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Effect of trandolapril on vascular responsiveness in cholesterol-fed rabbit-isolated arteries

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Abstract

According to the World Health Organisation, cardiovascular disorders are one of the main causes of morbi/mortality in the western world. The effect of trandolapril (0.3 mg kg^{-1} day^{-1}), a non-sulphydryl angiotensin-converting enzyme (ACE) inhibitor, on the vascular responsiveness in aorta isolated from hypercholesterolemic rabbits was examined. Three groups of rabbits ($n = 30$) were used: Group 0 (control group); Group 1 (hypercholesterolemic group, 0.5% (wt/wt) cholesterol-enriched diet) and Group 2 (hypercholesterolemic + trandolapril 0.3 mg kg^{-1} day^{-1}). After 18 weeks of treatment, the rabbits were killed and the thoracic aorta, proximal coronary and mesenteric (5th branch) arteries were isolated, cleaned off and mounted in an organ bath. Trandolapril had no significant effect on plasma cholesterol, high density lipoprotein (HDL) or low density lipoprotein (LDL). Despite the lack of effect of the drug on the above-mentioned parameters, treatment with trandolapril improved endothelium-dependent relaxation induced by acetylcholine in aortic and mesenteric rings from hypercholesterolemic rabbits treated with trandolapril. The relaxation induced by 10^{-5} M acetylcholine were $65.0 \pm 4.0\%$; $24.0 \pm 9.4\%$ ($P < 0.01$, $n = 10$) and $51.3 \pm 7.0\%$ ($P < 0.01$, $n = 10$) in aortic rings from Groups 0, 1 and 2, respectively, and $50.0 \pm 12.0\%$; $10.1 \pm 10.0\%$ ($P < 0.01$, $n = 10$); $61.0 \pm 9.7\%$ ($P < 0.01$, $n = 10$) in small mesenteric rings from Groups 0, 1 and 2, respectively. In addition, trandolapril treatment improved the increase in serotonin-induced contraction in proximal coronary arteries with respect to the hypercholesterolemic group. On the other hand, we did not find any differences among the group in endothelium-independent relaxation induced by sodium nitroprusside. These results provide evidence that trandolapril restores endothelium-dependent relaxation in hypercholesterolemic rabbit-isolated arteries. These data suggest that trandolapril might have beneficial action in the prevention of vascular alteration involved in atherosclerosis.

Keywords: Trandolapril; Angiotensin-converting enzyme inhibitor; Cholesterol-fed, rabbit; Aorta; Coronary artery; Mesenteric, 5th branch

1. Introduction

According to the World Health Organisation, cardiovascular disorders are one of the main causes of morbi/mortality in the western world. Among these disorders, atherosclerosis is a pathology, which is characterised by a progressive arterial stenosis of the large arteries. In patients, these lesions are composed of smooth muscle cells, extracellular matrix from these cells, variable amounts of lipid and lesser number of macrophages/monocytes (Ross, 1993). Several factors, such as platelet-derived growth factor (PDGF), endothelin-1 (ET-1) and angiotensin II (AII), are involved in the progression and in the changes in vascular reactivity found in atherosclerotic vessels. AII has many physiological roles, mainly related to the contraction of vascular smooth muscle (Catt et al., 1984), and it has been reported that AII is also involved in the migration and proliferation of arterial smooth muscle cells (Kawahara et al., 1988; Powell et al., 1989). In this way, some studies have indicated that angiotensin-converting enzyme (ACE) inhibitors may alter the arterial response to injury (Powell et al., 1989).

The present study was undertaken to assess whether the drug trandolapril non-sulfhydryl ACE inhibitor (Vidal et al., 1994) has antiatherosclerotic effects in the vascular reactivity in arteries isolated from cholesterol-fed rabbits.

2. Materials and methods

2.1. General procedure

Three groups ($n = 30$) of New Zealand White male rabbits (Biocentre, Barcelona, Spain), weighing 2.5 ± 0.5

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kg (3 months old) were used at the beginning of the study. Rabbits were housed identically in individual cages in an air-conditioned room under a 12 h light/dark cycle. Three groups ($n = 30$) of rabbits were used. Each group was fed according to the following scheme: Group 0, the normal group, was maintained on a standard diet; Group 1, the high-cholesterol control, was maintained on a diet containing 0.5% cholesterol (U.A.R., Paris, France); Group 2, the experimental group, received a diet containing 0.5% cholesterol plus trandolapril (0.3 mg kg^{-1} day^{-1}). The experiment lasted 18 weeks. All the animals were initially fed with a standard laboratory diet (Panlab, Barcelona, Spain) for at least 7 days after delivery to our laboratory. Tap water was available ''ad libitum''. Food intake was monitored daily for the first 7 days and each week thereafter.

Weight was determined before starting the treatment, and then weekly. A blood sample was collected from each rabbit before starting the treatment and then every 2 weeks. The blood samples were collected from the ear vein, and serum concentrations of cholesterol, high density lipoprotein (HDL) and low density lipoprotein (LDL) were determined with commercially available enzyme kits (Bio-Merieux, Marcy, France).

The animals were anaesthetised with ethyl ether and killed by exsanguination from the common carotid in the 18th week of the experiment. The thoracic aorta, proximal coronary and mesenteric arteries (5th branch) were rapidly removed and placed in Krebs–Henseleit solution of the following composition (mM): NaCl 119, KCl 4.7, $NaHCO_3$ 25, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5 and glucose 11.1. Adherent fat and surrounding tissue were cleaned off the aorta and proximal coronary, and then the arteries were cut into rings approximately 2–3 mm wide. The rings were then suspended between two stainless steel hooks in organ baths containing 10 ml of Krebs–Henseleit solution. The solution was kept at 36 ± 0.5°C and gassed continuously with a 95% O_2–5% CO_2 gas mixture (pH 7.35 ± 0.05). The aorta rings were mounted under 2 g tension and the coronary rings under 0.5 g. Each preparation was allowed to equilibrate for 90–120 min (aorta) or 30 min (proximal coronary). Contractile responses were measured isometrically by means of force-displacement transducers (Grass FT 03) and were recorded on a Grass polygraph as previously described (Tejerina et al., 1988). The isometric force was also digitalized by a MacLab A/D converter (Chart v3.2, A.D. Instruments, Castle Hill, Australia) and stored and displayed on a MacIntosh computer (Ruiz and Tejerina, 1998).

Using a dissecting microscope, a segment of small mesenteric artery, approximately 2 mm in length, corresponding to a 5th order branch of the superior mesenteric artery, was carefully dissected free from its vein. The artery was mounted in a small vessel myograph (Cauvin et al., 1984). Two 40 μm tungsten wires were passed through the lumen of an isolated cylindrical segment (approximately 175 μm inside diameter), one wire was fastened with screws to a fixed tissue mount and the other was pulled out by parallel hooks, which were attached to a strain-gauge force transducer (U-gauge, Shinko); the position of which was adjusted with a micromanipulator.

The vessel was set to a tension equivalent to that generated at 0.9 times the diameter of the vessel at 100 mm Hg transmural pressure (Mulvany and Warshaw, 1977). Vessels were allowed to equilibrate at 36 ± 0.5°C and gassed continuously with O_2. The isometric force was also digitalized by a MacLab A/D converter (Chart v3.2, A.D. Instruments) and stored and displayed on a MacIntosh computer (Ruiz and Tejerina, 1998).

After the equilibration period, aortic rings were contracted with noradrenaline (10^{-6} M) and exposed to acetylcholine (10^{-8}–10^{-5} M) or to sodium nitroprusside (10^{-8}–10^{-4} M) when contraction had reached a consistent maximum, in order to test endothelium-dependent and independent-relaxation. Other aortic rings were contracted with 80 mM KCl.

Proximal coronary arteries were initially contracted with 120 mM KCl; then, 30 min after being washed out, they were exposed to a concentration–response curve with 5-hydroxytryptamine (10^{-9}–10^{-4} M).

Mesenteric arteries were contracted with 80 mM KCl, and 30 min after washing out, the rings were exposed to a single concentration of noradrenaline (10^{-5} M), and when the plateau was reached, a concentration–response curve with acetylcholine (10^{-8}–10^{-5} M) was made.

2.2. Drugs

The following drugs were used: acetylcholine chloride (Sigma), noradrenaline bitartrate (Sigma), and sodium nitroprusside (Sigma). Trandolapril was a gift from Knoll Laboratories. Stock solutions were prepared by dissolving the compound in distilled water daily and keeping it on ice until used. The concentrations are reported as the final molar concentration in the organ chamber solution. Ascorbic acid was added to each daily-prepared solution of noradrenaline in order to avoid noradrenaline oxidation. Working solutions were made in Krebs–Henseleit solution.

All values used in the analyses represent mean ± S.E.M. of 10 rabbits in each group of experiments. Comparisons among the different groups were performed by two-way ANOVA test or Student's *t*-test and differences were considered significant when $P < 0.05$.

The Complutense University of Madrid (EEC official registration 28079-15ABC) approved all protocols concerning animals.

3. Results

3.1. General results

Acceptance of the diet supplemented with trandolapril was rapid and no significant differences among the groups

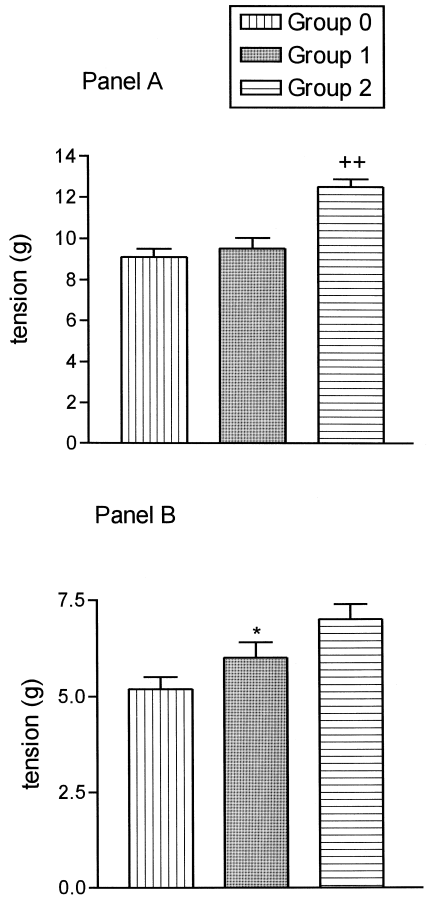

Fig. 1. Contractions induced by 80 mM KCl (panel A) or 10^{-6} M noradrenaline (panel B) in aortic rings. Each data shows the mean of each of the 10 experiments and vertical lines indicate the S.E.M. Group 0 (control group), Group 1 (hypercholesterolemic group, 0.5% cholesterol enriched-diet) and Group 2 (hypercholesterolemic + trandolapril 0.3 mg kg^{-1} day^{-1}). $^{*}P < 0.05$ vs. Group 0; $^{++}P < 0.01$ vs. Group 1.

in the daily consumption of the diet were observed. Moreover, no significant differences in body weights were observed during the experimental period; initial weights were 2500 ± 500 g and at the end of the treatments (18 weeks), the weights were 3556 ± 115 g (control group; Group 0), 3685 ± 93 g (hypercholesterolemic group, Group 1) and 3507 ± 72 g (hypercholesterolemic + trandolapril, Group 2).

Serum concentration of total cholesterol was 61.4 ± 5.7 mg/100 ml at the beginning of the study and increased gradually during the first 4 weeks in all the groups, except for the group fed with the standard diet. There were no significant differences in the increase of total serum cholesterol between the groups treated with trandolapril [Group 2 and Group 1 (high-cholesterol controls)], and no remarkable changes in the distribution of cholesterol in HDL or LDL (data not shown).

3.2. Contractile responses to KCl or noradrenaline in aortic rings

In the first group of experiments, the contractions induced by KCl (80 mM) or noradrenaline (10^{-6} M) were measured. In arteries obtained from Group 0 (control group), KCl induced a contraction of 9.1 ± 0.4 g, whereas in the hypercholesterolemic group (Group 1), this contraction was 9.5 ± 0.5 g. In Group 2 (trandolapril-treated group), the contraction induced by high-K^+ increased with respect to Group 1 and was 12.5 ± 0.4 g ($P < 0.01$, $n = 10$). In the same way, in Groups 0 and 1, noradrenaline (10^{-6} M) induced a contraction of 5.2 ± 0.3 and 6.0 ± 0.4 g, respectively ($P < 0.05$, $n = 10$), and in trandolapril-treated rabbits, this value was 7.0 ± 0.4 g (Fig. 1, panels A and B).

3.3. Responses to acetylcholine in aortic rings

Acetylcholine (10^{-8}–10^{-5} M) caused an endothelium-dependent relaxation in a concentration–response manner in all the groups studied. Endothelium-dependent relaxation strongly decreased in the hypercholesterolemic group (Group 1) with respect to the control group (Group 0), being the maximum relaxation induced by acetylcholine (10^{-5} M) $65.0 \pm 4.0\%$ and $24.0 \pm 9.4\%$ ($P < 0.001$, $n = 10$) in Groups 0 and 1, respectively. Trandolapril was able to restore endothelium-dependent relaxation to normal (maximal relaxation: $51.3 \pm 7.0\%$, $P < 0.01$ with respect to Group 1, $n = 10$) (Fig. 2).

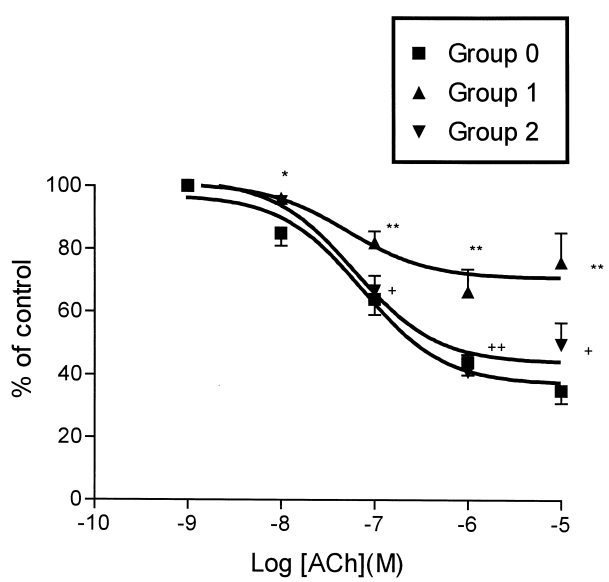

Fig. 2. Endothelium-dependent relaxation induced by acetylcholine (10^{-8}–10^{-5} M) in noradrenaline-precontracted aorta arteries. Each data shows the mean of each of the 10 experiments and vertical lines indicate the S.E.M. Group 0 (control group), Group 1 (hypercholesterolemic group, 0.5% cholesterol enriched-diet) and Group 2 (hypercholesterolemic + trandolapril 0.3 mg kg^{-1} day^{-1}). $^{*}P < 0.05$ vs. Group 0; $^{**}P < 0.01$ vs. Group 0; $^{+}P < 0.05$ vs. Group 1; $^{++}P < 0.01$ vs. Group 1.

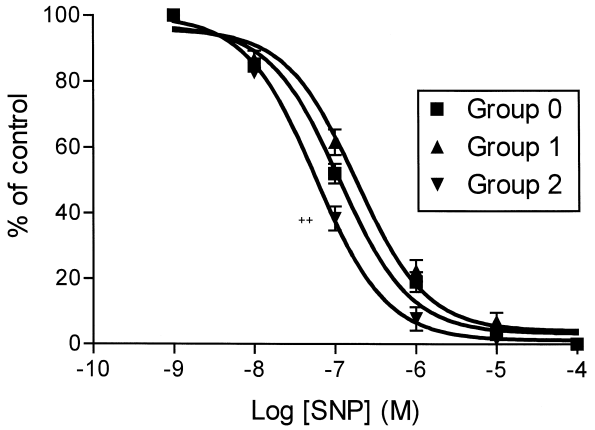

Fig. 3. Endothelium-independent relaxation induced by sodium nitroprusside (10^{-8}–10^{-4} M) in noradrenaline-precontracted aorta arteries. Each data shows the mean of each of the 10 experiments and vertical lines indicate the S.E.M. Group 0 (control group), Group 1 (hypercholesterolemic group, 0.5% cholesterol enriched-diet) and Group 2 (hypercholesterolemic + trandolapril 0.3 mg kg^{-1} day^{-1}). $^{++}P < 0.01$ vs. Group 1.

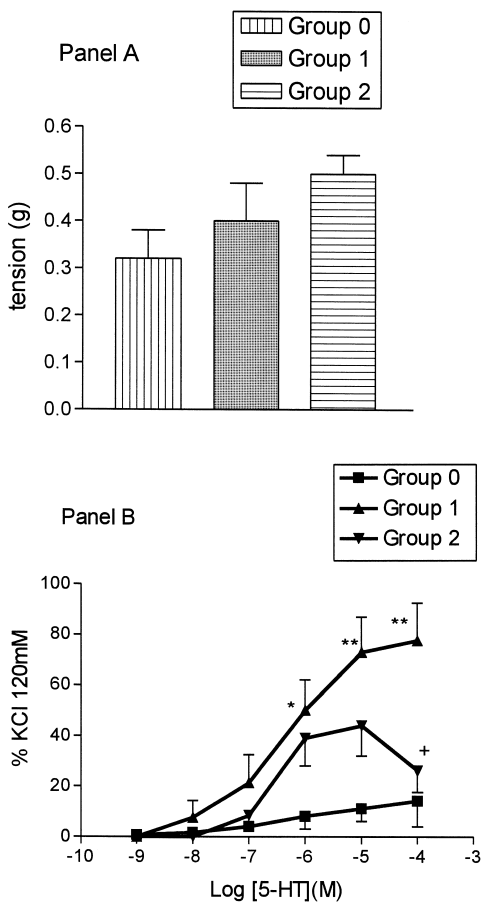

Fig. 4. Contractions induced by a single concentration of KCl (120 mM) (panel A) or by a cumulative concentration–response curve induced by 5-hydroxytryptamine (10^{-9}–10^{-4} M) (panel B) in proximal coronary arteries. Each data shows the mean of each of the 10 experiments and vertical lines indicate the S.E.M. Group 0 (control group), Group 1 (hypercholesterolemic group, 0.5% cholesterol enriched-diet) and Group 2 (hypercholesterolemic + trandolapril 0.3 mg kg^{-1} day^{-1}). $^{*}P < 0.05$ vs. Group 0; $^{**}P < 0.01$ vs. group 0; $^{+}P < 0.05$ vs. Group 1.

3.4. Responses to sodium nitroprusside in aortic rings

The endothelium-independent relaxation was also tested. Sodium nitroprusside induced a relaxation in all groups studied. Moreover, we did not find any differences in terms of maximum relaxation among the groups, although the concentration–response curve to sodium nitroprusside was slightly shifted to the right in the hypercholesterolemic group (Group 1) with respect to the control group, and in the trandolapril-treated group, this effect was prevented (Fig. 3).

3.5. Contractile responses to KCl and 5-hydroxytryptamine in proximal coronary arteries

The trandolapril-treated group (Group 2) showed an increase (but not statistically significant) in the contraction induced by 120 mM KCl in proximal coronary arteries with respect to the hypercholesterolemic group (Group 1) (Fig. 4, panel A).

On the other hand, in the hypercholesterolemic group, the concentration–response curve to 5-hydroxytryptamine

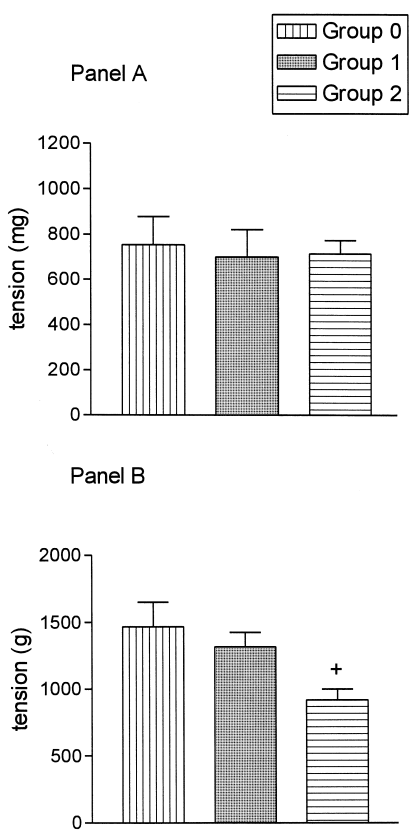

Fig. 5. Contractions induced by 80 mM KCl (panel A) or 10^{-5} M noradrenaline (panel B) in small mesenteric arteries. Each data shows the mean of each of the 10 experiments and vertical lines indicate the S.E.M. Group 0 (control group), Group 1 (hypercholesterolemic group, 0.5% cholesterol enriched-diet) and Group 2 (hypercholesterolemic + trandolapril 0.3 mg kg^{-1} day^{-1}). $^{+}P < 0.05$ vs. Group 1.

was shifted upward [$F(1) = 23.91,\ P < 0.00009$] with respect to the control group (Group 0). Moreover, the treatment with trandolapril partially restored this effect to normal. Thus, the maximum contraction induced by 10^{-4} M 5-hydroxytryptamine was $14.0 \pm 10.0\%$ (percentage of 120 mM KCl-induced contraction), $77.5 \pm 15.0\%$ and $26.0 \pm 8.3\%$ in Groups 0, 1 and 2, respectively (Fig. 4, panel B).

3.6. Contractile responses induced by KCl and noradrenaline in small mesenteric arteries

Fig. 5 (panels A and B) shows the values of contraction induced by high-K^+ or by a submaximal concentration of noradrenaline (10^{-5} M) in the different groups studied. The contractions obtained were similar in all the groups when the contraction was induced by high-K^+ (750 ± 125, 700 ± 121, 713 ± 63 g; Groups 0, 1, 2, respectively). However, when the contractions were induced by noradrenaline (10^{-5} M) in Groups 0 and 1, they were similar (1469 ± 185 and 1318 ± 397 g), while in trandolapril-treated rabbits, this value was decreased in a significant manner (919 ± 80 g; $P < 0.05$, $n = 10$).

3.7. Responses to acetylcholine in small mesenteric arteries

Cumulative addition of acetylcholine induced a concentration-dependent and sustained relaxation during contraction by noradrenaline (10^{-5} M). The concentration–relaxation curve of acetylcholine was shifted significantly [$F(1) = 8.71,\ P < 0.0061$] to the right in the small mesenteric arteries obtained from the hypercholesterolemic group compared with the control group (Fig. 6). The treatment with trandolapril completely restored endothelium-dependent relaxation in small mesenteric arteries [$F(1) = 24.45$, $P < 0.00009$] with respect to the hypercholesterolemic group.

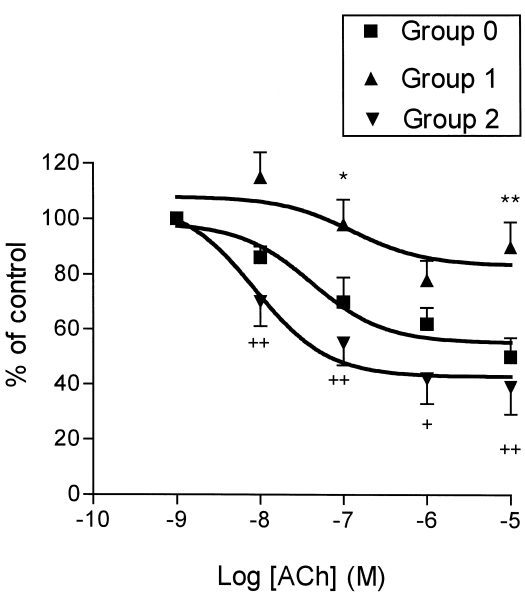

Fig. 6. Endothelium-dependent relaxation induced by acetylcholine (10^{-8}–10^{-5} M) in NA-precontracted small mesenteric arteries. Each data shows the mean of each of the 10 experiments and vertical lines indicate the S.E.M. Group 0 (control group), Group 1 (hypercholesterolemic group, 0.5% cholesterol enriched-diet) and Group 2 (hypercholesterolemic + trandolapril 0.3 mg kg^{-1} day^{-1}). $^{*}P < 0.05$ vs. Group 0; $^{**}P < 0.01$ vs. Group 0; $^{+}P < 0.05$ vs. Group 1; $^{++}P < 0.01$ vs. Group 1.

4. Discussion

The present study provides evidence that trandolapril, an ACE inhibitor, restores to normal the major changes in vascular responsiveness in arteries isolated from cholesterol-fed rabbits. Thus, endothelium-dependent relaxation in both aorta and small mesenteric arteries was completely restored to normal. Moreover, in proximal coronary arteries, the increasing contractions induced by 5-hydroxytryptamine were significantly decreased.

It is well known that vascular rings of atherosclerotic animals show an endothelium injury, which is the first step in atherosclerotic plaque development. This injury leads, among other things, to an alteration in the normal functionality of endothelium with a decrease in endothelium-dependent relaxation induced by agonists, such as acetylcholine or bradykinin.

Our data confirm the previously reported studies that show endothelial dysfunction resulting from hypercholesterolemia (Jayakody et al., 1985; Verburen et al., 1986; Sreeharan et al., 1986). Our data show that the relaxation in response to acetylcholine, an endothelium-dependent vasodilator, is clearly inhibiting (the dose–response curve to acetylcholine was shifted upward and left). In aorta and mesenteric arteries proceeding from rabbits fed with hypercholesterolemic diet plus trandolapril, the relaxation induced by acetylcholine was restored to normal in both arteries. These data are in agreement with the results previously reported (Becker et al., 1991; Finta et al., 1993; Riezebos et al., 1994) with ramipril (0.3 mg kg^{-1} day^{-1}), a non-sulphydryl angiotensin-converting enzyme inhibitor, in rabbits fed with an atherogenic diet plus ramipril for 8 or 12 weeks.

However, the dose–response curve with sodium nitroprusside was not affected by the atherogenic diet or the further addition of trandolapril and, therefore, the reduced responsiveness of the atherosclerotic arteries in response to acetylcholine is not a result of an inability of the vessels to relax. Acetylcholine acts via the receptor on the endothelium cell membrane to activate nitric oxide synthesis. Nitric oxide synthesis is a complex mechanism not fully understood. The process, like others in cells, seems to be ruled by $[Ca^{2+}]_i$ (Peach et al., 1987). Agonist stimulation of endothelial cells induces calcium release from intracellular stores. This is followed by an influx of external

calcium to maintain an increased level of cytosolic calcium (Colden-Standfield et al., 1987; Hallan et al., 1988). Membrane hyperpolarization in endothelial cells is induced as a consequence of activation of calcium-dependent potassium channels by an increase in cellular calcium (Busse et al., 1988). In addition, it has been reported that an N-terminal myristoylation of nitric oxide-synthase (Busconi and Michel, 1993) has been observed and it is plausible that the regulation of endothelial NO may affect the biological activity of this enzyme system.

Trandolapril, at first, might act on any of these steps in the NO synthesis.

We also studied the effects of trandolapril on contractile response induced by KCl and by noradrenaline in both resistance and capacitance rabbit-isolated arteries. Trandolapril inhibited the contraction induced by depolarization of the vascular smooth muscle cell membrane with KCl (or with increasing concentrations of extracellular Ca^{2+} in a high-K^+ solution) or that induced by the adrenergic agonist, noradrenaline in aorta arteries. These responses have been attributed to Ca^{2+} entry through voltage-operated channels (Bolton, 1979; Cauvin et al., 1983). Moreover, depolarization of smooth muscle cells through L-type Ca^{2+} channels has been implicated in the mechanism by which noradrenaline induces vascular smooth muscle contraction.

Taking into account the present data, we postulated that the enhanced effect of trandolapril on the contractile response in aorta arteries was due to the increase of Ca^{2+} fluxes. The increase in the relaxation induced by acetylcholine in arteries proceeding from rabbits fed with a hypercholesterolic diet plus trandolapril could be secondary to the enhanced responsiveness to noradrenaline. We did not find any difference among the groups in the contraction induced by high K^+ (80 mM) in mesenteric arteries (5th branch). The precise explanation for the tissue specificity is not yet known, but it may be due to the differences in the structure of the channels themselves from vessel to vessel (Quins et al., 1981, Cauvin et al., 1988) and/or to variations in the membrane potentials of the different arteries (Shibata et al., 1991; Tejerina et al., 1992). However, in the case of agonist-induced responses, the different efficacy of the drugs appears to be more related to the source of the Ca^{2+} activator of the contractile process.

In a previous work, trandolaprilat, the active diacid of trandolapril, did not modify the endothelium-dependent responses induced by acetylcholine or substance P in canine femoral arteries incubated ''in vitro'' with trandolaprilat. However, trandolapril indeed increases endothelium-dependent relaxation induced by bradykinin (Illiano et al., 1994) due to the reduction of the degradation of bradykinin by angiotensin-converting enzyme. The lack of effect on endothelium-dependent responses to acetylcholine in vitro rules out the possibility that trandolapril increases the release of endothelium-derived relaxing factor (EDRF) or of the effect of EDRF on vascular smooth muscle cells (Vidal et al., 1994). Likewise, the relaxation evoked by trandolapril in perfuse arteries with intact endothelium can be attributed to the reduction of metabolism of bradykinin originated by shear stress.

On the other hand, all proximal coronary arteries of hypercholesterolemic rabbits exhibited increased responses by 5-hydroxytryptamine with respect to control animals. This agrees with earlier studies of large coronary arteries of hypercholesterolemic rabbits (Vrints et al., 1990) and the coronary circulation of atherosclerotic non-human primates (Chilian et al., 1990). The abnormal responses to 5-hydroxytryptamine could be associated with an endothelium dysfunction in the form of impaired release of EDRF. In the trandolapril-treated group, the concentration–response curve of 5-hydroxytryptamine was shifted downwards with respect to the hypercholesterolemic group (Group 1). To our knowledge, this is the first report showing an effect of trandolapril treatment on contractile responses in coronary arteries. A mechanism of this action could be an interaction of trandolapril during the treatment of 5-hydroxytryptamine receptors in smooth muscle cells; another possible explanation is that again, the blood pressure-lowering effect of trandolapril improves the endothelium functionality, restoring the abnormal contractions induced by 5-hydroxytryptamine to normal.

In conclusion, these results confirm that endothelial dysfunction, as evidenced by the attenuated aortic relaxation response to acetylcholine, or by the increased responses to 5-hydroxytryptamine in proximal coronary arteries, results from the experimentally induced hiperlipidemia. Trandolapril normalises this endothelial dysfunction that results when rabbits are fed an atherogenic diet, implying that trandolapril protects against the functional degeneration of the endothelium caused by hypercholesterolemia. Trandolapril might be effective in the prevention of some of the pathophysiological alterations, which occur during atherosclerotic development.

Acknowledgements

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