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Remodelling of Septal Branch of Coronary Artery and Carotid Artery in L-NAME Treated Rats

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Summary

Coronary and carotid artery structure was studied in rats in order to analyze the processes in the cardiovascular system in NO-deficient hypertension model. Long-term inhibition of NO synthase was induced by L-nitro arginine methyl ester (L-NAME, 50 mg/kg/day p.o.) for a period of 8 weeks. An increase in blood pressure and heart/body weight ratio confirmed the reliability of the model. The wall thickness as well as the calculated wall area of the coronary artery increased by 70 % and 50 %, respectively, in comparison to control vessels. The wall thickness and the calculated wall area of the carotid artery increased by 73 % and 70 %, respectively. Further analysis indicated that both the tunica intima and tunica media in the coronary and the carotid artery increased quantitatively in a similar manner. Remarkable differences were found in the contribution of cellular and noncellular components in the tunica media of the coronary and carotid arteries of experimental animals. The calculated extracellular area increased by 116 % in comparison to the control coronary artery and by 97 % in comparison to the control carotid artery. The increase in extracellular matrix of the tunica media of coronary and carotid arteries seems to be basic cause of the remodelling of the vessels studied.

Key words

Nitric oxide – Coronary artery – Carotid artery – Morphometry

Introduction

Recently, a new model of hypertension has been developed (Bayliss *et al.* 1992, Jover *et al.* 1993, Delacretaz *et al.* 1994). Its pathogenetic background consisted of NO deficiency brought about by inhibition of NO synthase, the enzyme of crucial importance in almost ubiquitous pathway arginine –> citrulline + NO. Several authors studying the chronic NO-deficient model found that the developed hypertension is accompanied by cardiac hypertrophy (Morton *et al.* 1993, Kristek and Gerová 1996). Similarly, changes in vessel wall structure could be expected. However, the only data dealing with the mesenteric vascular bed are not unequivocal (Dunn and Wilson 1993, Morton *et al.* 1993).

We focused our attention on the coronary and carotid artery especially because the coronary artery has been shown to follow the geometry and also the metabolic processes in the myocardium very closely after loading the cardiovascular system (Gerová *et al.* 1992, 1996).

Methods

The experiments were carried out in Wistar male rats. Ten-weeks-old rats were divided into two groups. One group was given tap drinking water, the other group was administered NO synthase inhibitor L-nitro-arginine methyl ester in a dose 50 mg/kg body weight per day in the drinking water for a period of eight weeks.

Systolic blood pressure was measured by tail plethysmography each week.

The animals were sacrificed by an overdose of sodium pentobarbital. The chest was opened and the cardiovascular system was perfused under constant perfusion pressure 120 mm Hg with glutaraldehyde

fixative for 10 min *via* a cannula placed in the left ventricle. After perfusion, the middle part of the common carotid artery and upper third of the septal branch of the left descending coronary artery were excised, divided into 1 mm long segments and fixed in the same fixative. The samples were exposed to OsO_4, stained *en block* with uranyl acetate, dehydrated through a graded series of alcohols and embedded in Durcupan ACM. Subsequently, semithin sections were cut perpendicularly to the long axis of the vessels and stained with methylene blue. The arterial wall thickness (tunica intima and tunica media) of the respective vessel were measured in semithin sections in about 45° intervals around the vessel circumference. From the measured data the arterial wall area (tunica intima and tunica media) was calculated. Since the thickness of tunica adventitia might have been damaged by preparation of the arteries, it was not measured.

To express the individual structural components of the tunica media quantitatively, we employed the point counting method according to Weibel *et al.* (1966). The relative volume density of smooth muscle cells and of the extracellular matrix in controls and in experimental arteries were assessed. The grid was placed along the section randomly until 5 000 points were counted. Sections from three randomly selected blocks from both control and experimental vessels were processed in the same way. The relative contribution of smooth muscle cells and/or extracellular matrix was used for further calculation.

All results are expressed as means ± S.E.M. For statistical evaluation, one-way ANOVA and Bonferroni t-tests were used. The value of $p<0.05$ was considered significant.

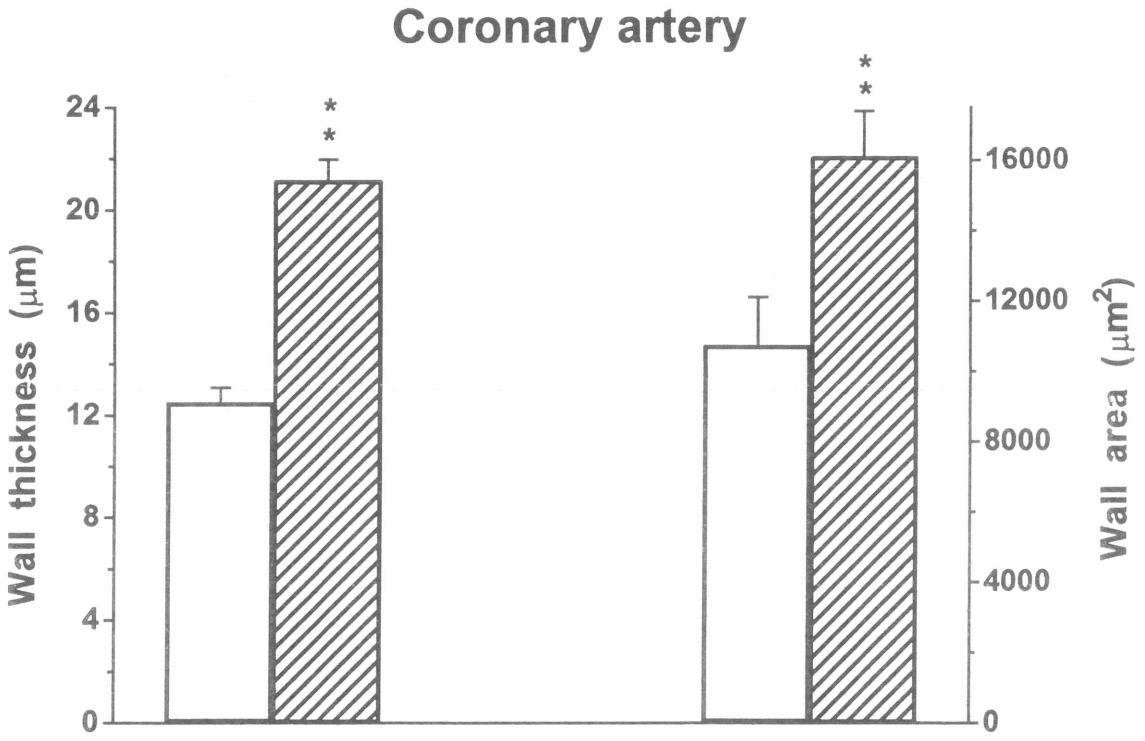

Fig. 1
*Wall thickness and wall area (tunica intima and tunica media) of the coronary artery. Open columns – control, hatched columns – L-NAME treated rats. ** $P<0.01$.*

Results and Discussion

The blood pressure in the group of rats treated for 8 weeks with NO synthase inhibitor L-NAME gradually increased. At the end of the experiment, their blood pressure was 187.3±4.2 mm Hg as compared to the control value 132.5±1.2 mm Hg. These findings are in agreement with those of Arnal *et al.* (1993) who used the same duration of NO synthase blockade and with other authors who treated rats for a shorter period with L-NAME (Bayliss *et al.* 1992, Ribeiro *et al.* 1992, Bernátová and Pecháňová 1994). Contrary to Arnal *et al.* (1993), our previous report indicated that concomitantly with the rise of blood pressure a significant increase of heart weight occurred (Kristek and Gerová 1996). Furthermore, the heart/body weight ratio markedly increased. Thus, similarly as in other types of hypertension models,

cardiac hypertrophy is a consequence of an increased cardiac load during NO-deficient hypertension. Having-in mind these findings, we studied the possible changes in the structure of coronary and carotid arteries.

The wall thickness (tunica intima and tunica media) of the coronary artery in the controls was 12.47±0.62 μm whereas it was 21.15±0.84 μm ($p<0.01$) in the experimental animals, i.e. a 50 % increase in wall thickness was found in NO-deficient hypertensive rats (Fig. 1). Since the wall thickness is influenced by the pressure during fixation we also calculated the wall area. Similar marked changes were found in this parameter (Fig. 1). Besides confirming the presence of cardiac hypertrophy, we have also demonstrated extensive remodelling of the coronary artery.

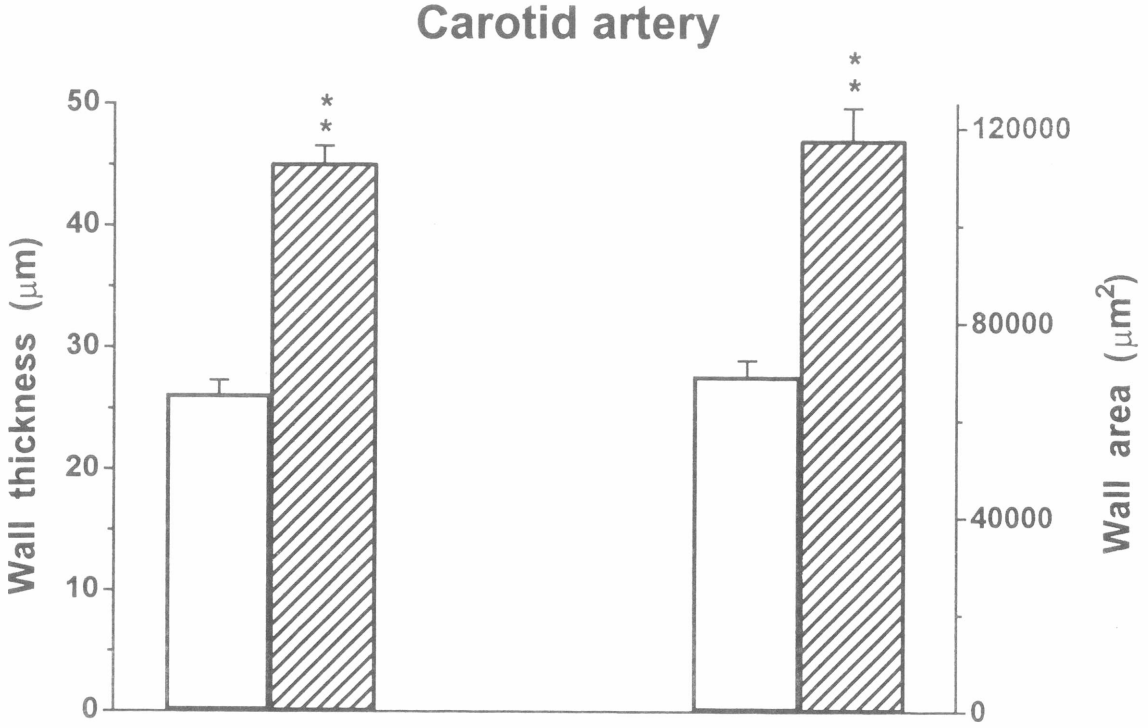

Fig. 2
*Wall thickness and wall area (tunica intima and tunica media) of the carotid artery. Open columns – control, hatched columns – L-NAME treated rats. ** $P<0.01$.*

The wall thickness of control carotid arteries was 26.08±1.23 μm. In the experimental carotid artery wall thickness increased to 45.14±1.41 μm, i.e. by 70 % (Fig. 2). These findings are in agreement with the data of Delacretaz *et al.* (1994) who observed an increase of arterial wall thickness in rats treated with L-NAME for 4 weeks; their measurements were performed, however, without pressurized fixation. The findings on structural changes in the mesenteric bed are ambiguous. Morton *et al.* (1993) observed an increase in arterial wall thickness after NO synthase inhibition. On the other hand, Dunn and Wilson (1993) did not find any changes in resistance mesenteric vessels after 3 weeks of NO synthase blockade. It could be supposed that the changes might become more apparent only at later stages. Pressure elevation itself affects the metabolic and proliferation processes in the vessel wall, however, they do not seem to depend solely on this. Since NO may have an antiproliferative effect on cellular hypertrophy (Garg and Hassid 1989, Nakaki *et al.* 1990, Cornwell *et al.* 1994, Arnal *et al.* 1994) NO synthase blockade would be expected to have an opposite effect.

To solve the question which part of the arterial wall is responsible for the increase in wall thickness, volume densities of tunica intima and tunica media were measured by electron microscopy. We found that the relative contribution of the tunica intima to the wall thickness of the coronary artery in control and experimental animals was not significantly different (Table 1). The same relations were found in the tunica media in both experimental and control animals (Table 1). This means that the volume densities of both tunica intima and tunica media did not significantly differ in control and experimental animals. However, when measuring and calculating the areas of tunica

intima and tunica media in absolute values, a significant increase of both layers was found (Table 1). These findings indicate that in NO-deficient hypertension both parts of the arterial wall increase in the same way.

Table 1 also presents the values concerning the carotid artery and documents the same relations which were found in the coronary artery.

Table 1
Volume densities and areas of tunica intima and tunica media in the coronary and carotid artery

	Volume density (%)		Areas Đμm^2)	
	Control	L-NAME	Control	L-NAME
Coronary artery				
Tunica intima	13.24±0.55	12.86±0.93	1393±156	2023±134**
Tunica media	86.51±0.60	87.11±0.95	9291±1255	14052±1226*
Carotid artery				
Tunica intima	11.17±0.30	10.10±0.30	7727±610	11337±752**
Tunica media	88.81±0.40	89.91±0.40	61260±2891	106078±6073**

*Values are means±S.E.M. * P<0.05. ** P<0.01*

Table 2
Volume densities and areas of extracellular matrix and smooth muscle of tunica media in the coronary and carotid artery

	Volume density (%)		Areas Đμm^2)	
	Control	L-NAME	Control	L-NAME
Coronary artery				
Extracellular space	11.76±1.83	17.90±1.58*	1378±491	2985±459*
Smooth muscle cells	74.76±1.70	69.21±1.37*	7912±882	11068±795*
Carotid artery				
Extracellular space	36.67±2.21	43.06±1.89*	25629±2860	50537±3536**
Smooth muscle cells	52.14±2.48	47.13±2.04	35630±1417	55542±4051**

*Values are means±S.E.M. * P<0.05. ** P<0.01.*

The next question concerned the contribution of the cellular and/or extracellular component to the increase in thickness of the tunica media. Table 2 indicates that the balance of the volume density of the cellular and extracellular component in the coronary artery of experimental animals changes substantially. The contribution of the extracellular components increased markedly. This finding indicates that the main contribution to the wall thickness is due to the noncellular component of the tunica media. A similar conclusion concerns the carotid artery (Table 2).

On the basis of the presented results, we can summarize that long-term NO synthase inhibition, resulting in an with consequent cardiac hypertrophy, is characterized by marked remodelling of the coronary and carotid arterial wall with predominant accumulation of extracellular matrix in the tunica media.

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Reprint Requests

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