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Antiapoptotic Effect of Heat Adaptation in Cultured Cells

E. A. Monastyrskaya, M. R. Duchen*, L. V. Andreeva*, F. Viegant, E. B. Manukhina, and I. Yu. Malyshev**

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Heat adaptation of cultured rat cardiomyoblasts and mouse myoblasts protected these cells from damage produced by staurosporine and attenuated metabolic disturbances. Heat adaptation inhibited apoptotic, but not necrotic cell death. The mechanism of antiapoptotic adaptive protection was not associated with prevention of mitochondrial depolarization, because heat adaptation not only induced depolarization of mitochondria, but also intensified this process under conditions of staurosporine-induced damage.

Key Words: *heat adaptation; cell culture; apoptosis; mitochondria*

Adaptation to environmental factors increases organism's resistance. The protective effects of adaptation are related to functional changes in the central neurohormonal mechanisms, on the one hand, and are associated with activation of the genetic apparatus and metabolic changes in organs involved in adaptation, on the other [9].

Published data suggest that the heart isolated from adapted animals is highly resistant to damage [1,2]. Moreover, the resistance of isolated hearts to ischemic and reperfusion damage increases after ischemic preconditioning [3]. These data indicate that environmental factors can directly activate the cellular protective mechanisms [8]. In the present work the possibility of protecting cultured cardiomyoblasts and myoblasts was evaluated by the nonspecific parameter of cell viability.

Energy balance determines the type of death for damaged cells. Apoptosis is an energy-dependent process, while necrosis proceeds during exhaustion of energy reserves in cells under acute conditions [12]. Mitochondria are the main structures responsible for energy production in eukaryotic cells. Mitochondria synthesize ATP using the energy of electrochemical proton gradient on their inner membrane. Therefore, the mitochondrial membrane potential ($m\Delta\psi$) reflects energy state of the cell. Mitochondria are involved in cascade reactions during apoptosis and secrete cytochrome *c* and other proapoptotic factors into the cytoplasm in the early stage of this process [13]. Changes in $m\Delta\psi$ play an important role in the release of proapoptotic factors and induction of apoptotic cascade reactions [5]. Here we evaluated whether the possible protective effect of adaptation in cultured cells is specific for the type of cell death and studied changes in $m\Delta\psi$ after cell adaptation.

MATERIALS AND METHODS

Experiments were performed on rat H9c2 cardiomyoblasts and mouse C2c12 myoblasts (ATCC). The cells were grown in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum and containing 1% penicillin-streptomycin at 37°C and 10% CO_2.

Heat adaptation was performed by daily heat exposure (1 h, 41.5°C, in a water bath) for 6 days. Ex-

Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow; *University College, London; **Utrecht University. ***Address for correspondence:*** igor.malyshev@mtu-net.ru. Malyshev I. Yu.

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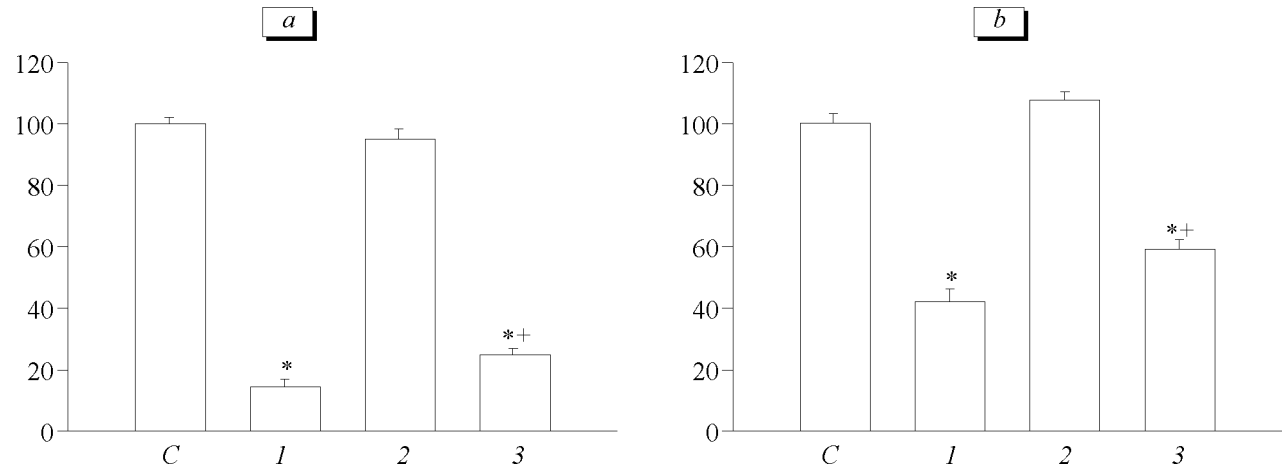

Fig. 1. Effect of heat preadaptation on metabolic activity of cells under staurosporine-induced damage. Here and in Fig. 2: H9c2 (*a*) and C2c12 cells (*b*). Control (*C*), staurosporine (*1*), adaptation (*2*), adaptation+SS (*3*). $p<0.05$: *compared to the control; $^{+}p<0.05$ compared to staurosporine.

periments with cells were started 24 h after the last exposure.

Apoptosis was induced with protein kinase inhibitor staurosporine (SS) in a concentration of 0.5 μM. SS was dissolved in 50% methanol. Control cells were treated with an equivalent volume of 50% methanol. Incubation was performed for 24 h.

Metabolic activity of cells was estimated by MTT-bioreduction. This method characterizes activities of NADH and NADPH dehydrogenases (respiratory complex I) and reflects total metabolic activity of cells. Cells were incubated with MTT in a concentration of 5 mg/ml at 37°C for 1 h. Violet crystals were dissolved in a solution containing isopropanol, 0.1 M HCl, and 10% Triton X-100. Light absorption at 590 nm was measured.

The cells were incubated with 10 μM Hoechst 33342 and 60 μM propidium iodide (PI) in dark for 30 min. Normal, apoptotic, and necrotic cells were counted under a fluorescence microscope (×400).

Confocal microscopy was used to measure $m\Delta\psi$ in individual cells [4]. The cells were incubated in 40 nM tetramethylrhodamine ester (TMRM) with 0.002% pluronic at room temperature for 20 min. Experiments were performed using a confocal visualization system (model 510, Carl Zeiss). Fluorescence was excited with a 543 He-Ne laser. Images were obtained at an emission wavelength >560 nm. The cell volume was estimated by multilayer scanning. Total images were analyzed using Lucida software (Kinetic Imaging).

The value of $m\Delta\psi$ in cell population of was determined by fluorescence cytometry. The cells were incubated with 0.2 μM TMRM at 37°C for 20 min. $m\Delta\psi$ was measured on a FASC device (Becton Dickinson) using CellQuest software. The geometric mean of a signal from TMRM in the histogram window for signal distribution was calculated to estimate $m\Delta\psi$.

The results were analyzed by Student's *t* test. The result were significant at two-way significance level $p<0.05$.

RESULTS

Incubation of H9c2 cells with 0.5 μM SS for 24 h decreased light absorption to 14±2% of the control. Adaptation had no effect on metabolic activity of cells. However, this procedure increased metabolic activity of cells treated with SS to 25±2% of the control (Fig. 1, *a*). Experiments with C2c12 cells revealed a similar

TABLE 1. Number of Viable, Apoptotic, and Necrotic Cells after Adaptation and Treatment with SS (*M*±*m*, %)

Group	Viable cells		Apoptosis		Necrosis	
	H9c2	C2c12	H9c2	C2c12	H9c2	C2c12
Control	82.3±5.2	86.8±2.3	10.7±5.1	10.4±1.5	6.6±0.9	2.8±0.8
Adaptation	87.4±5.4	90.8±4.0	7.6±4.9	7.1±3.0	5.0±2.7	2.1±1.0
SS	19.2±1.8*	46.1±5.0*	53.9±5.8*	43.7±2.6*	26.9±5.3*	10.2±2.9*
Adaptation and SS	28.1±2.2*+	65.3±3.5*+	43.2±3.5*+	23.7±1.7*+	28.7±3.8*	11.1±2.0*

Note. *$p<0.05$ compared to the control; $^{+}p<0.05$ compared to SS.

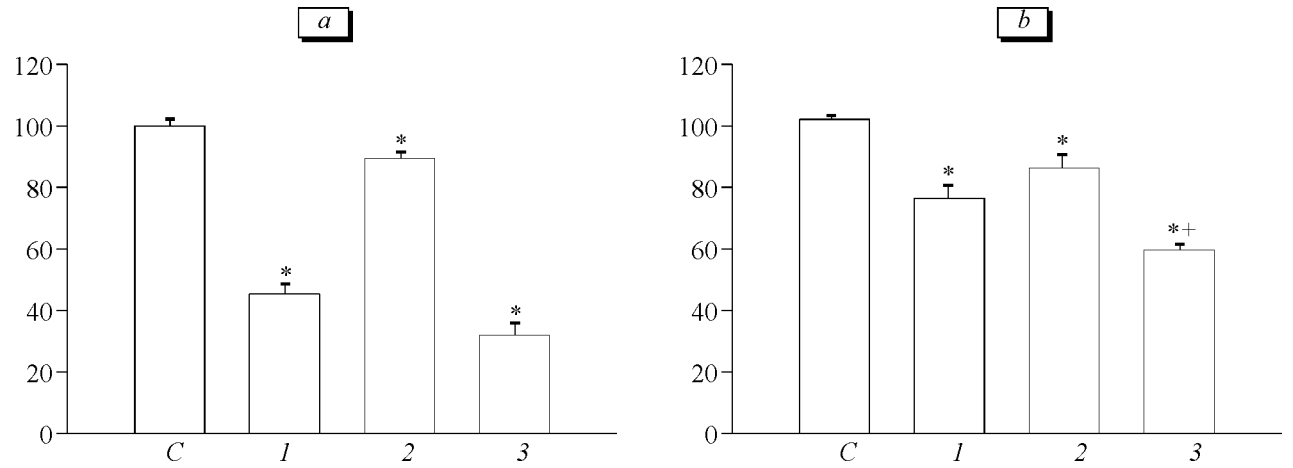

Fig. 2. Effect of heat adaptation on mitochondrial potential after treatment with staurosporine. Changes: flow cytofluorometer. Tetramethylrhodamine: % of the control.

effect of adaptation. Incubation with SS reduced metabolic activity of cells to 42±4% of the control. In adapted cell this parameter decreased only to 59±3% of the control (Fig. 1, *b*). These results show that SS produced a less pronounced toxic effect on adapted cells, which confirms the protective effect of adaptation at the cellular level.

SS in medium concentrations primarily induced apoptosis in cells. The percentage of necrotic cells was low. Adaptation had no effect on baseline parameters of necrosis and apoptosis in cultured cells. However, adaptation markedly increased the count of survived cells in SS-treated cultures due to selective inhibition of apoptosis (Table 1). It was typical of both H9c2 and C2c12 cells. Probably, activation of some physiological mechanisms during adaptation protect cells under condition of impaired energy supply (apoptosis), but not under conditions of acute energy deficiency when normal functioning of cellular systems is irreversibly impaired (necrosis) [12].

Confocal microscopy showed that adaptation stably decreased $m\Delta\psi$ in H9c2 and C2c12 cells (by 20±5 and 23±3%, respectively, compared to the control). The phenomenon of mitochondrial depolarization not accompanied by changes in metabolic activity of adapted cells indicates that they can maintain a constant level of energy production at low $m\Delta\psi$. This process can be interpreted as cell transition to a more economy regimen of functional activity. Another explanation of this phenomenon is activation of alternative pathways of energy production (*e.g.*, glycolysis) playing a role in energy supply to immortalized cells [11]. Previous studies showed that depolarization of mitochondria is preceded by their hyperpolarization. This process triggers the release of proapoptotic factors from the mitochondrial matrix [6]. Predepolarization can suppress this process and prevent apoptosis in individual cells.

Fluorescence cytometry assay of $m\Delta\psi$ confirmed depolarization of mitochondria in adapted cells. However, changes in $m\Delta\psi$ revealed by this method were less significant. Incubation with SS decreased $m\Delta\psi$ in H9c2 and C2c12 cells by 55±3 and 24±4%, respectively (Fig. 2, *a*, *b*). Preadapted H9c2 and C2c12 cells incubated with SS were characterized by greater depolarization (by 68±4 and 40±2%, respectively, Fig. 2, *a*, *b*). The data suggest that adaptation did not prevent changes in $m\Delta\psi$ in damaged cells, but promoted survival of seriously damaged cells.

Our experiments revealed the existence of adaptive protection at the cellular level, which does not involve neurohormonal factors. This protective effect is selective for apoptosis during SS-induced damage. Adaptation causes depolarization of mitochondria. The relationship between this effect and phenomenon of protection requires further investigations. Experiments with two cell lines produced similar results. Therefore, the protective effect of heat adaptation is relatively nonspecific for various types of cells.

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