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# Adaptive Stabilization of Structures and Adaptive Protection of the Heart in Rats of Two Different Genetic Strains: Role of Heat-Shock Proteins hsp70

**E. V. Malysheva, A. V. Zamotrinskii, and I. Yu. Malyshev**

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Periodic exposures of Wistar rats to a stressor resulted in the accumulation of five isoforms of heat-shock proteins (hsp70) in their myocardia and, as a consequence, in increased resistance to thermal damage shown by their isolated hearts, whereas in rats of the August strain such exposures did not lead to hsp70 accumulation and the heart's resistance was not increased. It is concluded that the ability of a given genetic strain to develop adaptive protection of the heart appears to depend on the ability of that strain to boost the expression of hsp70 genes in response to stressors.

**Key Words:** *stress; adaptation; heart; Wistar rats; August rats*

It has been established that adaptation to periodic stressful exposures is characterized by a broad spectrum of protective, notably cardioprotective, effects [1] and that important roles in adaptive protection of the heart are played, in addition to alterations in neurohumoral regulation, by mechanisms originating in the heart itself [2]. As a result, hearts isolated from stress-adapted animals have been found to exhibit greatly increased resistance to damage inducible by reperfusion, toxic concentrations of calcium and catecholamines, and heat [7,8]. Enhanced resistance to injurious factors is also displayed by intracellular structures isolated from such hearts, including sarcoplasmic reticulum elements [5] and mitochondria and nuclear DNA [7]. The set of alterations leading to enhanced

Institute of General Pathology and Pathological Physiology, Russian Academy of Medical Sciences, Moscow. (Presented by N. R. Paleev, Member of the Russian Academy of Medical Sciences)

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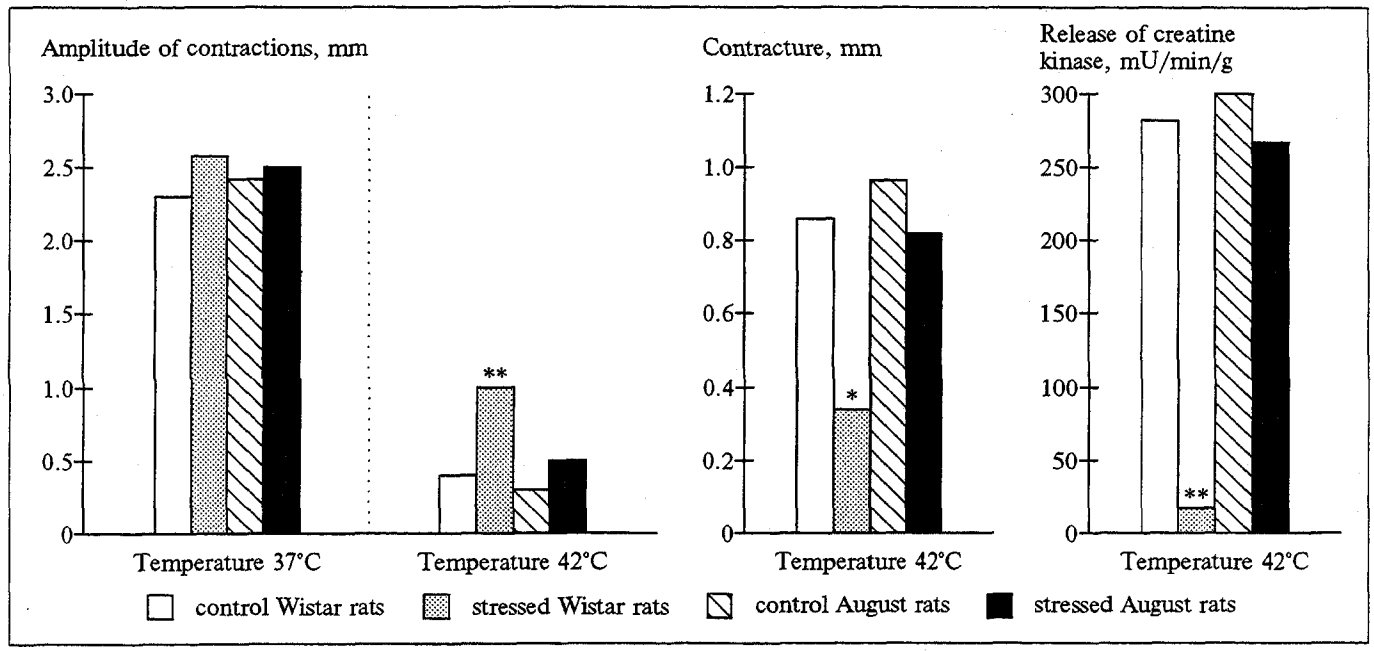


Fig. 1. Effect of periodic stress on the resistance to high temperature (42°C) shown by hearts isolated from Wistar and August rats. One and two asterisks: a significant difference from the control group at $p<0.05$ and $p<0.01$, respectively. Each group consisted of 6 rats.

resistance to stress has been termed the "phenomenon of adaptive stabilization of structures" (PASS) [6]. This phenomenon has been shown to be genetically determined and to depend largely on the extent to which the synthesis of heat-shock proteins from the hsp70 family is activated [2, 7, 8]. However, all previous experiments in which PASS and its mechanisms were studied used only Wistar rats, so that it was not known whether this phenomenon also develops in rats of other genetic strains and how different genetic strains differ with respect to the accumulation of heat-shock proteins during periodic exposures to stressors.

The objectives of the present study were, first, to compare quantitatively PASS in two different genetic strains of rats (Wistar and August) by measuring the resistance of their isolated hearts to thermal damage and, second, to see how these strains differ with regard to the accumulation of hsp70 isoforms upon periodic exposures to a stressor and then to consider these differences in relation to the magnitudes of PASS that developed in the two strains.

## MATERIALS AND METHODS

Wistar and August rats weighing 200-250 g were used. The periodic stress exposures comprised 12 sessions of short-term immobilization on the back once daily lasting 15 min on day 1, 30 min on day 2, 45 min on day 3, and 60 min on days 4 through 12 (all four limbs were tied, but the head was allowed to move freely [1]). After a course of such sessions, the rats did not develop edema in the limbs or stress-induced gastric mucosal lesions, nor did they show noticeable changes in behavior.

PASS was evaluated by a procedure in which an isolated heart is perfused according to Langendorf. The rats were heparinized (2000 U/kg intraperitoneally) and anesthetized with Nembutal (50 mg/kg intraperitoneally). Their hearts were then removed after thoracotomy and placed in a perfusion system with standard Krebs-Henseleit solution. The mechanical activity of the isolated hearts was recorded using a TD-112S isotonic transducer (Nihon Kohden, Japan) and modules of an RM-6000 polygraph (Nihon Kohden), which was also used for recording the ECG. Heat shock to an isolated heart was simulated by raising the perfusion solution's temperature from 37 to 42°C for 15 min [3]. The severity of thermal damage to the heart was rated by recording the amplitude of contractions, the degree of contracture, and abnormalities of cardiac rhythm. Damage to the sarcolemma was assessed spectrophotometrically according to Szasz [11] by the activity of creatine kinase (CK) in the solution outflowing from the heart. PASS was assessed by noting the extent to which the effects of the heat shock were prevented.

IEF-PAGE and monoclonal antibodies were used for identifying and characterizing hsp70 isoforms. Isoelectric focusing was carried out at 500 V for 18 h. Electrophoresis in the second direction was performed in 10% polyacrylamide gel [4]

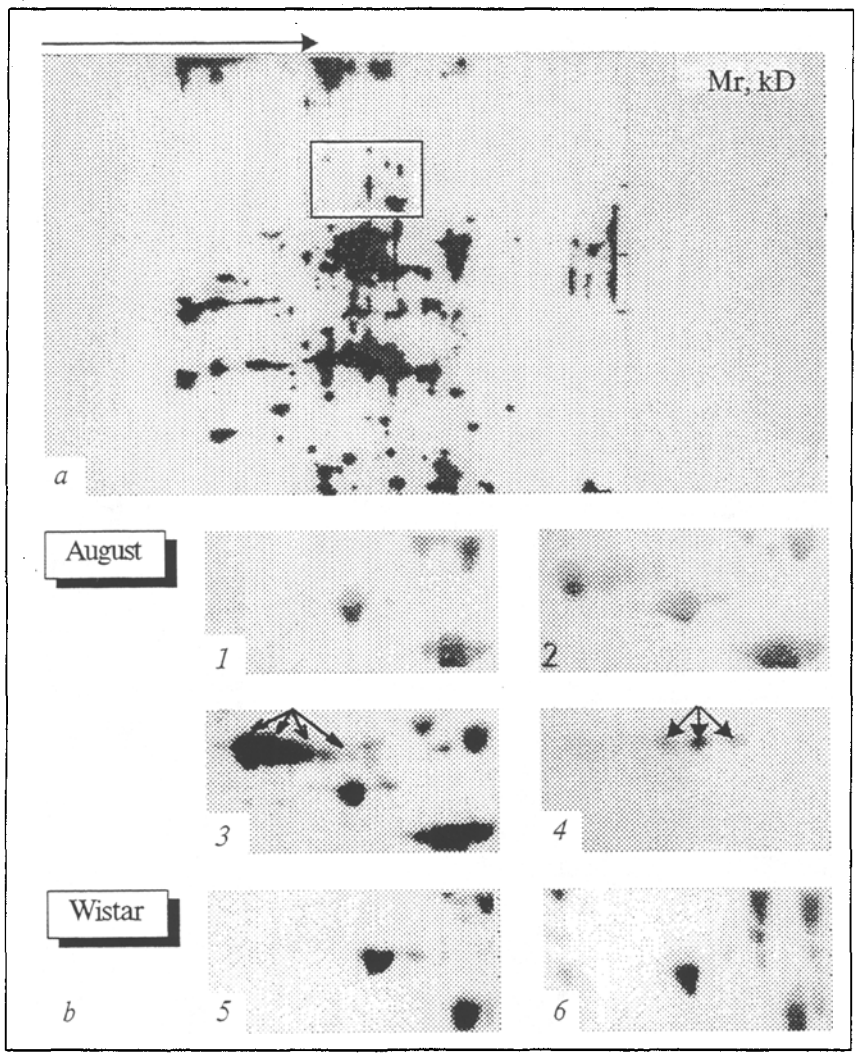


**Fig. 2.** Effect of periodic stress on heat-shock protein levels in the myocardium of Wistar and August rats. *a*) A typical electrophoregram of cytosol proteins from cardiac cells. The horizontal arrow shows the direction of isofocusing. The area of hsp70 location is boxed. *b*) Fragments of electrophoregrams covering the area boxed in Fig. 2, *a*: *1*) control Wistar heart; *2*) heat-shocked Wistar heart; *3*) hearts of Wistar rats periodically exposed to stress; *4*) hearts of heat-shocked Wistar rats – immunoblot with anti-hsp70-antibodies; *5*) control August hearts; *6*) hearts of August rats periodically exposed to stress. Arrows indicate hsp70 isoforms.

stained with silver nitrate by the method of Morrisey [9]. The hsp70 isoforms were identified and characterized by their molecular weights and isoelectric points. In addition, the gels obtained from the hearts of control and adapted rats were compared with those from heat-exposed (hyperthermic) rats, in which the inducible fraction is known to comprise heat-shock proteins [10,13]. To produce hyperthermia, the rats were first anesthetized with Nembutal (50 mg/kg intraperitoneally) and then placed in an incubator where they were exposed to 80°C for 20 min, which, in most rats, resulted in an elevation of the rectal temperature to 41-42°C after 5 min of exposure. This rectal temperature persisted for the remaining 15 min. Additionally, heat-shock (hsp70) proteins were identified by the Western blotting technique, in which the proteins were transferred to nitrocellulose at 20 mA, as described by Towbin *et al.* [12], followed by incubation of the blots in TBS containing 5% milk powder, to block the sites of nonspecific binding on the membrane. The blots were then incubated with N27F3-4 and C92F3A5 monoclonal antibodies to hsp70 at a 1:500 dilution (the antibodies were kindly donated by Dr. William Welch from the University of California) and finally with a peroxidase-conjugated goat antimouse IgG. Diaminobenzidine (Sigma) was used to visualize the reaction products.

## RESULTS

The histograms of Fig. 1 show the impact of periodic stress on the tolerance of heat shock exhibited by hearts of Wistar and August rats. The perfusion of hearts from control Wistar rats with a solution heated to 42°C depressed the amplitude of contractions significantly from 2.40±0.06 to 0.44± ±0.15 mm, reduced the contracture to 36% of the initial amplitude of contractions, and - most important - led to a massive release of CK into the perfusate, the activity of this enzyme reaching 295±110 mU/min/mg. Similar changes were recorded for hearts from control August rats.

In Wistar rats, the adaptation to periodic stress effectively limited the depression of contraction amplitudes, contracture, and CK release into the perfusate. For example, by minute 15 of exposure to the hot perfusion solution, the release of CK into the perfusate recorded for the hearts of adapted Wistar rats was 37 times less than that recorded for the hearts of control rats. This finding indicates that the adaptation to stress in this strain did involve PASS. In the August rats, the periodic stress exposures virtually did not alter the resistance of their hearts to thermal damage (Fig. 1), which indicates that the rats of this strain did not develop PASS as far as the parameters of cardiac resistance to thermal injury were concerned.

As shown previously, a key mechanism of PASS is associated with the accumulation of heat-shock proteins [1], and it was therefore of interest to evaluate differences in the accumulation of hsp70 in response to periodic stress exposures between Wistar and August rats and to consider these differences in relation to PASS in these two strains.

A typical two-dimensional electrophoregram of cardiac cytosol proteins is presented in Fig. 2, *a*. The distribution pattern of polypeptide fractions proved to be highly reproducible for specimens both from animals of the same strain and from those of different strains. In the stress-adapted Wistar rats, at least 5 polypeptides of molecular weight around 72 kD and having isoelectric points ranging from 6.3 to 5.7 were found to have accumulated in the myocardium (Fig. 2, *b*, *3*). The most representative polypeptide among those induced by the adaptation to the stressor was the most alkaline polypeptide. None of the fractions mentioned above was detectable for the control Wistar rats (Fig. 2, *b*, *1*).

To identify the proteins induced by the immobilization-produced stress, these proteins were compared with the fractions induced by the heat shock (Fig. 2, *b*, *2*). The identical positions of the inducible fractions after adaptation to the stress and after the heat shock and the observed ability of all these fractions to react with anti-hsp70-antibodies (Fig. 2, *b*, *4*) enabled us to conclude that the polypeptide accumulating in the Wistar rats during their adaptation to the immobilization stress were proteins from the hsp70 family.

In the August rats, in contrast to the Wistar rats (Fig. 2, *b*, *1* and *3*), periodic exposures to the stressor failed to result in the accumulation of any hsp70 isoforms in the myocardium (Fig. 2, *b*, *5* and *6*).

In summary, this study has shown that the accumulation of heat-shock proteins and the development of PASS in response to stress depends on the genetic strain of the animals. In Wistar rats, the adaptation to the immobilization-induced stress was accompanied by the abundant accumulation of hsp70 and by the development of a pronounced PASS, which was manifested, in particular, in an increased resistance of their hearts to thermal damage. In August rats, hsp70 did not accumulate and no PASS, as assessed by parameters of heart's resistance to thermal damage, developed.

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