Adaptogen ADAPT Modulates Stress-Induced HSP70 Synthesis and Improves Organism's Resistance to Heat Shock

P. A. Prodius, E. B. Manukhina, A. E. Bulanov, G. Wikman, and I. Yu. Malyshev

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 123, No. 6, pp. 629-631, June, 1997
Original article submitted April 10, 1996

The effect of the herbal adaptogen ADAPT on the basal and heat shock-induced synthesis of HSP70 and organism's resistance to heat shock is studied. It is shown that ADAPT decreases accumulation of these proteins in the myocardium and promotes their accumulation in the liver. ADAPT has no effect on arterial pressure but prevents its drop induced by heat shock and markedly increases the survival rate of experimental animals. These data agree with the hypothesis that the effect of ADAPT on the synthesis of HSP70 is an important component of its protective action.

Key Words: *ADAPT; adaptogens; heat shock; heat shock proteins; survival rate*

Medicinal plants such as *Schizandra*, *Leuzea*, *Panax*, *Aralia*, *Eleutherococcus* etc. are widely used in medicine for improving organism's resistance against damaging factors. Since 1958, these preparations have been referred to as "adaptogens" due to their ability to induce "the state of enhanced nonspecific resistance of the organism" [1]. However, the mechanisms of pharmacological activity of these drugs are poorly understood.

When analyzing the mechanisms of protective effects of adaptogens, the important role of heat shock proteins (HSP70) in the formation of nonspecific enhanced organism's resistance in adaptation to stress should be taken into account [2]. Organism's response to heat shock (HS) [7] and ischemia [3] also involves activation of HSP70 synthesis. The protective effects of HSP70 are realized through the following mechanisms: 1) binding of fatty acids and inhibition of their detergent action [5]; 2) strengthening of the antioxidant systems [8]; 3) disaggregation of denaturated proteins [7]; 4) binding of calmodulin and reduction of the damaging effects of calcium excess. Moreover, HSP70 improves cell resistance through induction of some structural genes [4].

In light of this, it can be assumed that adaptogens improve organism's resistance by modulating the synthesis of HSP70.

The aim of the present study was to evaluate the effect of ADAPT, a herbal adaptogen, on basal and HS-induced synthesis of HSP70 and on organism's resistance to HS. Heat shock was used as a common way of HSP70 induction.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats weighing 250-300 g. The animals were separated into 5 groups: group 1 were intact controls (n=7), group 2 rats were subjected to HS (n=11), group 3 rats were subjected to HS and treated with ADAPT 20 min before and immediately after HS (n=7), animals of group 4 received ADAPT without HS (n=6), and group 5 rats twice received 5% ethanol (n=6).

Institute of General Pathology and Pathological Physiology, Russian Academy of Medical Sciences, Moscow; Institute of Plants, Göteborg, Sweden

0007-4888/97/0006-0548$18.00

Herbal extract ADAPT (dry yellow powder) was provided by Swedish Institute of Plants. The preparation was dissolved in 5% ethanol (30 mg/ml) and administered *per os* in a volume of 0.5 ml (i.e., 60 mg/kg body weight).

For modeling heat shock the animals were heated in a special thermostat until the rectal temperature was 42°C and then for additional 15 min. The total duration of heating did not exceed 30 min [3].

The protective effect of ADAPT was estimated by the survival rate (number of survivors 24 after HS) and drop in arterial pressure (AD) after HS in control and ADAPT-treated rats. AP was measured on the caudal artery by the indirect method using a Physiograph DMP-4F setup.

The accumulation of HSP70 in the heart and liver was assessed by Western-blot analysis. To this end heart and liver specimens were minced with scissors and placed into hypotonic buffer: 10 mM Tris, 10 mM KCl, and 1 μM phenylmethylsulfonyl fluoride (Calbiochem), pH 7.4, at 4°C for 10 min and then homogenized at a 5:1 buffer:tissue w/w ratio. The homogenate was filtered through 8-fold gauze and centrifuged at 12,000*g* and 4°C for 10 min. The supernatant containing cytosol proteins was analyzed by electrophoresis [6] followed by blot analysis. The proteins were transferred from polyacrylamide gel to nitrocellulose membrane by electroelution [11]. The blots were incubated for 1 h in a buffer containing 50 mM Tris-HCl, 150 mM NaCl (pH 7.4) and 5% fat-free milk and then successively in the presence of mouse monoclonal anti-HSP70 antibodies (Amersham) and peroxidase-conjugated mouse antibodies (second antibodies). The second antibodies were developed with a HRP Color Development Reagent (BioRad). The content of HSP70 was assessed densitometrically from the width and intensity of antibody-binding band. The accumulation of HSP70 was measured 24 h after HS or (in groups without HS) ADAPT administration.

The data were processed statistically using the Student's *t* test.

RESULTS

In the control, no inducible HSP70 was detected in the liver and heart. Administration of ADAPT did not induce accumulation of HSP70, while HS stimulated HSP70 synthesis both in the liver and heart (Fig. 1). ADAPT attenuated the HS-induced accumulation of HSP70 in the myocardium and promoted it in the liver.

ADAPT had no effect on AP over the 24-h observation period. HS led to a sharp drop in AP from 110±2 to 79±3 mm Hg during the first hour after HS. ADAPT completely prevented the HS-induced drop of AP. Ethanol had no effect on both the initial AP and its drop after HS.

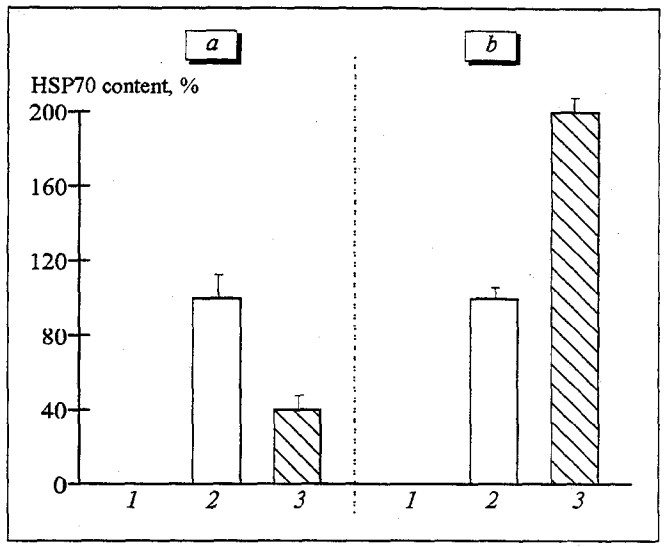

Fig. 1. Effect of ADAPT on heat-shock-induced HSP70 accumulation in the myocardium (*a*) and liver (*b*). *1*) control; *2*) heat shock; *3*) heat shock and ADAPT. The content of HSP70 after heat shock is taken as 100%.

The sharp drop in AP is the major cause of the post-HS mortality [9]. In our experiment, the post-HS mortality was 6 of 11 animals in the control group and only 1 of 7 in the ADAPT-treated group.

Thus, ADAPT modulates the HS-stimulated HSP70 synthesis and improves organism's resistance to HS.

Our experiments demonstrated that ADAPT decreased the accumulation of HSP70 in the myocardium. This implies that ADAPT reduced the activity of intracellular HSP70-inducing processes such as free radical processes and protein degradation. This results in inhibition of HSP70 synthesis and an increase in the survival rate after HS. The ability of ADAPT to prevent HS-induced drop in AP can also play a role in the protective effect of ADAPT.

The fact that ADAPT potentiates the HS-induced accumulation of HSP70 in the liver suggests that this preparation is effective against toxins, which are primarily inactivated in the liver.

Our data are insufficient to explain the specificity of the effect of ADAPT on different organs. However, it can be hypothesized that the modulation of HSP70 synthesis is an important mechanism underlying the protective effect of ADAPT.

REFERENCES

1. N. V. Lazarev, *Farmakol. Toksikol.*, **21**, No. 3, 81-85 (1958).
2. F. Z. Meerson and I. Yu. Malyshev, *Phenomenon of Adaptive Stabilization of Structures and Protection of the Heart* [in Russian], Moscow (1993).
3. R. W. Currie, *Circ. Res.*, **63**, 543-549 (1988).
4. Ph. Goelet, *Nature*, **322**, 419-422 (1986).

5. P. T. Guidon and C. E. Hightower, *Biochemistry*, **25**, 3231-3239 (1986).
6. V. K. Laemmli, *Nature*, **227**, 680-685 (1970).
7. H. R. B. Pelham, *Cell*, **46**, 959-961 (1986).
8. C. T. Privalle and I. Fridovich, *Proc. Natl. Acad. Sci. USA*, **84**, 2723-2726 (1987).
9. S. Shibolet, M. C. Lancaster, and Y. Danon, *Aviat. Space Environ. Med.*, **47**, 280-301 (1976).
10. M. A. Stevenson and S. K. Calderwood, *Mol. Cell. Biol.*, **10**, 1234-1238 (1990).
11. H. Towbin, *Proc. Natl. Acad. Sci. USA*, **76**, 4350-4354 (1979)

Effect of Lidocaine on Cholera Vibrio-Induced Changes in the Jejunum, Renal Medulla, and Lungs of Suckling Rabbits

E. A. Bardakhch'yan, Yu. M. Lomov, and N. G. Kharlanova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 123, No. 6, pp. 632-637, June,1997
Original article submitted April 10, 1996

Lidocaine injected into suckling rabbits infected with a virulent strain of *Vibrio cholerae* abolishes the development of hydropic and ballooning degeneration the jejunal enterocytes. Secretory granules and lipid inclusions accumulate in jejunal enterochromaffin cells and interstitial cells of renal medulla, respectively, and are not released into the vascular bed. In pulmonary tissue ultrastructural changes are mild, and capillary epithelium is undamaged, indicating that lidocaine stimulates pulmonary enzymes which inactivate biologically active substances implicated in the pathogenesis of cholera.

Key Words: *cholera; lidocaine; electron microscopy*

Activation of phospholipase A_2 (PLA_2) by cholera toxin stimulates the formation of platelet-activating factor, a potent mediator of inflammation and destruction, and probably a key factor of the cholera toxin-induced secretion [12]. Phospholipase A_2 is a key component of the pulmonary antisurfactant system, activation of which in cholera leads to intense destruction of the surfactant [3]. In association with Ca^{2+} PLA_2 cleaves arachidonic acid from membrane phospholipids, which is used in the synthesis of prostanoids (prostaglandins and thromboxanes) and eikosanoids (leukotrienes and fatty acid peroxides and hydroperoxides).

Particular attention has been focused on the role of prostaglandin E_2 (PGE_2) in the intestinal dehydration syndrome. In fact, choleragenic intoxication is accompanied by 3- to 5-fold rise of serotonin and PGE_2 [10]. It has been showed that cholera toxin causes dehydration by inducing the release of serotonin which stimulates PGE_2 production [10]. Biochemical findings correlate with ultrastructural changes in enterochromaffin (EC) cells of the small intestine in rats caused by choleragen [8] and, as we have shown, in interstitial cells of rabbits infected with *Vibrio cholerae*. Moreover, the development of experimental cholera strongly depends on the state of pulmonary vascular endothelium where serotonin and most of PG are inactivated [3]. Finally, lidocaine, a potent anesthetic, inhibits PLA_2 [11].

In this study we examined the effect of lidocaine on ultrastructural changes in cells producing serotonin and PGE_2 and on pulmonary tissue in experimental cholera.

MATERIALS AND METHODS

A total of 34 suckling rabbits were used, 25 of which were infected intragastrally with a 18-h culture of *Vibrio cholerae* strain El Tor 5879 [2]. Thirteen rab-

Laboratory of Pathomorphology and Pathophysiology, Antiplague Institute, Rostov-on-Don

0007-4888/97/0006-0550$18.00