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Energy Deficit as a Possible Factor for the Induction of Caspase-Dependent Apoptosis in Left Ventricular Myocardial Cells during Genetically Determined and Secondary Arterial Hypertension

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Activities of caspase-3 and caspase-8 in the left ventricular myocardium of Chinchilla rabbits with renovascular arterial hypertension and spontaneously hypertensive rats were measured after 10-day administration of a macroergic compound phosphocreatine. Treatment with phosphocreatine prevented activation of caspase-3, but had no effect on caspase-8 during secondary and genetically determined arterial hypertension. Our results indicate that the intrinsic mechanism of the induction of the caspase cascade in myocardial cells dominates over the extrinsic pathway during both types of arterial hypertension. Energy deficit is one of the inducing factors of these processes.

Key Words: *apoptosis; caspase; arterial hypertension; myocardium; phosphocreatine*

Apoptosis is a genetically determined programmed cell death. This process is induced by exogenous or intracellular stimuli and realized via various signal pathways. Initiator and effector caspases are the major components of these pathways.

Apoptosis is induced upon the impairment of the key processes that determine normal function of the cell, *e.g.* irreversible damage to DNA and energy deficit. Previous studies showed that the type of cell death depends on the degree of imbalance between cell energy requirements and energy production during mitochondrial oxidative phosphorylation [8]. Transient and reversible decrease in intracellular ATP concentration stimulates cell apoptosis, which is realized via p53 protein activation, formation of the apoptosome, and induction of the caspase cascade [10,11], while almost complete exhaustion of intracellular ATP is followed by necrosis. This phenomenon is related to the fact that apoptotic processes are energy-dependent and can be suppressed under conditions of severe energy deficit.

Much recent attention was paid to studying the mechanisms of induction and realization of apoptosis in terminally determined cells, including cardiomyocytes. Previous studies showed that arterial hypertension (AG) contributes to an increase in programmed cell death in the myocardium [1,3,5]. It can be suggested that energy deficit in cardiomyocytes serves as a factor that induces the apoptotic program during AG. This state is associated with the increase in ATP consumption by the cells due to myocardial overload and myocardial hypertrophy. Insufficient energy formation in the cell can also be a result of mitochondrial calcium overload, which is followed by uncoupling of the

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respiratory chain and oxidative phosphorylation [2]. ATP deficiency impairs normal function of ion channels. In addition to calcium overload, these changes contribute to a decrease in the mitochondrial membrane potential and, therefore, opening of the pores and release of proapoptotic factors [4,7,9]. The role of energy deficit in the induction of apoptotic death in myocardial cells during AG can be evaluated by administration of macroergic compounds to hypertensive animals. Here we studied the effect of macroergic compound phosphocreatine on activities of caspase-3 and caspase-8 in left ventricular (LV) myocardial cells during AG of different genesis.

MATERIALS AND METHODS

Experiments were performed on male Chinchilla rabbits (body weight 3.0-3.5 kg; *n*=24) and 15-week-old male SHR rats and Wistar-Kyoto rats (*n*=24).

The influence of phosphocreatine on some components of the caspase cascade in LV myocardial cells during secondary AG was studied on rabbits. The experiment was conducted in 2 series. In each series, the animals were divided into two groups. The treatment group 1 consisted of rabbits with renovascular AG of 4 weeks duration. The control group 2 was composed of intact rabbits. AG in rabbits of the treatment groups was modeled by the method of Goldblatt (constriction of the abdominal aorta by one-third of the initial diameter above the origin of the renal arteries) [6]. In series I, the animals received no treatment. In series II, all rabbits of the treatment group received intramuscular injections of Neoton (phosphocreatine as an active component) in a dose of 30 mg/kg for 10 days.

The effect of phosphocreatine on caspase activities in the LV myocardium during genetically determined AG was studied on rats. The treatment groups included spontaneously hypertensive SHR rats. The control groups consisted of normotensive Wistar-Kyoto rats. In series II, all rats of the treatment group were injected intramuscularly with Neoton in a daily dose of 30 mg/kg for 10 days (until the age of 15 weeks).

Each group consisted of 6 animals.

Thoracotomy and extirpation of the heart in all animals were performed under general anesthesia. The myocardial fragment (200-250 mg) was excised from the LV wall for further biochemical study.

The animal experiments were conducted in accordance with the Order No. 755 of the USSR Ministry of Health (12.08.1977).

Lysates of LV from rabbits and rats were obtained by homogenization of the myocardial fragment in the isolation medium containing 20 mM HEPES (pH 7.5), 10 mM KCl, 1.5 mM $MgCl_2$, and 1 mM dithiothreitol; protease inhibitor cocktail (104 mM AEBSF, 0.08 mM aprotinin, 1.5 mM pepstatin A, 2 mM leupeptin, 4 mM bestatin, and 1.4 mM E-64) was added at 100:1 ratio (Sigma reagents); the lysates were centrifuged at 15,000*g* and 4°C for 30 min. Activities of caspase-3 and caspase-8 in these lysates were measured colorimetrically using Caspase 3 Assay Kit Colorimetric and Caspase 8 Assay Kit Colorimetric (Sigma).

The study was performed on devices of the Collective Use Center (People's Friendship University of Russia) and Department of General Pathology and Pathophysiology (Medical Faculty, People's Friendship University of Russia).

The results were analyzed by Statistica 6.0 software (StatSoft Inc.). The significance of differences between the samples was evaluated by Mann–Whitney *U* test.

RESULTS

Caspase-3 activity in the LV myocardium increased significantly in untreated rabbits with 4-week AG (series I). Caspase-8 activity tended to increase in these animals, but did not differ significantly from the control. These data illustrate the prevalence of the mitochondrial mechanism of caspase cascade induction during renovascular AG (Table 1). In series II, caspase-3 activity in the myocardium of Neoton-treated rabbits did not differ from the control. Therefore, Neoton prevents activation of caspase-dependent apoptotic processes in LV myocardial cells. These data indicate that energy deficit serves as one of the major mechanisms of apoptosis induction in cardiomyocytes during secondary AG. Since caspase-8 activity did not differ from the control in series I, we did not measure this parameter in the myocardium of Neoton-treated rabbits with AG.

Activities of caspase-3 and caspase-8 in untreated SHR rats with genetically determined AG were shown to increase significantly at the age of 15 weeks (Table 2). Hence, the caspase cascade in LV myocardial cells of spontaneously hypertensive rats is induced by the extrinsic (receptor-mediated) pathway. Taking into account these data, activities of both caspases were measured in Neoton-treated animals of series II. No statistically significant differences were found in caspase-3 activity in the myocardium of Neoton-treated hypertensive rats and control animals. However, caspase-8 activity in animals of the treatment group was much higher than in the control. These data suggest that apoptotic signal transduction by the extrinsic (receptor-mediated) pathway is not related to energy deficit in the myocardium. The absence of changes in caspase-3 activity indicates that the extrinsic pathway of apoptotic signal transduction does not play the major role during genetically determined AG. Similarly to secondary AG, the intracellular (mitochondrial) signal

TABLE 1. Activities of Caspase-3 and Caspase-8 in the LV Myocardium of Rabbits (nmol/min×ml, *M*±*m*)

Parameter	Series I		Series II	
	control	AG 4 weeks	control	AG 4 weeks+Neoton
Caspase-3	0.27±0.03	0.36±0.02*	0.23±0.02	0.23±0.02
Caspase-8	0.99±0.16	1.39±0.11	N.d.	N.d.

Note. *$p \leq 0.05$ in comparison with the control. N.d.: not determined.

TABLE 2. Activities of Caspase-3 and Caspase-8 in the LV Myocardium of Rats (nmol/min×ml, *M*±*m*)

Parameter	Series I		Series II	
	control	SHR	control	SHR+Neoton
Caspase-3	0.18±0.02	0.27±0.03*	0.20±0.03	0.23±0.03
Caspase-8	1.06±0.13	1.61±0.06*	0.51±0.10	0.95±0.10*

Note. *$p \leq 0.05$ in comparison with the control.

due to energy deficit plays the main role in the induction of caspase-dependent apoptotic death of myocardial cells.

We conclude that the mitochondrial mechanism of the induction of the caspase cascade in LV myocardial cells predominates during genetically determined and secondary AG. Cell energy deficit is one of the inducing factors of these processes.

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