PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

Generalized Accumulation of Stress Proteins During the Organism's Adaptation to Stress

F. Z. Meerson, I. Yu. Malyshev, and A. V. Zamotrinskii

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Adaptation of the organism to repeated stress factors leads to the development of the phenomenon of structural adaptational stabilization (SAS) of structures [2,8]. This phenomenon manifests itself as a marked increase in the resistance to damaging factors not only of the whole body, but also of isolated organs and cell constituents. For example, isolated hearts of animals adapted to immobilization stress acquire a high resistance to reperfusion injury [8], toxic concentrations of catecholamines [5,6], the calcium paradox [2], and hyperthermia [8]. In addition, sarcoplasmic reticulum and mitochondria isolated from these hearts have been found to be resistant to autolysis [6] and nuclear DNA to be highly resistant to the action of single-strand exogenous DNA, which is known to induce lysis of nuclear proteins [7]. With respect to the heart, the stress protein hsp70 from the heat shock protein family plays an important role in the development of SAS [8]. After being accumulated in the cytoplasm and nuclei of cardiomyocytes during adaptation to stress agents, these proteins become involved in adaptational stabilization of cardiac structures due to the disaggregation of abnormal protein-protein interactions. At the same time, an important aspect remains unclear: whether hsp70 is accumulated in other organs, i.e., whether the adaptational activation of stress protein synthesis is of a generalized nature and, if so, what the specific features of this process are.

The aim of this study was to assess the influence of the adaptation to immobilization stress on the stress protein content of the heart, liver, and brain.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 200-250 g. The animals were adapted to stress by short-term immobilization on their backs during 12 days: the 1st day for 15 min, the 2nd day for 30 min, the 3rd day for 45 min, and the remaining days for 60 min every other day. The animals were fastened by all four limbs, the head being left free. The hsp70 content was determined in the cytosol and nuclear fractions of cardiomyocytes, hepatocytes and brain cells by two-dimensional electrophoresis [10]. The second-dimension electrophoresis was performed in 10% polyacrilamide gel [4]. Gels were silver stained using the procedure described by Morrisey [9]. The isoforms of inducible hsp70 were identified and characterized by molecular weight and isoelectric point [11,12]. In addition, we compared electrophore-

Research Institute of General Pathology and Pathological Physiology, Russian Academy of Medical Sciences, Moscow. (Presented by D. S. Sarkisov, Member of the Russian Academy of Medical Sciences)

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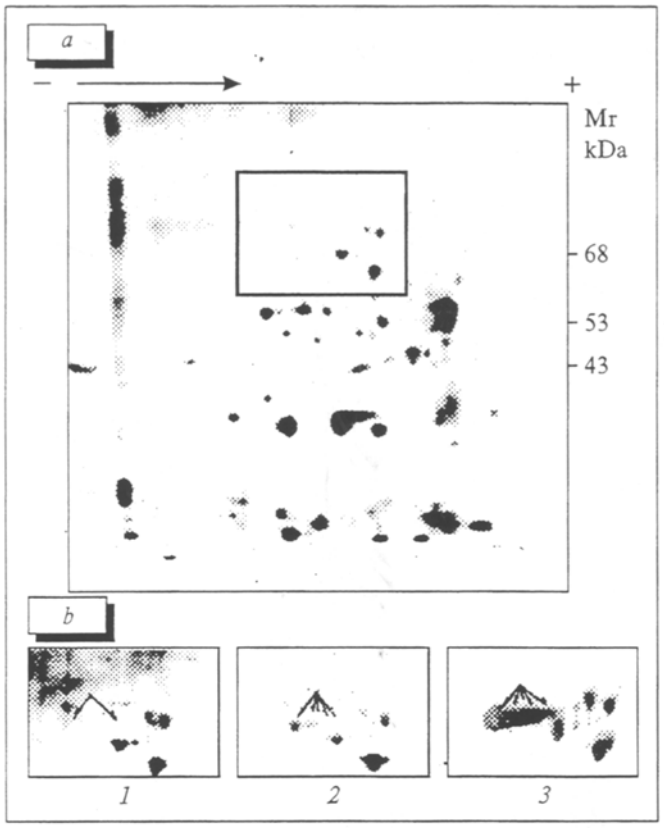

Fig. 1. Effect of adaptation to stress on heat shock protein content of cardiomyocyte cytosol fraction. *a*) typical electrophoregram of cytosol of rat cardiomyocytes. The direction of isoelectric focusing is indicated by a horizontal arrow. The localization of hsp70 is outlined with a rectangle. *b*) fragment of the area designated by the rectangle: *1*) control; *2*) heat shock; *3*) adaptation to stress. Arrows indicate hsp70 isoforms.

grams of proteins isolated from adapted rats with those obtained from rats subjected to hyperthermia, after which the stress-induced protein fraction in the 72 kD area is known to be represented by hsp70 [3,11,12].

RESULTS

Figure 1, *a* shows a typical electrophoregram of the cytosol fraction of cardiomyocytes. Adaptation to immobilization stress resulted in the accumulation of at least 5 isoforms of hsp70 with isoelectic points ranging from 6.3 to 5.7.

Two isoforms of hsp70 appeared in the 71-72 kD area at pI 6.2-6.3 on the electrophoregram of the nucleoplasm fraction of "adapted" cardiomyocytes and were not seen on control electrophoregrams.

Figure 2, *a* shows a two-dimensional electrophoregram of liver cytosol. Adaptation to immobilization stress led to the accumulation of only one isoform of inducible hsp70 (Fig. 2, *b*, *3*). This band was absent from the control electrophoregram (Fig. 2, *b*, *1*). Only one polypeptide with a molecular weight of about 72 kD was accumulated in the nuclei of hepatocytes after adaptation. Figure 3, a shows a typical electrophoregram of the cytosol fraction of brain cells. The influence of adaptation to immobilization stress on the hsp70 content in this fraction can be gauged from Fig. 3. It can be seen from the figure that polypeptides present in the 71-72 kD area on the electrophoregrams of "adapted" brain cells are absent from the control electrophoregrams (Fig. 3, *b*, *1*, *3*). An increase in the content of 2 isoforms of stress proteins with a molecular weight of 72 kD was observed in the nuclei of brain cells isolated from adapted animals.

A single exposure to immobilization stress did not result in the accumulation of hsp70 in heart, brain, or liver cells. Hence, the adaptational accumulation of stress proteins occurs not only in the heart, but also in other internal organs.

Accumulation of stress proteins provoked by adaptation to immobilization stress is most noticeable in the heart: 5 isoforms are accumulated in the cytoplasm and 2 isoforms in the nuclei; 2 isoforms are accumulated in the cytoplasm and 2

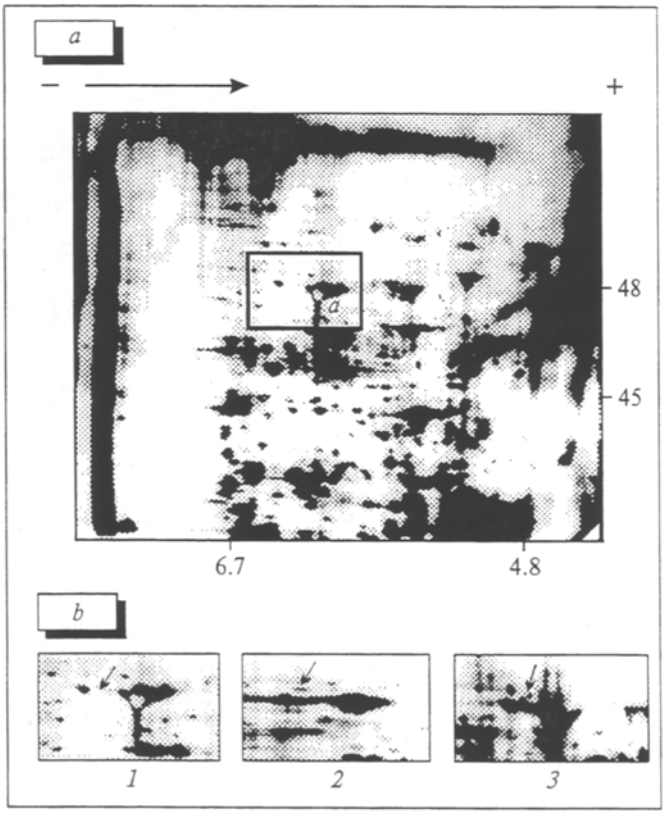

Fig. 2. Effect of adaptation to stress on heat shock protein content of hepatocyte cytosol fraction. *a*) typical electrophoregram of cytosol of rat hepatocytes. *b*) fragment of the area designated by the rectangle. A: albumin. Other notation as in Fig. 1.

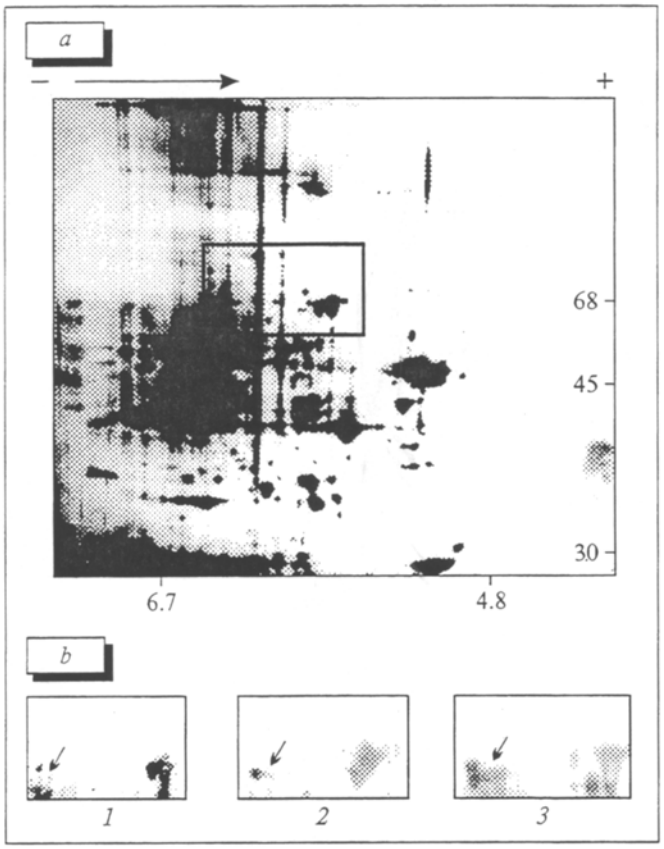

Fig. 3. Effect of adaptation to stress on heat shock protein content of brain cell cytosol fraction. *a*) typical electrophoregram of cytosol of rat brain cells. *b*) fragment of the area designated by the rectangle. A: albumin. Other notation as in Fig. 1.

isoforms in the nuclei of brain cells, while an insignificant accumulation of stress proteins - 1 isoform in the cytoplasm and 1 isoform in the nuclei - occurs in hepatocytes. Such a "gradient" of adaptational accumulation and isoform spectrum of hsp70 may reflect the extent of organ involvement in the adaptation to immobilization stress.

From our findings it can be concluded that accumulation of heat shock proteins is not an intrinsic property of the heart but is also characteristic of the brain and liver. This indicates that the activation of hsp70 synthesis during adaptation to stress might be quite an important phenomenon.

Previously we demonstrated that hsp70 participates in the formation of one of the important SAS mechanisms at the level of the heart and thus increases myocardial resistance to damaging factors [2,8]. Since during adaptation to stress heat stress proteins are accumulated both in the heart and in other organs, it can be assumed that SAS is a generalized phenomenon and, consequently, it may underlie the adaptive defense not only of separate organs but of the whole organism. Recently, this hypothesis was corroborated by experiments with sublethal hypoxia. It was found that adaptation of animals to repeated stress factors increases their resistance to acute hypoxia [1]. Inhalation of a gas mixture containing 6% oxygen for 120 min led to the death of 65% of the nonadapted animals, while only 10% of the animals adapted to stress died under the experimental conditions. Such a considerable increase (6.5-fold) in the resistance to acute hypoxia is consistent with the idea that SAS is not a local but rather a generalized phenomenon marshaling the organism's defenses even against sublethal hypoxia. Undoubtedly, one of the protective mechanisms is associated with generalized activation of stress-protein synthesis and the accumulation of stress proteins in internal organs of the adapted organism.

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