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Losartan Modifies Nitric Oxide-related Vasorelaxation in Isolated Aorta of Spontaneously Hypertensive Rat

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ABSTRACT

It is well known that angiotensin converting enzyme inhibitors(ACEIs) increase endothelium-dependent relaxation in aortic strips of spontaneously hypertensive rats(SHR) and this increase in relaxation may be due to altered endothelial nitric oxide breakdown. But there are few studies on the effect of the angiotensin II receptor blocker on the nitric oxide-mediated relaxation. So we attempted to investigate the effect of angiotensin II receptor blocker on the nitric oxide-dependent relaxation in isolated aorta of SHR.

Two week-treatment of losartan (30 mg/kg/day) increased the acetylcholine(10^{-9} to 10^{-5} M)-and histamine(10^{-8} to 10^{-4} M)-induced relaxation in endothelium intact strips but 90 minutes-treatment of losartan (10^{-4} M) showed no increase in relaxation. The phenylephrine (10^{-7} M)-induced contraction, repeated every 2 hours, was diminished gradually following lipopolysaccharide (LPS)-treatment (100 μg/ml) but there was no significant difference in enalapril- and losartan-treated group compared with control group.

These results suggest that activity of the endothelial constitutive NO synthase may be changed by chronic treatment of angiotensin II receptor blockers and ACEIs but angiotensin II antagonist and ACEI have no effect on the inducible NO synthase activity in the isolated aorta of SHR

Key Words: Nitric oxide, Losartan, SHR, Lipopolysaccharide, LNMMA, Aorta

INTRODUCTION

Nitric oxide (NO) synthesized from L-arginine by the vascular endothelium plays a role in the physiological regulation of blood flow and blood pressure (Moncada, 1992). In this vascular strips and other tissues, such as the brain, adrenal gland and platelets, NO is produced by a constitutive synthase which is Ca^{2+} and NADPH-dependent (Knowles *et al.*, 1989; Moncada, 1992; Radomski *et al.*, 1990). However, in macrophages, neutrophils, Kupffer cells and hepatocytes (Knowles *et al.*, 1990; Marletta *et al.*, 1988; Moncada, 1992; Rees *et al.*, 1990) NO is produced by an inducible enzyme which is Ca^{2+}-independent, and is induced by endotoxin (LPS) and cytokines. Excessive production of immunologically derived NO may be responsible for the hypotension and the reduced responsiveness to pressor agents (Beasley *et al.*, 1991; Mckenna, 1990; Parratt, 1976; Rees *et al.*, 1990) which is characteristic of endotoxic shock, Rees(1990) have shown that the vascular endothelium expressed an inducible, Ca^{2+}-independent NO synthase by LPS and interferon (IFN)-gamma in vitro and that vascular smooth muscle also expressed after administration of LPS in vivo.

Hypertension is associated with an endothelial

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dysfunction characterized by an imbalance between endothelium-dependent contraction and endothelium-dependent relaxation. In the aorta of spontaneously hypertensive rats(SHR), but not of normotensive Wistar-Kyoto rats, acetylcholine and serotonin may elicit endothelium-dependent contractions, and their endothelium-dependent vasodilating effect is depressed (Koga *et al.*, 1989; Luscher and Vanhoutte, 1986). Angiotensin converting enzyme inhibitors(ACEI) such as cilazapril and captopril markedly increased endothelium-dependent relaxation by acetylcholine and decreased endothelium-dependent contraction by serotonin (Clozel, 1991; Goldschmidt and Tallarida, 1991; Wiemer, 1991). Clozel and colleagues (1991) had shown that, in large vessels of SHR, endothelial dysfunction associated with the presence of subendothelial monocyte-macrophages was prevented by ACEI.

The present study was designed to investigate the effect of specific angiotensin receptor blocking agent, losartan (Chiu *et al.*, 1991; Fontoura *et al.*, 1991; Wong *et al.*, 1991), on regulation of nitric oxide synthase activity, especially whether could change nitric oxide-dependent relaxation in isolated aorta of SHR.

MATERIALS AND METHODS

Animals

Male SHR(18-week-old) were housed in a room with a 12-hr light/dark cycle. Standard rat chow diet and tap water were provided *ad libitum*.

Treatment groups: SHR were divided into three groups; control, losartan- and enalapril-treated group. The rats were treated with losartan (30 mg/kg/day) and enalapril (10 mg/kg/day) for two weeks.

Evaluation of nitric oxide mediated relaxation in isolated aorta

After checking the blood pressure and heart rate by Grass 79D polygraph connected with a Gould pressure transducer, the rats were sacrificed and the thoracic aortae were removed and placed in ice-cold modified Krebs' solution. Other experimental procedure was similar to that previously reported from our laboratory (Shim, 1990). The contracting responses were generated by phenylephrine (10^{-7} M) and relaxing responses to acetylcholine(10^{-9} to $^{-5}$) and histamine (10^{-8} to 10^{-4}) were determined after contraction of aorta. To induce inducible nitric oxide synthase lipopolysaccharide (LPS, 100 μg/ml) was added into the modified Krebs' solution during the experiment. To verify the relaxing responses mediated by nitric oxide N^G-monomethyl L-arginine (LNMMA, 100 μM), nitric oxide synthase specific inhibitor, was used.

Chemicals

Drugs were purchased from the Sigma Chemical Company. Losartan was kindly donated by DuPont-Merck Pharmaceuticals.

Statistical evaluation

Data were analyzed by using t-test. A P value of 0.05 or less was considered significant.

RESULTS

Effect of losartan and enalapril on the blood pressure and heart rate

A significant decrease in blood pressure was observed after two weeks of losartan and enalapril treatment in SHR (Table 1).

Effect of losartan and enalapril on the constitutive nitric oxide mediated relaxation

Endothelium-dependent relaxation induced by

Table 1. Systolic and diastolic blood pressure (SBP and DBP) and heart rate(HR) of control, losartan-treated (30 mg/kg/day, 2 weeks), and enalapril-treated (10 mg/kg/day, 2 weeks) group

	SBP (mmHg)	DBP (mmHg)	HR (beats/min)
Control (n=24)	230±6	178±4	429±10
Losartan (n=21)	168±5*	141±6*	424±18
Enalapril (n=15)	170±7*	132±8*	431±13

All values are represented as mean ±S.E.*, P<0.05 vs control.

acetylcholine and histamine was significantly enhanced in SHR treated with losartan and enalapril for two weeks (Fig. 1). But 90 minutes-in vitro treatment of losartan did not show any change in relaxation by acetylcholine and histamine (Fig. 2). LNMMA treatment inhibited the relaxation by acetylcholine and histamine in two weeks-treatment with losartan and enalapril (Table 2).

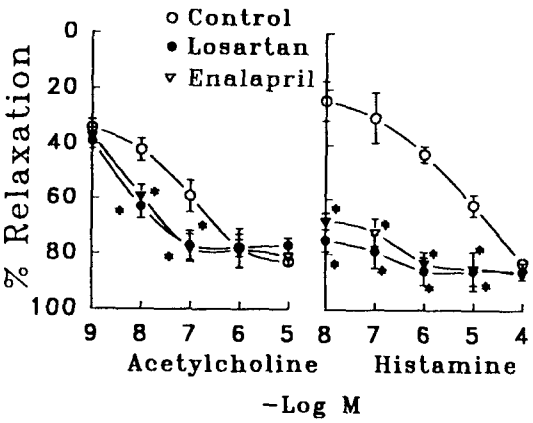

Fig. 1. Relaxations induced by acetylcholine(10^{-9} to 10^{-5} M) and histamine(10^{-8} to 10^{-4} M) were compared in control (n=16), losartam (30 mg/kg/day, 2 weeks)-treated (n=15) and enalapril-treated (n=11) group with intact endothelium. *, P<0.05 vs control.

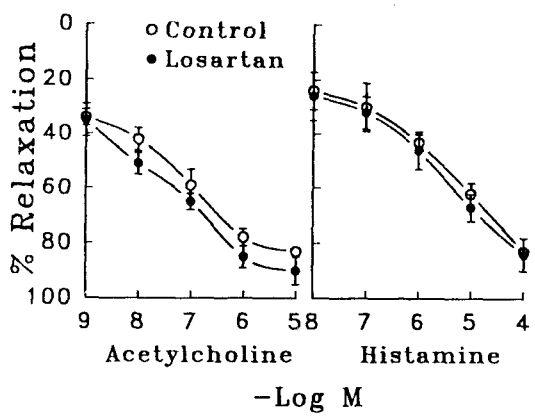

Fig. 2. Relaxations induced by acetylcholine(10^{-9} to 10^{-5} M) and histamine (10^{-8} to 10^{-4} M) were compared between control (n=16) and losartan (10^{-4} M, 90 min)-treated (n=15) group with intact endothelium.

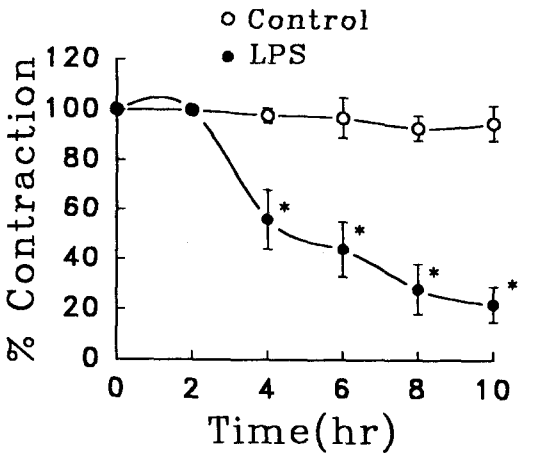

Fig. 3. Phenylephrine (10^{-7} M)-induced contractions, repeated every 2 hour, were compared between control (n=10) and LPS-treated (n=16) group in endothelium denuded aorta. *, P<0.05 vs control.

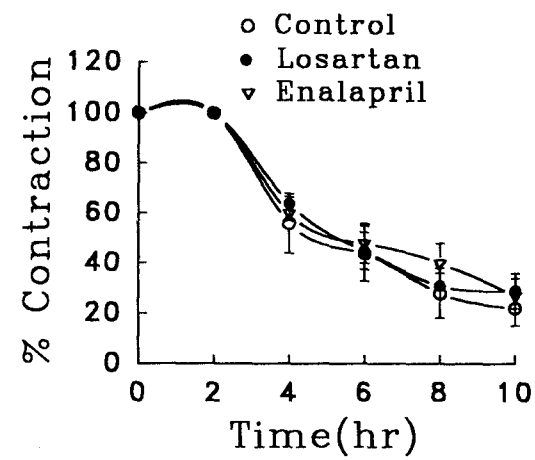

Fig. 4. Phenylephrine (10^{-7} M)-induced contractions, repeated every 2 hour, were compared among control (n=16), losartan-treated (n=15) and enalapril-treated (n=11) group in endothelium denuded aorta following LPS treatment.

Table 2. Acetylcholine-induced relaxation and phenylephrine-induced contraction were compared between treatment with LNMMA and not-treatment group in control, losartan-and enalapril-treated cases

	Relaxation(%)		Contracion(%)	
	LNMMA(−)	LNMMA(+)	(LNMMA(−)	LNMMA(+)
Control (n=16)	83±3	7±2*	24±9	89±5*
Losartan (n=15)	77±5	5±6*	39±8	87±8*
Enalapril (n=11)	80±3	6±7*	28±7	90±7*

Relaxation was evaluated in endothelium intact aortae and contraction in denuded aortae. All values are represented as mean ±S.E. *, $P<0.05$, vs LNMMA(−).

Table 3. Acetylcholine-or histamine-induced relaxation were compared before and after LPS treatment in endothelium intact aorta

	Relaxation(%)				
Acetylcoline(n=7)	−9	−8	−7	−6	−5
Before LPS	32 ± 7	41 ± 5	59 ± 4	79 ± 7	83 ± 4
After LPS	31 ± 6	44 ± 7	55 ± 4	77 ± 3	81 ± 2
Histamine (n=8)	−8	−7	−6	−5	−4
Before LPS	29±5	34±6	46±5	67±4	85±3
After LPS	30±8	32±4	42±3	64±5	83±1

All values are represented as mean±S.E. Concentration of acetylcholine and histamine are presented as log[M].

Effect of losartan and enalapril on the inducible nitric oxide mediated relaxation

Phenylephrine-induced contraction of isolated aorta reached to a plateau at 20 minutes after addition and was sustained. By treatment with LPS in denuded aorta, contraction induced by phenylephrine gradually decreased to about 20% at 10 hour (Fig. 3). After LNMMA treatment the decreased phenylephrine-induced contraction was restored (Table 2). Acetylcholine- and histamine-induced relaxations were not changed by the LPS treatment in endothelium intact aorta (Table 3). The decreased contractility by LPS treatment was not affected by two-week treatment of enalapril and losartan (Fig. 4).

DISCUSSION

It is generally accepted that structural and functional alterations in the vasculature contribute significantly to the elevation in peripheral vascular resistance in the established phase of hypertension(Folkow, 1982). The significant lowering of blood pressure to normotensive levels in SHR with losartan is not surprising in light of previous findings on the acute (Timmermans *et al.*, 1991; Wong *et al.*, 1990) and chronic (Mizuno *et al.*, 1992; Wong *et al.*, 1991) antihypertensive effect of losartan. What is particularly interesting, and of most importance in our study, is the significant endothelium-dependent relaxation in aortic strips of SHR treated with losartan for two weeks. It is well known that vasodilation of angiotensin converting enzyme inhibitors (ACEIs) in hypertensives is due to prevention of the formation of angiotensin II and protection of bradykinin from breakdown, which increase the amount of nitric oxide. In our study angiotensin II receptor blockade itself also enhanced endothelium-dependent vasodilation induced by acetylcholine and histamine which can be inhibited by LNMMA, inhibi-

tor of the synthesis of nitric oxide from L-arginine. These results suggest the possibility that angiotensin can act on the constitutive nitric oxide synthesis or metabolism directly, which may be affected by ACEI through functional chagne of endothelium.

Aortic contraction with phenylephrine was inhibited by LPS. The maximum inhibition at 10 hour is significantly restored by LNMMA. These observations indicated that the decrease in phenylephrine-induced contraction by LPS is due to formation of NO. Since these effects are independent of the presence of the endothelium, it is likely that the vascular smooth muscle itself is the major site of NO formation. Losartan and enalapril treatment had no effect on the LPS-induced relaxation and acetylcholine- and histamine-induced relaxation was not affected by LPS. These results leads to possibility that angiotensin system doesn't act directly on the inducible nitric oxide synthesis and its relaxation.

In summary, these results demonstrate the effectiveness of losartan for established hypertension in SHR and suggest that the effect may result from constitutive nitric oxide by losartan through significant functional alterations in constitutive nitric oxide.

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=국문초록=

선천성 고혈압흰쥐 적출대동맥에서 Nitric Oxide와 관련된 이완 반응에 Losartan이 미치는 영향

경북대학교 의과대학 약리학교실

박 봉 기·한 형 수·김 증 영

선천성고혈압흰쥐 (SHR)에서 angiotensin converting enzyme inhibitor (ACEI)를 처치하면 내피세포 의존적 이완이 증진된다고 알려져 있다. 본 실험은 angiotensin II가 nitric oxide (NO)와 관련되어 일어나는 적출 대동맥의 이완력에 변화를 주는지 관찰하고자 angiotensin II 작용 억제를 위해 angiotensin II 수용체 차단제인 losartan과 ACEI인 enalapril을 사용하였으며 혈관에서의 NO는 혈관내피세포에서 생성되는 constitutive NO와 주로 혈관 평활근에서 LPS에 생성되는 inducible NO가 있으므로 이들 양자에 대한 angiotensin II의 작용을 검토하였다. 2주간 losartan (30 mg/kg/day)과 enalapril (10 mg/kg/day)을 처치한 경우 acetylcholine (10^{-9} to 10^{-5} M)과 histamine (10^{-8} to 10^{-4} M)에 의한 이완 반응이 증가되었으나 90분간 적출 대동맥에 losartan (10^{-4} M)을 노출시킨 경우는 이완 반응에 변화가 없었다. Phenylephrine (10^{-7} M)을 2시간 간격으로 반복 투여하여 수축시킨 경우 LPS (100 μg/ml)처치에 의해 시간이 지남에 따라 수축력이 감소되었고 대조군에서는 수축력이 감소되지 않았다. LPS 처치에 따른 phenylephrine에 의한 수축력의 감소는 enalapril이나 losartan을 2주간 처치한 경우에도 영향을 받지 않았다. 이상의 결과로 미루어 아마도 losartan의 내피세포에 대한 작용은 constitutive NO 생성을 증가시키나 inducible NO생성에는 영향을 미치지 않을 것으로 여겨진다.