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INJURY AND REPAIR OF HEART MUSCLE DNA IN EMOTIONAL-PAIN-INDUCED STRESS

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In emotional-pain-induced stress (EPS) high catecholamine concentrations cause activation of lipid peroxidation in the myocardium and labilization of lysosomal enzymes, which damage the membranes of the myocardial cells [1]. It is possible that lysosomal enzymes and lipid hydroperoxides forming active free radicals injure not only membranes, but also DNA.

The object of this investigation was to study the state of polymerization of single DNA strands and the reparative synthesis of DNA in the myocardium of rats exposed to EPS.

EXPERIMENTAL METHOD

Male Wistar rats weighing 180-200 g were used. The animals were exposed for 6 h to EPS [4] and then decapitated at various times after the end of exposure. Control animals were killed at the same time. A group of five animals was used to isolate each preparation of nuclei [10] and DNA [8].

For sedimentation analysis, a suspension of nuclei in solution containing 0.1 M NaCl, 0.01 M EDTA, and 0.01 M Tris-HCl buffer, pH 7.4 ($4 \cdot 10^6$ nuclei in 1 ml) was applied in a volume of 1 ml to an equal volume of 1 N NaOH in a 36-ml centrifuge tube and allowed to stand for 12 h at 20°C. A linear (5-20%) sucrose gradient containing 0.2 N NaOH was poured in a volume of 34 ml into the bottom of the centrifuge tubes, starting with a low concentration. The nuclear lysate under these circumstances was displaced from the top of the gradient. The gradients were centrifuged in the SW-27 rotor of the Beckman L-65 centrifuge for 5 h at 25,000 rpm. Fractions, each of 2.4 ml, collected from the bottom of the centrifuge tube, were analyzed in UV light at 260 nm against the corresponding control solutions, which is a modification of the method in [9]. DNA of animal cells are known to contain alkaline-labile bonds, as a result of rupture of which stable DNA subunits are formed [7]. At the same time, deproteinization of the DNA and dissociation of the DNA-membrane complex take place [5], as confirmed by spectral data (Fig. 1), which point to liberation of DNA in the course of centrifugation from the other nuclear components remaining on the top of the gradient (fraction 15). During work with nondividing cells and tissues with a low mitotic index, incorporating radioactive precursors of DNA only weakly, sedimentation constants and molecular weight of the DNA can be determined by extrapolation to zero dilution, because of overloading of the gradient that is unavoidable even when a sensitive fluorescent label [6] is used. For this reason, in the present investigation the analysis was limited to qualitative evaluation of the change in the state of polymerization of the DNA, just as is usually done.

Reparative synthesis of DNA was assessed *in vivo* and *in vitro*. To determine reparative synthesis of DNA *in vivo* the animals were given an intraperitoneal injection of hydroxyurea

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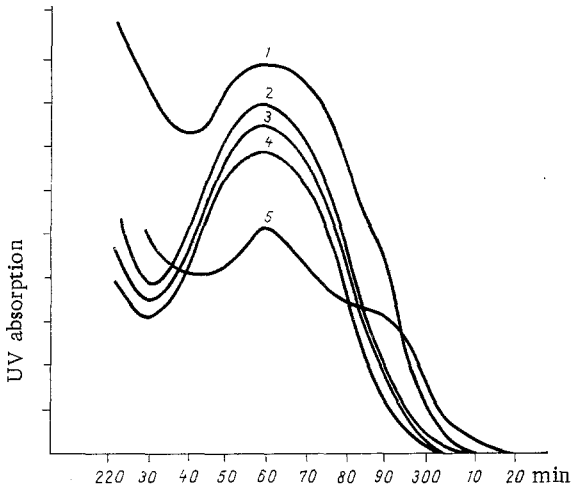

Fig. 1

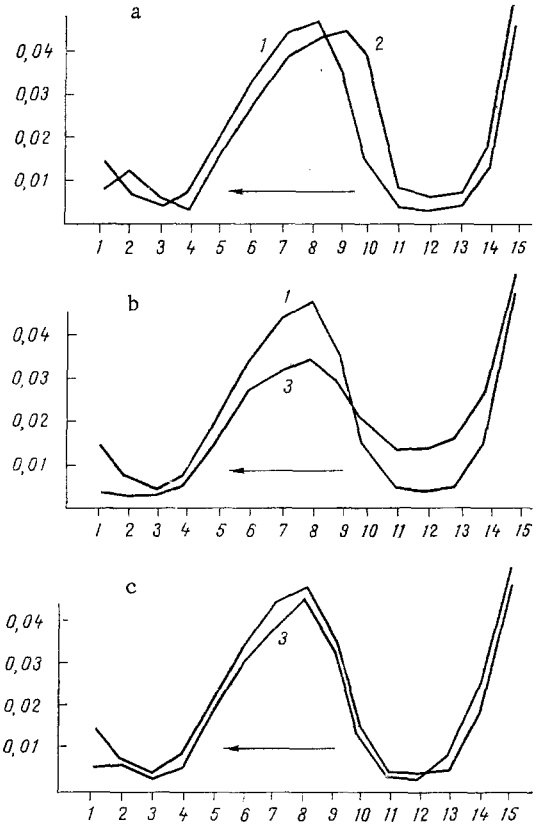

Fig. 2

Fig. 1. Absorption spectra of lysate of rat nuclei and contents of various fractions of gradients compared with spectrum of pure DNA (RNA content under 2%, protein under 1%) isolated from rat heart by Marmur's method [8]. 1) Nuclear lysate, 2) DNA, 3) fractions 5-9, 4) fractions 10-13 (2 h after stress), 5) fraction 15. Abscissa, wavelength; ordinate, UV absorption.

Fig. 2. Character of sedimentation of lysates of nuclei isolated from rat myocardium immediately after EPS (a), and 2 h (b) and 24 h (c) after EPS. 1) Control (peak corresponds to fraction 8 with probability of 95%), 2) nuclei isolated immediately after end of stress (peak corresponds to fraction 9 with probability of 70%), 3) nuclei isolated 2 h after end of stress (peak corresponds to fraction 8 with probability of 70%, total absorption of fractions 5-9 15% less than in the control with probability of 95%); 4) nuclei isolated 24 h after end of stress (peak corresponds to fraction 8 with probability of 70%). To obtain control sedimentation profiles 12 independent preparations of nuclei were used. Experimental sedimentation profiles plotted from data for three independent preparations for each variant. For each nuclear preparation 2 or 3 sedimentation profiles were obtained. Arrow indicates direction of sedimentation. Abscissa, Nos. of fractions; ordinate, UV absorption at 260 nm.

(500 mg/kg body weight), which inhibits semiconservative DNA synthesis but does not affect reparative DNA synthesis, and this was followed by injection of ^{3}H-thymidine (100 μCi/100 g body weight) [3]. A second injection of hydroxyurea was given after 1 h, and another hour later the animals were killed and DNA isolated.

To estimate reparative DNA synthesis *in vitro* incorporation of ^{3}H-thymidine triphosphate into DNA of heart muscle cell nuclei was determined by means of a system in which intensive incorporation of ^{3}H-thymidine triphosphate took place into DNA of hepatocyte nuclei in the course of reparative synthesis [11], in the absence of any appreciable replicative DNA synthesis [12], as was confirmed under our own experimental conditions also when myocardial nuclei were used, to judge from the absence of incorporation of ^{3}H-thymidine triphosphate in the same medium containing 2 mM hydroxyurea.

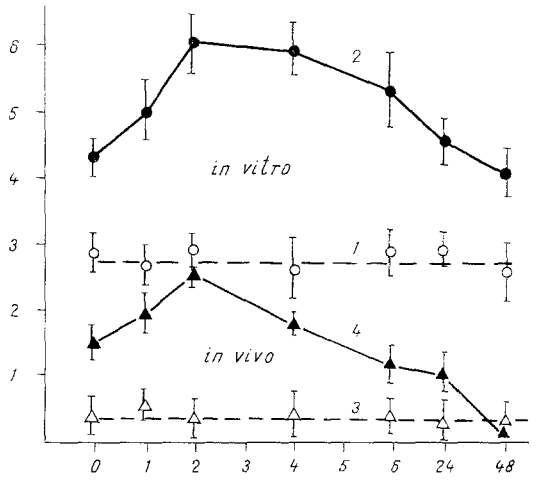

Fig. 3. Reparative synthesis of myocardial DNA induced in rats by EPS. 1) Control level of incorporation of ^{3}H-thymidine triphosphate into nuclear DNA *in vitro*, 2) incorporation of ^{3}H-thymidine triphosphate into nuclear DNA of animals taking part in experiment at specified time intervals after end of EPS. Time of isolation of nuclei 3 h (0°C). Nuclei used in DNA-polymerase system immediately after isolation; 3) control level of incorporation of ^{3}H-thymidine into rat myocardial DNA *in vivo*, 4) incorporation of ^{3}H-thymidine into rat myocardial DNA *in vivo* in animals receiving hydroxyurea at various time intervals after end of EPS. Time from injection of ^{3}H-thymidine to sacrifice 2 h. Degree of variation of cpm/mg DNA × 10^{-2} shown as standard deviation. Abscissa, time after EPS (in h); ordinate, radioactivity (in cpm/mg DNA × 10^{-2}).

Acid-insoluble residue obtained in the *in vitro* system, and the alcoholic precipitate of DNA obtained in investigations *in vivo*, were collected on Millipore HAWP 0.45 μ membrane filters, and radioactivity was counted with the aid of toluene scintillator in an Intertechnique (France) counter for 10 min. DNA was determined with diphenylamine [2].

EXPERIMENTAL RESULTS

Under the influence of EPS partial depolymerization of the myocardial DNA took place. Immediately after EPS (Fig. 2a) this was reflected in the fact that the peak of the sedimentation profile, which in the control coincided with fraction 8, was shifted to fraction 9, i.e., into the region of lower molecular weights. The most likely cause of this decrease in the degree of polymerization was the appearance of single-stranded DNA breaks.

It will be clear from Fig. 2b that 2 h after EPS the DNA sedimentation profile was substantially changed. The peak of the profile was back in the same position as in the control, but meanwhile an appreciable quantity of low-molecular-weight DNA fragments appeared in fractions 11-14. One of the possible mechanisms of this trend is that in most heart muscle cells which continued to function after exposure to stress, single-stranded DNA breaks were being rapidly repaired. Meanwhile, in cells located in micronecrotic foci, developing during stress [1], nuclease degradation of DNA was in progress, and as a result of alkaline denaturation of the DNA this led to the formation of low-molecular-weight single-stranded fragments.

As Fig. 2c shows, 24 h after EPS the sedimentation profile of DNA in the experimental group was indistinguishable from the control, and this can most likely be explained by repair of DNA in the living cells and elimination of DNA breakdown products in dying cells. This hypothesis was confirmed by the results of an investigation of reparative DNA synthesis in the myocardium obtained in experiments *in vivo* and *in vitro* on nuclear preparations, shown in Fig. 3.

Reparative DNA synthesis *in vivo* was virtually absent in the control but was found immediately after EPS; 2 h after EPS it reached a maximum and it remained at a certain level at least for 24 h. The same result in principle was obtained in the system *in vitro*: DNA synthesis in nuclei isolated from the heart of animals exposed to stress reached a maximum after 2 h and remained high for at least 2 days.

Incorporation of radioactive precursors in the experiments *in vitro* was considerably higher than *in vivo*. The difference can be attributed to the presence of injured DNA in the isolated nuclei — products of nuclease activity during isolation of the nuclei. An appreciable level of incorporation of ^{3}H-thymidine triphosphate, even 48 h after EPS, in the absence of incorporation of ^{3}H-thymidine at this point *in vivo* can be explained on the grounds that activity of the DNA-repair system is maintained for a certain length of time after actual cessation of repair of DNA injuries induced by EPS. On the whole the results are evidence that under the influence of EPS injuries arise in the heart muscle simultaneously with activation of free-radical processes and repair of the DNA chain is activated in the same way as happens under the influence of ionizing radiation, carcinogens, and mutagens.

It thus seems probable that repeated cycles of DNA injury—repair, taking place frequently under the influence of stress situations in the course of individual life, can lead to disturbances of the DNA nucleotide sequence, and this cumulation of errors of the genetic code may perhaps be one cause of aging.

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