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Effect of Phosphocreatine and Ethylmethylhydroxypyridine Succinate on the Expression of Bax and Bcl-2 Proteins in Left-Ventricular Cardiomyocytes of Spontaneously Hypertensive Rats

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We studied the effect of phosphocreatine and ethylmethylhydroxypyridine succinate on the expression of Bax and Bcl-2 proteins in left-ventricular cardiomyocytes of spontaneously hypertensive rats (SHR). Both drugs have no effect on the expression of Bcl-2, but significantly reduce the level of Bax protein (phosphocreatine produces more pronounced effect). These data attest to an important role of energy deficit and oxidative stress in the induction of cardiomyocyte apoptosis in genetically determined arterial hypertension.

Key Words: *apoptosis; hypertension; cardiomyocyte; phosphocreatine; mexidol*

Arterial hypertension (AH) of different genesis is associated with enhanced programmed death of cardiomyocytes (CMC) triggered by various factors including mechanical forces, oxidative stress, hypoxia, chronic persistence of growth factors, *etc.* [1,2,4,7,9]. High BP leads to moderate energy deficit in myocardial cells due to increased workload on the heart, its hypertrophy, and calcium overload of mitochondria. In turn, ATP deficit results in the opening of mitochondrial pores and release of pro-apoptotic factors [8]. Development of oxidative stress in hypertension is mainly related to activity of the mitochondrial respiratory chain [5] and angiotensin II-activated NADPH oxidase [9]. Since ROS are capable of inducing apoptosis, their involvement in the initiation of programmed CMC death observed in AH can be assumed.

Our previous studies have shown that high-energy compounds and, to a lesser extent, antioxidants are capable of reducing apoptosis in CMC [3], but their targets in the apoptotic signaling pathways are to be identified.

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Here we studied the effects of high-energy compound phosphocreatine and antihypoxant and antioxidant ethylmethylhydroxypyridine succinate on the expression of Bax and Bcl-2 proteins in CMC of the left ventricle (LV) in rats with genetically determined AH.

MATERIALS AND METHODS

The study was performed on 15-week-old male SHR rats. In series I, the rats received no treatment (n=4). In series II, the rats were daily intraperitoneally injected with neoton (active ingredient phosphocreatine) in a dose of 30 mg/kg (n=4) or mexidol (active substance ethylmethylhydroxypyridine succinate) in a dose of 5 mg/kg (n=4) 10 days before they reached 15 weeks of age. The control group included 15-week-old normotensive Wistar-Kyoto rats.

The thorax was opened under general anesthesia and the heart was extirpated. Animal experiments were conducted in accordance to the Order No. 755 of the Ministry of Health of the USSR (August 12, 1977) and European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986).

The specimens of LV myocardium were fixed in 10% neutral buffered formalin for 72 h, processed, and embedded in paraffin by the standard technique. Histological sections (5 μ) were mounted on poly-L-lysine-coated slides.

Immunohistochemical study of Bcl-2 and Bax were performed using primary rabbit polyclonal antibodies (Santa Cruz Biotechnology Inc.). The reaction was visualized with UltraVision Detection System Kit (Thermo Scientific). The slides were counterstained with Mayer's hematoxylin. The reaction was considered positive, when brown color was seen; 20 fields of view were examined at ×400 in each preparation using Avtandilov's grid. In this case, the ratio of the number of equidistant points falling to positively stained cytoplasm of CMC to the total number of points occupied by the cytoplasm of CMC was determined.

The data were processed using Statistica 6.0 software (StatSoft Inc.) Mann–Whitney *U* test was used to assess the significance of differences in the studied samples.

TABLE 1. Content of Bcl-2 and Bax Proteins (%) in LV CMC of Rat Heart (*M*±*m*)

Protein	Wistar-Kyoto Rats	SHR Rats		
		without therapy	neoton	mexidol
Bcl-2	2.27±0.24	0.72±0.13*	1.34±0.26*	0.75±0.13*
Bax	14.32±0.72	42.18±1.61*	22.27±1.46+*	32.02±1.30+*

Note. $p<0.05$ in comparison with *Wistar-Kyoto rats, +SHR rats without treatment.

RESULTS

Bcl-2 in CMC was weakly expressed and was detected in only some fields of view. Nevertheless, its level in LV myocardium of untreated SHR rats was significantly lower than in the controls. Weak expression of this protein in the myocardium of SHR and Wistar–Kyoto rats has been previously reported [6], but no significant differences between the strains were revealed. Proapoptotic protein Bax attracts more attention; its level in LV of SHR rats was almost 3-fold higher than in normotensive animals. Enhanced expression of Bax proves the importance of the mitochondrial signal transduction pathway in the implementation of the programmed death of CMC in hypertensive animals, because this protein participates in pore formation in the mitochondrial membrane, through which cytochrome C, endonuclease G, AIF, and other proapoptotic factors are released [10].

Exogenous phosphocreatine considerably reduced Bax level in LV CMC of SHR rats, which remained significantly higher than in normotensive animals, suggesting multifactorial nature of the enhanced expression of this protein in myocardial cells under conditions of high BP. Administration of neoton had no significant effect on the content of Bcl-2 in CMC, although some tendency to its increase was observed.

Administration of mexidol yielded similar results: Bcl-2 expression remained unchanged and Bax level significantly decreased, but was significantly higher than in controls. It is noteworthy that mexidol produced less pronounced effect on Bax expression in comparison with neoton. This explains less pronounced decrease in the total intensity of apoptosis after treatment with ethylmethylhydroxypyridine succinate in comparison with the effect of phosphocreatine reported earlier by us [3].

Thus, genetically determined AH in rats is accompanied by activation of mitochondrial signal transduction pathway probably triggered by energy deficit and to a lesser extent oxidative stress.

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