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Captopril Attenuates Proteosynthesis in the Aorta and Decreases Endothelaemia in Rabbits with Aortic Insufficiency

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Summary

The effect of the angiotensin converting enzyme (ACE) inhibitor, captopril, on proteosynthesis in the aorta, acetylcholine-stimulated aortic relaxation and endothelaemia (circulating endothelial cells) was investigated in rabbits with aortic insufficiency. The animals were studied 28 days after experimental intervention. Cardiac volume overload stimulated proteosynthesis in the aorta as reflected by increased ribonucleic acid (RNA) concentration and [^{14}C] leucine incorporation into proteins of the aorta. Moreover, the number of endothelial cells in the blood was increased. The administration of captopril starting from the second day of the haemodynamic overload, partially prevented the increase both in aortic proteosynthesis and in endothelaemia. Despite these alterations, the relaxing ability of the aorta to acetylcholine was not changed either by the haemodynamic overload or by captopril. We conclude that the increase of proteosynthesis in the aorta and of endothelaemia in the early period of chronic cardiac volume overload in rabbits were partially prevented by chronic captopril treatment. Neither aortic insufficiency nor captopril changed the acetylcholine-induced relaxation of the aorta.

Key words
Aortic insufficiency – Aorta – Proteosynthesis – Endothelaemia – Relaxation

Introduction

There is strong evidence that the protective effect of angiotensin converting enzyme (ACE) inhibition in different cardiovascular diseases is in part determined by the effect of ACE inhibition on the structure and function of central and peripheral vessels. However, data reported from different laboratories vary depending on the dose of ACE inhibitor used (antihypertensive vs. non-antihypertensive dose), on the time from the onset of the overload (prevention vs. regression of hypertrophy), on the type of vessel investigated and perhaps on the ACE inhibitor used. It was shown, for example, in spontaneously hypertensive rats (SHR) that hypertrophy of the mesenteric artery could only be prevented by early high-antihypertensive dose treatment with ramipril but not once hypertrophy had developed (Linz *et al.* 1995). In the same model lisinopril and angiotensin II receptor antagonist D 8731 prevented hypertrophy development of small arteries with no apparent dose-dependence (Shaw *et al.* 1995). In contrast, enalapril treatment did not prevent renal arterial hypertrophy in SHR despite the normalization of arterial pressure (Kett *et al.* 1995). On the other hand, both antihypertensive and non-antihypertensive dose of fosinopril reduced already established vascular hypertrophy, the effect being presumably dependent on the modulation of peripheral adrenergic transmission (Castellano *et al.* 1995). Normalization of media thickness to the lumen diameter ratio of peripheral resistance vessels was also achieved in patients with essential hypertension treated for one year with perindopril (Sihm *et al.* 1995).

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Moreover, trandolapril prevented deterioration of endothelium-dependent vasodilatation in rats with nitric oxide-deficient hypertension (Takase *et al.* 1996) and enalaprilat improved endothelium-dependent vasodilatation induced by cholinergic stimuli in the peripheral vasculature in chronic heart failure patients (Nakamura *et al.* 1994).

Despite a lot of data with respect to the effect of ACE inhibition on pressure overloaded vessels, information of this kind in volume overload are scarce. The aim of this study was to investigate whether inhibition of the renin-angiotensin system (RAS) by captopril can modify proteosynthesis in the aorta, endothelium-dependent relaxation of aorta and impairment of endothelial monolayer in the model of volume cardiac overload caused by aortic insufficiency in rabbits.

Materials and Methods

Aortic insufficiency was induced by methods described in detail elsewhere (Fízeľ and Fízeľová 1969, Šimko 1995). Four groups of animals were investigated 28 days after the experimental intervention: control (C) (n=11), control + captopril (Cc) (n=12), aortic insufficiency (A) (n=14) and aortic insufficiency + captopril (Ac) (n=16). Captopril was given twice daily in a single dose of 5 mg/kg body weight in 0.5 ml saline.

The RNA concentration was determined by a single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction (Chomczynski and Sacchi 1987). The DNA concentration was measured according to Davidson (1976). [^{14}C] leucine incorporation into aortic proteins was assessed by the method described by Gerová *et al.* (1996). The circulating carcasses of desquamated anuclear bodies of endothelial cells were concentrated by high-speed centrifugation and counted in a Bürker's chamber (Hladovec and Rossman 1973).

The abdominal aorta was dissected free and placed in an ice-cold Krebs solution, cleaned of connective tissue and cut into segments about 4 mm long (Holécyová *et al.* 1996). The individual segments were attached between an isometric force transducer (Sanborn FT 10) and a holder under a tension of 40 mN in a 20 ml organ bath containing Krebs solution. After a resting period of 60 min, the concentration-response curve to acetylcholine was obtained as described earlier (Holécyová *et al.* 1993).

Differences between groups were assessed by ANOVA and Duncan's multiple range test with $p<0.05$ taken as significant.

Table 1. Nucleic acid concentration in the aorta, and [^{14}C] leucine incorporation into aortal proteins

	Control	Control + captopril	Aortic insufficiency	Aortic insufficiency + captopril
RNA in aorta (mg/g w.w.)	0.70±0.025	0.72±0.05	1.13±0.04*	1.01±0.04*$^{+}$
DNA in aorta (mg/g w.w.)	1.51±0.11	1.55±0.15	1.84±0.16	1.71±0.10
[^{14}C] leucine incorporation into aorta (DPM/mg protein)	234±5.7	226±6.8	328±6.8*	303±7.9*$^{+}$

*Values are means ± S.E.M., * different from control group ($p<0.05$), $^{+}$ different from A group ($p<0.05$), $n=7$ in each experimental group.*

Results and Discussion

The DNA concentration in the aorta was not changed in either group. Aortic RNA concentration and [^{14}C]leucine incorporation into the aorta were increased in group A vs. group C. Captopril significantly attenuated these alterations ($p<0.05$, AC vs. A) (Table 1). The level of endothelial cells was increased in group A vs. C ($p<0.05$) and captopril almost completely prevented this change ($p<0.05$, Ac vs. A) (Fig. 1). Neither aortic insufficiency nor captopril changed the acetylcholine-induced relaxation of the aorta (Fig. 2).

The increased RNA concentration and incorporation of [^{14}C]leucine into proteins are indicators of stimulated proteosynthesis (Gerová *et al.* 1996). The concentration of DNA, on the other hand, reflects the amount of nucleic matter and should be responsible for the proliferation of vascular cells (Adams *et al.* 1989). It seems, therefore, that during one month lasting aortic insufficiency in rabbits, proteosynthesis in the aorta was stimulated without a significant increase in the proliferation of vascular cells. Hence, the increase in RNA concentration and [^{14}C] leucine incorporation into the aorta may reflect a hypertrophic process of aortic wall cells.

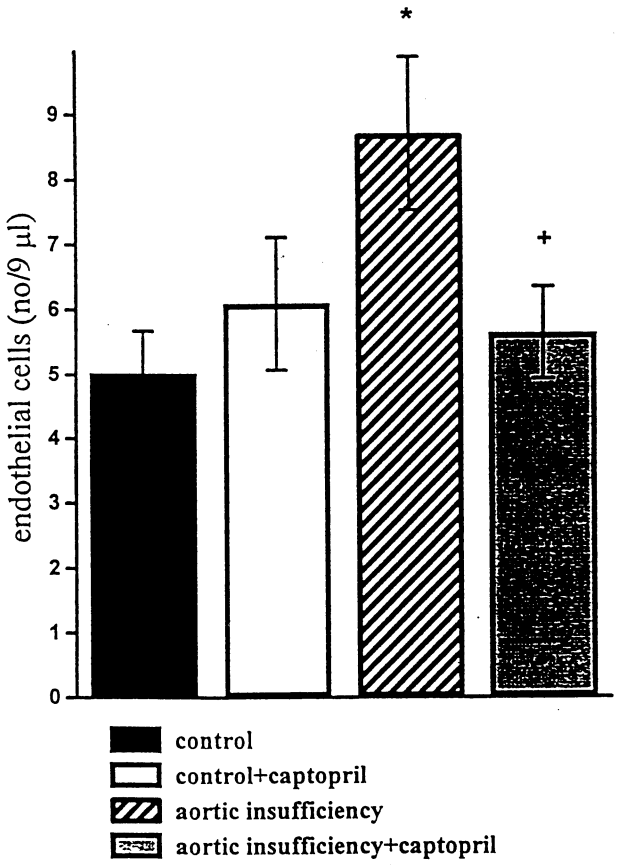

Fig. 1. *Endothelaemia (number of desquamated endothelial cells) in the blood (no=9 µl). Values are means ± S.E.M., * different from the control group, $p<0.05$, + different from A group, $p<0.05$.*

As the growth of endothelial cells is rigorously controlled, these cells are not supposed to be capable of responding to the haemodynamic stimulus by a hypertrophic growth (Schwartz *et al.* 1990). On the other hand, there are several reports on the hypertrophic process of smooth muscle cells in vessels. Saltis and Bobik (1992) showed hypertrophic growth of vascular smooth muscle cells in genetic hypertension and Kulik *et al.* (1991) demonstrated the hypertrophic process of vascular smooth muscle cells after cyclic tension deformation in culture. Thus, in our model of aortic insufficiency, the increase in RNA concentration and [^{14}C]leucine incorporation appear to reflect the hypertrophic growth of aortic smooth muscle cells. This consideration is in agreement with histological findings in pressure overloaded vessels, when increased thickness of the smooth muscle cell layer in the mesenteric artery was observed in spontaneously hypertensive rats (Gohlke *et al.* 1994).

The increased concentration of circulating endothelial cells observed in the aortic insufficiency group may reflect impairment of the endothelial monolayer of the vascular bed. Other authors showed increased endothelaemia in several conditions, such as smoking, hypertension, hypercholesterolaemia (Hladovec 1989), haemostasis (Hladovec and Kornalík 1992) or administration of norepinephrine (Babal *et al.* 1996). In the increased endothelial cell concentration, observed in our experiment, both enhanced pulse pressure, which is typical for aortic insufficiency (Šimko *et al.* 1997), and remodelling of the aorta and other blood vessels resulting from long-lasting volume overload may have been involved. Considering these facts it was surprising that endothelium-dependent relaxation of the aorta provoked by acetylcholine stimulation failed to be altered in rabbits with aortic insufficiency. Conceivably, either the potential loss was functionally compensated by the endothelial cells remaining in the aortic endothelial monolayer or endothelial cell desquamation had taken place in more distal parts of the vascular bed. It should also be taken into account that, under certain conditions, the increase in endothelaemia may reflect rather the alteration in the turnover of the endothelial monolayer than its injury.

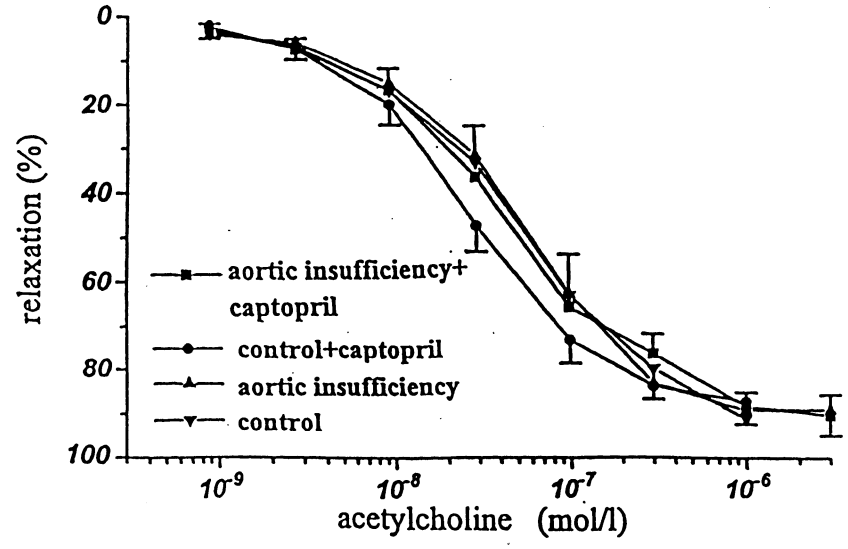

Fig. 2. *Relaxation of aorta (%) to acetylcholine (mol/l). Values are mean ± S.E.M.*

In keeping with the data on the pressure overloaded heart (Fish 1994), captopril partially prevented the increase in proteosynthesis in the aorta. The mechanism of this effect is probably not associated with interference of captopril with the haemodynamic overload. We previously found (Šimko *et al.* 1996, 1997) that the pulse pressure, which was increased in the group with aortic insufficiency, was not reduced by captopril administration. Hence, we suppose that attenuation of the hypertrophic growth of the aorta might have been determined by the interference of captopril with the tissue RAS. This effect of captopril may potentially be achieved by inhibition of angiotensin II formation, which is known to be a strong growth-promoting factor, or by enhancement of bradykinin levels stimulating the production of prostacyclin and nitric oxide (NO) having antigrowth properties (Linz *et al.* 1992). However, because the relaxation of the aorta provoked by acetylcholine (that is known to be NO-dependent) was not augmented by captopril, we do not presume that the L-arginine:NO pathway is activated. In agreement with this consideration, we have shown in the experimental model of NO-deficient hypertension in rats that captopril prevented the development of hypertension and hypertrophy without affecting NO-synthase activity (Bernátová *et al.* 1996). It remains to be elucidated, whether the effect of captopril is associated with activation of the arachidonic acid pathway or with inhibition of angiotensin II production.

Captopril, moreover, decreased endothelaemia in rabbits with the volume overloaded heart. The potential of ACE-inhibition to decrease endothelial cell concentration was also observed in patients with essential hypertension (Widimský *et al.* 1996) and may represent another protective effect of ACE-inhibition. The mechanism of this captopril effect remains unclear.

We conclude that the increase in proteosynthesis of the aorta and in endothelaemia were partially prevented by chronic captopril treatment in the early period of chronic cardiac volume overload in rabbits. Neither aortic insufficiency nor captopril changed the acetylcholine-induced relaxation of the aorta.

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