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Identification of α_1-adrenoceptor subtypes involved in the antinatriuretic response to intrarenal infusion of phenylephrine

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Abstract

It is reported that α_1-adrenoceptors located in the renal vasculature and renal tubules play a major role in mediating antinatriuretic response to renal nerve stimulation as well as to the infusion of α_1-adrenoceptor agonist. In the present study intrarenal infusion of α_1-adrenoceptor agonist phenylephrine (0.25 μg/kg/min) to Inactin-anesthetized Sprague-Dawley rats produced approximately 50% reduction in urine output, Na^+ excretion and glomerular filtration rate without causing significant alterations in mean blood pressure, heart rate and fractional Na^+ excretion. These changes were completely abolished by prior intrarenal infusion of prazosin (0.5 μg/kg/min for 30 min). In separate groups of experiments, the animals received either a selective irreversible α_{1A}-adrenoceptor antagonist, SZL-49 [1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(2-bicyclo[2,2,2]octa-2,5-diene-2-carbonyl)piperazine], or an α_{1B}-adrenoceptor antagonist, chloroethylclonidine, intrarenally prior to phenylephrine infusion. Neither SZL-49 nor chloroethylclonidine alone significantly altered glomerular filtration rate and renal electrolyte excretion. However, SZL-49 (0.15 μg/kg/min), but not chloroethylclonidine (50 μg/kg/min), completely abolished phenylephrine-induced changes in urine output, Na^+ excretion and glomerular filtration rate. These results demonstrate that phenylephrine decreases urine output and Na^+ excretion, mainly due to a reduction in glomerular filtration rate resulting from activation of α_{1A}-adrenoceptors, and that proximal tubular α_{1A}- or α_{1B}-adrenoceptors do not appear to contribute to this response.

Keywords: α_1-Adrenoceptor; Renal function; Phenylephrine; Prazosin

1. Introduction

An increase in renal nerve activity is reported to cause antinatriuresis via α_1-adrenoceptor mediated alterations in both renal hemodynamics and tubular transport (DiBona, 1985). Recent studies show that there are multiple subtypes of α_1-adrenoceptors present in the kidney (Han et al., 1990a; Minneman et al., 1988; Feng et al., 1991; Jackson et al., 1992). The rat renal artery has been shown to contain only α_{1A}-adrenoceptors (Han et al., 1990b). We have recently reported that both α_{1A}- and α_{1B}-adrenoceptors are present approximately in equal density in the proximal tubules (Gopalakrishnan et al., 1993), where renal nerves exert their major influence on renal function (DiBona, 1985). Although it is clear that renal nerve activation leads to a decrease in renal Na^+ excretion via both vasoconstriction (hence decrease in renal blood flow and glomerular filtration rate) and enhanced tubular transport, the subtype(s) of α_1-adrenoceptors involved in each case is not clear. It has been proposed that α_{1A}-adrenoceptor is involved in vasoconstriction, since the inactivation of rat renal α_{1A}-adrenoceptors abolished α_1-adrenoceptor-mediated renal vasoconstriction (Elhawary et al., 1992). It is also recently reported that α_{1B}-adrenoceptors mediate renal tubular Na^+ and water reabsorption (Elhawary and Pang, 1994). Therefore, we thought it would be of interest to know which subtype(s) of α_1-adrenoceptor is involved in the antinatriuretic response to α_1-adrenoceptor activation.

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2. Materials and methods

2.1. In vivo functional studies

Surgical procedures

All experiments were performed in male Sprague-Dawley rats (Harlan, Indianapolis) weighing between 300–350 g. The animals were anesthetized with Inactin (5-ethyl-5-(1-methyl-propyl)-2-thio-barbiturate sodium; 100 mg/kg i.p.). After tracheostomy a cannula was inserted to facilitate spontaneous respiration. The right jugular vein and the left carotid artery were catheterized for saline/drug administration and measurement of arterial pressure. In addition, the right femoral artery was catheterized for blood sampling. The left and right ureters were exposed via a midline abdominal incision and cannulated for the collection of urine samples. The left kidney was exposed with care being taken not to damage the renal nerves. To facilitate intrarenal infusion, stretched PE-10 tubing was inserted from the aorta into the left renal artery and the tip was positioned rostral to the exit of the renal artery from the aorta. The right side served as sham-operated control. After the completion of surgery, isotonic saline was infused at a rate of 1 ml/h via the jugular vein and at a rate of 0.25 ml/h via the renal artery. All drugs were given intrarenally. Blood pressure was monitored with a Statham pressure transducer and heart rate derived from the pulse using a cardiotachograph. Both blood pressure and heart rate were recorded on a Grass polygraph. Hemodynamic and renal parameters were allowed to stabilize over a period of 60 min before beginning the experiment.

Experimental protocol

The protocol consisted of four consecutive 30 min urine collection periods. The initial two periods served as control during which only saline was infused. During the third period, phenylephrine at a dose of 0.25 μg/kg/min was infused intrarenally for 30 min. After the termination of phenylephrine infusion, saline alone was infused for additional 30 min.

The following four series of experiments were performed.

Series 1. Phenylephrine infusion (0.25 μg/kg/min) alone ($n = 6$).

Series 2. Phenylephrine infusion in animals pretreated with the α_1-adrenoceptor antagonist prazosin ($n = 6$). Prazosin (0.5 μg/kg/min) was infused intrarenally for 30 min starting at the onset of the second period.

Series 3. Phenylephrine infusion in animals pretreated with the irreversible α_{1A}-adrenoceptor antagonist ($n = 6$). SZL-49 (0.15 μg/kg/min) was given intrarenally for 30 min starting at the onset of the second period.

Series 4. Phenylephrine infusion in animals pretreated with the irreversible α_{1B}-adrenoceptor antago-

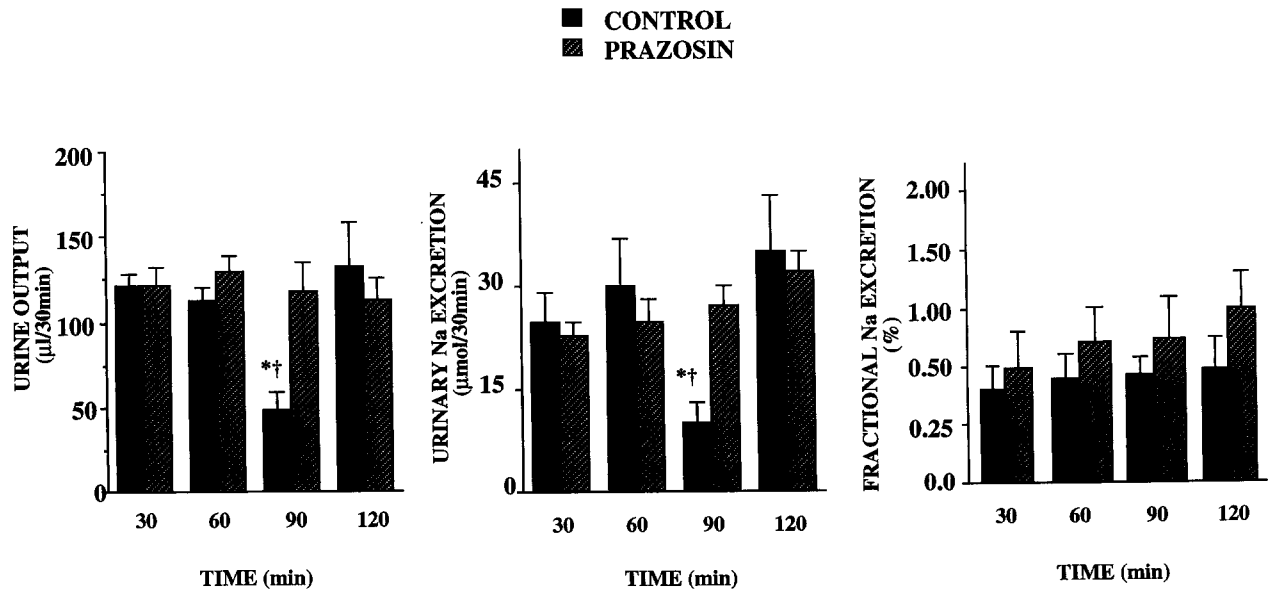

Fig. 1. Influence of prazosin on phenylephrine-induced changes in urinary sodium (Na) excretion, urine output and fractional sodium (Na) excretion. Phenylephrine (0.25 μg/kg/min) was intrarenally infused for 30 min and the infusion began at the end of the second urine collection period (60 min) in both 'CONTROL' (the group of rats receiving saline (vehicle) pretreatment) and 'PRAZOSIN' (the group receiving prazosin pretreatment). Prazosin (0.5 μg/kg/min) was intrarenally infused for 30 min and the infusion began at the end of the first urine collection period (30 min). $n = 6$ rats in each group. * Significantly different as compared to the corresponding control value (obtained during the first 30 min urine collection period). † Significant difference between these two groups at the same urine collection period.

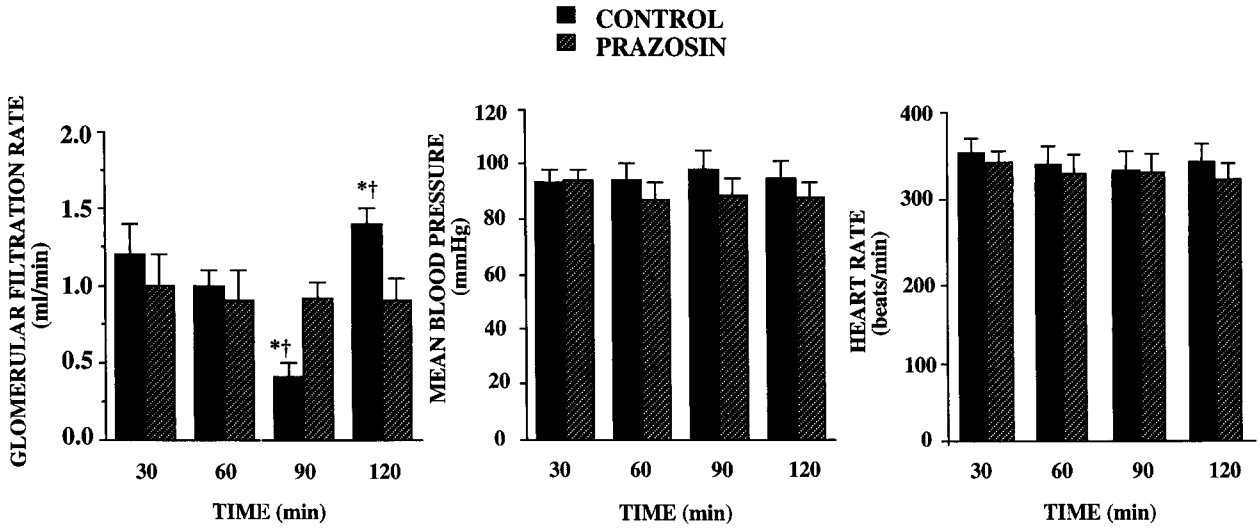

Fig. 2. Influence of prazosin on phenylephrine-induced changes in glomerular filtration rate, mean blood pressure and heart rate. Phenylephrine (0.25 μg/kg/min) was intrarenally infused for 30 min and the infusion began at the end of the second urine collection period (60 min) in both 'CONTROL' (the group of rats receiving saline (vehicle) pretreatment) and 'PRAZOSIN' (the group receiving prazosin pretreatment). Prazosin (0.5 μg/kg/min) was intrarenally infused for 30 min and the infusion began at the end of the first urine collection period (30 min). $n = 6$ rats in each group. * Significantly different as compared to the corresponding control value (obtained during the first 30 min urine collection period). † Significant difference between these two groups at the same urine collection period.

nist chloroethylclonidine ($n = 6$). Chloroethylclonidine (50 μg/kg/min) was given intrarenally for 30 min starting at the onset of the second period.

Analytical measurements

Na^+ concentrations in the plasma and urine samples were measured using a Beckman electrolyte analyzer. Urine and plasma creatinine concentrations were measured using a Beckman creatinine analyzer 2 (Beckman Instruments, Fullerton, CA, USA). Glomerular filtration rate was calculated from the clearance of creatinine.

2.2. Statistical analysis

All data are presented as mean ± S.E.M. The repeated measurements followed by the protected least significant difference multiple range test were used for comparison of data within groups, and one-way analysis of variance was used for comparison between groups. A value of $P < 0.05$ was considered statistically significant.

2.3. Drugs and chemicals

Chloroethylclonidine and SZL-49 [1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(2-bicyclo[2,2,2]octa-2,5-diene-2-carbonyl)piperazine] were purchased from Research Biochemicals (Natick, MA, USA). Other drugs and chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All the drugs were dissolved in saline.

3. Results

It was established in our preliminary study that all hemodynamic and renal parameters were stable throughout the experimental periods when saline alone was infused.

Table 1
Effects of various doses of phenylephrine, infused directly into the renal artery of the anesthetized rat on urine output, urinary Na^+ excretion and glomerular filtration rate

Dose (μg/kg/min)	Urine output (μl/30 min)		Urinary Na^+ excretion (μmol/30 min)		Glomerular filtration rate (ml/min)	
	Control	Drug	Control	Drug	Control	Drug
0.10	138 ± 8	120 ± 4 *	23 ± 2	19 ± 3 *	1.3 ± 0.3	1.0 ± 0.1 *
0.15	135 ± 6	100 ± 5 *	25 ± 4	15 ± 3 *	1.4 ± 0.2	0.9 ± 0.2 *
0.20	140 ± 8	85 ± 12 *	26 ± 8	12 ± 5 *	1.3 ± 0.4	0.6 ± 0.3 *

Data are presented as mean ± S.E.M. These were 4–6 animals per dose group. * $P < 0.05$ from control.

Table 2
Influence of prazosin (0.5 μg/kg/min), SZL-49 (0.15 μg/kg/min) and chloroethylclonidine (50 μg/kg/min) on urine output, urinary Na^+ excretion and glomerular filtration rate in anesthetized rats

Antagonist	Urine output (μl/30 min)				Urinary Na^+ excretion (μmol/30 min)				Glomerular filtration rate (ml/min)			
	C	A60	A90	A120	C	A60	A90	A120	C	A60	A90	A120
Prazosin	120 ± 6	115 ± 10	112 ± 15	108 ± 15	22 ± 3	20 ± 6	23 ± 4	19 ± 5	1.2 ± 0.3	1.0 ± 0.3	1.3 ± 0.3	1.0 ± 0.4
SZL-49	132 ± 10	125 ± 15	120 ± 16	140 ± 16	26 ± 6	24 ± 3	21 ± 3	20 ± 4	1.5 ± 0.1	1.3 ± 0.3	1.1 ± 0.4	1.5 ± 0.4
Chloroethyl-clonidine	127 ± 12	119 ± 10	120 ± 11	130 ± 15	25 ± 3	24 ± 4	20 ± 4	22 ± 4	1.4 ± 0.2	1.3 ± 0.3	1.2 ± 0.3	1.3 ± 0.4

Data are presented as mean ± S.E.M. There were 4–6 animals for each antagonist. The antagonist was adminsitered immediately after collecting the first 30 min urine and blood samples, and three subsequent samples collected, each 30 min apart after giving the antagonist (A60, A90 and A120).

3.1. Effect of phenylephrine on renal function

As shown in Figs. 1 and 2, intrarenal infusion of phenylephrine (0.25 μg/kg/min) produced marked decreases in urine output, urinary Na^+ excretion and glomerular filtration rate. With this dose of phenylephrine no significant alterations in mean blood pressure, heart rate or fractional Na^+ excretion were observed (Figs. 1 and 2). All renal parameters returned to control values immediately after the termination of phenylephrine infusion. It is important to mention that, in our preliminary study, various doses of phenylephrine (0.05, 0.1, 0.15, 0.2, 0.25, 0.5 μg/kg/min) had been employed in an attempt to find a dose which would cause minimal change in glomerular filtration rate while producing marked reductions in urine output and Na^+ excretion. However, even with the use of a wide dose range, we could not separate glomerular hemodynamic changes from tubular effects. At the dose of 0.05 μg/kg/min of phenylephrine none of the changes was statistically significant. At doses of 0.1, 0.15 and 0.2 μg/kg/min, small but statistically significant changes were observed in urine output, urinary Na^+ excretion as well as glomerular filtration rate

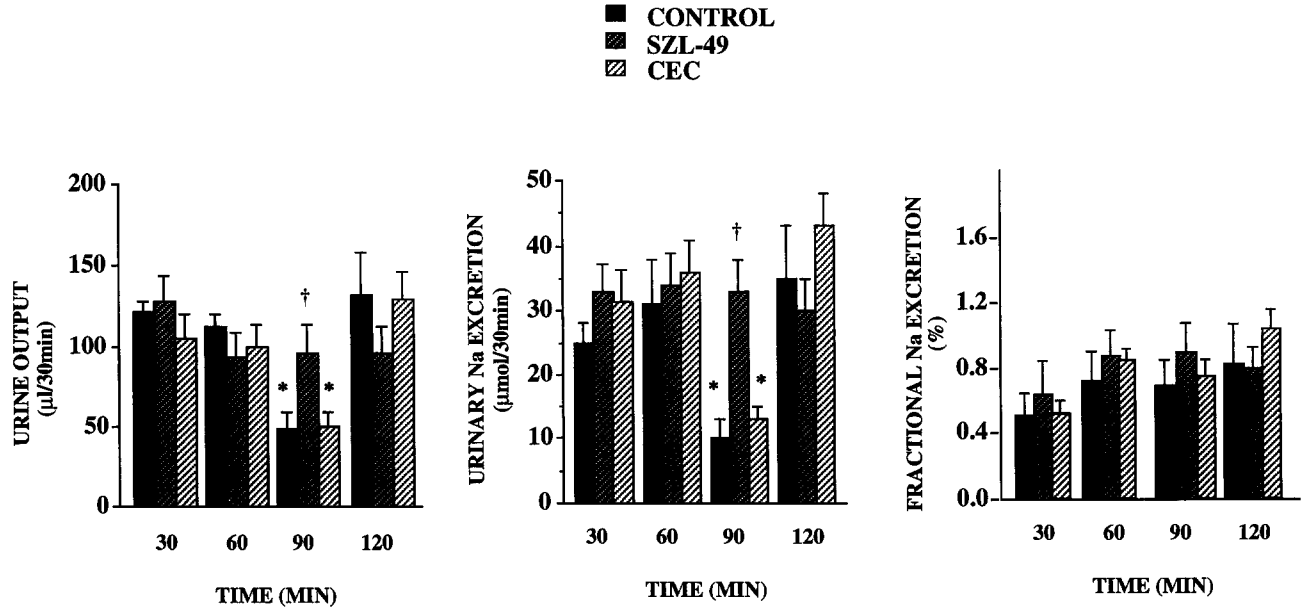

Fig. 3. Influence of SZL-49 and chloroethylclonidine on phenylephrine-induced changes in urine output, urinary sodium (Na) excretion, fractional sodium (Na) excretion. Phenylephrine (0.25 μg/kg/min) was intrarenally infused for 30 min and the infusion began at the end of the second urine collection period (60 min) in all three groups. 'CONTROL' = the group of rats receiving saline (vehicle) pretreatment. 'SZL-49' = the group receiving SZL-49 pretreatment. 'CEC' = the group receiving chloroethylclonidine pretreatment. Both SZL-49 (0.15 μg/kg/min) and CEC (50 μg/kg/min) were infused intrarenally for 30 min and the infusion began at the end of the first urine collection period (30 min). $n = 6$ rats in each group. * Significantly different as compared to the corresponding control. † Significantly different as compared to the group receiving phenylephrine alone.

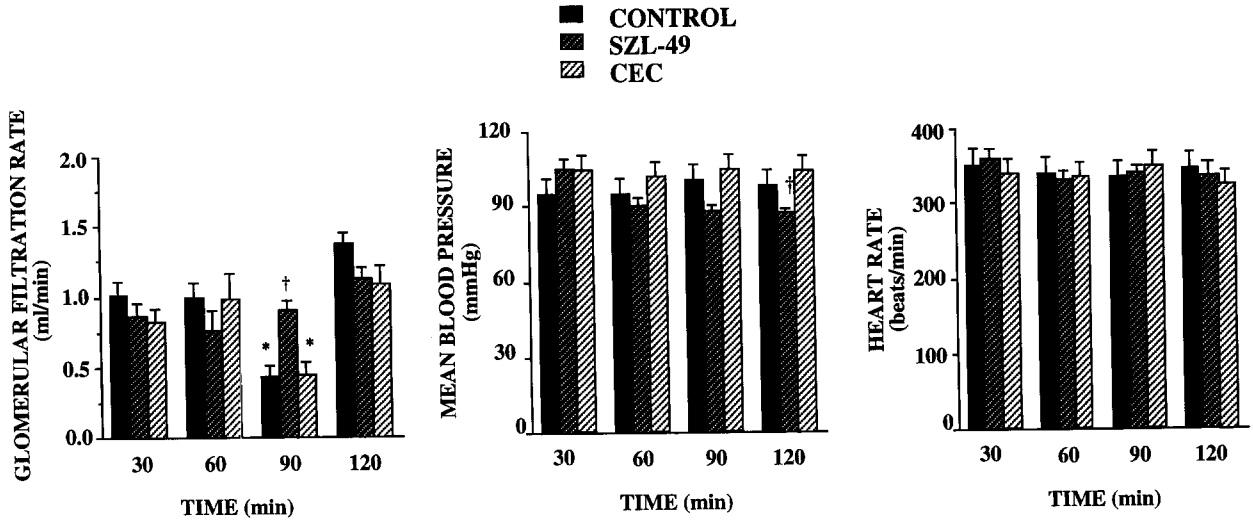

Fig. 4. Influence of SZL-49 and chloroethylclonidine on phenylephrine-induced changes in glomerular filtration rate, mean blood pressure and heart rate. Phenylephrine (0.25 μg/kg/min) was intrarenally infused for 30 min and the infusion began at the end of the second urine collection period (60 min) in all three groups. 'CONTROL' = the group of rats receiving saline (vehicle) pretreatment. 'SZL-49' = the group receiving SZL-49 pretreatment. 'CEC' = the group receiving chloroethylclonidine pretreatment. Both SZL-49 (0.15 μg/kg/min) and CEC (50 μg/kg/min) were infused intrarenally for 30 min and the infusion began at the end of the first urine collection period (30 min). $n = 6$ rats in each group. * Significantly different as compared to the corresponding control. † Significantly different as compared to the group receiving phenylephrine alone.

(Table 1). The important observation was that the magnitude of decreases in all of these three parameters was similar, albeit smaller, in comparison with 0.25 μg/kg/min (Table 1). We observed anuria in 3 out of 5 rats tried with the dose of 0.5 μg/kg/min. Also shown in Figs. 1 and 2, all changes induced by phenylephrine were abolished by prazosin infused intrarenally prior to administration of phenylephrine. In addition, when prazosin was infused alone, no significant changes in any of the measured parameters were observed (Table 2).

3.2. Effects of α_1-adrenoceptor subtype antagonists on phenylephrine-induced alterations in renal function

As presented in Figs. 3 and 4, in rats pretreated with the irreversible α_{1B}-adrenoceptor antagonist chloroethylclonidine, urine output, urinary Na^+ excretion and glomerular filtration rate during phenylephrine infusion were not different from those receiving saline control. It is important to note that doses of both SZL-49 and chloroethylclonidine are reported to be sufficient to inactivate corresponding α_1-adrenoceptor subtypes (Elhawary et al., 1992). We did not find any significant alterations in either hemodynamic or renal parameters when chloroethylclonidine was administered alone (Table 2). In contrast, in rats pretreated with the irreversible α_{1A}-adrenoceptor antagonist SZL-49, phenylephrine at this dose did not produce any significant changes in urine output, urinary Na^+ excretion and glomerular filtration rate. Similar to chloroethylclonidine, no significant changes in any hemodynamic or renal parameters were observed when SZL-49 alone was intrarenally infused (Table 2).

4. Discussion

It is generally known that activation of α_1-adrenoceptors either by renal nerve stimulation or exogenously administered α_1-adrenoceptor agonists leads to antidiuresis and antinatriuresis (DiBona, 1985; Jeffries et al., 1987). The factors proposed to be involved in mediating α_1-adrenoceptor activation induced antinatriuresis are renal vasoconstriction (Cooper and Malik, 1985), decreased ultrafiltration coefficient, and increase in tubular transport (DiBona, 1985; Osborn and Harland, 1988). The relative contribution of each of these factors under any particular experimental condition depends largely on the dose of agonists used or intensity of renal nerve stimulation employed. For example, it was demonstrated in anesthetized rabbits that when relatively smaller doses of phenylephrine and methoxamine were infused intrarenally, modest, but significant decreases in urine output, absolute and fractional Na^+ excretion were seen without any changes in renal blood flow or glomerular filtration rate (Hesse and Johns, 1985). However, with increasing doses of these agonists, marked reductions in renal blood flow and glomerular filtration rate were also observed, which contributed to further decrease of urine flow and Na^+ excretion (Hesse and Johns, 1985). Since α_1-adrenoceptors are heterogeneous, the recent availability of selective α_1-adrenoceptor subtype antagonists has made

it possible to differentiate the relative contribution of these receptor subtype(s) in mediating each of the α_1-adrenoceptor mediated effects. The results of the present study show that phenylephrine at the dose used produced marked antidiuresis and antinatriuresis. There are several points indicating that these renal effects of phenylephrine are caused predominantly, if not solely via alterations in glomerular hemodynamics. First, marked reductions in urine output and Na^+ excretion during phenylephrine infusion were associated with a similar degree of decrease of glomerular filtration rate in terms of percentage changes over the corresponding control. Second, the fractional Na^+ excretion, which is a rather good indicator of tubular function, was not significantly altered. Furthermore, those antagonists, i.e., prazosin and SZL-49, which abolished the phenylephrine-induced change in glomerular filtration rate, also effectively eliminated the changes in urine output and Na^+ excretion caused by phenylephrine administration. The results of the present study also suggest that α_{1A}-, but not α_{1B}-adrenoceptors are involved in mediating the hemodynamic effect of phenylephrine (i.e., decrease in glomerular filtration rate), since this effect was completely abolished by the relatively selective α_{1A}-adrenoceptor antagonist SZL-49, and the α_{1B}-adrenoceptor antagonist chloroethylclonidine had no detectable effect. This observation is consistent with a previous report, which showed in an in vitro study that α_1-adrenoceptors located in the rat renal resistance vessels are predominantly of the α_{1A}-adrenoceptor subtype (Elhawary et al., 1992).

The results from our control study suggest that under basal condition in our preparations, the influence of endogenous catecholamines on glomerular hemodynamics or tubular transport, if any, is negligible, since no significant alterations in glomerular filtration rate and renal parameters were observed in the presence of the antagonists, prazosin, chloroethylclonidine or SZL-49. This could be due to the influence of anesthesia and caution should be used in extrapolating this observation to other conditions.

In addition to hemodynamic influence, α_1-adrenoceptor activation also causes antinatriuresis via a direct influence at the level of renal tubules including proximal tubule. Intrarenal infusion of phenylephrine in anesthetized rabbits (Hesse and Johns, 1985) and rats (Elhawary and Pang, 1994) at a dose which did not alter renal hemodynamics, significantly reduced urine flow and absolute and fractional Na^+ excretion. However, we did not find a dose of phenylephrine which consistently produced significant antinatriuresis and antidiuresis without significantly altering glomerular filtration rate in our pilot study. One reason for this could be that the basal urine output and urinary Na^+ excretion are relatively low in our rats and further decreases in urine output and Na^+ excretion are very difficult, if not impossible to achieve without significant reduction in glomerular filtration rate.

In summary, our results indicate that the decrease in glomerular filtration rate seen during phenylephrine infusion was mediated via activation of α_{1A}-adrenoceptors, and it is this glomerular hemodynamic change which is responsible for the antidiuretic and antinatriuretic response to phenylephrine.

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