ADAPTATION TO STRESS INCREASES THE RESISTANCE OF NUCLEAR DNA OF HEART CELLS THROUGH HEAT SHOCK PROTEIN ACCUMULATION IN THE NUCLEUS

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Repeated exposure to short-term stress situations leads to the development of adaptation of the individual, which not only increases his resistance to severe stress, but also brings about a broad spectrum of cross protective effects, i.e., protects the body against direct ischemic, chemical, cold-induced, and even radiation damage [4]. This adaptation of protection has been shown to be realized not only at the level of neuroendocrine mechanisms, but also at the level of the target organs themselves. It has been shown, for instance, that the isolated heart of adapted animals has sharply increased resistance to reperfusion injuries [8], to high calcium concentrations [9] and toxic doses of catecholamines [3], and that organelles isolated from them (elements of the sarcoplasmic reticulum and mitochondria) differ from controls in being more resistant to autolysis [10]. This combination of phenomena has been described as the "adaptive stabilization of structures phenomenon" (ASSP) [5]. The most evidence is that ASSP is accompanied by marked accumulation of heat shock proteins hsp70, with molecular weight of about 70 kD, in the cytoplasm of the cells [11]. They play an important role in poststress recovery of cellular structures through disaggregation of abnormal protein—protein interactions [13]. Taken as a whole, it can be concluded from these data that hsp70 may not only perform its protective functions in the stressed cell, but may also play an important role in the development of ASSP and in the formation of adaptive protection of cytoplasmic structures [5, 10]. However, some important problems still remained unsolved: is ASSP realized only in cytoplasmic structures or does it develop also at the level of the genetic matrix (DNA), and if ASSP is realized at the DNA level, what is the role of hsp70 in this phenomenon.

The aim of the present investigation was to assess the effect of adaptation to stress, first, on the resistance of DNA of heart cells to a known damaging factor, namely single-stranded exogenous DNA and, second, on the concentration of hsp70 in cardiomyocyte nucleoplasm.

EXPERIMENTAL METHOD

Male Wistar rats weighing 200-250 g were adapted to stress-inducing immobilization by the scheme described by the writers previously [9]. Cardiomyocytes were isolated by the usual method [6], using type I collagenase ("Sigma"). The cells were next treated with 0.1% Triton X-100, causing lysis of the cytoplasmic membrane. Under these circumstances the nuclei remained stable. The suspension of nuclei was incubated for 15 min at 37°C with or without the addition of exogenous single-stranded DNA. After incubation the nuclei were fixed with 0.5% glutaraldehyde and stained with the DNA-binding dye ethidium bromide (10 μg/ml). The cytofluorometric analysis of DNA was carried out on a laser flow fluorometer, by the usual method [15]. The hsp concentration was determined in the nucleoplasm of heart cells of control and adapted animals. To obtain nuclei, hypotonic shock and treatment with Triton X-100 were used. To obtain nucleo-

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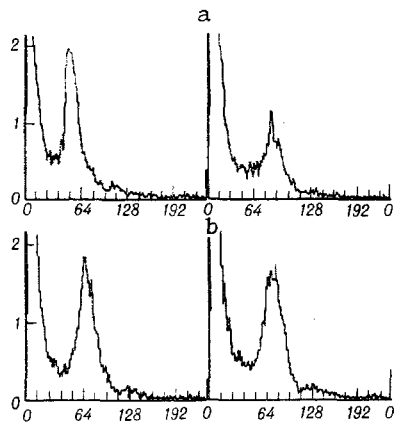

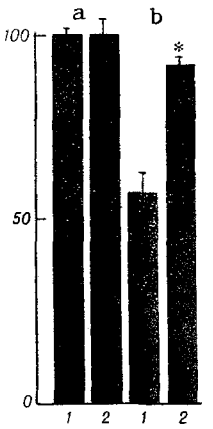

Fig. 1 Fig. 2

Fig. 1. Effect of adaptation to stress on resistance of nuclear DNA of heart cells to damaging action of single-stranded exogenous DNA. a) histograms of distribution of nuclear DNA in control; b) the same after adaptation. Abscissa, intensity of fluorescence of DNA-bound dye (in relative units — analyzer channels); ordinate, number of nuclei (in thousands). Nuclei containing normal and diploid set of DNA within the range from the 48th to the 112th channel. On left of arrow — histograms of nuclear suspensions in control and with adaptation, without the addition of single-stranded DNA; on right — histograms of nuclei after addition of single-stranded DNA in a concentration of 50 μg/ml.

Fig. 2. Effect of adaptation to stress on resistance of nuclear DNA of heart cells to damaging action of single-stranded exogenous DNA. a) number of nuclei containing normal set of DNA without addition of single-stranded DNA; b) the same, after addition of single-stranded DNA in a concentration of 50 μg/ml. 1) Control, 2) Adaptation. Amplitude of peak of fluorescence of nuclei without addition of single-stranded DNA taken as 100% and corresponds to columns in Part a. *$p < 0.01$) significance of differences from control.

plasm the nuclear residue was lysed by triple resuspension and centrifugation (20,000*g*, 30 min) in 10 volumes of buffer containing 1 mM Tris-HCl, 2 mM EDTA, pH 7.4, and 1 mM PMSF [2]. The supernatants were pooled and used for two-way electrophoresis as in [12] with minor modifications. The tubes were prefocused for 20 min with a current of 0.5 mA to the tube. Isoelectric focusing was carried out with a voltage of 500 V for 18 h. Electrophoresis in the second direction was carried out in 10% polyacrylamide gel. The gels were stained with silver nitrate [7]. Molecular weight marker proteins were commercially pure proteins from "LKB," and "Pharmacia." As the standard for isoelectric focusing we used a preparation of carbamoylated carboanhydrase from the same firm.

The significance of differences of adaptation from the control was determined by Student's test.

EXPERIMENTAL RESULTS

Histograms recorded in one typical experiment are shown in Fig. 1. In the control, addition of single-stranded DNA to the nuclear suspension in a concentration of 50 μg/ml led to a significant decrease in amplitude of the fluorescence peak of nuclei with a normal DNA content. This indicated considerable destruction of nuclear DNA under the influence of exogenous single-stranded DNA. The bottom part of Fig. 1 shows the same experiment on a suspension of cell nuclei isolated from the heart of an adapted animal. Clearly virtually no destruction of DNA took place.

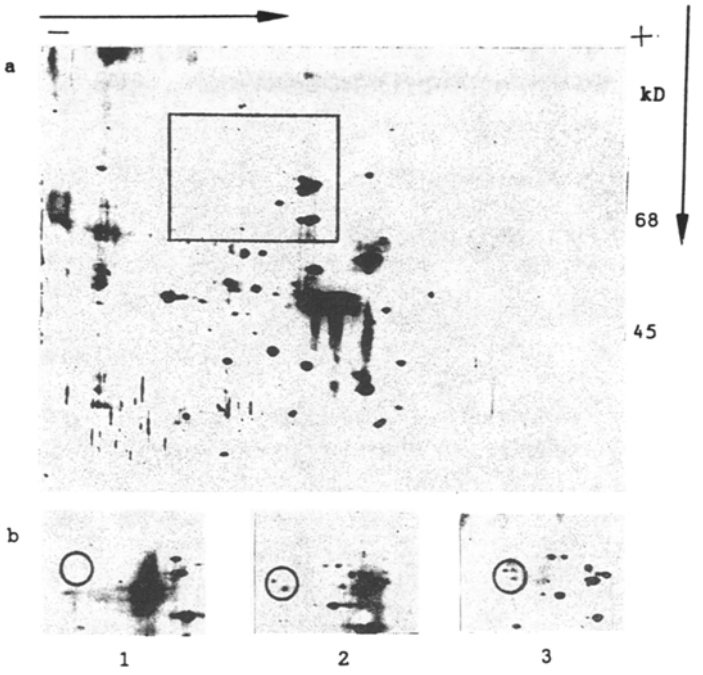

Fig. 3. Effect of adaptation to stress on heat shock protein accumulation in nucleoplasm of heart cells. a) Typical result of electrophoresis of nucleoplasmic proteins of rat heart cells. Horizontal arrow indicates direction of isoelectric focusing. Rectangle surrounds region in which hsp70 was located; b) fragment of gel corresponding to region indicated in Fig. 3a by rectangle. 1) Control, 2) Heat shock, 3) Adaptation to stress. Locations of hsp70 isoforms circled.

Dependence of DNA breakdown as a function of concentration of exogenous DNA is shown quantitatively in Fig. 2. With an increase in the concentration of single-stranded DNA from 0 to 50 μg/ml in the control, about 43% of the nuclei were degraded, compared with only 8% of nuclear DNA in the case of adaptation. Thus the nuclear DNA of the heart cells of animals adapted to short-term periodic stress is more resistant to the damaging action of exogenous single-stranded DNA.

When an attempt is made to explain this DNA-protective effect of adaptation it must be recalled that single-stranded DNA can activate nuclear proteases which specifically hydrolyse histone $H_1$60 [14], and thus increase the accessibility of particular regions of DNA for external agents and, in particular, for nucleases [1]. This may ultimately lead to degradation of DNA.

With this in mind, an attempt can be made to explain the protective effect of adaptation by accumulation of heat shock proteins. After exposure to stress the concentration of these proteins increases in both cytoplasm and nucleus [13]. It has also been shown that hsp70 can bind with damaged proteins [13] and protect them against the action of proteases. Taken together, these data suggest that hsp70 could play a definite role in the antiproteolytic mechanism of adaptive protection of nuclear DNA. However, the question whether hsp70 accumulate in heart cell nuclei during adaptation to stress has not yet been answered. Accordingly, we went on to analyze changes in the spectrum of nuclear proteins during adaptation to stress. The typical result of electrophoresis of the nuclear sap is shown in Fig. 3a. The nucleoplasm of the cardiac nuclei in the control, during heat shock, and during adaptation to stress contains similar protein fractions. In heat shock and adaptation to stress, however, at least two polypeptides appear in the 71-72 kD region (Fig. 3b, gels 2, 3), which are absent in the heart of control animals (Fig. 3b, gel 1). Depending on the results of coelectrophoresis with albumin and carbamoylated carboanhydrase, the isoelectric point of these polypeptides lies within the 6.2-6.4 region. On the basis of these parameters these proteins can be identified as an inducible form of hsp70 [13].

Accumulation of hsp70 of this kind was not observed after a single exposure to stress.

We are thus dealing with adaptive accumulation of this stress protein simultaneously with the development of ASSP. The adaptive stabilization of structures phenomenon is manifested not only in the cytoplasm, but also at DNA levels. An important role in the adaptive protection of DNA is probably played by hsp70 accumulation in the nucleoplasm. This suggests that methods of adaptive medicine under development at the present time may enable the pathological states to be corrected at the molecular-genetic level also.

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