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Prevention of intimal thickening after endothelial removal by a nonpeptide angiotensin II receptor antagonist, losartan

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1 The present experiments were designed to investigate the role of local angiotensin II receptors in the myointimal proliferative response of the vascular wall after endothelial removal, by use of a novel, nonpeptide, angiotensin II receptor antagonist, losartan.

2 When administered 1 week before endothelial removal from the rabbit carotid artery and then continuously until animals were killed 6 weeks later, losartan in a dose of 10 mg kg^{-1} daily, p.o. had no significant effects on the carotid blood flow (CBF), mean arterial blood pressure (MBP) and heart rate (HR).

3 A full endothelial lining with increased density of regenerated endothelial cells was observed 6 weeks after the endothelial removal. These changes were unaffected by treatment with losartan.

4 Six weeks after endothelial removal, acetylcholine (ACh)- and adenosine diphosphate (ADP)-induced relaxations were greatly reduced though endothelial cells had regenerated. The reduction of the relaxations to these agonists were significantly restored by chronic treatment with losartan. The endothelial-independent, sodium nitroprusside (SNP)-induced relaxation remained unaffected in all groups.

5 There were no differences in the noradrenaline (NA)- and endothelin-1 (ET-1)-induced contractions of the carotid artery strips between vehicle and losartan-treated groups. In contrast, the contractile response of the strips to angiotensin II was significantly decreased in the losartan group, indicating the specific antagonism by chronic losartan against the angiotensin II receptor.

6 Six weeks after endothelial removal, marked myointimal proliferation resulting from new accumulation of proliferating smooth muscle cells and connective tissue was observed in the vehicle group. Losartan treatment greatly suppressed the myointimal proliferative response.

7 These results suggest that the local angiotensin II receptors play a role in the myointimal proliferative response of the vascular wall to removal of the endothelium.

Keywords: Intimal thickening; endothelial removal; nonpeptide angiotensin II receptor antagonist; losartan; endothelial-derived relaxing factor (EDRF); regrowth of endothelial cells

Introduction

The mechanisms causing the myointimal proliferative response of the vascular wall to removal of the endothelium are complex and poorly understood, but initially quiescent smooth muscle cells must be activated either directly by the injury or indirectly by a variety of factors. Recently, Powell *et al.* (1989) have reported that the local angiotensin system may participate in modulating the proliferation of the vascular wall after arterial injury, since inhibitors of angiotensin-converting enzyme prevent the myointimal proliferation. There has been evidence indicating that angiotensin-converting enzyme (Nakamura *et al.*, 1988), angiotensinogen mRNA (Cassis *et al.*, 1988) and angiotensin II receptors (Penit *et al.*, 1983) are involved in the vascular wall.

On the other hand, we have demonstrated that the myointimal proliferative response after endothelial removal may be brought about partly by the decreased release/production of the endothelium-derived relaxing factor (EDRF, Furchgott & Zawadzki, 1980) by the regenerated endothelial cells (Azuma *et al.*, 1990). According to Garg & Hassid (1989), the endogenous EDRF (nitric oxide, Palmer *et al.*, 1987) may function as an inhibitory modulator of vascular smooth muscle cell mitogenesis and proliferation. In addition, EDRF is an antiaggregating substance (Azuma *et al.*, 1986b; Furlong *et al.*, 1987; Radomski *et al.*, 1987) which may also modulate the release of platelet-derived growth factor.

The present experiments were designed to investigate the role of local angiotensin II receptors and endothelial cells in the vascular response to endothelial removal by use of a newly developed, orally active, nonpeptide angiotensin II receptor antagonist, losartan {2-n-butyl-4-chloro-1-[(2′-(tetrazol-5-yl)-1,1′-biphenyl-4-yl)methyl]-1H-imidazole-5-methanol, potassium salt} (Chiu *et al.*, 1990; Wong *et al.*, 1990). The agent was chronically administered p.o. to rabbits in which the right carotid artery was subjected to removal of the endothelium.

Methods

Animals

Japanese White male rabbits, 10 weeks of age, were used. These rabbits were purchased at 8 weeks of age and housed individually for 2 weeks before the experiments in a temperature (23 ± 1°C)- and humidity (50 ± 20%)-controlled room and were fed regular chow (CE-2, Japan Clea) throughout the experimental periods.

Seventeen rabbits were divided randomly into two groups consisting of 8 and 9. The body weight changes (Table 1) and general behaviour of all rabbits before and after the endothelial removal and during the administration of losartan appeared to be normal.

Administration of losartan

Losartan was dissolved in distilled water at a concentration of 0.05 mg ml^{-1} and given as a drinking water in a dose of

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10 mg 200 ml^{-1} kg^{-1} daily for 7 weeks (1 week before and 6 weeks after the endothelial removal). Losartan did not influence drinking behaviour; 200 ml kg^{-1} of water was sufficient for intake of the drug and was completely consumed in a day. Rabbits given distilled water without losartan served as controls.

Endothelial removal of the carotid artery

Rabbits were anaesthetized with 25 mg kg^{-1}, i.v. of sodium pentobarbitone. An arterial embolectomy catheter (12-040-3F/40 cm/3F, American Edwards Laboratories, Santa Ana, California, U.S.A.) was inserted into the right common carotid artery through the incision made on the vessel wall, as described elsewhere (Azuma *et al.*, 1990). The intraluminal surface of the artery was then gently rubbed by means of one stroke of a balloon (filled with 0.15 ml air and approximately 2.5 mm in diameter) so as not to damage underlying smooth muscle cells and other tissues. Complete denudation of the endothelium was achieved throughout the common carotid artery as assessed by the morphological examination (scanning electron microscopy) of random controls (4 rabbits) at 2 h after the procedure. For the left carotid artery, a sham operation without the balloon was carried out and the artery of this side served as a control.

Measurement of carotid blood flow (CBF), mean arterial blood pressure (MBP) and heart rate (HR)

At 6 weeks after endothelial removal, rabbits were anaesthetized with sodium pentobarbitone (25 mg kg^{-1}, i.v.). After a midline incision in the ventral cervical region, the common carotid arteries of both sides were exposed; special care was taken not to disturb the vascular wall and the CBF was measured by means of a FR-015T probe (Nihon Kohden Kogyo Co., Tokyo) and an electromagnetic flow meter (MFV-1200, Nihon Kohden Kogyo Co.). In order to measure MBP, the right femoral artery was exposed and cannulated with polyethylene tubing filled with saline containing heparin. The other end of the tubing was connected to a pressure transducer (TP-400T, Nihon Kohden Kogyo Co.). MBP was recorded on a pen-writing oscillograph (CP-642G, Nihon Kohden Kogyo Co.) via an amplifier (AP-620G, Nihon Kohden Kogyo Co.). HR was measured with an ECG pulse counter (AC-611G, Nihon Kohden Kogyo Co.) and bioelectric amplifier (AB-620G, Nihon Kohden Kogyo Co.).

Organ chamber experiments

After measurement of CBF, MBP and HR, rabbits were exsanguinated from the femoral arteries. The common carotid arteries were rapidly excised and kept in modified Krebs solution. After removal of fat and connective tissue, a 2-mm wide transverse ring was cut off with a razor blade, and a transverse strip was made and mounted vertically in an organ bath containing 20 ml of modified Krebs solution continuously bubbled with 95% O_2 and 5% CO_2 at 37°C. Special care was taken to avoid unintentional rubbing of the intraluminal surface. Mechanical responses were measured according to the method described previously (Azuma *et al.*, 1986a,b; 1989; 1990). The composition of modified Krebs solution was as follows (mM): NaCl 115.0, KCl 4.7, $MgSO_4 \cdot 7H_2O$ 1.2, $CaCl_2 \cdot 2H_2O$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25.0 and glucose 10.0. After 60 min of equilibration, 10^{-6} M ACh was given to all strips during contraction caused by 10^{-6} M NA to test for the presence or absence of the functional endothelial cells. After this, relaxations to ACh ($10^{-8} \sim 10^{-5}$ M), ADP ($3 \times 10^{-8} \sim 10^{-5}$ M) and sodium nitroprusside (SNP, $10^{-9} \sim 3 \times 10^{-5}$ M) were examined during a contraction caused by 10^{-6} M NA as follows: In groups a and c, responses of the control left and the previously denuded right carotid arteries prepared from rabbits given vehicle were tested, respectively. In groups b and d, responses of the control left and the previously denuded right carotid arteries prepared from rabbits given losartan were tested, respectively. Relaxations caused by ACh, ADP and SNP were given as a percentage of the 10^{-6} M NA-induced contraction. Contractions to NA ($10^{-9} \sim 3 \times 10^{-5}$ M), ET-1 ($10^{-10} \sim 3 \times 10^{-8}$ M) and angiotensin II ($10^{-10} \sim 10^{-7}$ M) in the quiescent left and the right carotid arteries prepared from rabbits which had been treated with vehicle or losartan were examined in different experiments. The E_{max} (the maximum response) and ED_{50} (the concentration producing 50% of the E_{max}) were obtained from the log concentration-response curves.

Electron microscopy

Scanning electron microscopic examinations were performed according to the methods described in a previous paper (Azuma *et al.*, 1990). The ultrastructure of the intraluminal surface was observed by means of a scanning electron microscope (ESM-3200, Elionix).

Light microscopy

Left and right carotid arteries were isolated and fixed in a 10% neutral solution of formaldehyde. The transverse specimen was embedded in paraffin after dehydration with ethanol. Thin sections were stained with haematoxylin-eosin (HE), Elastica-Van Gieson method (Weigert, 1898) or Sudan III for light microscopic analysis. The initimal thickness of the cross-sections was evaluated by measuring the area of intima and media on the picture at final magnification × 40 by means of a KP-90N planimeter (Uchida Co.). Results are expressed as a percentage of intimal area to that of medial one.

Chemicals

The following chemicals were used: acetylcholine chloride (ACh, Ovisot for injection, Daiichi Pharmaceutical Co.), indomethacin (Merck-Banyu), N^G-nitro-L-arginine (L-NNA), angiotensin II (human) and endothelin-1 (ET-1, human) (all from Protein Research Foundation), (−)-noradrenaline bitartrate (NA), adenosine 5′-diphosphate sodium salt (ADP), methylene blue and sodium nitroprusside (SNP) (all from Sigma), 2-n-butyl-4-chloro-1-[(2′-(tetrazol-5-yl)-1,1′-biphenyl-4-yl)methyl]-1H-imidazole-5-methanol, potassium salt (losartan, generous gift from Banyu Pharmaceutical Co.), which is a specific antagonist of angiotensin II receptors and is orally active (Chiu *et al.*, 1990; Wong *et al.*, 1990). All chemicals were dissolved in distilled water immediately before use except for indomethacin and L-NNA which were dissolved in dimethylsulphoxide (DMSO) and kept frozen at −20°C until use (10^{-2} M stock solution). DMSO was present in a final concentration of 0.5% in the experiments with these agents, and this concentration had no effect on any parameters tested.

Statistical analysis

All data are expressed as the mean ± s.e.mean. The statistical significance between two means was determined by Student's *t* test.

Results

Baseline data

Carotid blood flow (CBF) was not significantly different in the control left and the previously denuded right carotid arteries both in the vehicle- and the losartan-treated groups. The chronic administration of losartan in a dose of 10 mg kg^{-1} daily for 7 weeks did not affect the CBF in the arteries

of either sides. The mean arterial blood pressure (MBP) was slightly lower (approximately 10 mmHg) in the losartan group, but not significantly different from the vehicle group. There was no difference in the heart rate (HR) between the two groups. These results are shown in Table 1. Wong *et al.* (1990) have reported that a bolus oral dosing at 10 mg kg^{-1} of losartan produced marked inhibition of the angiotensin II pressor response in conscious normotensive rats, although it did not alter basal MBP and HR.

Electron microscopic findings

In the sham-operated left carotid arteries from both groups, scanning electron microscopy revealed that the endothelial cells were regularly arranged parallel to the direction of the blood stream. A representative micrograph is shown in Figure 1a. Six weeks after endothelial removal, a full endothelial lining on the intraluminal surface was observed in the previously denuded right carotid arteries both in vehicle- and losartan-treated groups. The shape, direction and type of junctions of the regenerated endothelial cells were, however, different from control arteries (Figure 1b and c).

The cell density was determined by means of scanning electron microscopy. Results are shown in Table 2. The density of regenerated endothelial cells in the right carotid artery was significantly increased compared with that of the corresponding controls, and was unaffected by the administration of losartan.

Influence of losartan on the relaxation responses

In the control left and the previously denuded right carotid artery strips, 10^{-6} M ACh and 10^{-6} M ADP produced relaxation in arteries precontracted with 10^{-6} M NA. Relaxation was abolished after deliberate denudation of the endothelial cells (complete endothelial removal without damage of the smooth muscle layer was confirmed morphologically), or greatly reduced by pretreatment with 10^{-5} M methylene blue and 10^{-5} M L-NNA, an inhibitor of EDRF synthesis (Kobayashi & Hattori, 1990), but unaffected by 10^{-5} M indomethacin (data not shown), indicating that the ACh- and ADP-induced relaxations are mediated by EDRF (Furchgott & Zawadzki, 1980), but not by prostaglandin I_2.

The concentration-dependent relaxation induced by ACh in the control left carotid artery of the vehicle-treated group did not significantly differ from that of losartan-treated group (Figure 2). The E_{max} values were estimated to be 74.4 ± 7.5% ($n = 6$) in the former and 81.8 ± 4.9% ($n = 7$) in the latter. In the right carotid artery of the vehicle-treated group 6 weeks after endothelial removal, the ACh-induced relaxation was greatly reduced although endothelial cells had regenerated. It should be noted that this reduced relaxation was significantly restored toward the control level by the chronic treatment with losartan. Similar results were obtained for ADP-induced relaxation (data not shown).

In contrast, the SNP-induced relaxation in arteries precontracted with NA remained unaffected by chronic losartan

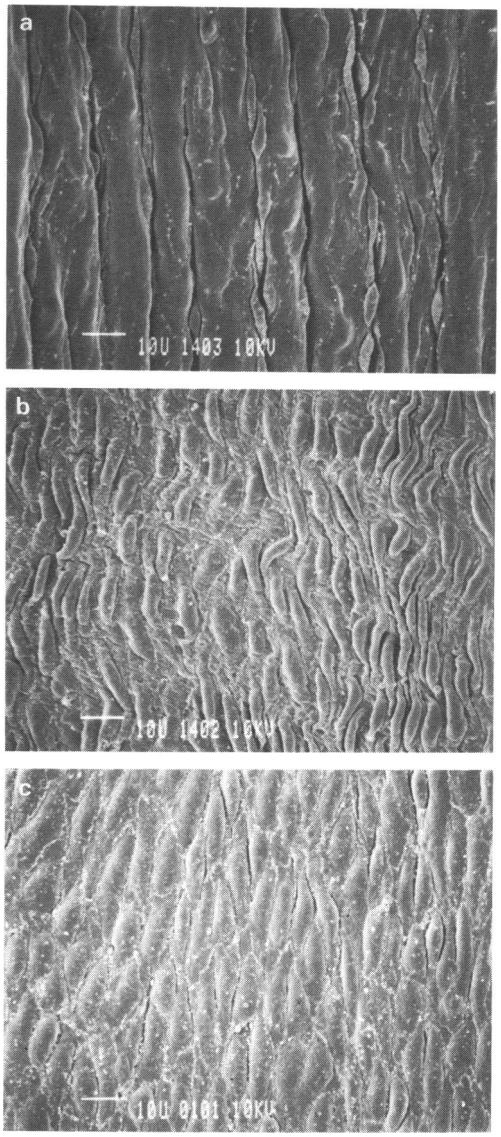

Figure 1 Scanning electron microscopic findings on the intraluminal surface of rabbit carotid arteries. (a) Normal appearance of endothelial cells in the sham-operated left carotid artery (vehicle group). The endothelial cells were regularly arranged, parallel to the direction of the blood stream. (b) (Vehicle group) and (c) (losartan group): 6 weeks after endothelial removal, the luminal surface was entirely covered with the regenerated endothelial cells, which were morphologically different from native ones and irregularly oriented. Scale bar: 10 μm.

Table 1 Baseline data on body weight (BW), carotid blood flow (CBF), mean arterial blood pressure (MBP) and heart rate (HR) of rabbits

	BW (kg)		*CBF* (ml min^{-1})			
Treatment	*1w before surgery*	*6w after surgery*	*Control side*	*Denuded side*	*MBP* (mmHg)	*HR* (beats min^{-1})
Vehicle	1.82 ± 0.08 (8)	2.61 ± 0.06 (6)	23.9 ± 2.8 (6)	20.5 ± 2.3 (6)	114.7 ± 7.0 (6)	276 ± 13 (5)
Losartan	1.85 ± 0.08 (9)	2.60 ± 0.05 (7)	21.9 ± 1.0 (6)	21.2 ± 2.2 (6)	104.0 ± 3.4 (6)	279 ± 26 (5)

Results are given as mean ± s.e.mean. Losartan was dissolved in distilled water and given as a drinking water at a dose of 10 mg 200 ml^{-1} kg^{-1} daily for 7 weeks (1 week before and 6 weeks after endothelial removal) (see text). Measurements of CBF, MBP and HR were performed under sodium pentobarbitone anaesthesia 6 weeks after the endothelial removal (immediately before the rabbits were killed) (see text). Figures in parentheses indicate the number of rabbits. In order to assess morphologically the completeness of endothelial removal, 2 rabbits each in vehicle and losartan groups were killed 2 h after the operation at week 0.

Table 2 Influence of losartan on regeneration of endothelial cells

Vessel	Treatment	Number of endothelial cells ($\times 10^3$ mm^{-2})	
Control artery	Vehicle	8.5 ± 0.7 (5)	***
	Losartan	8.1 ± 0.8 (7)	
Denuded artery	Vehicle	12.5 ± 0.2 (5)	**
	Losartan	12.0 ± 0.5 (5)	

Number of endothelial cells was determined by means of scanning electron microscopy 6 weeks after removal of endothelium from the rabbit carotid artery (see text). Six weeks after the endothelial removal, the luminal surface was entirely covered with regenerated endothelium. Results are given as mean ± s.e.mean.
*$P<0.01$ and **$P<0.005$. Figures in parentheses indicate the number of rabbits.

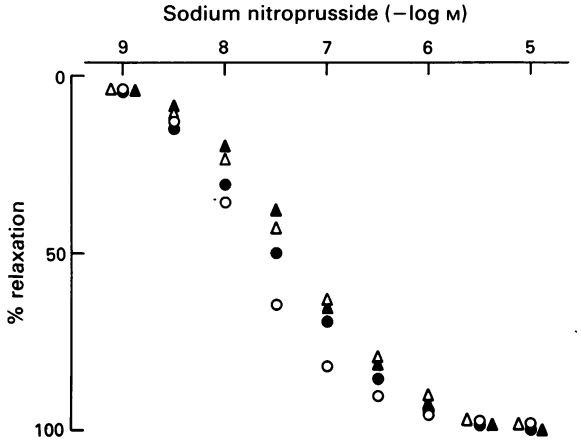

Figure 3 Comparison of endothelium-independent relaxation response of control left (△, ▲) and the prevously denuded right carotid arteries (○, ●) to sodium nitroprusside between vehicle (open symbols) and losartan (closed symbols) groups. Each point represents the mean of 5 to 7 experiments. Relaxation is given as a percentage (percent relaxation) of the 10^{-6} M noradrenaline-induced contraction.

treatment both in the control left and the previously denuded right carotid arteries (Figure 3).

Influence of losartan on contractile responses

In the control left carotid artery and the previously denuded right carotid artery, NA, ET-1 and angiotensin II produced a concentration-dependent contraction. The concentration-response curves and contraction parameters (E_{max} and ED_{50}) for each agonist, and influence of losartan administration are shown in Figure 4 and Table 3. As can be seen in Figure 4, there were no differences in the NA- and ET-1-induced contractions of the previously denuded right carotid arteries between vehicle and losartan groups. In contrast, the contractile response of the strips to angiotensin II was significantly decreased in the losartan group.

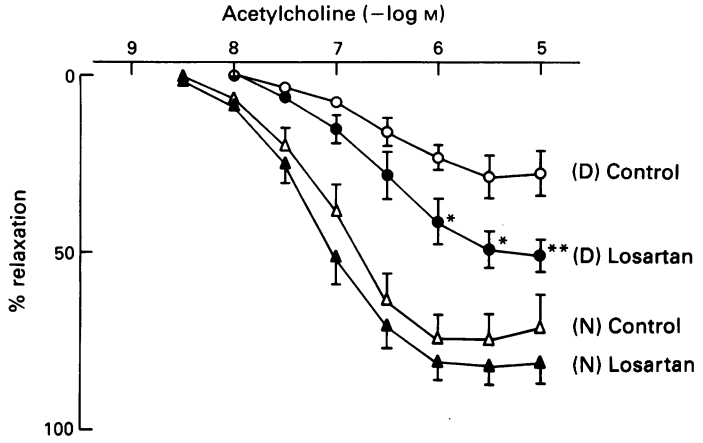

Figure 2 Comparison of endothelium-dependent relaxation response of control left (N) and previously denuded right (D) carotid arteries to acetylcholine between vehicle and losartan groups. Each point represents the mean of 6 to 7 experiments. Vertical bars show s.e.mean. Significant difference at *$P<0.05$ and **$P<0.01$ vs. the corresponding value in the denuded right carotid artery of vehicle group. Relaxation is given as a percentage (percent relaxation) of the 10^{-6} M noradrenaline-induced contraction.

Table 3 Comparison of the contractile responses of control and denuded carotid artery strips to noradrenaline, endothelin-1 and angiotensin II in vehicle and losartan-treated groups

		Noradrenaline		Endothelin-1		Angiotensin II	
Vessel	Treatment	E_{max} (mg)	ED_{50} ($\times 10^{-8}$ M)	E_{max} (mg)	ED_{50} ($\times 10^{-9}$ M)	E_{max} (mg)	ED_{50} ($\times 10^{-9}$ M)
Control artery	Vehicle	821 ± 43 (5)	2.3 ± 0.1 (5)	622 ± 26 (5)	1.8 ± 0.2 (5)	308 ± 15 (5) ***	2.0 ± 0.4 (5)
	Losartan	864 ± 97 (7) **	2.0 ± 0.2 (7)	586 ± 89 (7)	1.5 ± 0.1 (7)	* 109 ± 36 (7) ***	2.8 ± 1.2 (5)
Denuded artery	Vehicle	1108 ± 75 (5)	3.6 ± 0.8 (5)	645 ± 34 (6)	2.0 ± 0.3 (6)	450 ± 64 (6) **	2.5 ± 0.4 (6)
	Losartan	1013 ± 102 (6)	2.4 ± 0.2 (5)	649 ± 97 (7)	1.8 ± 0.2 (7)	181 ± 53 (7) **	3.2 ± 0.5 (5)

Results are given as mean ± s.e.mean. The E_{max} (maximum response) and ED_{50} (concentration to produce 50% of E_{max}) values were obtained from concentration-response curves. Figures in parentheses indicate the number of rabbits. Losartan was dissolved in distilled water and given as a drinking water at a concentration of 10 mg 200 ml^{-1} kg^{-1} daily for 7 weeks (1 week before and 6 weeks after endothelial removal) (see text). Rabbits were killed 6 weeks after the operation and carotid artery strips were prepared to analyze the mechanical responses (see text).
*$P<0.05$; **$P<0.01$ and ***$P<0.005$, respectively.

The E_{max} values for NA, ET-1 and angiotensin II were significantly higher or tended to be higher in the previously denuded right carotid artery strips than those values in the corresponding controls. The ED_{50} values, however, remained unchanged in all groups (Table 3).

Histological findings

In the sham-operated left carotid artery of the vehicle and the losartan groups, no noticiable changes could be detected (Figure 5a). Six weeks after endothelial removal, marked myointimal proliferative response resulting from new accumulation of proliferating smooth muscle cells and connective tissue was observed in the vehicle group (Figure 5b and Table 4). Losartan significantly suppressed the myointimal proliferative response to ballooning (Figure 5c and Table 4). The decreased amount of neointima appeared to reflect a reduction in all components of the intimal thickening, with fewer smooth muscle cells and less matrix formation (Figure 5c).

Discussion

Six weeks after removal of the endothelium from the rabbit carotid artery, the endothelial lining was reestablished with significantly increased density of the regenerated endothelial cells. Significant impairment of the endothelium-dependent relaxation was accompanied by myointimal proliferation. All these findings were similar to those in our previous paper (Azuma *et al.*, 1990). The exact level at which the impairment occurs is not known. It has been suggested that the site of the impairment occurs beyond the level of the receptors (Azuma *et al.*, 1990; Flavahan & Vanhoutte, 1990), but may also be due to reduction in the synthesis/release of EDRF by the regenerated endothelial cells and that the capacity to synthesize/release EDRF per cell greatly reduces, since the cell density had significantly increased over the normal (Azuma *et al.*, 1990). In addition, it is well established that EDRF is an anti-aggregating substance (Azuma *et al.*, 1986b; Furlong *et al.*, 1987; Radomski *et al.*, 1987) and it has been suggested by Garg & Hassid (1989) that endogenous EDRF (NO, Palmer *et al.*, 1987) may act as an inhibitory modulator of vascular smooth muscle cell mitogenesis and proliferation. These findings support the hypothesis that the injury or dysfunction of endothelial cells, together with resultant platelet aggregation and release of platelet products, is one of the important mechanisms of myointimal proliferative res-

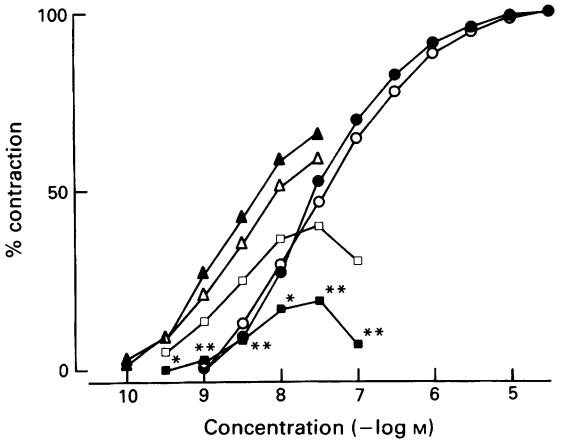

Figure 4 Comparison of contractile responses of the previously denuded right carotid artery to noradrenaline (○, ●), endothelin-1 (△, ▲) and angiotensin II (□, ■) between vehicle- (open symbols) and losartan-treated (closed symbols) groups. Each point represents the mean of 6 to 7 experiments. Significant difference at $*P<0.05$ and $**P<0.01$, respectively vs. corresponding controls. The maximum contraction (E_{max}) to 3×10^{-5} M noradrenaline was taken as 100% and determined to be 1108 ± 75 mg ($n = 6$) in the vehicle group and 1013 ± 102 mg ($n = 7$) in the losartan group.

Table 4 Influence of losartan on myointimal proliferative response to endothelial removal of right carotid artery

Treatment	*Intima/Media* (%)
Vehicle	59.4 ± 5.9 (6)
Losartan	24.9 ± 3.7 (7)***

Results are given as mean ± s.e.mean. Figures in parentheses indicate the number of rabbits. Intima/Media (%) in the sham-operated left carotid artery was estimated to be 4.7 ± 0.4 ($n = 6$).
Significantly different from vehicle group at $***P<0.005$.

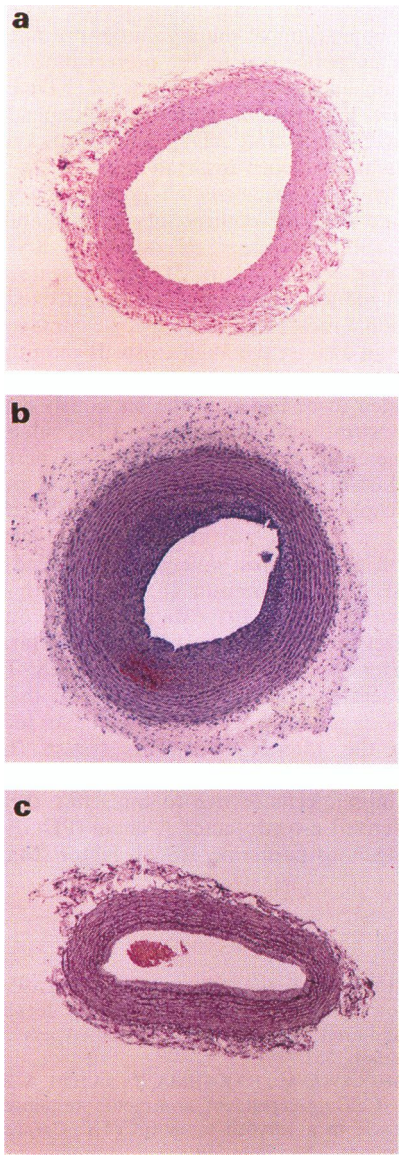

Figure 5 Light microscopic findings in rabbit carotid arteries. (a) Almost normal appearance of the cross-section of control left carotid artery (vehicle group). HE-staining (× 40). (b) Six weeks after endothelial removal, myointimal proliferation resulting from new accumulation of smooth muscle cells and connective tissue, and a slight accumulation of lipid can be observed. Sudan III -staining (× 40). (c) Less myointimal proliferation can be observed in losartan-treated group. Elastica-Van Gieson-staining (× 40).

ponse (Schwartz *et al.*, 1981; Ross, 1986).

Thus, the suppression of the myointimal proliferation with losartan may be partly due to the preservation of the endothelial function in the release/action of EDRF with this agent, because the reduced endothelium-dependent relaxations induced by ACh and ADP were significantly restored by chronic treatment with losartan (Figure 2).

Although, in the vehicle-treated group as well as in the losartan-treated one, the contractility to NA and ET-1 and the endothelium-independent relaxation to SNP remained unaltered (Table 3 and Figure 3), the exogenously applied angiotensin II-induced contraction was greatly reduced in the losartan group, indicating the specific antagonism with chronic losartan against the angiotensin II receptor. The E_{max} values for NA, ET-1 and angiotensin II were significantly higher or tended to be higher in the previously denuded right carotid artery strips without change in ED_{50} values (Table 3). We have some evidence indicating that the hyperreactivity of the thickened carotid artery may be due to the increased and sustained phosphorylation of the myosin light chain in the neointima. These results will be published elsewhere.

Studies with cell cultures have generally failed to support the hypothesis that angiotensin II is a smooth muscle cell mitogen. However, the *in vitro* data do not rule out an *in vivo* mitogenic effect for angiotensin II (Emmet & Harris-Hooker, 1986; Geisterfer *et al.*, 1988; Lyall *et al.*, 1988; Taubman *et al.*, 1989). Daemen *et al.* (1991) have shown that the mitogenic effect of angiotensin II *in vivo* may be an indirect result of activating the adrenergic nervous system (Luft *et al.*, 1989), since the α_1-adrenoceptor agonists can directly stimulate smooth muscle cells *in vivo* to transcribe the mRNA of the platelet-derived growth factor A-chain (PDGF-A) gene, a possible endogenous paracrine growth factor (Majeski *et al.*, 1990). It has also been demonstrated that angiotensin II increases the induction of ET mRNA expression and synthesis of functional ET peptide in cultured human vascular smooth muscle cells and these effects of angiotensin II are blocked by a specific receptor antagonist, [Sar^1,Ala^8]-angiotensin II (Resink *et al.*, 1990). ET is a potent mitogen and may play a potential role in the development of vascular diseases (Hirata *et al.*, 1989). Thus losartan may prevent the myointimal proliferative response through blocking angiotensin II receptors which mediate direct and indirect mitogenic effects of angiotensin II. In support of this hypothesis, Powell *et al.* (1989) have provided evidence showing that inhibitors of angiotensin-converting enzyme prevent the myointimal proliferation after vascular injury. Therefore, the present observations, taken together with observations demonstrating angiotensin-converting enzyme (Nakamura *et al.*, 1988), angiotensinogen mRNA (Cassis *et al.*, 1988) and angiotensin II receptors (Penit *et al.*, 1983) in the vascular wall, lead us to suggest that local angiotensin II receptors may play a role in the myointimal proliferative response of the vascular wall to endothelial removal.

In conclusion, the nonpeptide and specific angiotensin II receptor antagonist, losartan, may be a useful tool for investigation of the mechanisms of myointimal thickening after endothelial removal and may have therapeutic applications in preventing the proliferative response occurring after coronary angioplasty and vascular surgery.

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