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Effects of Subchronic Treatment with AT_1 Receptor Antagonists on Endothelium-dependent and -independent Relaxation

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To investigate whether AT_1 receptor antagonists are acting by increasing endothelium-dependent and -independent relaxation of aortas in normotensive rats, AT_1 receptor antagonists, losartan and KR-30988, and angiotensin converting enzyme inhibitor, captopril, were orally administered for two weeks (50 mg/kg, b.i.d.). The blood pressure, heart rate and body weight were not significantly changed by losartan, KR-30988 and captopril compared to the control group. In aortic preparations, the pD_2 of KR-30988 for ACh-induced relaxation was 8.33 ± 0.16, significantly ($p<0.05$) lower than that of control group (7.71 ± 0.15). ACh-induced relaxation was significantly increased in losartan-treated group ($p<0.01$) at 10^{-6} M of ACh, and in captopril-treated group ($p<0.05$) at the range of 10^{-7}-10^{-5} M of ACh. The pD_2 values for histamine-induced relaxation of losartan, KR-30988 and captopril were 5.57 ± 0.10, 5.85 ± 0.21 and 5.60 ± 0.01, respectively, with significant differences in all groups ($p<0.01$) compared to that of control group (5.13 ± 0.09). ACh-induced relaxations of aortic preparations were not changed by pretreatment of indomethacin (10^{-5} M), and completely blocked by pretreatment of L-NAME (10^{-5} M) in all groups. Sodium nitroprusside-induced relaxations were not significantly changed by all drugs tested in this experiments. These results suggest that AT_1 receptor antagonists, losartan and KR-30988, enhance the endothelium-dependent relaxation on aortic preparations through the release of, or increase sensitivity, to nitric oxide in normotensive rats.

Key words : AT_1 receptor, Losartan, KR-30988, Endothelium-dependent relaxation

INTRODUCTION

Hypertension is associated with an endothelial dysfunction characterized by an increased endothelium-dependent contraction and a decreased endothelium-dependent relaxation. Antihypertensive therapy with reserpine, hydralazine (Luscher *et al.*, 1987) and angiotensin converting enzyme inhibitors, such as captopril (Liao and Chen, 1992), silazapril (Young *et al.*, 1995), trandopril (Pourageaud and Freslon, 1995), fosinopril (Rizzoni *et al.*, 1995) and SQ29852 (Sunano *et al.*, 1992), reverses the depressed endothelium-dependent relaxation in hypertensive rats, but not with artenolol (Schiffrin, 1995). In this antihypertensive therapy, normalization of arterial pressure (Luscher *et al.*, 1987; Sunano *et al.*, 1992) and reduction in pulse pressure (Schiffrin, 1995) appears pivotal in reversing the dysfunctional endothelium of hypertensive rats. While Shultz and Raij (Shultz and Raij, 1989) strongly suggested that some antihypertensive agents including captopril have direct effects on endothelium-dependent relaxation in rat aorta which is not simply due to the lowering of systemic blood pressure. The modulation of endothelium-dependent relxation may vary from one class of antihypertensive agents to another and even within the same class of agents. It is very important to elucidate whether some antihypertensive agents will have direct effect on endothelium, not by lowering of blood pressure, because these agents are widely used not only in hypertension, but also in many other purposes. However, there are little information regarding whether angiotensin AT_1 receptor antagonists themselves, when administered chronically, influence or modulate the response of vessels to agonists of endothelium-dependent and -independent relaxation or not. The present study was performed to investigate the possible correlation between AT_1 receptor antagonists and their effects on endothelium-dependent and -independent relaxation in aortas from normotensive rats.

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MATERIALS AND METHODS

Materials

Norepinephrine hydrochloride, acetylcholine chloride, histamine dihydrochloride, sodium nitroprusside, indomethacin and NG-nitro-L-arginine methylester (L-NAME) hydrochloride were purchased from Sigma Chemical Co. Indomethacin was dissolved in distilled water containing 5% $NaHCO_3$. Captopril, obtained from Boryung Pharmaceutical Co., was dissolved in distilled water. Losartan and KR-30988, synthesized at Korea Research Institute of Chemical Technology (KRICT), were dissolved in 0.05 N KOH. Other drugs were dissolved in distilled water. All were prepared just before use.

Animals

The experiments were performed on male Sprague-Dawley rats weighing 350-400 g, provided by the Department of Experimental Animals, KRICT and kept in a storage room under the conditions of constant temperature, relative humidity and illumination (12 h light, 12 h dark) until the day of experiment with free access to food and tap water.

Experimental protocol

Animals were divided into four groups at random : the control group received 0.05 N KOH solution orally with volume of 3 ml/kg; the other 3 groups were given each 50 mg/kg b.i.d. of losartan, KR-30988 and captopril by oral route, respectively. A little higher dose was chosen in this experiments compared to the other chronic experiments using captopril (Shultz and Raij, 1989; Gardiner and Bennett, 1983; Antonaccio *et al.*, 1981), because the effects of drugs may not be persisted, if administered twice a day, compared to the continuous administration with tap water. In addition, losartan and KR-30988 are less potent than captopril in lowering the blood pressure in renin-dependent hypertensive rats (Lee and Shin, 1994). However, in this experiments same dose in all groups was used to compare the effects of drugs on endothelium-dependent and -independent relaxation.

After 2 weeks of treatments, blood pressure and body weight were measured on all rats using tail cuff method with Multichannel 8000 (TSE, Germany) the day prior to sacrifice. On the day of the experiment, the rats were sacrificed by a blow on the head and exsanguination. Thoracic aorta was isolated and cleaned of adhering fat and connective tissue. Each artery was cut into rings of 2-3 mm width, with extreme care to preserve endothelium intact. The preparations were suspended between wire hooks located in an tissue bath containing 20 ml of Kreb's buffer solution bubbled with mixed gas (95 % O2, 5 % CO_2) and maintained at 37°C. Isometric contractile activity was measured with a force displacement transducer (Grass FT03) and displayed on a chart recorder (Multicorder MC 6625, Hugo Sachs Electronik, Germany). The preparations were allowed to equilibrate for 60 minutes under the resting tension of 2 gram. After the preparations reached submaximal contraction with 10^{-7} M norepinephrine (NE), washed out 3 times for 45 minute, and rechallenged with NE to get the optimal condition of the length-tension relationship. After reaching the plateau with NE, acetylcholine (10^{-10}-10^{-5} M), histamine (10^{-8}-10^{-3} M) and sodium nitroprusside (10^{-10}-10^{-6} M) were added to the each tissue bath. In separate experiments, each aortic preparations were pretreated with indomethacin (10^{-5} M), cyclooxygenase inhibitor, and L-NAME (10^{-5} M), nitric oxide synthase inhibitor (Rees *et al.*, 1990) 30 minute prior to the addition of NE. The results are expressed as the percent of decrease in tension of the NE contraction.

Statistical analysis

Data were expressed as mean±S.E.M. The difference between groups were evaluated by unpaired Student's t test, with $p<0.05$ to be considered significant statistically.

RESULTS

The blood pressure and heart rate were measured in all rats 5 hours after the administration of drugs prior to sacrifice. There was no significant change of systolic blood pressure in the groups treated with losartan, KR-30988, captopril and normotensive control groups (Table I). Also, the systolic blood pressure was not decreased 1 hour after the administration of drugs (data not shown). Heart rate and body weight did not differ significantly among the four groups.

In aortic preparations acetylcholine (ACh) caused a dose-dependent decrease in tension precontracted with NE (10^{-7} M) in all groups (Fig. 1). ACh relaxed the aortic preparations maximally at the concentration of 10^{-5} M in all groups, and the maximal effects of losartan,

Table 1. Effects of subchronic treatment with losartan, KR-30988 and captopril on systolic blood pressure, heart rate and body weight in normotensive rats

Group	n	Dose (mg/kg, b.i.d., p.o.,two weeks)	Systolic blood pressure (mmHg)	Heart rate (beats/min)	Body weight (g)
Control	10	-	141±6	375±8	378±8
KR-30988	6	50	146±3	377±20	371±5
Losartan	10	50	139±5	358±17	374±8
Captopril	10	50	145±3	377±10	376±10

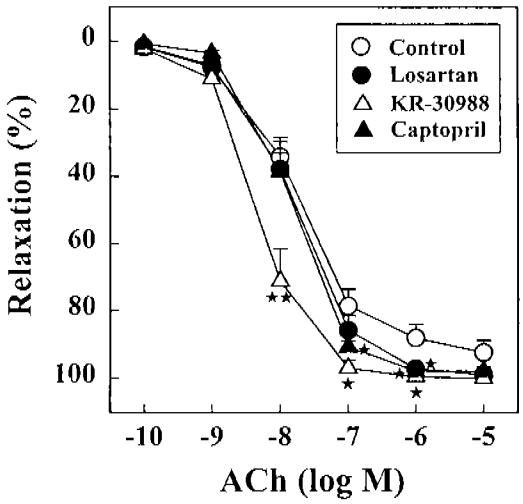

Fig. 1. Acetylcholine-induced relaxation precontracted with norepinephrine (10^{-7} M) in thoracic aortic preparations from normotensive rats subchronically treated with losartan, KR-30988 and captopril (50 mg/kg, b.i.d., p.o., two weeks). Data are expressed as mean±S.E.M. from 6-10 rats. *p<0.05, **p<0.01; Significantly different from control

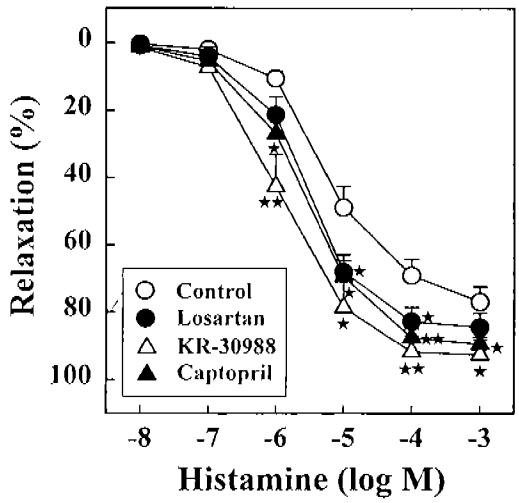

Fig. 2. Histamine-induced relaxation precontracted with norepinephrine (10^{-7} M) in thoracic aortic preparations from normotensive rats subchronically treated with losartan, KR-30988 and captopril (50 mg/kg, b.i.d., p.o., two weeks). Data are expressed as mean±S.E.M. from 6-10 rats. *p<0.05, **p<0.01; Significantly different from control

KR-30988 and captopril were slightly increased with no significance compared to control group. At the concentration of 10^{-8} M, ACh produced a decrease in tension of aortic preparations by 71.2±9.8% in the KR-30988 treated group (p<0.01 vs control), but only 34.1±5.7% in the control group. The 10^{-7} M of ACh relaxed the aortic preparations by 96.9±2.3% in the KR-30988, 90.8±1.9% in the captopril and 78.6±5.0% in the control group (both KR-30988 and captopril groups p<0.05 vs control). In all drug-treated groups, ACh (10^{-6} M) -induced relaxation was significantly increased (p<0.01) compared to that of control group. Histamine produced a dose-dependent relaxation of the aortic preparation precontracted with NE (10^{-7} M) in all groups (Fig. 2). The maximal relaxation by histamine was reached with the concentration of 10^{-3} M in all groups, and the maximal effects with losartan, KR-30988 and captopril were 84.5±4.2, 92.7±4.5 and 89.5±2.1%, respectively, with significant increase compared to control group (77.0±4.6 %). The 10^{-6} M of histamine produced relaxation of the aortic preparations to a 10.5±2.0% in control group, 21.3±5.5% in losartan, 42.6±9.5% (p<0.01) in KR-30988 and 26.9±6.4% (p<0.05) in captopril. At 10^{-5} M of histamine, the effects of losartan, KR-30988 and captopril on histamine-induced relaxation were 68.0±5.1%, 78.8±10.2% and 69.5±4.9%, respectively (all groups p<0.05 vs control group of 48.9±6.2%). The pD2 of KR-30988 for ACh-induced relaxation was 8.33±0.16, significantly (p<0.05) higher than that of control group (7.71±0.15), although losartan and captopril did not significantly differ with control group (Table 2). The pD2 for histamine-induced relaxation in losartan, KR-30988 and captopril were 5.57±0.10, 5.85±0.21 and 5.60±0.01, respectively,

Table 2. The pD_2 values for acetylcholine (ACh), histamine (His) and sodium nitroprusside (SNP)-induced relaxation precontracted with norepinephrine (10^{-7} M) in thoracic aortic preparations from normotensive rats subchronically treated with losartan, KR-30988 and captopril (50 mg/kg, b.i.d., p.o., two weeks). Data are expressed as mean+S.E.M. from 6-10 rats (mean±S.E.M.).

Group	ACh	His	SNP
Control	7.71±0.15	5.13±0.09	8.08±0.13
KR-30988	8.33±0.16*	5.85±0.21**	8.13±0.12
Losartan	7.79±0.14	5.57±0.10**	8.38±0.13
Captopril	7.83±0.06	5.60±0.01**	8.42±0.15

*p<0.05, **p<0.01; Significantly different from respective control

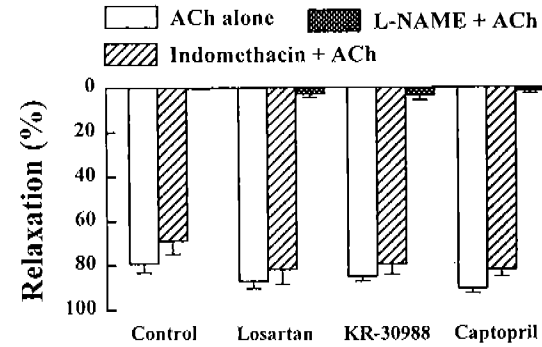

Fig. 3. Effects of indomethacin (10^{-5} M) and L-NAME (10^{-5} M) on acetylcholine (10^{-7} M)-induced relaxation precontracted with norepinephrine (10^{-7} M) in thoracic aortic preparations from normotensive rats subchronically treated with losartan, KR-30988 and captopril (50 mg/kg, b.i.d., p.o., two weeks). Data are expressed as mean±S.E.M. from 6 rats.

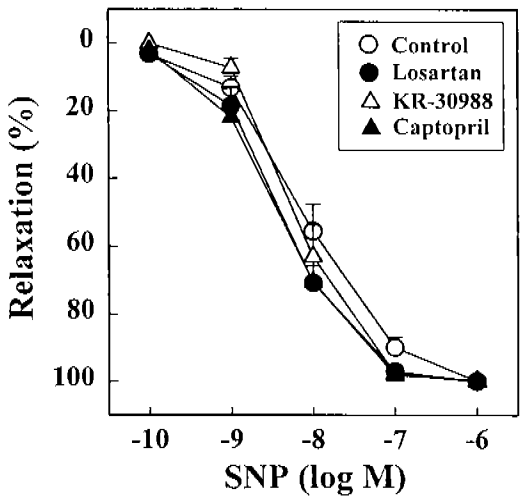

Fig. 4. Sodium nitroprusside (SNP)-induced relaxation precontracted with norepinephrine (10^{-7} M) in thoracic aortic preparations from normotensive rats subchronically treated with losartan, KR-30988 and captopril (50 mg/kg, b.i.d., p.o., two weeks). Data are expressed as mean±S.E.M. from 6-10 rats.

with significant differences in all groups ($p<0.01$) compared to that of control group (5.13±0.09).

In separate experiments, indomethacin (10^{-5} M) had no effect on the relaxation induced by 10^{-7} M ACh on aortic preparations in all groups (Fig. 3). L-NAME completely blocked ACh-induced relaxations in all groups (Fig. 3). In order to examine the endothelium-independent relaxations, sodium nitroprusside (SNP) was used in this experiments. SNP produced a dose-dependent relaxation of the tension in all drug-treated groups (Fig. 4). In all groups, SNP-induced relaxations were increased slightly, but not significantly changed by the drugs tested compared to control group.

DISCUSSION

Losartan has been well known as a nonpeptidic angiotensin AT_1 receptor antagonist (Timmermans *et al.*, 1993; Wong *et al.*, 1991; DeGraaf *et al.*, 1993; Fenoy *et al.*, 1991). KR-30988 has also been characterized as a potent, orally active and highly selective AT_1 receptor antagonist (unpublished data), which reduces arterial pressure in renin-dependent hypertensive rats (Lee and Shin, 1994). The antihypertensive effect of losartan was attenuated by pretreatment with L-NAME in renal hypertensive rats (Guan *et al.*, 1996) and dogs (Sudhir *et al.*, 1993), suggesting a contribution of nitric oxide on the blood pressure reduction by interrupting the renin-angiotensin system in renin-dependent hypertension. Moreover, vasoconstrictive response of L-NAME was attenuated in kidney of rat pretreated with losartan (Ohishi *et al.*, 1992), suggesting a contribution of losartan on the vasoconstriction by inhibiting of nitric oxide. Although there are much information about the interaction of nitric oxide with the renin-angiotensin system, little has been known whether losartan itself may influence or modulate nitric oxide derived function in normotensive rats.

To investigate the correlation between the nonpeptidic AT1 receptor antagonists and the function of endothelium, each 50 mg/kg (b.i.d.) of losartan and KR-30988 was administered orally for 2 weeks in normotensive rats. The blood pressure, heart rate and body weight were not significantly changed by the administration of drugs compared to control group, however, blood pressure was significantly decreased by all drugs tested in this experiments in renal hypertensive rats (unpublished data). ACh caused a dose-dependent relaxation in aortic preparations precontracted with NE in all groups. Compared to control, Losartan and KR-30988 had significantly enhanced endothelium-dependent relaxations in response to ACh at the concentration of 10^{-6} M, and 10^{-8}-10^{-6} M, respectively. Similary, captopril significantly increased it at 10^{-7}-10^{-6} M of ACh. The effect of KR-30988 on ACh-induced relaxations was stronger than those of losartan and captopril (The potency order was KR-30988>captopril>losartan). This order is not agreed with the antihypertensive effects of those drugs; the order of blood pressure lowering effect was captopril>KR-30988>losartan in renin- dependent hypertensive rats (Lee and Shin, 1994). The effect of captopril on ACh-induced relaxation was smaller than that in previous report (Schultz and Raij, 1989), which could be due to different administration. Because the effect of drug may not be persisted, if administered twice a day, compared to the continuous administration with tap water. Histamine-induced relaxation in aortic preparation (10^{-6}-10^{-3} M) was significantly enhanced by losartan, KR-30988 and captopril. The potency order on histamine-induced relaxation in aortic preparation was KR-30988>captopil=losartan. These results suggest that chronic treatment of AT1 receptor antagonist in normotensive rats may facilitate the endothelium-dependent relaxation, which indirectly supported by Sigmon *et al.* (Sigmon *et al.*, 1992) suggested that the inhibition of systemic nitric oxide systhesis with L-NAME resulted in the decreased renal blood flow in anesthetized rats, which can be reversed by losartan.

Angiotensin converting enzyme inhibition with captopril or cilazapril can remarkably improve the endothelial function in spontaneous hypertensive rats (Clozel, 1991) and essential hypertensive patients (Schiffrin, 1995). The mechanism of such effect could be associated with scavenging of oxygen-derived free radicals by sulphydryl group of captopril (Shultz and Raij, 1989), increasing the release or action of nitic oxide (Clozel, 1991) and facilitating the release of

prostacyclin (Liao and Chen, 1992). In this experiment, however, the relaxation by ACh in aortic preparations from all rats was not affected by indomethacin (10^{-5} M), suggesting that the product of cyclooxygenase (prostacyclin) was not responsible for modulating this relaxation. In contrast, L-NAME (10^{-5} M) completely abolished the ACh-induced relaxation of aortic preparations of all groups. These results suggest that the facilitation of the endothelium-dependent relaxation by chronic treatment with losartan could be due to the increased action of nitric oxide. The mechanism that losartan and KR-30988 enhance relaxation mediated by nitric oxide is not clear. It could be due to the increased synthesis or release of nitric oxide, a decreased destruction of nitric oxide, or the facilitated effect of nitric oxide upon the vascular smooth muscle. SNP produced a dose-dependent relaxation in aortic preparation. However, all drugs used in this experiments did not significantly enhance the relaxation with SNP at all doses used in this experiments, suggesting that AT_1 receptor antagonist does not facilitate the endothelium-independent relaxation in aortic preparations from normotensive rats. Conclusively, AT_1 receptor antagonists, losartan and KR-30988, may enhance the endothelium-dependent relaxation through release or increase sensitivity of nitric oxide in normotensive rats.

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