GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Thermal Adaptation Activates HSP70 Synthesis, Inhibits Overproduction of Nitric Oxide, and Protects the Body from Acute Hypotension during Heat Shock

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 128, No. 11, pp. 507-510, November, 1999
Original article submitted April 5, 1999

The role of HSP70 and nitric oxide in antihypotensive effects of thermal adaptation was studied. Western blot analysis and electron paramagnetic resonance were used to determine the contents of HSP70 and nitric oxide. Protective effect of adaptation was evaluated by the limitation of blood pressure drop after heat shock. The formation of protective effects, accumulation of HSP70, and development of the ability to decrease nitric oxide overproduction had similar dynamic patterns and appeared at the same period. Quercetin, an inhibitor of HSP70 synthesis, prevented the development of protective effects. The data suggest that HSP70 accumulated during adaptation prevents heat shock-induced hypotension by restricting NO overproduction and interfering with its cytotoxic effects.

Key Words: *nitric oxide; heat-shock proteins; hypotension; adaptation*

Acute damage to the cardiovascular system that manifests in low blood pressure (BP) and myocardial damage is the chief cause of death during heat shock (HS) [13]. It was shown that preliminary heat preconditioning diminishes damage to cells, tissues, and the body caused by subsequent heat exposure [9,11]. Preconditioning stimuli induce the synthesis of protective HS proteins of the HSP70 family associated with various damages to cell structures. The protective action of HSP70 is assumed to be realized via dissociation of denatured protein aggregates [11,12] and involvement in utilization of irreversibly damaged proteins [6,11].

Adaptation to periodic mild heating inducing accumulation of HSP70 in organs [11] can also improve the resistance to HS. The advantage of this procedure over preconditioning is that mild thermal factors, each of which does not have damaging abilities, induce protective effects.

However, the role of HSP70 in protective effects of thermal adaptation remains poorly understood. Recent data suggest that HS-induced drop of BP is associated with overproduction of nitric oxide (NO) [2,3] and that HSP70 suppresses activity and synthesis of NO synthase [5]. Adaptation to mild stress, hypobaric hypoxia, or physical exercises also prevents NO-dependent hypotension [1]. These data suggest that thermal adaptation induces antihypotensive effects and that HSP70 is involved in this process.

Here we studied the effects of thermal adaptation on the HS-induced decrease in BP and NO overproduction, compared the dynamics of HSP70 accumulation and formation of protective effects of thermal adaptation, and analyzed the influence of the HSP70

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0007-4888/99/0011-1097$22.00

synthesis inhibitor on protective effects of thermal adaptation.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 220-250 g. HS was induced by heating the animals in a thermostat for 15 min after attaining rectal temperature of 41.5±0.5°C (the total duration of heating did not exceed 35 min). One hour after HS, BP in the caudal artery was measured by the indirect method using a DMP-4F physiograph (Narco Bio-Systems). Thermal adaptation was performed by heating the animals for 5, 7, and 10 min on days 1, 2, and 3-6, respectively. Protective effect were analyzed 24 h after the last procedure (day 6) by restriction of the HS-induced drop of BP.

Accumulation of HSP70 in the heart was estimated by Western blot analysis. Tissue samples from the heart, liver, and brain were cut with scissors and placed into a hypotonic buffer (pH 7.4) containing 10 mM Tris, 10 mM KCl, and 1 μM phenylmethylsulfonyl fluoride (Calbiochem) at 4°C for 10 min. The tissue was then homogenized in the same solution at a buffer:tissue ratio of 3:1 (w/w). The homogenate was filtered through 6 layers and centrifuged at 12,000*g* and 4°C for 10 min. The supernatant containing cytosolic proteins was analyzed by Western blotting and electrophoresis [10]. The proteins were transferred from polyacrylamide gel to the nitrocellulose membrane by electroelution. Blots were preincubated with 5% delipidated milk in a buffer containing 50 mM Tris-HCl and 150 mM NaCl (pH 7.4) for 1 h. These blots were then consecutively incubated with mouse monoclonal antibodies against HSP70 (Amersham) and second peroxidase-conjugated mouse antibodies (Sigma) for 1 h. Second antibodies were developed with diaminobenzidine (BIO-RAD) in the presence of 10% H_2O_2 and 0.3% $CoCl_2$. Changes in HSP70 content were analyzed by the intensity of staining of monoclonal antibody band.

Production of NO was analyzed by its incorporation into Fe-diethyldithiocarbamate complexes using electron paramagnetic resonance (EPR) [15].

The inhibitor of HSP70 gene transcription quercetin [7] was injected intraperitoneally in a daily dose of 5 mg/kg for 6 days 30 min before heating.

The results were analyzed by Student's *t* test and expressed as $M \pm m$.

RESULTS

Thermal adaptation affected BP in conscious rats before and after HS (Fig. 1, *a*). Single HS decreased BP from 113.7±0.2 to 88.9±0.2 mm Hg ($p<0.05$). Adaptation had no effect on BP but abolished the HS-induced drop of BP. Protective effects were observed only after 6 days of adaptation but not after the 1st or 3rd thermal procedure.

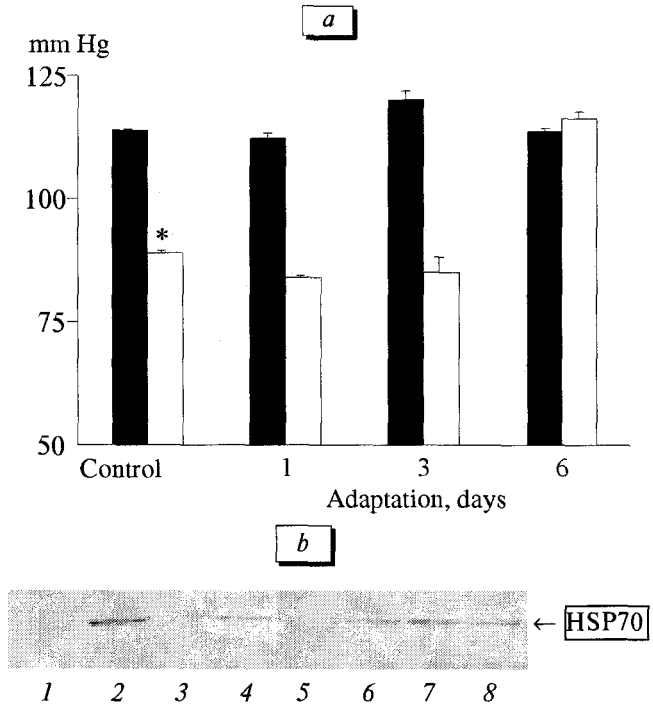

Fig. 1. Effects of thermal adaptation on blood pressure (*a*) before (dark bars) and after heat shock (HS, light bars) and HSP70 accumulation (*b*, Western blot analysis). The intensity of staining and the width of bands characterize HSP70 accumulation. Control (*1*); HS (*2*); adaptation, 1 day (*3*); adaptation, 1 day+HS (*4*); adaptation, 3 days (*5*); adaptation, 3 days+HS (*6*); adaptation, 6 days (*7*); and adaptation, 6 days+HS (*8*).

Blots shown in Fig. 1, *b* allowed us to compare the development of protective effects of adaptation with the dynamics of HSP70 accumulation in the heart. On day 6 of adaptation, the resistance of animals to HS increased, and HSP70 were accumulated in the heart, but no protective effects, neither accumulation of HSP70 in the myocardium were found on days 1 and 3 of adaptation.

Thus, the formation of protective antihypotensive effects during thermal adaptation was accompanied by activation of HSP70 synthesis.

Injections of quercetin did not affect BP but prevented the development of protective antihypotensive effects of thermal adaptation (Fig. 2).

These results confirm role of HSP70 in the adaptation-induced protection.

Then, we analyzed the influence of adaptation on HS-induced NO overproduction. HS enhanced the synthesis of NO in the myocardium from 0.2±0.1 to 3.0±0.7 ng NO/g tissue ($p<0.05$). A 3-day course of adaptation had no effect on NO overproduction, while adaptation for 6 days completely inhibited this pro-

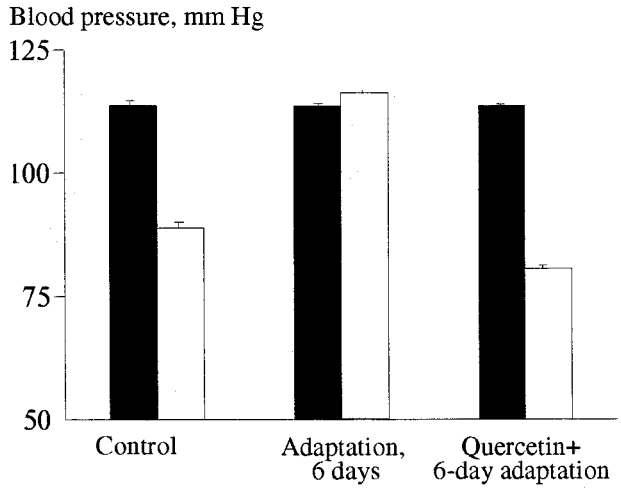

Fig. 2. Effects of quercetin on formation of thermal adaptation before (dark bars) and after (light bars) heat shock.

cess. Thermal adaptation during 3 or 6 days had no effects on NO production in the myocardium.

Thus, the formation of protective effects, accumulation of HSP70, and the restriction of NO overproduction had similar dynamics. These results agree with the hypothesis that HSP70 are involved in adaptive prevention of NO-dependent hypotension.

The involvement of HSP70 in heat preconditioning and its ability to inhibit NO synthase should be considered when analyzing the role of HSP70 in adaptive antihypotensive effects [5]. However, there are some differences in the mechanisms of preconditionning- and adaptation-induced activation of HSP70 synthesis. During preconditioning, activation of HSP70 synthesis primarily results from cell damage [9], while during adaptation this activation is associated with the effects of such physiological factors as high level of blood hormones and local changes in blood flow.

It is important that NO overproduction in the endothelium and vascular smooth muscles damages protein structures and inhibits mitochondrial respiration. Such processes cause redistribution of regional blood flow and severe hypotension [14]. HSP70 attenuate cytotoxic effects of NO and prevent acute circulatory disturbances due to their cytoprotective properties.

Another mechanism of the protective antihypotensive action of thermal adaptation is probably associated with its modulatory effects on metabolism of cell Ca^{2+} and macroergic phosphates [8] and the structure and functions of vasoactive molecules including prostaglandins, leukotrienes, and glucocorticoids [4].

Our findings and published data suggest that HSP70 accumulated during adaptation attenuate HS-induced hypotension by preventing hyperactivation of NO synthesis and reducing cytotoxic effects of NO.

This study was supported by the Russian Foundation for Basic Research (grants 97-04-48370 and 97-04-48371), INTAS-OPEN CALL, 1977 (grant 524), and the Netherlands Organization for Scientific Research (grant 047.006.006).

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