

## Full-length article

**Chiral selective effects of doxazosin enantiomers on blood pressure and urinary bladder pressure in anesthetized rats<sup>1</sup>**Shi-ping MA<sup>2</sup>, Lei-ming REN<sup>2,4</sup>, Ding ZHAO<sup>2</sup>, Zhong-ning ZHU<sup>2</sup>, Miao WANG<sup>2</sup>, Hai-gang LU<sup>3</sup>, Li-hua DUAN<sup>3</sup><sup>2</sup>Hebei Medical University School of Pharmacy, Shijiazhuang 050017, China; <sup>3</sup>Hebei Professional College in Chemical & Pharmaceutical Sciences, Shijiazhuang 050031, China**Key words***R*-doxazosin; *S*-doxazosin; blood pressure; ventricular pressure; urinary bladder pressure; rat<sup>1</sup> Project supported by the Hebei Natural Science Foundation (No. C2006000802).<sup>4</sup> Correspondence to Prof Lei-ming REN.

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**Abstract**

**Aim:** To study chiral selective effects of doxazosin enantiomers on blood pressure and urinary bladder pressure in anesthetized rats. **Methods:** In anesthetized rats, the carotid blood pressure, left ventricular pressure of the heart and the urinary bladder pressure were recorded. **Results:** Administration of *S*-doxazosin at 0.25, 2.5, 25, and 250 nmol/kg iv produced a dose-dependent decrease in blood pressure, but its depressor effect was significantly weaker than that induced by *R*-doxazosin and racemic-doxazosin (*rac*-doxazosin), and the ED<sub>30</sub> values (producing a 30% decrease in mean arterial pressure) of *R*-doxazosin, *rac*-doxazosin and *S*-doxazosin were 15.64, 45.93, and 128.81, respectively. *Rac*-doxazosin and its enantiomers administered cumulatively in anesthetized rats induced a dose-dependent decrease in the left ventricular systolic pressure and  $\pm dp/dt_{\max}$ , and the potency order of the 3 agents was *R*-doxazosin > *rac*-doxazosin > *S*-doxazosin. *Rac*-doxazosin and its enantiomers decreased the vesical micturition pressure dose-dependently at 2.5, 25, and 250 nmol/kg, and the inhibitory potency among the 3 agents was not significantly different. **Conclusion:** *S*-doxazosin decreases the carotid blood pressure and left ventricular pressure of the heart less than *R*-doxazosin and *rac*-doxazosin, but its effect on the vesical micturition pressure is similar to *R*-doxazosin and *rac*-doxazosin, indicating that *S*-doxazosin has chiral selectivity between cardiovascular system and urinary system in anesthetized rats.

**Introduction**

Racemic-doxazosin (*rac*-doxazosin), a highly selective  $\alpha_1$ -adrenoceptor antagonist, can block the over-contraction of smooth muscle in the prostate and urethral tract and improve the urinary dynamics of benign prostatic hyperplasia (BPH) and their clinical symptoms in patients<sup>[1]</sup>. It has also been reported that adrenaline produces a contractile response, presumably mediated by the stimulation of  $\alpha$ -adrenoceptors instead of the normal  $\beta$ -adrenoceptor response on the detrusor muscle obtained from the patient with BPH and outflow obstruction<sup>[2]</sup>. The pharmacological properties of doxazosin in the human prostate were characterized by Hatano *et al* who did not find a significant difference among the pA<sub>2</sub> values of doxazosin and its enantiomers against phenylephrine-induced contraction via  $\alpha_1$ -adrenoceptors in the

human prostate<sup>[3]</sup>.

We previously reported that *rac*-doxazosin reduced urethral pressure increased by the hypogastric nerve stimulation in an anesthetized cat<sup>[4]</sup>. We also found that the efficacy of *S*-doxazosin against the noradrenaline-induced contraction in the isolated rabbit thoracic aorta and carotid artery was much lower than that of *R*-doxazosin and *rac*-doxazosin<sup>[5]</sup>. These results might imply that *S*-doxazosin is an agent preferentially acting on the lower urinary tract tissues with minor cardiovascular side effects. In order to identify the chiral selective effects of doxazosin enantiomers between the cardiovascular system and the urinary system, we attempted to observe the effects of *rac*-doxazosin, *R*-doxazosin and *S*-doxazosin on the blood pressure, left ventricular pressure of the heart and the urinary bladder pressure in anesthetized rats.

## Materials and methods

**Rats** Male Wistar rats (200–300 g) were obtained from the Experimental Animal Center of Hebei Medical University (Certificate No. DK0412-0012).

**Chemicals** Rac-doxazosin methane sulphonate, *R*-doxazosin hydrochloride and *S*-doxazosin hydrochloride are white crystalline powder, synthesized by and obtained from the Center for Drug Research and Development, North China Pharmaceutical Corporation. All chemicals were dissolved in distilled water.

**Arterial blood pressure and heart rate** Rats were anesthetized with urethane (1 g/kg sc and 1 g/kg ip), and a catheter was inserted into the trachea to allow drainage of bronchial secretion and to facilitate breathing. Polyethylene catheters were inserted into the right femoral vein for drug administration and into the left common carotid artery for blood pressure measurement, respectively. Blood pressure was monitored via the arterial catheter connected to a MLT0380/D Reusable BP Transducer (PowerLab, ADInstruments, Castle Hill, Australia), and displayed on PowerLab/8sp (ADInstruments, Castle Hill, Australia) through an IBM computer running the PowerLab Chart 4.0 software (ADInstruments, Castle Hill, Australia). The heart rate (HR) was monitored via an electrocardiogram recorded on PowerLab/8sp. After an equilibration period of 30 min, doxazosin and its enantiomers were injected into the femoral vein. Changes produced by rac-doxazosin, *R*-doxazosin and *S*-doxazosin in systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial blood pressure (MAP) and HR were recorded. Each agent was administered at 0.25, 2.5, 25, and 250 nmol/kg, respectively. Forty rats were randomly divided into 2 groups (*S*-doxazosin group and *R*-doxazosin group) and only 1 dose of *S*-doxazosin or *R*-doxazosin was administered (0.5 mL/kg, iv) to each animal; an additional 5 rats were given normal saline as the control group. The purpose of a single administration of doxazosin enantiomers was to observe the time-response course of each dose of *S*-doxazosin and *R*-doxazosin, and we found that the responses to doxazosin enantiomers at 2.5 nmol/kg reached a peak value about 20 min after administration; those of higher doses reached a peak value within 5 min after administration. The other 24 rats were randomly divided into 3 groups (*S*-doxazosin group, *R*-doxazosin group and rac-doxazosin group) and 1 agent at 4 doses (0.25, 2.5, 25, and 250 nmol/kg) was administered cumulatively to each rat. An additional 8 rats were given normal saline as the control group.

**Left ventricular pressure** Rats were anesthetized with urethane (2 g/kg, 1 g/kg sc and 1 g/kg ip) and a catheter was

intubated into the trachea to facilitate breathing. A polyethylene catheter (OD 1.2 mm, ID 1.0 mm) was filled by heparin (12500 U/500 mL), and its outer wall was lubricated with paraffin liquid. The pulse pressure dropped suddenly as soon as the catheter was inserted into the left ventricle through the right carotid artery. At the end of experiment, the aortic valve was examined<sup>[6]</sup>. After the left ventricular pressure was recorded for 30 min, rac-doxazosin, *R*-doxazosin or *S*-doxazosin was injected into the femoral vein, and the left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP),  $\pm dp/dt_{\max}$  and HR were recorded on PowerLab/8sp. Twenty-four rats were randomly divided into 3 groups (*S*-doxazosin group, *R*-doxazosin group and rac-doxazosin group), and 1 agent at 4 doses (2.5, 25, 250, and 2500 nmol/kg) was administered cumulatively to each rat. We observed and compared the effects of *S*-doxazosin, *R*-doxazosin and rac-doxazosin using a cumulative administration design in the experiments of blood pressure and ventricular pressure. The second dose (25 nmol/kg) was given 20 min after the first dose (2.5 nmol/kg), and other higher doses were given 5 min after the front administrations. An additional 8 rats were given normal saline as the control group.

**Urinary bladder pressure** The rats were anesthetized with urethane (1.2 g/kg sc). The bladder was exposed through a suprapubic longitudinal incision and a small hole was made at the dome of the bladder<sup>[7,8]</sup> in order to intubate a double lumen catheter into the bladder. Then the double lumen catheter was fixed with silk ligation. An inner catheter (OD 0.7 mm) of the double lumen catheter was connected to an infusion pump for perfusion with Tyrode's solution without sugar at 10 mL/h, and intravesical pressure was continuously recorded via the outer catheter (OD 1.4 mm) on PowerLab/8sp. To eliminate the influence by the pump perfusion on the intravesical pressure, the top of inner catheter was 2 mm longer than that of outer catheter of the double lumen catheter. After obtaining control cystometrograms, we injected drugs through the femoral vein, then the micturition pressure (MP; maximal bladder pressure during micturition), basal pressure (BP; the lowest bladder pressure during filling), intercontraction interval (ICI; the interval between voids) and micturition volume (MV; the volume of expelled urine) were recorded. Seventy-two rats were divided into 3 groups (*S*-doxazosin group, *R*-doxazosin group and rac-doxazosin group) randomly, and 3 doses (2.5, 25, and 250 nmol/kg) of each agent were given. Only 1 dose of an agent was administered (0.5 mL/kg) to 1 animal. An additional 8 rats were given normal saline as the control group. After an equilibration period of 15 min, 10 continuous micturition cycles were recorded before drug administration, and

their mean values were calculated as the control. Ten continuous micturition cycles were then immediately recorded after administration and their mean values were calculated as drug effects.

**Statistical analysis** Data were expressed as mean±SD, and ED<sub>30</sub> values (producing a 30% decrease in MAP) were calculated with weighted probit analysis (Bliss and Finney, using NDST version 4.2, 1996, Sun RY, *et al*, editors). One-way ANOVA was used to evaluate any differences among the concentration-dependent responses. Comparison of a pair of responses before and after treatment was analysed by paired *t*-test in the same preparation. Comparisons between the 2 groups were made by unpaired *t*-test, and comparisons among 3 or more groups were made by Dunnett's multiple comparisons test (using GraphPat InStat V2.05a, San Diego, CA, USA). *P* values less than 0.05 were considered statistically significant.

**Results**

**Influence of single dose of doxazosin enantiomers on the carotid blood pressure and HR in anesthetized rats** SBP, DBP and MAP in the control animals did not change significantly during the period 60 min after giving normal saline (*P*>0.05, *n*=5; data not shown). Doxazosin enantiomers decreased the carotid blood pressure in a dose-dependent manner. *R*-doxazosin at 0.25, 2.5, 25, and 250 nmol/kg decreased the SBP, DBP and MAP more potently than *S*-doxazosin (*P*<0.01; Table 1; Figure 1). *R*-doxazosin at 250 nmol/kg decreased SBP by 50.2%, DBP by 60.6% and MAP by 56.6%, while *S*-doxazosin at the same dose decreased

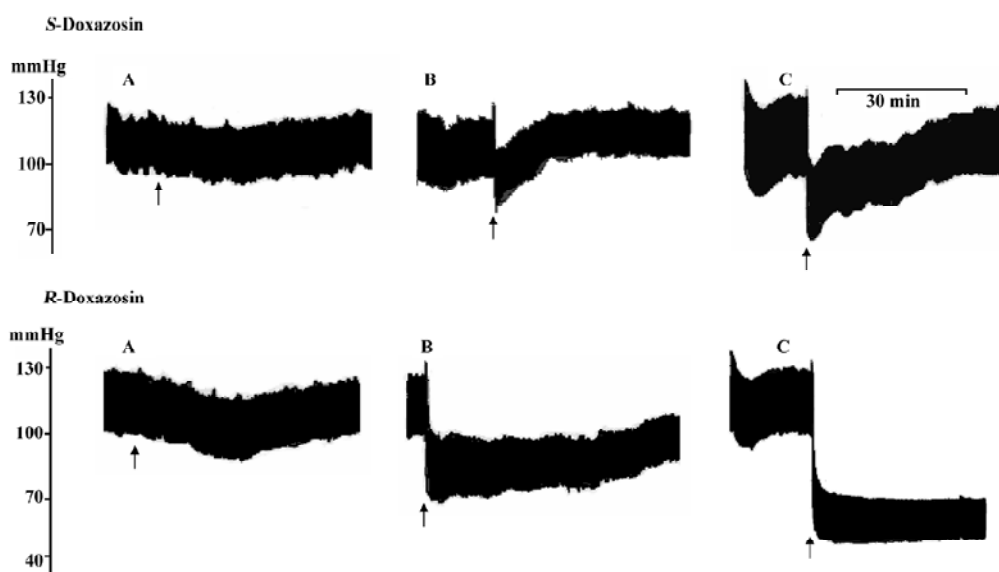
SBP by 31.8%, DBP by 41.1% and MAP by 37.3%. *R*-doxazosin at 25 and 250 nmol/kg and *S*-doxazosin at 250 nmol/kg decreased the HR slightly (by 7.8%, 11.0% and 7.3%), but significantly (*P*<0.05 and 0.01, *n*=5).

**Influence of cumulative administration of doxazosin and its enantiomers on the carotid blood pressure and HR in anesthetized rats** SBP, DBP and MAP in the control animals did not change significantly at 30 min, 60 min, 90 min, and 120 min after giving normal saline 4 times (*P*>0.05, *n*=8; data not shown). Cumulative administration of doxazosin enantiomers at 0.25, 2.5, 25, and 250 nmol/kg produced similar effects on the SBP, DBP and MAP like single dose administration (Table 2). The ED<sub>30</sub> values (producing a 30% decrease in MAP) of *R*-doxazosin, rac-doxazosin and *S*-doxazosin were 15.6±9.4, 45.9±20.6 and 128.8±35.7 (*n*=8), respectively and their ratio was 1:3:8. *R*-doxazosin at 250 nmol/kg, but not *S*-doxazosin and rac-doxazosin, decreased the HR slightly (by 10.9%), but significantly (*P*<0.05, *n*=8).

**Influence of cumulative administration of doxazosin and its enantiomers on the left ventricular pressure in anesthetized rats** LVSP, LVEDP and ±dp/dt<sub>max</sub> in the control animals did not change significantly at 30 min, 60 min, 90 min, and 120 min after giving normal saline 4 times (*P*>0.05, *n*=8; data not shown). Rac-doxazosin and its enantiomers at 250 and 2500 nmol/kg decreased the LVSP (*P*<0.01) and -dp/dt<sub>max</sub> (*P*<0.01), and the inhibitory effects by *S*-doxazosin were significantly weaker than those by *R*-doxazosin (Table 3). *R*-doxazosin and rac-doxazosin at 250 and 2500 nmol/kg significantly decreased the +dp/dt<sub>max</sub>, but the 3 agents did not significantly change the LVEDP in anesthetized rats (Table 3).

**Table 1.** Effects of a single dose of doxazosin enantiomers on the carotid blood pressure of rats. *n*=5. Mean±SD. <sup>c</sup>*P*<0.01 vs control. <sup>e</sup>*P*<0.05, <sup>f</sup>*P*<0.01 vs *R*-doxazosin.

	<i>S</i> -doxazosin				<i>R</i> -doxazosin (nmol/kg)			
	0.25	2.5	25	250	0.25	2.5	25	250
<b>SBP</b>								
Control (mmHg)	116±12	123.4±7.8	123.4±8.6	130.2±9.9	118.6±7.7	122.0±7.4	128.8±6.4	124±12
Treatment (mmHg)	112±14	116±11	105.0±6.7 <sup>ef</sup>	89±11 <sup>ef</sup>	111±11	102.6±7.3 <sup>c</sup>	90.6±5.2 <sup>c</sup>	61.4±4.6 <sup>c</sup>
Decrease (%)	3.8±3.9	6.2±6.4	14.8±3.4	31.8±6.6	6.5±5.4	19.4±2.0	29.7±2.4	50.2±2.2
<b>DBP</b>								
Control (mmHg)	90.8±9.7	98.8±5.6	93.0±9.3	98±12	92±10	94.6±4.7	98.4±6.7	9±10
Treatment (mmHg)	86.4±9.7	91.4±6.8 <sup>e</sup>	75.0±9.6 <sup>ef</sup>	57.6±9.1 <sup>ef</sup>	85±12	76.2±3.3 <sup>c</sup>	61.6±5.4 <sup>c</sup>	38.8±5.3 <sup>c</sup>
Decrease (%)	4.8±4.4	7.3±7.8	19.3±4.6	41.1±7.3	7.7±7.0	19.4±2.0	37.4±3.0	60.6±3.2
<b>MAP</b>								
Control (mmHg)	99±10	107.0±5.9	103.1±8.6	109±11	100.7±9.2	103.7±5.2	108.5±6.2	107±10
Treatment (mmHg)	95±11	99.6±8.0 <sup>e</sup>	85.0±7.3 <sup>ef</sup>	68±9 <sup>ef</sup>	94±12	85.0±4.4 <sup>c</sup>	71.3±5.0 <sup>c</sup>	46.3±4.9 <sup>c</sup>
Decrease (%)	4.4±4.2	6.9±7.3	17.5±4.0	37.3±6.9	7.2±6.3	18.0±2.1	34.3±2.7	56.6±2.6



**Figure 1.** Influence of single doses of doxazosin enantiomers on the carotid blood pressure in anesthetized rats. (A) 2.5 nmol/kg; (B) 25 nmol/kg; (C) 250 nmol/kg. Arrows indicate injection of compounds.

**Table 2.** Effects of cumulative administration of doxazosin and its enantiomers on the carotid blood pressure of rats.  $n=8$ . Mean $\pm$ SD. <sup>b</sup> $P<0.05$ , <sup>c</sup> $P<0.01$  vs before drugs. <sup>e</sup> $P<0.05$ , <sup>f</sup> $P<0.01$  vs *R*-doxazosin.

Dose (nmol/kg)	<i>S</i> -doxazosin			<i>R</i> -doxazosin			Rac-doxazosin		
	SBP	DBP	MAP	SBP	DBP	MAP	SBP	DBP	MAP
Before drugs	124.7 $\pm$ 7.8	79.13 $\pm$ 9.21	94.1 $\pm$ 8.4	129 $\pm$ 10	81 $\pm$ 12	96.6 $\pm$ 12	134 $\pm$ 9	84 $\pm$ 11	101 $\pm$ 10
After drugs									
0.25	123.2 $\pm$ 5.3 (0.9 $\pm$ 6.4)	78.6 $\pm$ 9.8 (0.5 $\pm$ 7.1)	93.8 $\pm$ 7.1 (0 $\pm$ 7)	121 $\pm$ 13 (5.6 $\pm$ 6.7)	74 $\pm$ 14 (8.9 $\pm$ 8.3)	90 $\pm$ 13 (7.3 $\pm$ 8.0)	129 $\pm$ 11 (4.2 $\pm$ 4.0)	80 $\pm$ 11 (6.0 $\pm$ 6.8)	96 $\pm$ 11 (5.2 $\pm$ 4.8)
2.5	122.3 $\pm$ 7.3 <sup>f</sup> (1.8 $\pm$ 4.5)	77 $\pm$ 10 <sup>f</sup> (2.2 $\pm$ 6.3)	92.1 $\pm$ 8.8 <sup>f</sup> (2 $\pm$ 5)	108 $\pm$ 14 <sup>b</sup> (16.2 $\pm$ 5.6)	62 $\pm$ 14 <sup>b</sup> (23.7 $\pm$ 7.5)	78 $\pm$ 13 <sup>b</sup> (19.9 $\pm$ 6.3)	122 $\pm$ 11 <sup>c</sup> (8.66 $\pm$ 5.16)	75.9 $\pm$ 8.2 <sup>f</sup> (10.6 $\pm$ 6.9)	91.8 $\pm$ 8.0 <sup>f</sup> (9.9 $\pm$ 5.9)
25	109 $\pm$ 10 <sup>cf</sup> (12.4 $\pm$ 3.8)	64.3 $\pm$ 7.9 <sup>cf</sup> (18.7 $\pm$ 4.0)	78.3 $\pm$ 7.4 <sup>cf</sup> (15.6 $\pm$ 3.1)	93 $\pm$ 14 <sup>c</sup> (28.1 $\pm$ 6.2)	47 $\pm$ 13 <sup>c</sup> (43.2 $\pm$ 8.0)	62 $\pm$ 13 <sup>c</sup> (36.5 $\pm$ 6.8)	111 $\pm$ 11 <sup>cf</sup> (17.0 $\pm$ 4.8)	62.0 $\pm$ 8.6 <sup>cf</sup> (26.4 $\pm$ 6.0)	78.4 $\pm$ 8.0 <sup>cf</sup> (22.6 $\pm$ 4.7)
250	86.9 $\pm$ 10.6 <sup>cf</sup> (30.5 $\pm$ 5.2)	41.5 $\pm$ 7.0 <sup>cf</sup> (47.8 $\pm$ 3.8)	56.4 $\pm$ 6.6 <sup>cf</sup> (40.2 $\pm$ 3.2)	74.0 $\pm$ 8.5 <sup>c</sup> (42.5 $\pm$ 3.0)	31.1 $\pm$ 9.4 <sup>c</sup> (62.2 $\pm$ 6.5)	45.2 $\pm$ 8.8 <sup>c</sup> (53.5 $\pm$ 4.6)	81.28 $\pm$ 8.09 <sup>c</sup> (39.4 $\pm$ 4.8)	34.6 $\pm$ 4.0 <sup>c</sup> (58.7 $\pm$ 4.3)	50.0 $\pm$ 4.0 <sup>c</sup> (50.3 $\pm$ 4.1)

**Influence of a single dose of doxazosin and its enantiomers on the urinary bladder pressure in anesthetized rats** MP, BP, ICI, and MV in the control animals did not change significantly during the period of 60 min after giving normal saline ( $P>0.05$ ,  $n=8$ ; data not shown). *S*-doxazosin at 2.5, 25, and 250 nmol/kg significantly decreased the MP; *R*-doxazosin and rac-doxazosin at 25 and 250 nmol/kg decreased the MP (Table 4). There was no significant difference among the 3 agents for their inhibitory effects on the MP ( $P>0.05$ ,  $n=8$ ). The 3 agents did not significantly affect the values of BP, ICI, and MV ( $P>0.05$ ,  $n=8$ , Figure 2).

## Discussion

$\alpha_1$ -adrenoceptors mediate some of the main actions of the natural catecholamines, such as epinephrine and nor-epinephrine, and have a crucial role in the regulation of arterial blood pressure<sup>[9]</sup>. Their antagonists can relax the arteriole and vein so as to decrease the standing and lying blood pressure. There are functional  $\alpha_{1A}$ - and  $\alpha_{1D}$ -adrenoceptor subtypes in response to the vasoconstriction of the rat common carotid arteries, but no  $\alpha_{1B}$ -adrenoceptor subtype<sup>[10]</sup>. The analysis of mutant mice has provided some insight into the physiological role of each  $\alpha_1$ -adrenoceptor subtype in the regu-

**Table 3.** Effects of cumulative administration of doxazosin and its enantiomers on the left ventricular pressure of the rat.  $n=8$ . Mean $\pm$ SD. <sup>b</sup> $P<0.05$ , <sup>c</sup> $P<0.01$  vs before drug. <sup>e</sup> $P<0.05$ , <sup>f</sup> $P<0.01$  vs *R*-doxazosin.

	Before drugs	Drug dose (nmol/kg)			
		2.5	25	250	2500
<b>LVSP (mmHg)</b>					
<i>S</i> -doxazosin	117 $\pm$ 10	115 $\pm$ 12	113 $\pm$ 12 <sup>f</sup>	94 $\pm$ 11 <sup>cf</sup>	91.8 $\pm$ 9.0 <sup>cf</sup>
<i>R</i> -doxazosin	113.6 $\pm$ 7.3	106.6 $\pm$ 8.2	100 $\pm$ 10 <sup>b</sup>	76 $\pm$ 11 <sup>c</sup>	76 $\pm$ 11 <sup>c</sup>
Rac-doxazosin	115.6 $\pm$ 5.7	113.0 $\pm$ 9.4	105.1 $\pm$ 9.6	85.3 $\pm$ 9.0 <sup>c</sup>	87.1 $\pm$ 7.5 <sup>ce</sup>
<b>LVEDP (mmHg)</b>					
<i>S</i> -doxazosin	0 $\pm$ 6	-0.3 $\pm$ 5.6	-1 $\pm$ 6	-3.0 $\pm$ 4.8	-3.7 $\pm$ 5.3
<i>R</i> -doxazosin	-1.99 $\pm$ 4.11	-2.97 $\pm$ 3.21	-3.84 $\pm$ 3.22	-5.4 $\pm$ 2.8	-6.4 $\pm$ 2.4
Rac-doxazosin	-2.5 $\pm$ 3.0	-3.0 $\pm$ 3.3	-3.6 $\pm$ 3.3	-5.1 $\pm$ 2.7	-5.9 $\pm$ 3.1
<b>+dp/dt<sub>max</sub> (mmHg/s)</b>					
<i>S</i> -doxazosin	5509 $\pm$ 704	5329 $\pm$ 675	5290 $\pm$ 665	4898 $\pm$ 522 <sup>f</sup>	4810 $\pm$ 466 <sup>f</sup>
<i>R</i> -doxazosin	5217 $\pm$ 956	5067 $\pm$ 928	4771 $\pm$ 819	4136 $\pm$ 777 <sup>b</sup>	4077 $\pm$ 648 <sup>b</sup>
Rac-doxazosin	5953 $\pm$ 709	5733 $\pm$ 654	5551 $\pm$ 751	5017 $\pm$ 724 <sup>b</sup>	5017 $\pm$ 622 <sup>b</sup>
<b>-dp/dt<sub>max</sub> (mmHg/s)</b>					
<i>S</i> -doxazosin	4564 $\pm$ 571	4522 $\pm$ 528	4424 $\pm$ 550 <sup>f</sup>	3615 $\pm$ 601 <sup>ce</sup>	3494 $\pm$ 547 <sup>c</sup>
<i>R</i> -doxazosin	4149 $\pm$ 604	3912 $\pm$ 596	3596 $\pm$ 564	2812 $\pm$ 496 <sup>c</sup>	2844 $\pm$ 419 <sup>c</sup>
Rac-doxazosin	4701 $\pm$ 614	4535 $\pm$ 721	4328 $\pm$ 744	3448 $\pm$ 621 <sup>c</sup>	3615 $\pm$ 516 <sup>c</sup>

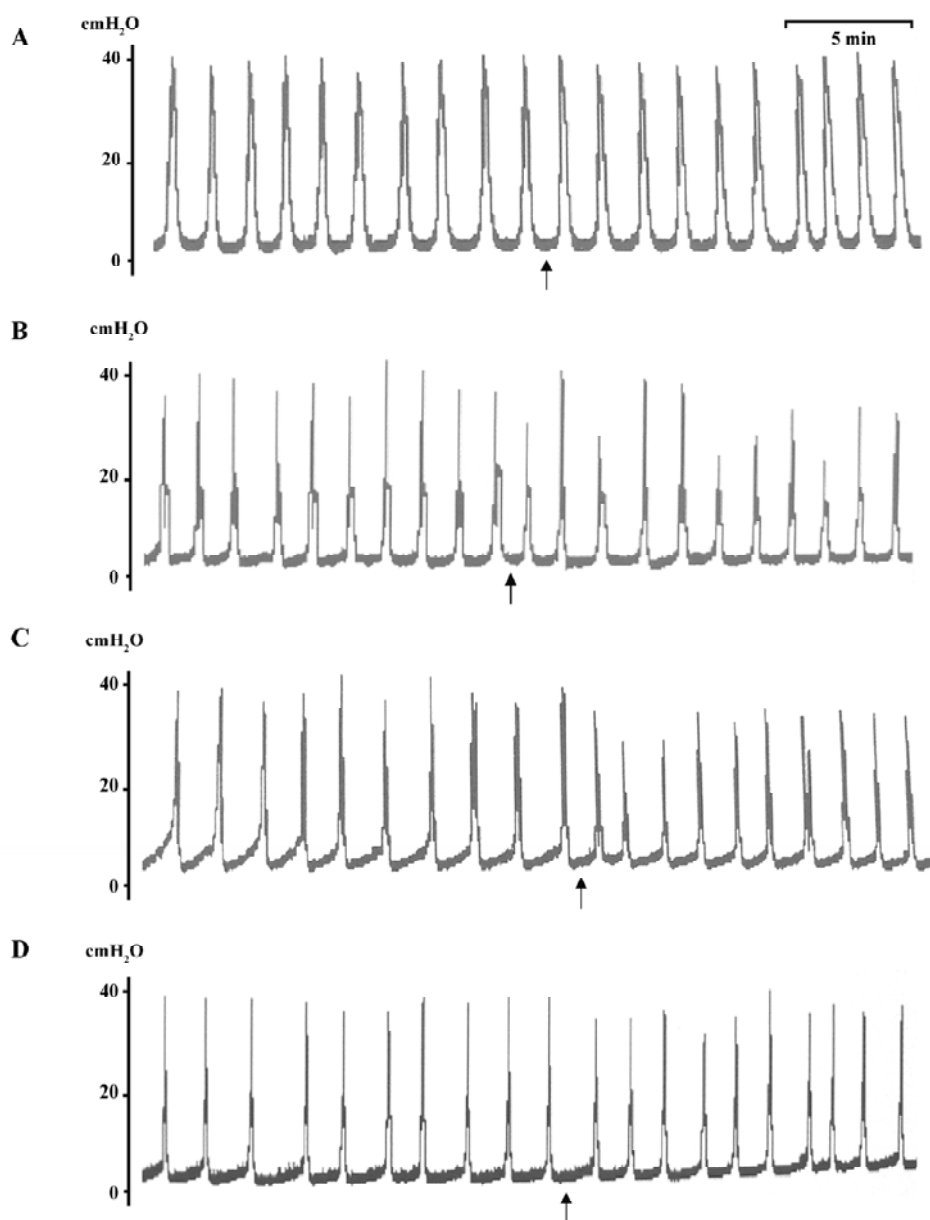
**Table 4.** Effects of single doses of doxazosin and its enantiomers on the urinary bladder pressure of rats.  $n=8$ . Mean $\pm$ SD. <sup>b</sup> $P<0.05$ , <sup>c</sup> $P<0.01$  vs control.

Drugs (nmol/kg)	MP (cmH <sub>2</sub> O)		BP (cmH <sub>2</sub> O)		MV (mL)	
	Control	Treatment	Control	Treatment	Control	Treatment
<b><i>S</i>-doxazosin</b>						
2.5	35.7 $\pm$ 4.0	33 $\pm$ 4.4 <sup>b</sup>	3.6 $\pm$ 1.1	3.6 $\pm$ 1.8	0.18 $\pm$ 0.05	0.19 $\pm$ 0.05
25	36.1 $\pm$ 4.6	32.3 $\pm$ 5.4 <sup>c</sup>	3.0 $\pm$ 1.2	2.9 $\pm$ 1.4	0.23 $\pm$ 0.07	0.22 $\pm$ 0.05
250	39.2 $\pm$ 3.9	31.8 $\pm$ 4.3 <sup>c</sup>	2.4 $\pm$ 1.0	1.7 $\pm$ 1.2	0.17 $\pm$ 0.04	0.18 $\pm$ 0.03
<b><i>R</i>-doxazosin</b>						
2.5	34.9 $\pm$ 4.4	32 $\pm$ 5	3.6 $\pm$ 2.3	3.5 $\pm$ 2.4	0.20 $\pm$ 0.09	0.17 $\pm$ 0.05
25	36.1 $\pm$ 4.6	30.8 $\pm$ 3.6 <sup>c</sup>	2.7 $\pm$ 1.5	2.0 $\pm$ 0.9	0.21 $\pm$ 0.07	0.18 $\pm$ 0.04
250	39.2 $\pm$ 3.9	32.4 $\pm$ 4.8 <sup>c</sup>	3.1 $\pm$ 1.9	2.4 $\pm$ 1.4	0.17 $\pm$ 0.06	0.19 $\pm$ 0.06
<b>Rac-doxazosin</b>						
2.5	36.7 $\pm$ 5.3	34.0 $\pm$ 5.4	3.4 $\pm$ 1.7	3.0 $\pm$ 1.4	0.17 $\pm$ 0.05	0.17 $\pm$ 0.04
25	36.2 $\pm$ 3.2	29.7 $\pm$ 3.8 <sup>c</sup>	2.4 $\pm$ 1.7	1.5 $\pm$ 0.7	0.22 $\pm$ 0.09	0.20 $\pm$ 0.04
250	40.2 $\pm$ 4.0	32.7 $\pm$ 3.1 <sup>c</sup>	2.3 $\pm$ 1.6	2.1 $\pm$ 1.3	0.21 $\pm$ 0.06	0.23 $\pm$ 0.04

lation of blood pressure.  $\alpha_{1A}$ -adrenoceptors play an extremely significant role in maintaining basal blood pressure, whereas  $\alpha_{1B}$ -adrenoceptors are important in the pressure response to catecholamines and  $\alpha_{1D}$ -adrenoceptors play a role in both physiological responses<sup>[9]</sup>.

Doxazosin is an  $\alpha_1$ -adrenoceptor antagonist with pharmacological properties similar to those of prazosin. Recently, we found that the  $pA_2$  values of *S*-doxazosin, concerning the vasoconstrictive responses to noradrenaline in the rabbit

isolated thoracic aorta and common carotid artery, were significantly lower than those of *R*-doxazosin and rac-doxazosin<sup>[5]</sup>. The present study further showed that doxazosin and its enantiomers administered intravenously obviously decreased the carotid blood pressure, but the inhibitory effects by the 3 agents on blood pressure were significantly different and their potency order was *R*-doxazosin>rac-doxazosin>*S*-doxazosin. *S*-doxazosin decreased the HR slightly only at the highest dose, and its action on the HR



**Figure 2.** Influence of single doses of doxazosin and its enantiomers at 250 nmol/kg on the bladder micturition in anesthetized rats. (A) Control; (B) *S*-doxazosin; (C) *R*-doxazosin; (D) rac-doxazosin. Arrows indicate injection of compounds.

was significantly weaker than *R*-doxazosin.

In adult rat tissues, the  $\alpha_{1A}$ -adrenoceptor protein is most marked in the brain, intermediate in the heart, aorta, liver, vas deferens, and minimal in the kidney and prostate<sup>[11]</sup>. It has been confirmed that the 3 subtypes of  $\alpha_1$ -adrenoceptors are all expressed in the heart, but their expression extent in mRNA and protein levels is different. In this study, doxazosin and its enantiomers administered cumulatively to the anesthetized rats decreased the LVSP and  $-dp/dt_{\max}$  significantly at 250 and 2500 nmol/kg, and the inhibitory effects by *S*-doxazosin were significantly weaker than those by *R*-doxazosin. At 250 and 2500 nmol/kg, *R*-doxazosin and rac-

doxazosin, but not *S*-doxazosin, significantly decreased the  $+dp/dt_{\max}$ . Results obtained from the above studies in the carotid blood pressure and left ventricular pressure indicate that *S*-doxazosin has a weak inhibitory effect on the cardiovascular system in comparison with *R*-doxazosin and rac-doxazosin. It is well known that blood pressure is regulated primarily by cardiac output, total peripheral resistance and blood volume, and it is also controlled by central and peripheral sympathetic activity. Further experiments should be designed to investigate the effects of doxazosin enantiomers on the sympathetic nerve activity, blood vessel resistance and isolated heart tissues to clarify the different mechanism

between *S*-doxazosin and *R*-doxazosin.

BPH is a common cause of urinary flow obstruction in ageing men and may lead to lower urinary tract symptoms. At present,  $\alpha_1$ -adrenoceptor antagonists are usually considered as the first-line therapy for BPH patients<sup>[12]</sup>. Therefore, we further investigated the influence of doxazosin and its enantiomers on the urinary bladder pressure in anesthetized rats.

The affinities of doxazosin and its enantiomers for the  $\alpha_1$ -adrenoceptor subtypes have been determined in radioligand-binding studies using the membrane preparations isolated from rat fibroblast expressing the human  $\alpha_{1A}$ -,  $\alpha_{1B}$ - and  $\alpha_{1D}$ -adrenoceptor subtypes, and rac-doxazosin, *R*-doxazosin and *S*-doxazosin are potent antagonists with balanced activity across all three cloned human  $\alpha_1$ -adrenoceptor subtypes<sup>[3]</sup>. Ishizuka *et al*<sup>[13]</sup> reported that in conscious rats, intrathecal administration of rac-doxazosin at 60 mg/kg decreased the micturition pressure of the urinary bladder by 13 cmH<sub>2</sub>O when the bladder was perfused with saline constantly by means of a pump, and its effect on micturition pressure was much more pronounced in the animal with post-obstruction bladder hypertrophy induced by partial ligation of the urethra. They also indicated that doxazosin enantiomers administered intrathecally produced qualitatively similar effects on micturition pressure to rac-doxazosin<sup>[13]</sup>. In the present study, we demonstrated that in the anesthetized rats, intravenous administration of *S*-doxazosin at 2.5, 25, and 250 nmol/kg significantly decreased the urinary bladder MP, and there was no significant difference among rac-doxazosin, *S*-doxazosin and *R*-doxazosin for their inhibitory effects on MP of the urinary bladder. The 3 agents administered intravenously did not significantly affect the other cystometric parameters recorded.

Although Hatano *et al* reported that *R*-doxazosin and *S*-doxazosin were potent antagonists with balanced activity across all 3 cloned human  $\alpha_1$ -adrenoceptor subtypes, we were not able to confirm the similar results in the experiments of the carotid blood pressure and left ventricular pressure of the heart in the anesthetized rats when the drugs were given intravenously, except for the experiment of urinary bladder micturition. The possible reasons for the conflicting results are not clear, but some underlying mechanisms can be considered. First, the pharmacological profile of the wild-type of  $\alpha_1$ -adrenoceptor subtypes in tissues or *in vivo* might be different from that of cloned type of  $\alpha_1$ -adrenoceptor subtypes in cultured cells. Second, functions of the cardiovascular and urinary system are regulated by many factors including  $\alpha_1$ -adrenoceptors; some unknown effects might be involved in doxazosin enantiomers. It has been well es-

tablished that sympathetic and parasympathetic nuclei in the lumbosacral cord receive inputs from noradrenergic neurons in the brainstem, and these pathways have been implicated in the supraspinal control of micturition<sup>[14]</sup>. Antagonism at the  $\alpha_1$ -adrenoceptors on the detrusor muscle or at prejunctional  $\alpha_1$ -adrenoceptors facilitating acetylcholine release in the bladder<sup>[15]</sup>, would produce decreased MP. Recently Szell *et al*<sup>[16]</sup> reported that cholinergic nerve terminals  $\alpha_{1A}$ -adrenoceptors mediated prejunctional facilitation, whereas postjunctional  $\alpha_{1B}/\alpha_{1D}$ -adrenoceptors mediated smooth muscle contraction of the rat urinary bladder. Further experiments should be done with the isolated detrusor muscle, prostate smooth muscle and urethral smooth muscle to observe the precise effects of doxazosin enantiomers.

In summary, the current results suggest that in anesthetized rats, *S*-doxazosin administered intravenously at the used dosages reduces the carotid blood pressure and left ventricular pressure of the heart less than *R*-doxazosin and rac-doxazosin, but its effect on the vesical micturition pressure is similar to *R*-doxazosin and rac-doxazosin, indicating that *S*-doxazosin has chiral selectivity between cardiovascular system and urinary system in anesthetized rats.

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