Dynamics of Formation and Reversion of the Phenomenon of Adaptational Stabilization of Structures Correlates with Changes in HSP70 Content in the Myocardium

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

UDC 616.127-008.9-02:613.863]-092.9-07

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 116, № 8, pp. 134-137, August, 1993
Original article submitted March 10, 1993

Key Words: *stress proteins; adaptation; myocardium*

The phenomenon of adaptational stabilization of structures (PASS) implies that adaptation of the organism to stress influences enhances the resistance not only of the whole organism but also of isolated organs, primarily of the heart, to a broad spectrum of damaging factors from toxic concentrations of catecholamines to the reperfusion paradox and heat damage [2,3,8]. The resistance of cell structures such as the sarcoplasmic reticulum, mitochondria [3,7], and nuclei [1] also increases. PASS has been found to be realized against the background of a considerably increased content of heat-shock proteins (hsp70) in the myocardium, together with alterations in their isoform composition and subcellular distribution [1,8]. However, some essential questions are not yet answered. How rapid is the rate of formation of PASS and of the cardioprotective effects and how long do they persist after termination of the adaptation sessions? What is the pattern of dynamics of hsp70 isoform composition during adaptation and after its termination? Does the dynamics of the protective adaptation effect follow the changes in the hsp70 content in the myocardium? Answers to these questions are necessary not only for understanding the dynamics of the cardioprotective effects of adaptation, but also for a more detailed analysis of the role of stress proteins in the development of PASS.

The aims of the present study were, first, to analyze the dynamics of formation and reversion of PASS by studying the changes in resistance of the isolated heart to reperfusion and thermal damage during and after adaptation to stress; and second, to compare the obtained dynamics of PASS with changes in the content and isoform composition of hsp70 in the myocardium.

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. Both the control and the adapted animals were heparinized (2000 IU/kg intraperitoneally) and narcotized with nembutal (50 mg/kg intraperitoneally). The heart was then promptly removed and mounted onto a Langendorf perfusion system with standard Krebs-Henseleit solution containing (in mM): 120 NaCl, 20 $NaHCO_3$, 4.8 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 1.2

Research Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow. (Presented by G. N. Kryzhanovskii, Member of the Russian Academy of Medical Sciences)

0007-4888/93/0008-0919$12.50

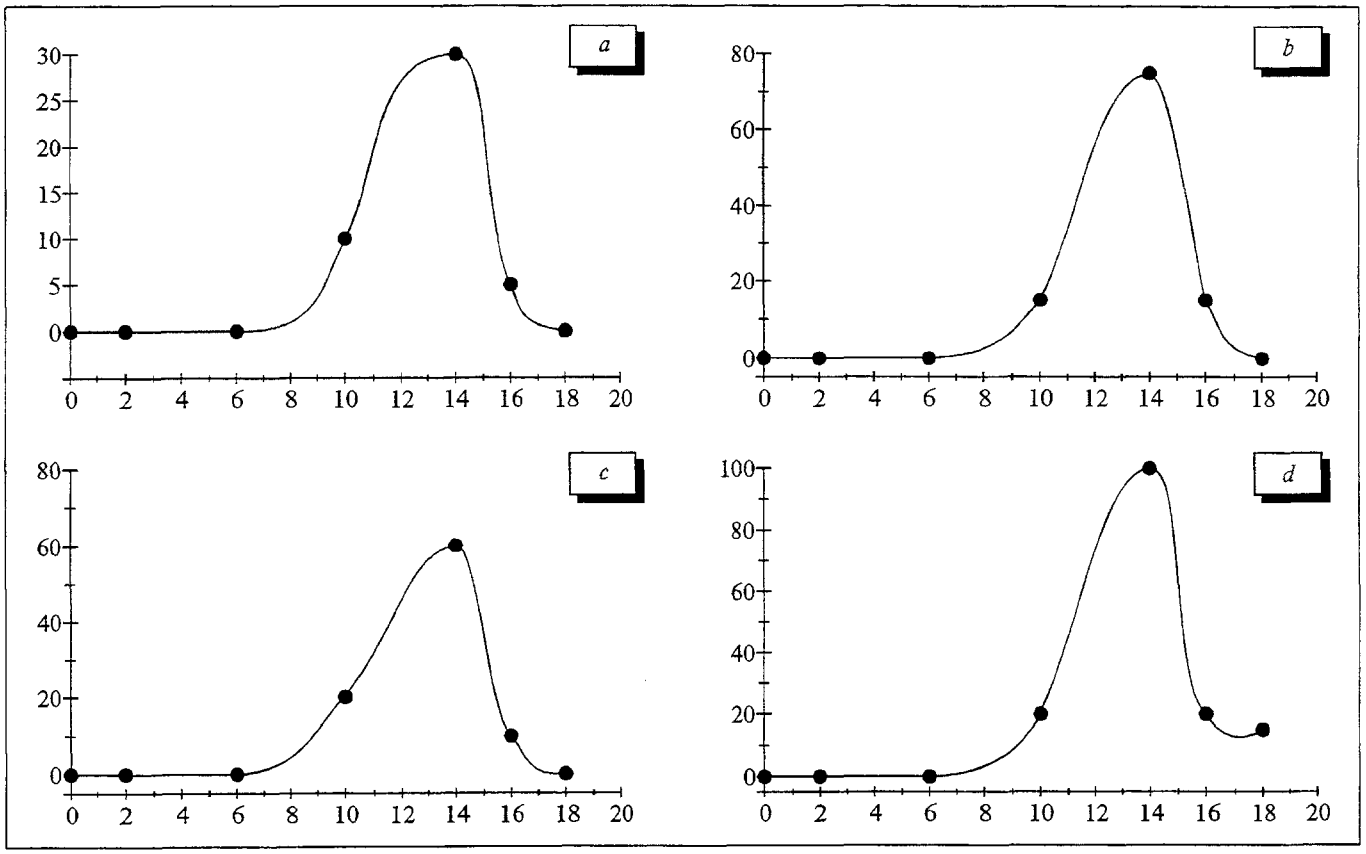

Fig. 1. Dynamics of formation and reversion of increased resistance of isolated heart to reperfusion damage during and after course of adaptation to immobilization stress. (Comparison with the content of hsp 70 in the myocardium.) Here and in Fig. 2: ordinate: *a*) diminution of contraction amplitude depression, in % in comparison with control; *b*) reduction of contracture in the heart of adapted animals, in % in comparison with control; *c*) shortening of arrhythmia duration, in % in comparison with control; *d*) content of hsp70 in the heart of adapted animals, %. Maximum accumulation after completion of the course of adaptation was taken as 100%. Abscissa: days after beginning of adaptation and after its termination; *: days of immobilization stress.

KH_2PO_4, and 11 glucose. The solution was aerated at 37°C with a mixture of 95% O_2 and 5% CO_2 (pH 7.3-7.4). The perfusion pressure was 9.5 kPa (97 cm H_2O). Mechanical activity of the isolated heart was evaluated using a TD-112S isotonic transducer (Nihon Kohden, Japan), attaching the sensor element to the apex of the heart by means of a sharp steel hook [4]. Electrodes for ECG recording were placed on the aorta and on the left ventricle. The mechanical activity and ECG were recorded with specialized units of an RM-6000 polygraph and a VC-9 oscilloscope (Nihon Kohden, Japan). Ischemic and reperfusion injuries were produced as described previously [5] by complete occlusion of the coronary flow over 15 min, after which the perfusion was restored and observations were performed for an additional 20 min. Heat shock on the isolated heart was modeled by raising the temperature of the perfusion solution from 37°C to 42°C, followed by observation for 15 min. The extent of reperfusion- and heat-induced injury to the isolated heart was assessed from the following parameters: depression of the contraction amplitude, contracture, rhythm disturbances, and release of creatine kinase (CK) into the perfusion solution. The contracture was measured in millimeters by the absolute change in diastolic length of the heart along the apex-base axis in relation to the diastolic length of the heart recorded at the end of the stabilization period [4]. Disturbances in the cardiac rhythm were assessed from the ECG. Damage to the sarcolemma was evaluated by spectrophotometric determination of CK activity in the perfusion solution with Labsystems kits. The content of hsp70 was determined in the cytoplasm of cardiomyocytes. To this end, the heart was thoroughly drained of blood in a special perfusion setup and a piece of tissue weighing approximately 240-260 mg was excised. For isolation of the cytoplasmic fraction of proteins, the specimen was placed in hypotonic buffer solution containing 10 mM Tris (pH 7.4), 10 mM KCl, and 1 mM PMSF at 4°C for 10 min and then homogenized in this solution at a 1:5 tissue:buffer ratio. The homogenate was filtered through 8 layers of gauze and centrifuged at 12,000 g for 10 min. The su-

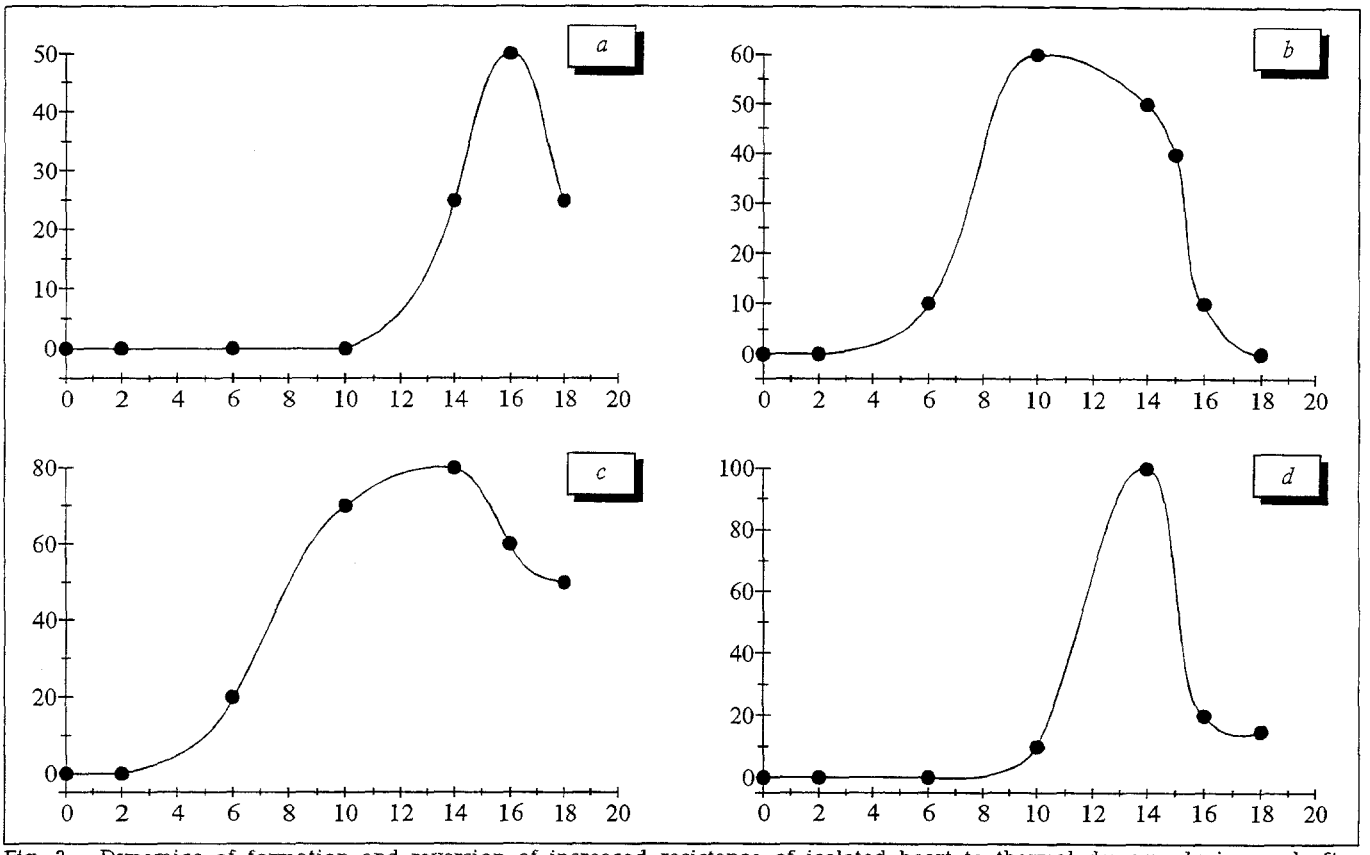

Fig. 2. Dynamics of formation and reversion of increased resistance of isolated heart to thermal damage during and after course of adaptation to immobilization stress. (Comparison with the content of hsp70 in the myocardium.) Other notation as in Fig. 1.

pernatant was the solution of cytoplasmic proteins. Two-dimensional electrophoresis was carried out as described previously [10]. For preparation of gels for isoelectric focusing LKB Pharmacia ampholines of pH ranges 5-8 (for 1.6% gel) and 3.5-9.5 (for 0.4% gel) were used. Isoelectric focusing was carried out at 500 V for 18 h. The second dimension-electrophoresis was performed in 10% PAGE as described elsewhere [6]. Preparations of carbamoyl carbonic anhydrase (LKB Pharmacia) served as pI marker. Purified proteins (LKB Pharmacia) were used as molecular weight standards: bovine serum albumin, 68 kD; ovalbumin, 45 kD; carbonic anhydrase, 30 kD. The gels were stained with silver nitrate as described elsewhere [9]. Identification and characterization of hsp70 isoforms were performed by their molecular weight and isoelectric point.

RESULTS

The dynamics of the formation and reversion of PASS during and after adaptation to stress is shown in Figs. 1 and 2.

As seen from the figures, the formation of a high resistance to reperfusion injuries during adaptation has a certain latent period, which in our case was 10 days (Fig. 1). On day 14 a pronounced cardioprotective effect was achieved, which manifested itself in a diminution of the depression of the contraction amplitude by 31%, a reduction of contracture by 78%, a shortening of arrhythmia duration by 60%, and a reduced release of CK into the perfusate by 58% (Fig. 1). After termination of the adaptation sessions this protective effect disappeared rapidly and the resistance of the heart to reperfusion damage was equal to that observed in the control after just 4 days (Fig. 1).

The adaptational resistance of the heart to thermal injury also developed after a latent period of 6-10 days starting from the beginning of adaptation (Fig. 2). The maximal protective effect was found to occur on day 4 after termination of the adaptation sessions, judging from the "diminution of the contraction amplitude depression" parameter, whereas according to the other two parameters, "restriction of contracture" and "reduction of CK release", the maximal resistance of the heart was achieved on day 10 of adaptation and remained at this level during 4 days. During this period, heat shock-induced contracture was decreased by about 50% and the release of enzymes was reduced by

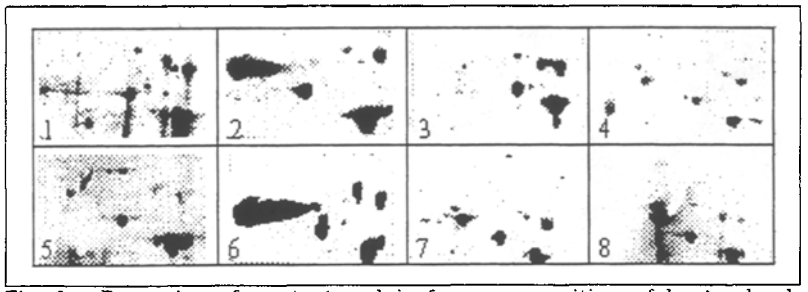

Fig. 3. Dynamics of content and isoform composition of heat-shock proteins in cytoplasm of cardiomyocytes during and after course of adaptation to immobilization stress. *1*) control; *2*) heat shock; *3*) day 2 of adaptation; *4*) day 8 of adaptation; *5*) day 10 of adaptation; *6*) day 2 after termination of adaptation; *7*) day 4 after termination of adaptation; *8*) day 6 after termination of adaptation. Arrows: hsp70 isoforms.

almost 80% (Fig. 2). After termination of the adaptation course the thermoprotective effect persisted considerably longer then the increased resistance to reperfusion injuries (Figs. 1, 2). Thus, even 6 days after adaptation, the hearts of adapted animals showed a higher resistance to heat shock than the hearts of the controls.

Next, we studied the changes in the hsp70 content during and after the course of adaptation and compared it with the dynamics of PASS. The following interesting relationships were thereupon revealed.

Hsp70 isoforms began to be detected in the cytoplasm starting from day 6 of adaptation (one isoform) (Fig. 3, *4*) and, correspondingly, the cardioprotective effects did not appear until then (Figs. 1 and 2). The maximal accumulation of 5 inducible stress proteins with m.w. 72 kD and pI 5.7-6.3 was observed on day 2 after completion of a 12-day course of adaptation to immobilization (Fig. 3, *6*). This corresponded to the time of development of the most powerful protective effects, such as an increased resistance of the isolated heart to reperfusion injury and heat shock (Figs. 1 and 2). Termination of adaptation led to a gradual decrease of hsp70 isoforms, and after 6 days their content was considerably reduced though still higher then before adaptation (Fig. 3, *8*). On this day, just the thermoprotective effect of adaptation was reliably detectable, while the resistance to reperfusion was equal to that before adaptation (Fig. 2).

Thus, the dynamics of the content of heat-shock proteins in the myocardium during and after adaptation was very close to the dynamics of formation and reversion of the PASS protective effects, namely, in both cases a certain latent period precedes a rapid activation of the synthesis of stress proteins and formation of protective effects. After termination of the adaptation course, the content of stress proteins decreases, which corresponds to a reversion of the protective effects. This suggests again the important role of stress proteins in the mechanisms of adaptational stabilization of structures and demonstrates the pattern of changes in hsp70 content depending on the duration of adaptation and the period elapsintg after it. In other words, there is a real possibility of analyzing the sequence of activation of gene expression of particular hsp70 isoforms in the course of formation and "extinction" of PASS.

Our investigation showed that during adaptation an accumulation of basic polypeptides with a pI of about 6.3 is followed by an accumulation of acidic peptides with a pI from 6.0 to 5.7. After the termination of adaptation, the isoforms disappear in the opposite order: acidic before basic (Fig. 3). This dynamics of the isoform composition of hsp70 in the myocardial cells probably reflects the time course of activation of gene expression of different hsp70 isoforms during and after adaptation of the organism to stress interventions.

Moreover, close inspection of the time-coordinated activation of stress proteins shows up one very interesting relationship, namely, in the adaptation of the organism to periodic stress interventions, equal in strength and duration, there is a moment when the next intervention evokes a sharp increase of stress protein gene expression. This situation is clearly depicted in Figs. 1-3. As seen from the figures, stress interventions caused no accumulation of stress proteins in the myocardium until the 6th day of adaptation. On day 6 just a negligible amount of one isoform of hsp70 was detected in the cytoplasm of cardiomyocytes. On day 10 of adaptation, despite additional stress influences, the picture did not differ from that observed on day 6 (Fig. 3). Surprisingly, on day 14 the next standard stress exposure led to a vigorous trigger activation of hsp70 synthesis (Fig. 3), when 5 isoforms of the inducible proteins were detected in the cells. A peculiar phenomenon of explosive adaptation is realized. This trigger adaptation-induced accumulation of hsp70 after a certain latent period suggests that during adaptation to stress a peculiar mechanism of activation of hsp70 synthesis comes into play, which probably differs in many feature from stress-induced activation of these proteinss.

REFERENCES

1. I. Yu. Malyshev, A. V. Zamotrinskii, and F. Z. Meerson, *Byull. Eksp. Biol. Med.*, **112**, № 8, 126-128 (1991).

2. F. Z. Meerson, *Kardiologiya*, № 3, 3-12 (1990).
3. F. Z. Meerson and I. Yu. Malyshev, *Phenomenon of Adaptational Stabilization of Structures and Heart Protection* [in Russian], Moscow (1993).
4. W. H. Gilst and J. M. Koomen, *Basic. Res. Cardiol.*, **82**, 233-240 (1987).
5. D. J. Hearse. S. M. Humphrey, and E. B. Chain, *J. Molec. Cell. Cardiol.*, 5, 395-401 (1973).
6. V. K. Laemmli, *Nature*, **227**, 680-685 (1970).
7. F. Z. Meerson, T. G. Sazontova, and Yu. V. Arkhipenko, *Biomed. Sci.*, 1, 373-378 (1990).
8. F. Z. Meerson, I. Yu. Malyshev, and A. V. Zamotrinsky, *Molec. Cell Biol.*, **111**, 87-95 (1992).
9. J. H. Morrisey, *Analyt. Biochem*, **117**, 307-310 (1981).
10. P. H. O'Farrell, *J. Biol. Chem.*, **250**, 4007-4021 (1975).

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

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

UDC 616.153.96-02:613.863]-092.9-07

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 116, № 8, pp. 137-139, August, 1993
Original article submitted March 10, 1993

Key Words: *stress proteins; adaptation to stress; adaptation to hypoxia*

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.

Research Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow. (Presented by G. N. Kryzhanovskii, Member of the Russian Academy of Medical Sciences)

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

0007-4888/93/0008-0923$12.50