Cardiac Membrane Proteins and Phospholipids in L-NAME Induced Hypertension

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Abstract. This study deals with qualitative alterations of membranes in cardiac cells after chronic inhibition of NO synthesis in rats induced by administration of N^G-nitro-L-arginine methyl ester (L-NAME) in a dose 40 mg/kg/day for 4 weeks Concentration of proteins did not change either in the cytosolic fraction or in the particulate fraction of the cardiac homogenate from L-NAME treated rats The concentration of phospholipids and consequently the ratio of phospholipids to membrane proteins increased by 100% and 88%, respectively The concentration of conjugated dienes (CD), often used as an indirect marker for the production of free oxygen radicals, increased by 141% after calculation per gram of tissue However, evaluation of CD concentration directly in phospholipids revealed no change, suggesting that phospholipids in cardiac tissue after L-NAME treatment were not damaged additionally, by increased level of free oxygen radicals The increase of the CD concentration in the cardiac tissue is therefore, a consequence of the elevated phospholipid concentration

Key words: L-NAME — Hypertension — Phospholipids — Conjugated dienes

Introduction

Synthesis of NO from L-arginine in endothelial cells has been shown to be responsible for endothelium-dependent vascular relaxation, inhibition of platelet aggregation, reduction of cardiac output and regulation of blood pressure and heart rate (Moncada *et al* 1991, Amrani *et al* 1992, Pecháňová and Bernátová 1996) Chronic inhibition of NO-synthesis by L-arginine analogues induced deterioration of these functions which lead to sustained hypertension, development of left ventricular hypertrophy, myocardial fibrosis and hyperplasia in the media of coronary arteries and the aorta (Babál *et al* 1997)

The present study deals with qualitative alterations of membranes in myocardial cells after long lasting inhibition of NO synthesis in rats

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Materials and Methods

The hypertension in 15 weeks old male Wistar Kyoto rats was induced by inhibition of NO-synthase The arginine analogue N^G-nitro-L-arginine methyl ester (L-NAME) was applied daily in a dose 40 mg/kg/day in the drinking water for 4 weeks

In all individual animals the systolic blood pressure (SBP) was measured by a non-invasive method – tail cuff pletysmography The protein content in total homogenate of cardiac tissue, in cytosolic fraction and in the particulate fraction was estimated by the method of Lowry *et al* (1951) The phospholipid concentration (taken as 25 times the weight of lipid phosphorus) was estimated in lipid extract via measuring the inorganic phosphorus in the mineralized sample (Taussky and Shorr, 1953) The concentration of conjugated dienes in lipid extracts was estimated according to Kogure *et al* (1982)

All results were expressed as means ± SEM The significance of differences between the individual groups was determined with the use of unpaired Student's *t*-test A value of $P < 0\ 05$ was regarded as significant

Results and Discussion

In agreement with previous studies in our experiment the long lasting administration of NO-synthase inhibitor L-NAME induced a significant increase of the systolic blood pressure from 120 ± 3 to 170 ± 8 mm Hg Despite the proved fact that the NO-deficient hypertension is accompanied by left ventricular hypertrophy and by an increased concentration of nucleic acids as well as by an increased proteosynthesis (Pecháňová *et al* 1997), the concentration of proteins did not change significantly either in the cytosolic fraction or in the particulate fraction of the cardiac homogenate from L-NAME treated rats as shown in Table 1 The protein distribution in the cytosolic or particulate fraction was similar in both investigated groups In control animals 21% of proteins and after L-NAME treatment 23% of proteins were found in the cytosol In membraneous, or particulate fraction there was localized 77% of proteins in controls and in L-NAME rats it was 78% Our findings suggest that for keeping the similar concentration of proteins in the cardiac tissue during the deficit of NO in the organism the hypertrophy and the increased degradation of proteins might be responsible

Table 1. Proteins and phospholipids (PL) in myocardial cells after long lasting inhibition of NO synthesis in rats The inhibition of NO synthase was induced by 4 weeks lasting administration of L NAME

	Proteins			Phospholipids		Conjugated dienes	
	Total (mg/g)	Cytosol (mg/g)	Membr (mg/g)	Tissue (mg/g)	Membr (mg/g)	Tissue (nmol/g)	PL (nmol/mg)
Controls	122 ± 4	28 ± 1	94 ± 3	18 ± 2	177 ± 30	91 ± 4	5 1 ± 0 1
L NAME	141 ± 4	30 ± 2	110 ± 3*	37 ± 3*	333 ± 30*	218 ± 7*	6 0 ± 0 6

The number of rats in each experimental group was $n = 6$ $^*p < 0\ 05$

The concentration of phospholipids and consequently the ratio of phospholipids to membrane proteins increased by 100% and 88%, respectively during the L-NAME induced hypertension

The concentration of conjugated dienes (CD), often used as an indirect marker for the production of free oxygen radicals, increased by 141% (from 91 to 218 nmol) after calculation per gram of tissue (Table 1) However, evaluation of CD concentration directly in phospholipids revealed no change, suggesting that phospholipids in cardiac tissue after L-NAME treatment were not damaged additionally, by increased level of free oxygen radicals The increase of the CD concentration in the cardiac tissue is therefore a consequence of the elevated phospholipid concentration

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Hypokalemia-Induced Ultrastructural, Histochemical and Connexin-43 Alterations Resulting in Atrial and Ventricular Fibrillations

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Abstract. Perfusion of the isolated guinea pig heart with hypokalemic solution provide simple model for examination of the molecular mechanisms involved in the incidence

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