PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

Role of Heat Shock Proteins in the Formation of Stress Resistance in Different Animal Strains

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In response to heat shock five isoforms of hsp70 are accumulated in the myocardium of Wistar rats highly resistant to stress in comparison with only 3 isoforms of hsp70 in August rats with a lower resistance to stress. This suggests that genetic mechanisms which determine the stress resistance of a particular strain are probably related to transcription of hsp70 genes.

Key Words: *stress; heat shock; myocardium; hsp70; genetic strains*

Different organisms from procaryotic to high eucaryotic react to environmental stress factors in different ways. At the same time a common feature of cell response to stress in many cases is quick synthesis of so-called heat shock proteins, hsp [13,15]. The main members of the above family are proteins with a molecular weight around 70 kD (hsp70). Hsp70 are known to participate in the restriction of stress-induced damage by disintegrating abnormal protein-protein interactions [13,15]. For instance, experiments on cell cultures from *Drosophila melanogaster* [1], Syrian hamster [9,10], and rat liver [6,7] have demonstrated a correlation between increased resistance to stress, namely to heat shock, and hsp70 accumulation. At the same time Widelitz *et al.*, using various rat cells [16], showed that complete inhibition of synthesis of hsp70 did not affect thermotolerance. Similar data on the absence of any relation between the synthesis of hsp70 and increased resistance to stress were also obtained by other authorities [3,4,8,12]. Thus, the role of heat shock proteins in the formation of stress resistance of the organism remains unclear. One possible approach is the study of peculiarities of accumulation of hsp70 in response to a single stress factor in various animal strains with different resistance to stress.

In this context the aim of the present study was, first, to compare quantitatively stress resistance in different animal strains by comparing their resistance to heat shock and, second, to evaluate the differences in the accumulation of hsp70 and their isoforms in response to heat shock in rats with different resistance to stress and to compare the differences in hsp70 accumulation with the differences in stress resistance. Rats of the Wistar and August strains were used.

MATERIALS AND METHODS

Experiments were carried out on Wistar and August rats weighing 200-250 g. Heat shock reproduced after Currie *et al.* [2] was used as the stress influence. To this end the rats were first narcotized with Nembutal (50 mg/kg, i.p.) and then exposed to 80°C for 20 min in a special incuba-

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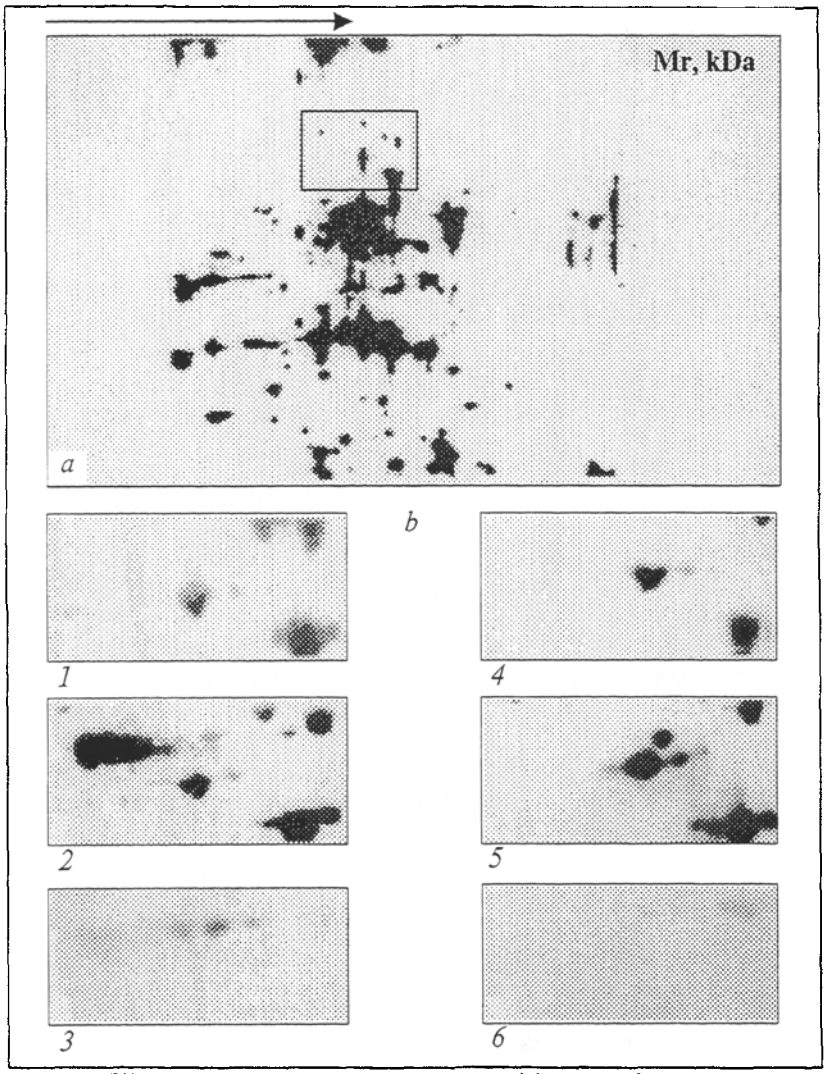

Fig. 1. Effect of heat shock on the content of heat shock proteins in the myocardium of Wistar and August rats. *a*) typical electrophoregram of cytosol proteins from rat cardiomyocytes. Direction of isoelectrofocusing is shown with an arrow. Location of hsp70 is outlined with a rectangle. *b*) fragments outlined with rectangles: *1*) Wistar controls; *2*) heat shock, Wistar; *3*) heat shock, Wistar, immunoblot with hsp70-specific antibodies; *4*) August controls; *5*) heat shock, August; *6*) heat shock, August, immunoblot with hsp70-specific antibodies.

tor. Under these condition the rectal temperature in the majority of rats attained 41-42°C after 5 min and was maintained at this level during the next 15 min. After this procedure some animals died during the first few hours. The resistance to stress of a particular strain was quantitatively assessed from the number of survivors.

Hsp70 isoforms from the myocardium were identified and characterized by IEF-PAGE using monoclonal antibodies. Isoelectrofocusing was carried out at 500 V for 18 hours. The second direction 10% PAGE was performed after Laemmli [5]. The gels were stained with silver nitrate after Morrisey *et al.* [11]. Hsp70 isoforms were identified and characterized by molecular weights and isoelectric points. IEF-PAGE analysis of heat shock proteins was supplemented with Western blotting. To this end the proteins were transferred to nitrocellulose at 200 mA after Towbin *et al.* [14]. The blots were incubated in TBS containing 5% dry milk for saturation of nonspecific binding sites on the membrane and then with N27F3-4 and C92F3A5 monoclonal antibodies to hsp70 (generous gift from Dr. J. Welch, University of California) diluted 1:500. The blots were then incubated with peroxidase-conjugated goat antimouse IgG. The reaction was visualized with diaminobenzidine.

RESULTS

Two of 20 Wistar rats (10%) and 11 of 18 August rats (61%) died after heat shock. Thus, the resistance to stress evaluated by the resistance to heat shock was 6-fold higher in Wistar than in August rats. In view of this it was interesting to compare the accumulation of hsp70 in response to heat shock in Wistar and August rats.

Figure 1, *a* illustrates a typical electrophoregram of cytosol proteins from the heart. The distribution pattern of polypeptide fractions was highly reproducible for both the same sample and samples from different animals. Heat shock induced the accumulation of no less than 5 polypeptides with a molecular weight around 72 kD and isoelectric points ranging from 6.3 to 5.7 (Fig. 1, *b*, *2*), the most abundant being the maximally alkaline polypeptide. No fraction was detected in the control (Fig. 1, *b*, *1*). The ability of these fractions to interact with specific antibodies to hsp70 allowed us to identify the polypeptides accumulating due to heat shock in Wistar rats as heat shock proteins.

In August rats heat shock induced the accumulation of no more than 3 of 5 isoforms of hsp70 detected in Wistar rats (Fig. 1, *b*, *4-6*). The maximally alkaline isoform most expressed in Wistar rats was utterly absent in August rats.

Thus, heat shock induces considerable accumulation of hsp70 in the myocardium of Wistar rats, and correspondingly these rats are more resistant to stress, which particularly manifests itself in a higher resistance to heat shock; in August rats, on

the other hand, the accumulation of hsp70 is less expressed than in Wistar rats and the resistance to heat shock is also lower.

These data corroborate the assumption that activated synthesis of hsp70 plays an important role in increased resistance to heat shock and that genetic mechanisms determining the stress resistance of a particular animal strain may be related to transcription of hsp70 genes. We still cannot explain why heat shock induced 5 isoforms of hsp70 in Wistar in comparison with just 3 isoforms in August rats. This calls for further studies of differences in the structure of the promoter region of the genes and of the mechanisms of action of factors activating hsp70 expression in different animal strains.

One key aspect in the understanding of the mechanisms of stress-resistance formation in different animal strains has to do with targeted modulation of the genetic apparatus aimed at achieving stress resistance in previously nonresistant organisms. The genes of heat shock proteins are just a part of the complex mechanism which determines the stress resistance of an organism.

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