE and Methox were not due to alterations in systemic haemodynamics, since these drugs caused negligible systemic effects. It was also unlikely that CEC inhibited the tubular effects of PE via actions on the renal vasculature since CEC only weakly suppressed renal vasoconstriction elicited by α_1-adrenoceptor stimulation (Elhawary *et al.*, 1992). Furthermore, SZL-49 (Elhawary *et al.*, 1992) and the Ca^{2+} entry blocker, nifedipine (Han *et al.*, 1990) produced marked rightward shifts of the dose-renal pressor response curve of PE but SZL-49 did not inhibit renal tubular effects of PE in the present study. Therefore, the subtype of α_1-adrenoceptors mediating renal vasoconstriction are different from those mediating tubular effects.

The effect of α_1-adrenoceptor activation, either by renal nerve stimulation or selective agonists, on renal function has been extensively studied (for review see DiBona, 1985) and this includes: increased Na^+ and water reabsorption in the proximal tubules, increased Na^+ and Cl^- reabsorption in the thick ascending limb of the Loop of Henle (DiBona & Sawin, 1982), increased Ca^{2+} (Jones & Manitius, 1986) and bicarbonate reabsorption (Osborn & Harland, 1988), stimulation of gluconeogenesis (Kessar & Saggerson, 1980) and, increased renovascular resistance (Cooper & Malik, 1985; Wolff *et al.*, 1987; DiBona & Sawin, 1987; Jeffries *et al.*, 1987). Intrarenal arterial infusion of PE in anaesethetized rabbits, at a dose which did not alter renal haemodynamics, significantly reduced urine flow and absolute and fractional Na^+ excretion by about 15, 23, and 23% respectively (Hesse & Johns, 1985). Higher doses of PE, which caused 11% decrease of blood flow and 15% decrease in filtration rate,

were found to decrease urine flow, absolute and fractional Na^+ excretion by 43%, 49% and 42% respectively. In the same study, a low dose of Methox, which had no effect on MAP, HR, renal blood flow or glomerular filteration rate, significantly reduced urine flow, absolute and fractional Na^+ excretion by about 33%, 30 and 28% respectively. The high dose of Methox, which significantly reduced blood flow (by 15%) and filtration rate (by 26%), decreased urine flow, absolute and fractional Na^+ excretion by 79%, 76% and 65%, respectively. These results are in accordance with our present results in anaesthetized rats of PE ($30\ \mu g\ kg^{-1}$) and Methox ($10\ \mu g\ kg^{-1}$), in reducing urine flow, by 64 and 74%, and Na^+ excretion, by 52 and 64%, respectively.

Our results of the involvement of α_{1b}- but not α_{1a}-adrenoceptors in renal tubular Na^+ and water resorption are in accordance with the anatomical distribution of these receptors in the kidney. Feng *et al.* (1991) reported a distinct pattern of distribution of the two subtypes of α_1-adrenoceptors in the rat kidney. Both α_{1a}- and α_{1b}-adrenoceptors are found in equal proportions in the renal cortex and outer strip of the medulla; however, the α_{1b}-subtype predominates in the inner strip of the medulla. According to the anatomical structure of the nephron, the vascular tissue mass diminishes from the cortex to the medulla, the proximal convoluted tubules exist in the cortex and outer strip of the medulla (Sundaresan *et al.*, 1987), and the thick ascending limb of the loop of Henle predominates in the inner strip of the medulla (Kriz, 1981). Therefore, the regional distribution of subtypes of α_1-adrenoceptors and the anatomical distribution of vascular and tubular tissues is consistent with the results of functional studies which show that renal vasoconstrictor and tubular effects are mediated by α_{1a}- and α_{1b}-adrenoceptors, respectively.

In conclusion, our results suggest discrete physiological function for each subtype of α_1-adrenoceptors in rats: α_{1a}-adrenoceptors for renal vasoconstriction and α_{1b}-adrenoceptors for renal tubular Na^+ and water reabsorption.

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