Comparative Analysis of Polypeptides in the Heat Shock Proteins Synthesized in the Myocardium of Wistar and August Rats

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The use of two-dimensional electrophoresis to study the polypeptide composition of heat shock proteins 70 synthesized in the myocardium of Wistar and August rats in response to thermal stress and repetitive immobilization stress revealed interstrain differences in the composition of stimulated polypeptides in these proteins. The differences are mainly due to differential expression of the heat shock protein gene(s) encoding polypeptides with pI 6.2-6.0 in the two strains. The drastically reduced levels of the synthesis and/or accumulation of some heat shock proteins 70 in the hearts of August rats are associated with their failure to develop cardioprotective phenomena in response to repetitive immobilization stress.

Key Words: *major stress proteins; heat shock proteins 70; polypeptide composition; rat heart; interstrain differences*

Cells of all organisms from bacteria to man respond to thermal and certain other physiological forms of stress by inducing *de novo* synthesis of heat shock proteins (hsp) that have a molecular weight of about 70 kD under denaturing conditions (hsp-70) [12]. The hsp-70 family contains several polypeptides having similar amino acid sequences but differing in the degree to which their synthesis is induced [11]. To date, the structure of the hsp-70 gene family has been best studied in yeasts and, among higher eukaryotes, in man [4,6]. It has been established that the genomes of these species each contain several distinct genes which are stimulated by hsp-70 and which encode polypeptides with similar molecular weights but different isoelectric points [4]. However, there remain large gaps in our knowledge of how individual genes from this family are expressed and what the physiological significance of their protein products is in eukaryotes. The described mutations of hsp-70-stimulated genes manifest themselves at the biochemical level in altered polypeptide (isomorphic) makeups of the synthesized hsp-70, and the cells carrying such mutations usually also have markedly modified physiological parameters [5]. In searching for rat populations with altered expressions of hsp-70-stimulated genes, it is useful to consider how protein products of these genes are synthesized in the tissues of August rats. Rats of this strain are known to be more susceptible to the damaging action of a number of stressors than rats of other strains, including Wistar rats.

The aim of the present investigation was to compare the induction and accumulation of hsp-70 polypeptides synthesized in the myocardium of Wistar and August rats in response to thermal stress and to repetitive immobilization stress.

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MATERIALS AND METHODS

Male Wistar and August rats weighing 200-250 g were used. Thermal stress was produced by plac-

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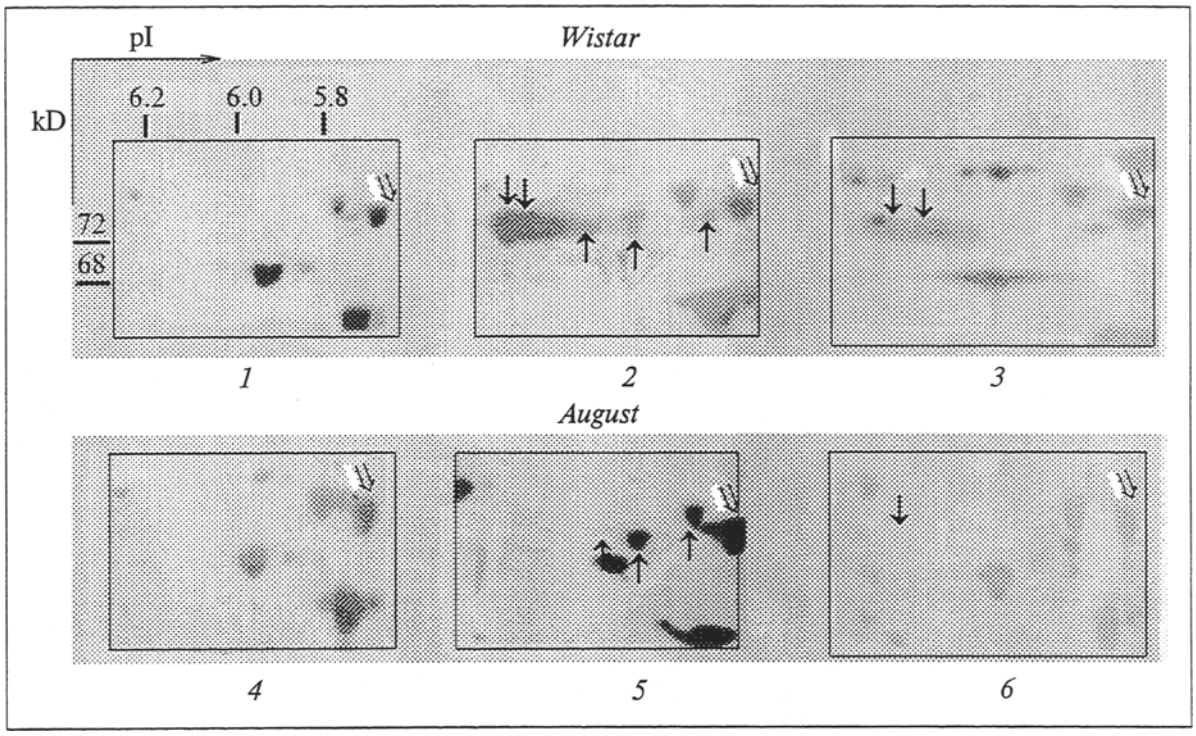

Fig. 1. Differences in the polypeptide composition of hsp−70 synthesized in Wistar (*1*, *2*, and *3*) and August (*4*, *5*, and *6*) rat myocardia. Shown are photographs of two−dimensional electrophoregram fragments corresponding to the locations of hsp−70 family proteins. *1* and *4*) myocardial protein profiles for control rats; *2* and *5*) myocardial protein profiles obtained for rats 48 h after thermal stress; *3* and *6*) myocardial protein profiles obtained for rats after 14 days of adaptation to immobilization stress. The positions of stimulated hsp−70 with pI 5.7−5.9 are shown by up−pointing arrows and the positions of those with pI 6.0−6.2, by down−pointing ones. The white arrow indicates the location of the constitutive hsp−70 (hsc−73).

ing Nembutal-anesthetized rats (50 mg/kg intraperitoneally) in an incubator and heating them to a rectal temperature of 42°C, which was maintained for 15 min; the rats were then returned to ambient temperature conditions, and their hearts, removed 48 h later, were used for analysis.

For adaptation to immobilization stress, rats were repeatedly immobilized in the supine position by fixation of all four limbs (the head was not fixed). They were immobilized in this manner once a day for 15 min on day 1, 30 min on day 2, 45 min on day 3, and 60 min on days 4 through 12. After the last (12th) immobilization session, the rats were killed by decapitation and their hearts were quickly removed and washed free of blood traces in the Langendorff system [3]. The hearts from 5 heat-stressed August and Wistar rats and from 5 immobilization-stressed rats of these strains were examined. Hearts from nonstressed August and Wistar rats served as controls.

For the analysis of myocardial proteins, we used cytosol obtained after centrifugation of heart homogenates for 15 min at 12,000 g. Samples for isoelectric focusing and two-dimensional gel electrophoresis were prepared as described by O'Farel, with modifications [2]. After electrophoresis, the gels were stained with silver nitrate [9]. A model of reperfusion-caused damage to an isolated heart was obtained by complete interruption of coronary blood flow for 15 min, followed by resumption of perfusion and observation for 20 min. The degree of heart damage was assessed by recording contraction amplitudes, contractures, cardiac rhythm abnormalities, and the release of creatine kinase to the perfusate, as previously described [3].

RESULTS

For our comparative study of hsp-70 gene expression in the myocardium of rats from two genetically distinct populations we chose to analyze the polypeptide compositions of hsp-70 synthesized in August and Wistar rat myocardia in response to thermal stress because this is the most common inducer of all hsp-70 genes [4], and also in response to repetitive immobilization stress because this had been shown to induce selectively the synthesis of particular hsp-70 polypeptides [7].

Figure 1 shows pooled results obtained with the two-dimensional gel electrophoresis of hsp-70 proteins synthesized in the myocardia of Wistar (*2* and *3*) and August (*5* and *6*) rats in response to the thermal and immobilization stress. The gel fragments presented in this figure correspond to the

electrophoregram areas where proteins of the hsp-70 family are located. The patterns of electrophoretic fractionation were well reproducible for the myocardial preparations from rats of the tested groups, and the patterns shown here are representative of these groups as a whole.

The myocardia of unstressed (control) Wistar and August rats did not contain hsp-70 in demonstrable amounts (*1* and *4* in Fig. 1). The two-dimensional fractionation patterns of myocardial proteins contained in the control rats from these two populations were so similar that we failed to detect differences between their polypeptide compositions (data not shown).

The exposure of Wistar and August rats to thermal stress resulted in the synthesis and accumulation in their hearts of several polypeptides with molecular weights of 71-72 kD, which had previously been identified as hsp-70 [7]. In the Wistar myocardia, hsp-70 were represented by at least five polypeptides isofocusing in a pH gradient range of approximately 5.7-6.2, and the polypeptides present in greatest amounts were those with pI values of 5.8 and 6.2 (Fig. 1, *2*). The hsp-70 synthesized in the August myocardia were also represented by several fractions, but these differed in composition from Wistar myocardial hsp-70 in that hsp-70 polypeptides with pI 6.0-6.2 were much less well represented. The representation of more alkaline hsp-70 ranged from the complete absence of their fractions to the presence of minor fractions hardly detectable after staining with silver nitrate. In contrast, the Wistar and August myocardial hsp-70 with pI 5.7-5.9 did not differ either in composition (3 polypeptides were detected) or in the quantitative representation of their fractions (Fig. 1, *2* and *5*).

In Wistar rats, the repetitive immobilization stress led, on the one hand, to a phenomenon of adaptive stabilization of cardiac structures [7,8] and, on the other, to an accumulation of hsp-70, mainly of polypeptides with pI 6.0-6.2 (Fig. 1, *3*). The cardioprotective effect of this stabilization phenomenon was manifested, in particular, in a heightened ability of the heart to resist reperfusion-inducible injuries. On day 14 of adaptation, the isolated Wistar hearts showed reduced suppression of contraction amplitudes (by 31% as compared to the control Wistar hearts), decreased durations of arrhythmias (by 60%), diminished contractures (by 78%), and reduced creatine kinase release into the perfusate (by 58%) [3]. The hearts of August rats, unlike those of their Wistar counterparts, did not exhibit statistically significant differences from the control hearts in any of the above-mentioned four parameters of resistance to the reperfusion-caused damage. The failure of August rats to develop an adequate phenomenon of adaptive stabilization of cardiac structures is associated with the low level of hsp-70 accumulation in the myocardium, where polypeptides with pI 6.0-6.2 were almost undetectable (Fig. 1, *6*).

To summarize, the levels to which synthesis of hsp-70 with pI 6.0-6.2 is induced and/or these proteins accumulate in the heart of August rats are much lower than in Wistar rats, whereas the expression of the hsp-70 gene(s) coding for polypeptides with pI 5.7-5.9 appears to be the same in these two strains. The defective accumulation of alkaline hsp-70 in the myocardium of August rats is associated with their failure to develop cardioprotective phenomena similar to those developed by the Wistar rats in response to repetitive immobilization stress. The results of this study indicate, first, that the rat genome apparently contains several hsp-70 genes which are subject to differential regulation and, second, that different hsp-70 polypeptides make unequal contributions to the development of cardioprotective phenomena.

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REFERENCES

1. N. N. Dygalo, *Izv. Sibirsk. Otdel. Akad. Nauk SSSR, Ser. Biol.*, № 3, 116-119 (1982).
2. A. V. Zamotrinskii and S. S. Shishkin, *Biokhimiya*, **56**, 863-873 (1991).
3. I. Yu. Malyshev, F. Z. Meerson, A. V. Zamotrinskii, and O. N. Budanova, *Byull. Eksp. Biol. Med.*, **116**, № 8, 134-137 (1993).
4. T. K. C. Leung, M. Y. Rajaendran, C. Monfries, *et al.*, *Biochem. J.*, **267**, 125-132 (1990).
5. S. Lindquist and B. Didomenico, in: *Changes in Eucaryotic Gene Expression in Response to Environmental Stress*, B. G. Atkinson and D. B. Walden (eds.), Orlando (1985), pp. 72-87.
6. S. Lindquist and E. A. Craig, *Ann. Rev. Genet.*, **22**, 631-677 (1988).
7. F. Z. Meerson, I. Y. Malyshev, and A. V. Zamotrinsky, *Molec. Cell. Biochem.*, **111**, 87-95 (1992).
8. F. Z. Meerson, I. Y. Malyshev, and A. V. Zamotrinsky, *Molec. Cell. Biochem.*, **86**, 87-96 (1991).
9. J. H. Morrisey, *Analyt. Biochem.*, **117**, 307-310 (1981).
10. P. H. O'Farel, *J. Biol. Chem.*, **250**, 2347-2360 (1975).
11. H. R. B. Pelham, *Cell*, **46**, 959-961 (1986).
12. M. J. Schlesinger, *J. Biol. Chem.*, **265**, 12111-12114 (1990).