Biphasic Character of the Phenomenon of Adaptive Stabilization of Structures during Long-Term Adaptation of the Organism to Stress

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In 1989-1990 [2,9], the phenomenon of adaptive stabilization of structures (PASS), manifested in an increased resistance not only of the whole organism but also of isolated organs (primarily, of heart) to a broad spectrum of damaging factors, ranging from toxic concentrations of catecholamines to reperfusion and heat injury [2,3,9], was discovered for adaptation of the organism to stress. Simultaneously, the resistance of such cell structures as the sarcoplasmic reticulum, mitochondria [3,8], and nucleus [1] to autolysis increases. PASS has been found to be realized against the background of a significant increase of the content of heat shock proteins (hsp 70) in the myocardium [1,9]. Thus, PASS emerged as the cellular component of adaptation, boosting the resistance of structures to damaging factors due to the activation of the genetic apparatus [3]. A marked PASS has been shown to appear as soon as after a 14-day course of adaptation to stress [1,2,3,8]. However, the possibility of maintaining PASS on a high level by prolonging the course of adaptation has not been studied; in other words, the dynamics of PASS is still to be clarified in long-term adaptation to stress.

Accordingly, the aim of the present study was, first, to compare the magnitude of PASS judging by the changes in the resistance of the isolated heart to reperfusion injury after 14-day and 28-day courses of adaptation to stress, and, second, to compare the data obtained and the changes of the content and isoform composition of hsp 70 in the myocardium.

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MATERIALS AND METHODS

The experiments were carried out on male Wistar rats weighing 200-250 g. Over 14 and 28 days the animals were adapted to stress by short-term immobilizations in the supine position: 15 min on day 1, 30 min on day 2, 45 min on day 3, and 60 min every other day on the remaining days. The control and adapted animals were heparinized (2000 IU/kg, i.p.) and narcotized with nembutal (50 mg/kg, i.p.). The heart was then excised and placed in a Langendorff perfusion system with standard Krebs-Henseleit solution of the following composition (mM): NaCl 120, $NaHCO_3$ 20, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, glucose 11. The solution was aerated with a gas mixture containing 95% O_2 + 5% CO_2 at a temperature of 37°C. The pH was fixed in the range 7.3-7.4. The perfusion pressure was 9.5 kPa (97 cm water). The mechanical activity of the isolated heart was assessed using a TD-112S Nihon Kohden isotonic transducer (Japan), its sensitive element applied to the apex of the heart with the aid of a sharp steel hook [4]. The electrodes for ECG recording were applied to the aorta and to the left ventricle. The mechanical activity and the ECG

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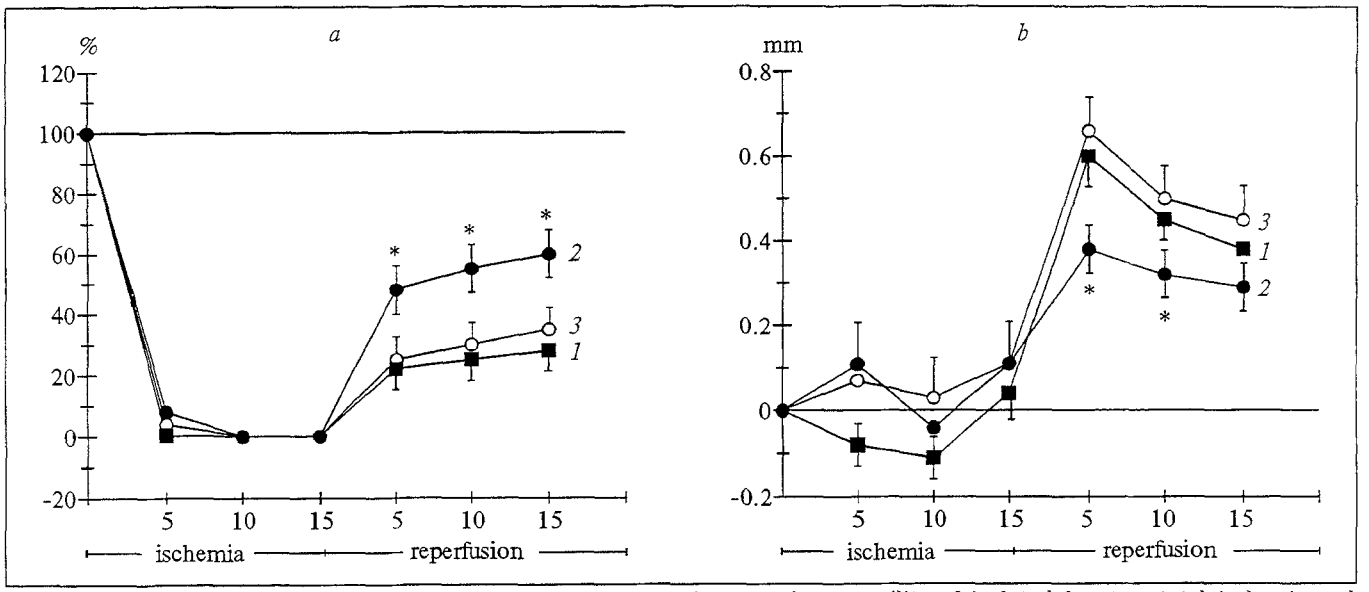

Fig. 1. Effect of various durations of adaptation to stress on resistance of contractility of isolated heart to total ischemia and reperfusion. Abscissa: time, min; ordinate: *a*) amplitude of heart contractions, % of initial; *b*) cardiac constriction, mm of apico–basal shortening. *1*) control; *2*) adaptation to stress (14 days; *3*) adaptation to stress (28 days); asterisk: reliable differences vs. control ($p<0.05$).

were recorded with the aid of specialized modules: an RM-6000 polygraph and a VC-9 oscilloscope (Nihon Kohden). The models of ischemic and reperfusion injuries were reproduced after Hearse et al. [5] by completely occluding the coronary vessel for 15 min, after which perfusion was commenced and observations were performed for 20 min during reperfusion. The amount of damage to the isolated heart for reperfusion injury was assessed from the following: the depression of the amplitude of contraction, contracture, the disturbances of the rhythm, and the release of creatine kinase (CK) into the perfusion fluid. The manifestations of contracture were measured in mm according to the absolute changes of the diastolic length of the heart in the apico-basal direction versus the diastolic apico-basal length of the heart recorded at the end of the period of stabilization [4]. The disturbances of the heart rhythm were judged by the ECG. The lesions in the sarcolemma were assessed in accordance with the spectrophotometrically assayed activity of CK in the solution outflowing from the heart, using Labsystems kits.

The content of hsp 70 was measured in the cytoplasm of cardiomyocytes. For this purpose, the blood was first washed out of the heart, and approximately 240-260 mg of tissue were excised. To obtain the fraction of cytoplasmic proteins the tissue was placed in hypotonic buffer (10 mM Tris pH 7.4, 10 mM KCl, and 1 mM phenylmethylsulfonylfluoride) for 10 min at 4°C, and then homogenized in the same solution at a tissue/buffer ratio of 1:5. The resulting homogenate was filtered through 8 layers of gauze and centrifuged for 10 min at 12,000 g. Cytoplasmic proteins were found in the supernatant. Two-dimensional electrophoresis was performed after O'Farrell [11]. Ampholines (LKB Pharmacia) for pH ranges 5-8 (1.6%) and 3.5-9.5 (0.4%) were used to prepare the gel for isoelectrofocusing. Isoelectrofocusing was performed at 500 V for 18 h. The second dimension of electrophoresis was carried out in 10% polyacrylamide gel after Laemmli [6]. The preparation of carbamoylated carboanhydrase (LKB Pharmacia) was used as an electrofocusing marker. Bovine serum albumin (68 kD), ovalbumin (45 kD), and carboanhydrase (30 kD) were used as molecular weight markers. The gels were silver-stained after Morrisey [10]. Isoforms of hsp 70 were identified and characterized according to the molecular weight and isoelectric point.

The content of heat shock proteins was determined by Nosern assay after Sambrook [12]. Here, RNA was obtained from the rat heart after Maniatis et al. [7]. RNA was fractionated by electrophoresis in 1.5% agarose and in 2.2% formaldehyde gel. Human cDNA for inducible hsp 70, kindly supplied by Prof. A. Gudkov (Institute of Oncology, Russian Academy of Medical Sciences), was used as cDNA. cDNA was labeled at α-P dCTP [7].

RESULTS

The data in Fig. 1 show the effect of a prolonged course of adaptation to stress on the resistance of

the isolated animal hearts to reperfusion paradox. It is evident that during the period of reperfusion the amplitude of contraction (Fig. 1, *a*) was proportionally reduced with respect to the constriction (Fig. 1, *b*). The preliminary adaptation to stress, lasting 14 days, produced a marked anticonstrictive effect and accordingly provided for a far higher level of contraction amplitude in the case of reperfusion than in the control. After 28 days of adaptation its anticonstrictive effect and the ability to maintain a high amplitude of contraction were lost.

During the study of the effect of adaptation of various duration on the intensity of arrhythmia for reperfusion paradox, ventricular fibrillation and ventricular tachycardia were observed in 86% of cases during reperfusion, whereas on the 14th day of adaptation arrhythmias of such a kind were noted in just 25%, and on the 28th day in just 50% of cases. Significant differences were also observed for the duration of severe arrhythmias. For instance, in the control hearts the total duration of ventricular fibrillation and ventricular tachycardia over 15 min of reperfusion was 89.5 sec on the average, while on the 14th day of adaptation this value was only 2.4 sec, and on the 28th day it was somewhat increased and constituted 36.6 sec.

Thus, the anticonstrictive and antiarrhythmic effects of adaptation, being the major manifestations of PASS, are pronounced on the 14th day of adaptation. On the 28th day of adaptation the anticonstrictive effect disappears and the antiarrhythmic effect falls off slightly, though remaining pronounced. These results are summarized in Fig. 2.

Electrophoregrams presented in the same figure enable us to assess the effect of the duration of adaptation to stress on the content of heat shock proteins in the myocardium. It is seen that on the 28th day of adaptation just two isoforms (out of the five discovered on the 14th day) of hsp 70 are still present in the myocardium. Such a dynamics of hsp 70 content satisfactorily correlates with the magnitude of PASS (Fig. 2) and, thereby, once again confirms the important role of stress proteins in the adaptive increase of cardiac resistance to injury.

Figure 3 shows that the maximum accumulation of hsp 70 on the 14th day was attended by an increase of the content of mRNA for hsp 70 (2.6 kb). This provides evidence that during adaptation both the accumulation of heat shock proteins and PASS per se are genetically determined.

Thus, during prolonged adaptation of the organism to stress (28 days), PASS exhibits a marked biphasic character: a first phase of development over approximately 14 days and a second phase of reduction, which takes place regardless of whether the course of adaptation is continued.

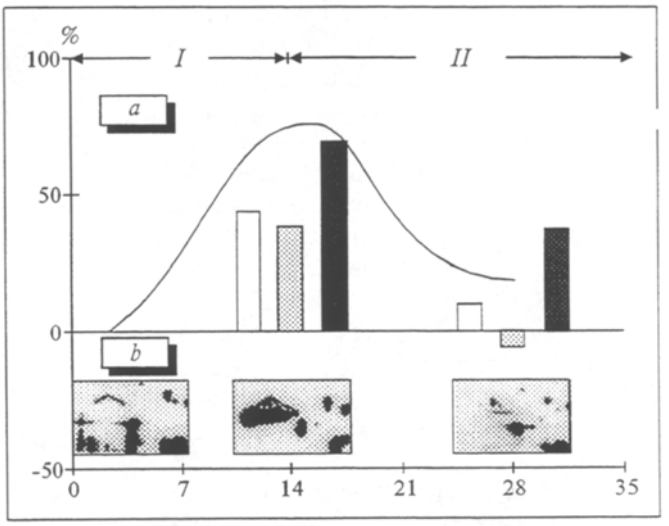

Fig. 2. Dynamics of PASS and changes of content of heat shock proteins during long-term adaptation to stress. *a*) ordinate: protective effect of adaptation assessed as resistance of isolated heart to reperfusion injury, %; complete prevention of damage is taken as 100%; light bar: inhibition of depression of amplitude of contraction; dark bar: prevention of contracture; hatched bar: restriction of arrhythmias. *I*) first phase: development of PASS; *II*) second phase: reduction of PASS. Dynamics of PASS is shown by dashed line. *b*) ordinate: changes of content and isoform composition of hsp 70 (shown by arrows). *a* and *b*) abscissa: time of adaptation to stress, days.

In an analysis of the mechanisms possibly underlying the biphasic character of PASS, one must take into account the most recent data obtained in our laboratory. Activation of phospholipase C, the crucial link of the inositoltriphosphate-diacylglycerol (ITP-DAG) regulator cascade, is observed on the 14th day of adaptation [3]. This

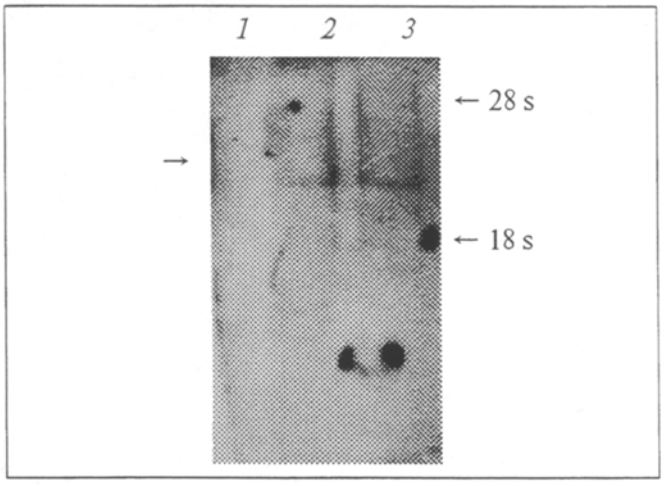

Fig. 3. Effect of adaptation to stress on content of inducible hsp 70 mRNA in cells of myocardium. *1*) control; *2*) adaptation to stress; *3*) heat shock. Arrow at left shows localization of hsp 70 mRNA.

inevitably leads to an increased ITP and DAG synthesis in the cell. An enhancement of ITP- and DAG-induced inotropic responses for phenylephrine-induced stimulation, as was testified in the same study, also attests to the increased production of ITP and DAG. On the 14th day of adaptation both of the above effects - an increase of phospholipase C activity and an enhancement of inotropic effects - are attended by a marked elevation of the hsp 70 content in the myocardium and an increased resistance of the heart to reperfusion paradox (Fig. 2), the latter being a well-known component of PASS. Activation of phospholipase C and enhanced responses to phenylephrine proved to be absent on the 28th day of adaptation [3], this, as was shown by our experiments, being accompanied by a marked drop of the content of heat shock proteins and the disappearance or diminishment of PASS (Fig. 2).

On the whole, these experimental data imply a relationship between one of the mechanisms underlying the biphasic character of PASS and the ITP-DAG cycle participating in PASS development by regulating the synthesis of heat shock proteins.

It is also worthy of note that adaptation to repeated or prolonged exposure to stress makes it possible to follow up the changes in regulatory mechanisms contributing to the defense of the heart. In fact, during a study of adaptation to continuous moderate stress, it was discovered that on the 5th day of this process the tone of the vagus nerve markedly increases in the animals, and this shift in the nervous regulation of the heart plays an important role in preventing ischemic and reperfusion arrhythmias [3]. Later, the increased tone of the vagus disappears, but the resistance of the heart to ischemic and reperfusion injuries is preserved not only for the whole organism, but also for isolated organs. This occurs due to the development of PASS, which peaks on the 14th day of adaptation. Precisely at this time a high resistance to reperfusion paradox, accumulation of hsp 70 in the cytoplasm and nuclei of cardiomyocytes, and an increase in the resistance of the SPR nuclei and structures to autolysis were observed in our experiments [1,2,9]. In the later stage of adaptation to stress, as was shown above, activation of the ITP-DAG regulatory cycle disappears and a decrease in the content of stress proteins is noted in the myocardium, as well as a reduction of PASS. It is important that during this period, yet unknown mechanisms acting at the stage of PAS reduction maintain the increased resistance of the heart to acute anoxia and subsequent reoxygenation under conditions of the whole organism [3].

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