Direct and Cross-Protective Effects of Heat Adaptation in Cultured Cells

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The model of 6-day dosed heat adaptation was developed on cultured rat H9c2 cardiomyoblasts and mouse C2c12 myoblasts. Heat adaptation produced a direct protective effect and increased the resistance of cells to heat shock. The cross-protective effect of heat adaptation was manifested in an increase in the resistance to staurosporine-induced damage. Heat adaptation did not protect cells from oxidative damage. As differentiated from adaptation of the organism, heat adaptation produces direct and cross-protective effects that develop due to the action of environmental factors in the absence of neurohumoral agents. It should be emphasized that the cross-protective effect of heat adaptation in cultured cells is specific to a certain type of damage.

Key Words: *heat adaptation; cell culture; heat shock; staurosporine; oxidative stress*

Adaptation to environmental factors increases the resistance of organisms to adverse factors. The protective effect of adaptation manifesting in an increase in organism's resistance to the adaptive factor is designated as direct protective effect [10]. Adaptation increases organism's resistance not only to the adaptive factor, but also to other factors. This phenomenon is designated as cross-protective effect of adaptation [10]. On the one hand, the protective effects of adaptation are related to functional changes in the central neurohumoral mechanisms. On the other hand, they are associated with activation of the genetic apparatus and metabolic changes in cells of organs involved in adaptation. Studies of the mechanism underlying adaptation of cultured cells would elucidate the development of direct and cross-protective effects of adaptation in the absence of neurohumoral factors.

The protective effects of environmental factors on organisms and cultured cells were studied on the model of preconditioning [4]. The protective effect of preconditioning develops after a single exposure to environmental factors that produce moderate damage to cells and activate the mechanisms protecting organisms or cultured cells from adverse factors. Preconditioning produces direct [14] and cross-protective effects [6,9]. The adaptive increase in the resistance of organs is associated with repeated exposure to moderate factors that do not induce functional and structural disturbances in cells.

Here we studied the cellular mechanisms of adaptation, developed the model of dosed adaptation to heat in cultured cells, and evaluated its direct and cross-protective effects.

MATERIALS AND METHODS

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

Heat adaptation was performed by daily 1-h exposure of sealed dishes with cells in a water bath at 41.5°C. The model of heat adaptation was selected after repeated exposure to heat for 1, 3, and 6 days.

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Experiments with cells started 24 h after the last hyperthermia. The protective effect of adaptation was determined by an increase in the resistance of cells to various adverse factors, including treatment with apoptosis inductor staurosporine (SS), heat shock, and oxidative stress.

SS in a concentration of 0.5 μM was dissolved in 50% methanol. An equivalent volume of 50% methanol was added to control cells. Cells were incubated with SS for 24 h.

Heat shock was induced by incubation of sealed dishes with cells in a water bath at 45°C for 1 h followed by 24-h incubation at 37°C.

Oxidative stress was produced by incubation of cells with 250 μM tert-butyl hydroperoxide for 2 h followed by 22-h incubation in fresh culture medium.

Metabolic activity of cells was estimated by bioreduction of 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) [12]. This method characterizes activities of NADH and NADPH dehydrogenases (respiratory complex I) and reflects total metabolic activity of cells. The cells were incubated with MTT in a concentration of 5 mg/ml at 37°C for 1 h. Violet crystals were dissolved in the solution containing isopropanol, 0.1 M HCl, and 10% Triton X-100. Light absorption of the solution was measured on a spectrophotometer at 590 nm. Metabolic activity of cells was directly proportional to the degree of light absorption.

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

RESULTS

One-hour exposure of H9c2 cells to heat (41.5°C) for 1, 3, and 6 days at 24-h intervals had no effect on their metabolic activity (Fig. 1, *a*). After 24-h incubation of H9c2 cells with 0.5 μM SS light absorption of the metabolic product decreased to 14±2% of the control. Heat exposure (41.5°C) for 1 and 3 days did not affect the severity of damage produced by SS. Heat exposure for 6 days increased metabolic activity of cells treated with SS to 25±2% of the control level (Fig. 1, *a*). Experiments with C2c12 cells showed that heat exposure for 6 days produces a similar protective effect. Incubation of C2c12 cells with SS reduced their metabolic activity to 42±4% of the control. In cells preheated for 6 days this parameter decreased only to 59±3% of the control (Fig. 1, *b*).

Repeated exposure to heat for 6 days not reducing metabolic activity of cells produced an adaptive protective effect. The development of this effect reflects adaptation of cultured cells. A single exposure to heat during adaptation produced no protective effects. The adaptive effect observed in our experiments differs from the protective influence of repeated preconditioning and reflects an independent adaptive phenomenon.

Heat exposure for 3 days did not increase the resistance of cells. Therefore, adaptation of cells is similar to heat adaptation of the organism that produces the protective effect only after 6 days.

Heat shock in H9c2 cells decreased their metabolic activity to 46±3% of the control. Six-days adaptation increased metabolic activity of cells subjected to heat shock to 63±3% of the control (by 17%, Fig. 2, *a*). Heat shock in C2c12 cells decreased their metabolic activity to 35±7% of the control. Adaptation abolished the inhibitory effect of heat shock and increased metabolic activity of cells to 94±10% of the control (Fig. 2, *a*).

Our results show that heat adaptation increases the resistance of cultured cells not only to heat shock, but also to SS-induced damage. Therefore, adaptation of cultured cells produces the direct and cross-protective effects.

Oxidative stress decreased metabolic activity of H9c2 and C2c12 cells to 9±1 and 76±8% of the control, respectively (Fig. 2, *b*). Preadaptation of cells did not modulate the severity of damage after oxidative stress and, therefore, had no protective effect.

Thus, the adaptive effect of heat adaptation is specific foro the type of cell damage. Heat adaptation prevents damage produced by SS and heat shock, but has no protective effect during oxidative stress induced by tret-butyl hydroperoxide. It should be emphasized that this specificity is not associated with the severity of damage. Adaptation produces no protective

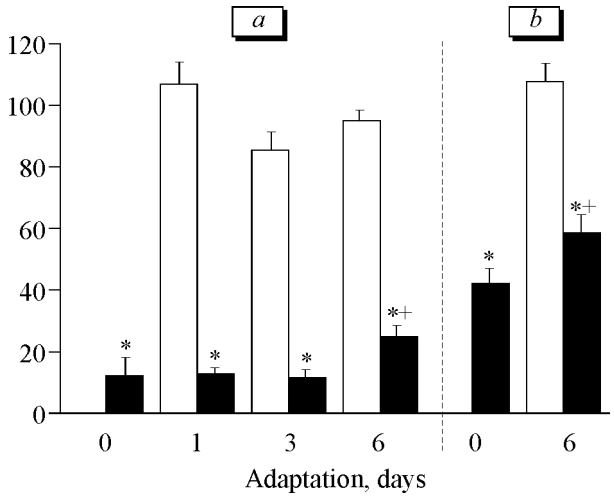

Fig. 1. Metabolic activity of H9c2 (*a*) and C2c12 cells (*b*) after heat preadaptation (light bars) and staurosporine-induced damage (dark bars). $^{+}p<0.05$ compared to staurosporine. Here and in Fig. 2: ordinate: MTT reductase activity, % of the control (100%); $^{*}p<0.05$ compared to the control.

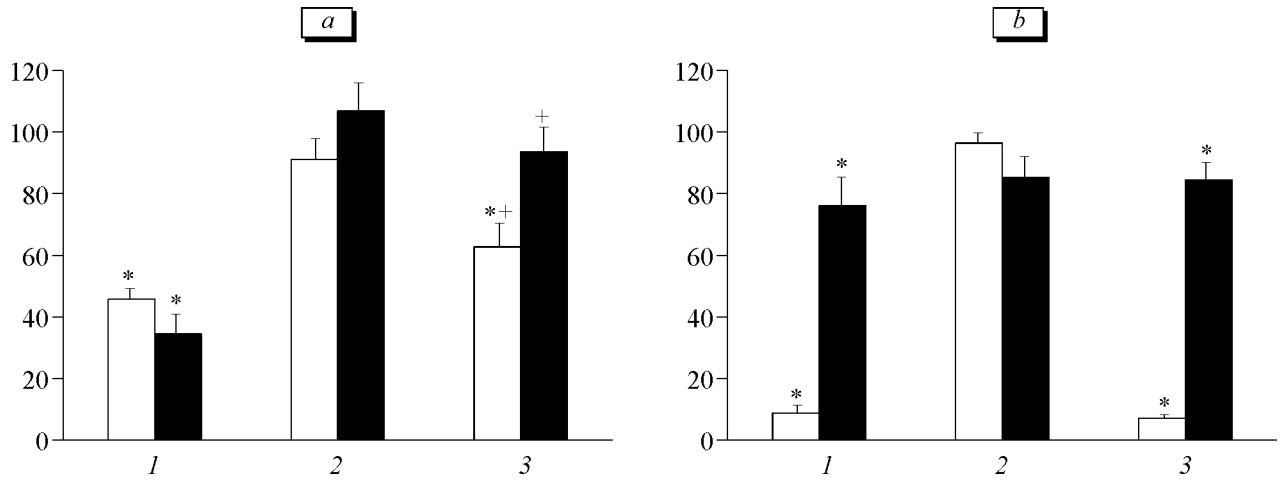

Fig. 2. Metabolic activity of H9c2 (light bars) and C2c12 cells (dark bars) after heat shock (*a*) and oxidative stress (*b*): treatment (*1*), adaptation (*2*), and treatment after adaptation (*3*). $^{+}p<0.05$ compared to heat shock.

effects during severe and moderate oxidative damage to various cells. It cannot be excluded that the specific protective effect of adaptation is associated with the type of cell death induced by different damaging factors. SS induces apoptosis in cultured cells [4]. Oxidative stress primarily induces cell necrosis [13]. These data suggest that the mechanisms of adaptive protection primarily interfere with apoptosis, but not with necrosis. Our recent experiments showed that preadaptation to heat reduces the incidence of apoptosis in cells, but does not affect the count of necrotic cells [3].

No differences were revealed in the direct and cross-protective effects of adaptation on H9c2 and C2c12 cells. It was probably associated with the fact that these cells belong to muscle cells.

Our results indicate that the direct and cross-protective effects of adaptation to environmental factors result from their influence on cells in the absence of neurohumoral factors (as differentiated from adaptation of the organism).

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