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A Comparative Study of Generalized Activation of the Synthesis of Stress Proteins in Adaptation to Stress and Hypoxia

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Generalized activation of the synthesis of stress proteins, members of the heat-shock protein family (hsp70), plays an important role in the development of protective effects of adaptation to stress [3]. The adaptation-induced accumulation of stress proteins in different organs occurs not only in adaptation to stress, but also in other types of adaptation, in particular, to hypoxia. However, the character and peculiarities of the generalized activation of the synthesis of heat-shock proteins for adaptation to hypoxia have remained unclear until now. Such an analysis is especially interesting since the stressor component in moderate hypoxia is considerably less pronounced than in adaptation to stress [1], and the protective effects of these two forms of adaptation differ markedly on the level of both isolated organs and the whole organism [2,4].

The aim of the present study was to evaluate the effect of adaptation to hypoxia on the content of hsp70 in different organs and to compare it with stress-induced adaptation.

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MATERIALS AND METHODS

Experiments were carried out on male Wistar rats weighing 200-250 g. Adaptation to stress was performed by fixing the animals in the supine position once a day over 12 days: on the 1st day for 15, on the 2nd for 30, on the 3rd for 45 min, and then for 60 min every other day. Immobilization was performed by fixing all four extremities leaving the head free. Adaptation to hypoxia was conducted by stepwise elevations in a pressure chamber: in the first session the animals were elevated to 1000 m, in the second to 2000 m, in the third to 3000 m, and in the others to 4000 m above sea level. The entire course of adaptation to graduated hypoxia consisted of 40 daily sessions lasting 5 hours each. The content of hsp70 was determined in the cytosol and nuclear fractions from the myocardium, liver, and brain cells employing two-dimensional electrophoresis after O'Farrell

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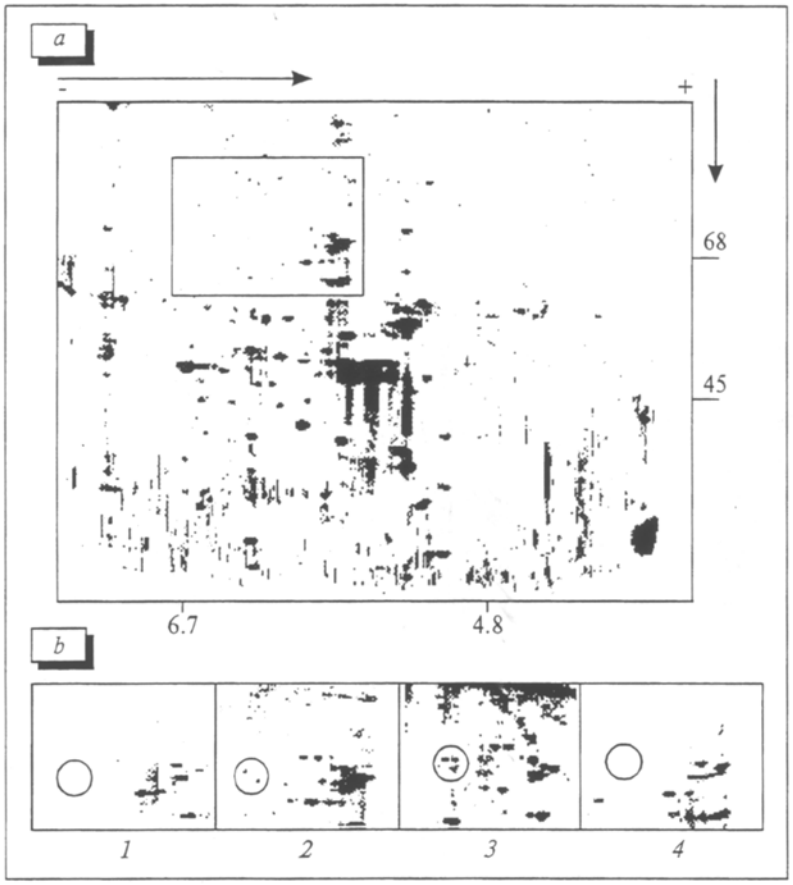

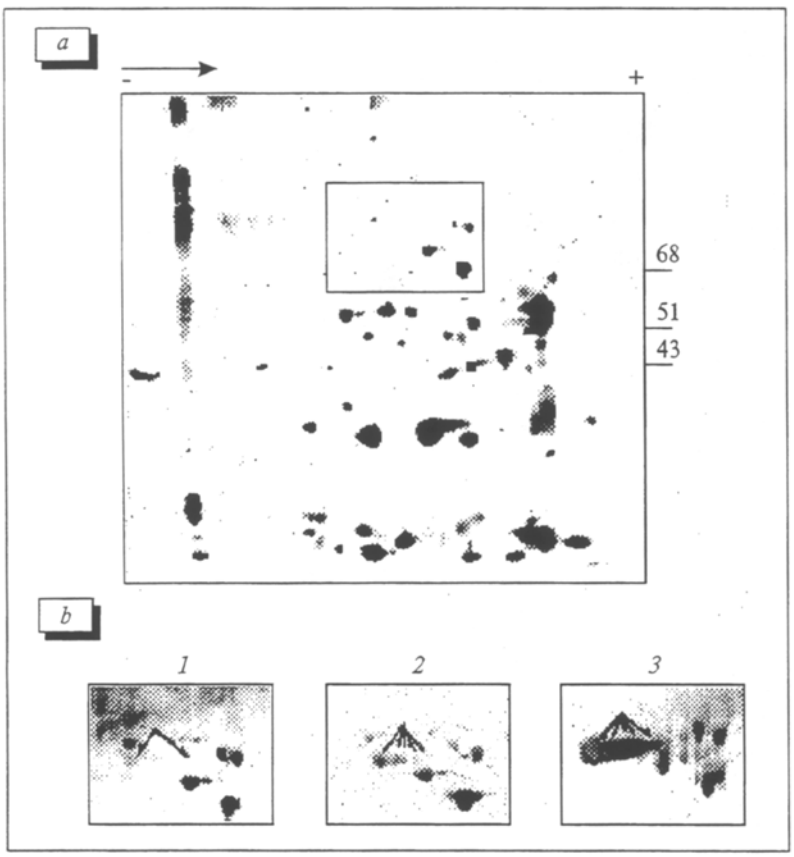

Fig. 1. Effect of adaptation to stress and to hypoxia on the content of heat–shock proteins in cytosol of heart cells. *a*) typical electrophoregram of cytosol proteins from rat cardiomyocytes. The horizontal arrow shows the direction of isoelectric focusing. Here and in Figs 2. and 3 the rectangle shows the location of hsp 70 isoforms; *b*) fragment of electrophoregram outlined by rectangle (*a*). *1*) control; *2*) heat shock; *3*) adaptation to stress; *4*) adaptation to hypoxia; arrows: location of hsp70 isoforms.

[11]. The second-dimension electrophoresis was carried out in 10% PAGE after Laemmli [6]. The gels were then stained with silver [10]. Isoforms of inducible hsp70 were identified and characterized by their molecular weight and pI [12,14]. In addition, we compared the gels from adapted animals with the gels from animals subjected to hyperthermia, where the inducible fraction in the region of 72 kD is known to correspond to hsp70 [5,12,14].

RESULTS

The gels presented in Fig. 1 allow us to compare the influence of adaptation to stress and to hypoxia on the content of heat-shock proteins in cytosol from heart cells. As seen from the figure, the adaptation to high-altitude hypoxia resulted in the accumulation of just 2 acidic isoforms of the hsp70 family (pI $\approx$ 5.8) out of 5 proteins detected after adaptation to immobilization stress (Fig. 1, *3, 4*). The adaptation to stress was also found to cause the accumulation of two isoforms of hsp70 in the nucleoplasm, whereas the adaptation to hypoxia was not accompanied by the accumulation of inducible hsp70 in any nuclear fraction (Fig. 2, *3, 4*).

It becomes evident that the changes in isoform composition and subcellular distribution of hsp70 in the myocardium of adapted animals depend strictly on the factor to which the organism is adapted: adaptation to stress leads to the accumulation of 5 cytoplasmic and 2 nucleoplasmic forms of hsp70, while adaptation to hypoxia induces just 2 cytoplasmic isoforms.

Fig. 2. Effect of adaptation to stress and to hypoxia on the content of heat–shock proteins in nucleoplasm of cardiomyocytes. *a*) typical electrophoregram of nucleoplasmic proteins from rat cardiomyocytes.

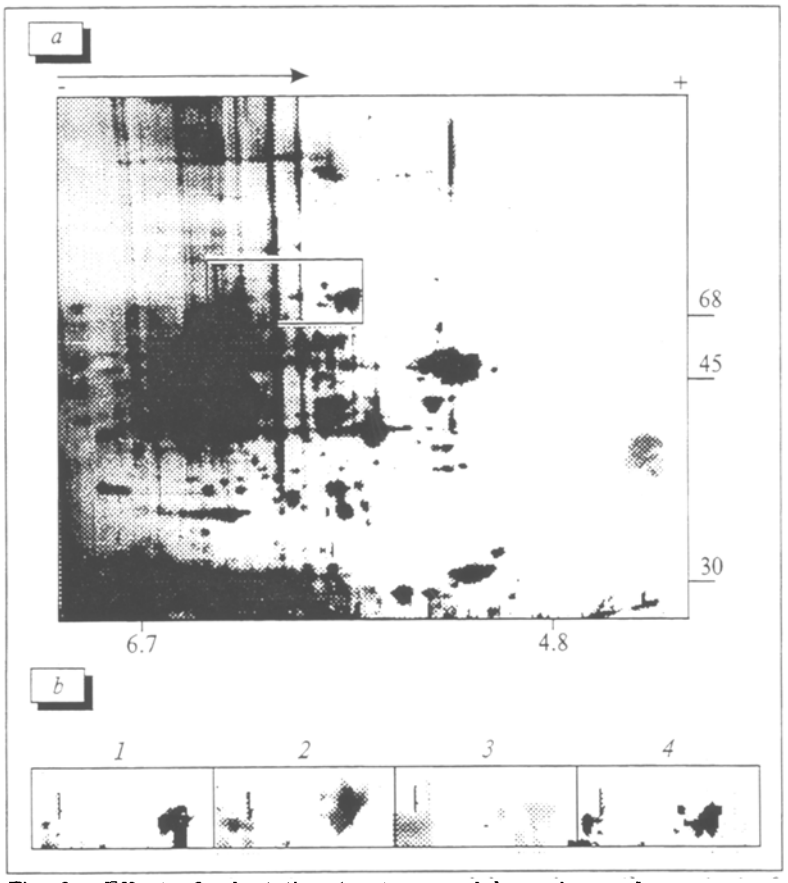

Fig. 3. Effect of adaptation to stress and hypoxia on the content of heat-shock proteins in brain cell cytosol. *a*) typical electrophoregram of cytosol proteins from rat brain.

The data presented in Fig. 3 shows that an analogous pattern is observed also in the brain: the adaptation to stress induces two or more isoforms of hsp70, whereas adaptation to periodic hypoxia induces just one isoform.

Thus, in both types of adaptation, the accumulation of stress proteins of the hsp70 family occurs not only in the heart but also in other organs, notably, the brain, which implies its generalized character. However, the character and the peculiarities of the generalized activation of the synthesis of stress proteins depend to a marked extent on the type of adaptation: the adaptation to stress induces a more pronounced accumulation of stress proteins in different organs than that induced by adaptation to hypoxia.

At present, we cannot answer specifically why adaptation to immobilization stress induces more isoforms of hsp70 in diverse organs then adaptation to hypoxia. However, the actual possibility of the induction of a different number of isoforms in response to different interventions is well established. For example, in the rabbit heart exposed to ischemia the accumulation of 3 isoforms of hsp70 with pI values of 6.0, 6.1, and 6.15 occurs [9], while in the rat myocardium after short-term heat shock just one isoform of hsp70 accumulates [5]. The possibility of differential regulation of different inducible hsp70 genes has been clearly demonstrated in man [7] and is also well documented in yeast [13]. It may be assumed that in adaptation to such diverse factors as immobilization stress and hypoxia, differences in the hormonal spectrum, as well as in the sequence and duration of the action of hormones may predetermine the unequal expression of various heat-shock proteins and the accumulation of different isoforms.

The facts demonstrated here concerning a more generalized activation of the synthesis of stress proteins after adaptation to stress than to hypoxia allow us to conclude that not only the cardioprotective effect [2,8], but also the protective mechanisms on the organismic level are more powerful in adaptation to stress than in adaptation to hypoxia.

On the whole, there is one important aspect to understanding the differences in the development of protective effects in adaptation to immobilization stresses and to hypoxia. It has to do with the fact that variations and peculiarities of interorgan activation of the synthesis of heat-shock proteins seem to be as varied as the environmental factors to which the adaptation may be formed. Therefore, further investigation of the molecular and, in particular, the genetic mechanisms of the protective effect of adaptation to other factors such as physical loads, cold, heat, and so on holds great promise. At the same time, it is evident that heat-shock proteins represent just one component in the complex mechanism of long-term adaptation of the organism.

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Changes of the Efficacy of Vasoconstrictive Stimuli in Acute and Chronic Hypotension in the Microcirculatory Bed of Rat Skeletal Muscle

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Changes of intravascular pressure may modulate the sensitivity and responsiveness of blood vessels to constrictive stimuli. An increase in the perfusion pressure to 140 mm Hg raises the sensitivity and responsiveness of the small vessels of dog kidneys to norepinephrine [5]. According to the data obtained by us earlier, the responses to stimulation of the sympathetic tract in the microcirculatory bed of rat skeletal muscle become weaker in acute and chronic hypertension [1,2]. One may assume that regional hypotension elicits the opposite effect. For instance, in experiments performed on the mesentery of rats, a decrease of perfusion pressure to 30 mm Hg caused an enhancement of constrictive responses to angiotensin II and phenylephrine [6]. At the same time, it has been demonstrated on the perfused extremities of rats with hypotension lasting 1-2 months that vascular responses to neuroergic stimuli, as well as the blood supply of the tissues (but not the perfusion pressure) initially drop and recover later on [3].

In the present work we studied vasoconstrictive responses of the microcirculatory bed of rat skeletal muscle to stimulation of the sympathetic tract in control (sham-operated) rats and in rats with chronic regional hypotension lasting 1-2 months, as well as these responses against the background of a short-term decrease of perfusion pressure in the posterior part of the rat body.

MATERIALS AND METHODS

The experiments with a short-term decrease of the arterial pressure (AP) were carried out on 11 male Wistar rats weighing 250±35 g. Pretreatment of the animal, preparation of the muscle (*m. extensor hallucius proprius*) on the left hind paw, and biomicroscopy were performed as in the previous investigation [1]. The AP was recorded in the right femoral artery with the aid of an RE-10 catheter hooked up to a manometer. The perfusion pressure in the posterior part of the body was reduced by occluding the abdominal aorta with a hydraulic

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