ADAPTATION TO STRESS INCREASES THE RESISTANCE OF HEART CELL NUCLEI TO THE DAMAGING ACTION OF SINGLE-STRANDED EXOGENOUS DNA

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During the last decade it has been shown that in response to repeated exposures to short-term stress situations the body develops adaptation which not only increases its resistance to severe stress, but also possesses a broad spectrum of crossed protective effects, i.e., it protects the body against direct ischemic [3], chemical [15], cold [9], and even radiation-induced damage [4]. This adaptive 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. Thus it has been shown that the isolate heart of adapted animals possesses sharply increased resistance to reperfusion injuries [11], to high Ca^{2+} concentrations [5], and to toxic doses of catecholamines [2], whereas organelles isolated from it, namely elements of the sarcoplasmic reticulum and mitochondria — differ from the controls in their higher degree of resistance to autolysis [5]. This combination of phenomena has been described as the phenomenon of adaptive stabilization of structures (PASS) [6]. However, until recently one fundamental question remained open: is PASS realized only in cytoplasmic structures or does it develop also at the level of the genetic template (DNA). When this problem is studied it must be recalled that induction of nuclear proteases by single-stranded DNA regions can lead to irreversible damage to DNA [14]. It has been shown that such single-stranded DNA regions may arise as a result of free-radical injury to DNA during ischemia or severe stress [3].

The aim of this investigation was to assess the effect of preliminary adaptation of the body to stress on resistance of heart cell nuclei to the damaging action of exogenous single-stranded DNA.

EXPERIMENTAL METHOD

Male Wistar rats weighing 200-250 g were adapted to immobilization stress by the method described by the writers previously [5]. Cardiomyocytes were isolated by the usual method [7], using type I collagenase ("Sigma"). The cells were next treated with 0.1% Triton X-100, which caused lysis of the cytoplasmic membrane. The nuclei remained stable under these circumstances. The suspension of nuclei was incubated for 15 min at 37°C with or without the addition of exogenous single-stranded DNA. The single-stranded DNA was obtained by boiling a commercial preparation of double-stranded DNA from bovine spleen in water [10]. After incubation the nuclei were fixed with 0.5% glutaraldehyde and stained by the DNA-binding dye ethidium bromide (10 μg/ml). Cytofluorometric analysis of the DNA was carried out on a continuous flow laser fluorometer by the usual method [16]. Fluorescence of the dye was excited by a Spectra-Physica 2000 argon laser (USA) with a wavelength of 488 nm. Experimental programs were provided by IBM PC. By this method it is possible to record nuclei with the normal (diploid) DNA content in the form of a fluorescence peak, which with our technique lay between the 48th and 112th channel of the fluorescence intensity analyze (Fig. 1). Degradation of nuclear DNA by the action of single-stranded DNA was estimated correspondingly on the basis of the decrease in amplitude of this peak.

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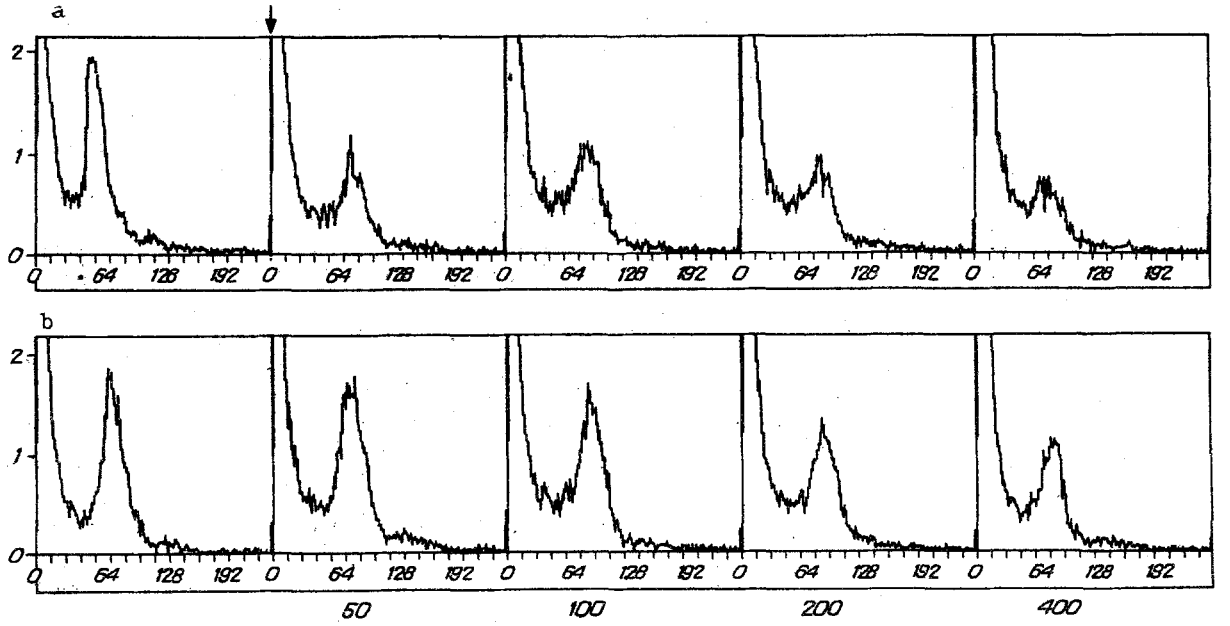

Fig. 1. Effect of adaptation to stress on resistance of nuclear DNA of heart cells to the damaging action of single-stranded exogenous DNA. a) Histograms of distribution of nuclear DNA in control, b) the same, after adaptation. Abscissa, intensity of fluorescence of DNA-bound dye (in relative units — analyzer channels); ordinate, number of nuclei (in thousands). Nuclei containing normal (diploid) set of DNA lie between 48th and 112th channels. Arrow indicates addition of single-stranded DNA. On left of arrow — histograms of nuclear suspensions in control and during adaptation without addition of single-stranded DNA; on right — histograms of nuclei after addition of single-stranded DNA in concentrations of 50, 100, 200, and 400 μg/ml (indicated below beneath histograms).

EXPERIMENTAL RESULTS

Histograms recorded in one typical experiment are shown in Fig. 1. Clearly in the control (Fig. 1a) addition of single-stranded DNA to the nuclear suspension in concentrations of 50, 100, 200, or 400 μg/ml led to a significant decrease in amplitude of the fluorescence peak of the nuclei with a normal DNA content. This indicates significant degradation of nuclear DNA by the action of exogenous single-stranded DNA.

Histograms recorded in the same experiment on nuclear suspensions from cells isolated from the heart of an adapted animal are shown in Fig. 1b. Degradation of DNA begins to be clearly manifested only with single-stranded DNA in a concentration of 200 μg/ml, whereas in the control marked DNA degradation took place with exogenous DNA in a concentration of only 50 μg/ml.

DNA degradation as a function of exogenous DNA concentration is shown quantitatively in Fig. 2. It is interesting to note that in the control, two different region can be distinguished on the curve of nuclear DNA degradation: 1) a region of rapid degradation of nuclear DNA corresponding to an increase in the concentration of single-stranded DNA from 0 to 50 μg/ml and disintegration of about 43% of the nuclei, and 2) a region of slow degradation, corresponding to an increase in the concentration of single-stranded DNA from 50 to 400 μg/ml and to disintegration of 24% of the nuclei. Thus in the second region, for every increase of the concentration of single-stranded DNA by 50 μg/ml there was an increase of 3-4% in the number of degraded nuclei, an order of magnitude less than in the first region.

The reason may probably be that two pools were present in the suspension of nuclei isolated from heart cells: the first — highly sensitive to the damaging action of single-stranded DNA and, judging by the number of degraded nuclei, this pool did not exceed 43% of the total number of nuclei isolated from the heart cells; the second pool — with low sensitivity to single-stranded DNA. Evidently the nuclei of these pools belong to different types of cells: the first pool was formed by nuclei of highly specialized muscle cells (cardiomyocytes), the second by nuclei of nonmuscular cells, such as fibroblasts, endothelial cells, and so on. This hypothesis is in agreement with two facts. First, we know that the number of cardio-

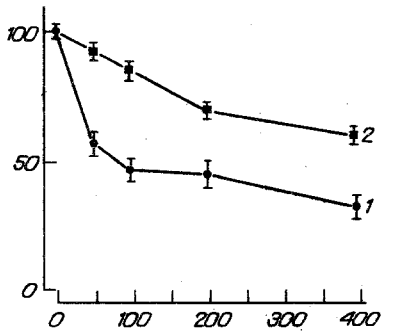

Fig. 2. Effect of adaptation to stress on resistance of nuclear DNA of heart cells to damaging action of single-stranded exogenous DNA. Abscissa, concentration of single-stranded DNA (in μg/ml); ordinate, amplitude of fluorescence peak of nuclei containing normal amount of DNA (in %). Amplitude of peak without addition of single-stranded DNA taken as 100%. 1) Control; 2) adaptation to stress.

myocyte nuclei does not exceed 20-30% of the total number of nuclei isolated from heart cells [12], and accordingly, the relative percentages of nuclei of the pools with high and low sensitivity were similar to the ratio of the number of cardiomyocyte nuclei to the number of nuclei from other heart cells. Second, it has been shown that phenomena of DNA degradation in monosuspensions of nonmuscular cells (lymphocytes or fibroblasts) do not begin to be observed until the concentration of single-stranded DNA is high — more than 600-800 μg/ml [8].

The main result of these experiments was that preliminary adaptation of the animal increased the resistance of the nuclear DNA to the damaging action of single-stranded exogenous DNA. Essentially, the protective effect of adaptation was realized mainly in relation to the easily damaged pool, conjecturally formed by cardiomyocyte nuclei. With an increase in exogenous DNA concentration from 0 to 50 μg/ml, only 8% of the nuclei DNA was degraded during adaptation compared with 43% in the control, whereas in the second region, with an increase in the exogenous DNA concentration from 50 to 400 μg/ml the increase in the number of degraded nuclei was the same as in the control — about 4% for every 50 μg/ml. Meanwhile, the amplitude of the fluorescence peak corresponding to normal nuclei was higher during adaptation over the whole range of damaging concentrations of single-stranded DNA. For instance, in concentrations of 50 and 100 μg/ml the number of undamaged nuclei during adaptation was almost 40% greater than in the control, whereas in concentrations of 200 and 400 μg/ml it was 22 and 28% higher respectively than in the control.

Thus the heart cell nuclei of animals adapted to short-term periodic stress are more resistant to the damaging action of exogenous single-stranded DNA.

If an explanation is to be given of this nuclear-protective and, more especially, DNA-protective effect of adaptation to stress, it will have to be recalled that single-stranded DNA can activate nuclear proteases specifically hydrolyzing histone H_i [14], and can thus increase the accessibility of certain regions of DNA for external agents, and for nucleases in particular [1]. This may ultimately lead to degradation of DNA. The results of special series of experiments in fact showed that, first, double-stranded exogenous DNA in a concentration of up to 400 μg/ml did not lead to degradation of nuclear DNA, and second, phenylmethylsulfonyl fluoride, an inhibitor of serine proteases, in a concentration of 2 mM, completely prevented degradation of nuclear DNA induced by the action of exogenous single-stranded DNA. This confirms the hypothesis of the specific protease-dependent character of damage to nuclear DNA and gives a possible explanation of the protective effect of adaptation by the action of so-called heat shock proteins (HSP). It is well known that after exposure to stress these proteins accumulate both in the cytoplasm and in the nucleus. It has also been shown that HSP are able to bind with damaged proteins [13], and to screen them from the action of proteases. These results as a whole suggest that HSP may play a definite role in the antiproteolytic mechanism of adaptive protection of nuclear DNA. However, this hypothesis requires experimental verification.

PASS is thus manifested not only in the cytoplasm, but also at the DNA level. This suggests that methods of adaptive medicine being developed at the present time will enable pathological states to be corrected at the molecular-genetic level also.

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