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REGULATION OF SUPEROXIDE DISMUTASE ACTIVITY DURING DEEP HYPOTHERMIA BY SIMULTANEOUS ADMINISTRATION OF WATER- AND LIPID-SOLUBLE ANTIOXIDANTS

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In connection with the use of an assisted circulation (AC) in contemporary heart surgery deep hypothermia (DH) of the myocardium is frequently carried out. The use of DH enables the vessels of the heart to be occluded for a comparatively long time during an operation. DH reduces the consumption of oxygen and the energy reserves, but it also disturbs the coordination of metabolism [7, 8, 10]. The leading causes of this are increased secretion of catecholamines during hypothermia [5] and subsequent activation of free radical generation and of lipid peroxidation [10]. Restoration of perfusion potentiates the action of these aggressive factors, and this may be the cause of unfavorable results of operations on the heart. Protection of the heart under both normal and extremal conditions is effected by enzymic and nonenzymic antioxidative systems. Under conditions of DH the heart is protected by maintenance of activity predominantly of superoxide dismutase (SOD) [1-3, 9].

In this investigation the action of panthetin and α-tocopherol was studied on activity of free radical generation during DH in the myocardium and blood.

EXPERIMENTAL METHOD

SOD activity and secretion of adrenalin and noradrenalin were investigated during cooling and long-term DH lasting from 30 to 60 min, at intervals of 10 min. The work was done on male Wistar rats weighing 250-270 g. The animals

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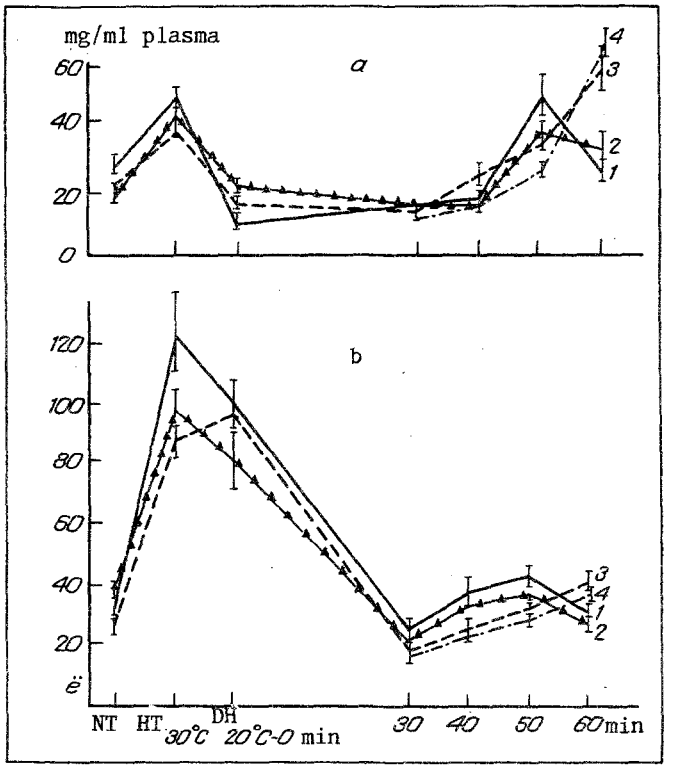

Fig. 1. Plasma levels of adrenalin (a) and noradrenalin (b). 1) Control; 2) α-tocopherol; 3) panthetin; 4) α-tocopherol + panthetin. Abscissa (I-VII) stages of experiment (explanation in text); ordinate, catecholamine concentration (in mg/ml plasma). NT) Normothermia, HT) hypothermia, DH) deep hypothermia.

were kept on a synthetic diet for the week before the experiment, The rats were divided into four groups: 1) control, 2) rats protected by α-tocopherol, 3) protected by panthetin, and 4) animals with combined protection (half the dose of α-tocopherol + half the dose of panthetin). The experiments were carried out in seven stages: I) normothermia; II) cooling to 30°C; III) cooling to 20°C; IV) maintenance of the animal for 30 min during DH; V) for 40 min; VI) for 50 min, and VII) for 60 min All the animals were anesthetized 30 min before cooling by intraperitoneal injection of a mixture of thiopental sodium, 20% hydroxybutyrate solution, 0.1% atropine solution, and physiological saline in the ratio of 1:1.6:0.4:1. The anesthetic mixture was injected in a dose of 0.5 ml/100 g body weight. An additional injection of morphine in a dose of 0.5 mg/100 g body weight was given. The animals were cooled by the immersion method at the rate of 0.67 degC/min. Panthetin (from Daiichi, Japan) was injected in a dose of 50 mg/kg 24 h before the experiment began, and α-tocopherol (in the form of the dipotassium salt of D,L,α-tocopheryl phosphate) was injected in a dose of 30 mg/kg 48 and 24 h before the experiment began. The antioxidants were given in combination: α-tocopherol in a dose of 15 mg/kg 48 h and 24 h before, and panthetin in a dose of 25 mg/kg 24 h before the beginning of the experiment.

Blood samples after decapitation of the animals were collected in cold test tubes with heparin and kept in the cold. The myocardium was homogenized with 1.15% KCl 3 times, for 20 sec each time, with an interval of 30 sec; for determination of SOD, the hemoglobin of the erythrocytes and myoglobin of the tissue were precipitated with a mixture of alcohol and chloroform in the ratio of 25:15 on an alcohol—acetone bath at a temperature of between —25°C and —20°C (the low temperatures were produced by the addition of liquid nitrogen).

Catecholamines (free and bound with plasma proteins) were determined by the method in [4] on a UT-7110 fluorometer at 365-515 nm for adrenalin and 405-515 nm for noradrenalin. SOD activity was determined on a "Specord M 40" spectrofluorometer (East Germany) by the method in [11], α-tocopherol by the method in [6], and malonic dialdehyde (MDH) as in [12]. Adrenalin came from "Fluka" (Switzerland), noradrenalin from "Hoechst" and NADH from "Boehringer" (West Germany), nitro-BT and phenazine metasulfate from "Sigma" (USA), and other reagents from "Reanal" (Hungary).

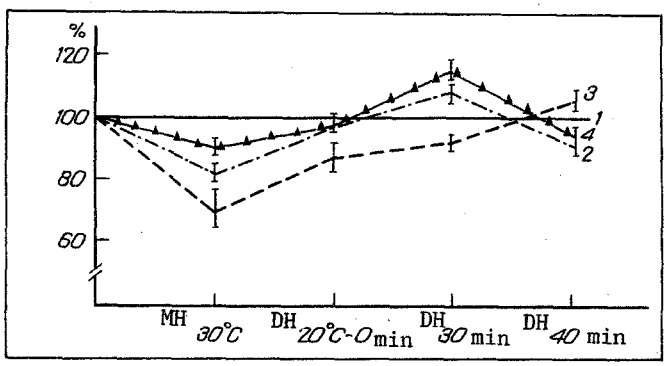

Fig. 2. Changes in SOD activity and concentrations of α-tocopherol and MDA during cooling and DH in the myocardium. 1) Control; 2) α-tocopherol; 3) MDA; 4) SOD. Abscissa, stages of experiment; ordinate, changes in SOD activity and α-tocopherol and MDA concentrations (in % of control). MH) Moderate hypothermia.

Statistical analysis of the data and correlation analysis were carried out by the usual methods. Altogether 260 animals were used.

EXPERIMENTAL RESULTS

A study of the action of cooling of the animals to a temperature of moderate hypothermia (stage II) showed marked activation of the adrenergic system. Secretion of adrenalin was increased by 2.5 times and of noradrenalin by 4 times (Fig. 1). These compounds penetrate the myocardium instantly, and as a result metabolism is sharply activated: the ability of the myocardium to take up oxygen is increased, β-oxidation of fatty acids and CoA formation are activated, and oxidation of energy yielding substrates in the Krebs' cycle is increased [13].

The results of the study of the myocardial antioxidative system showed a decrease in the α-tocopherol concentration and of SOD activity in the myocardium as the temperature fell to 30°C. The MDA concentration fell simultaneously (Fig. 2). This synchronized change of the two parameters is due to the fact that at this stage the hydroperoxides formed are used as energy substrates, as the writers showed previously [14].

As the temperature continued to fall to 20°C the adrenalin concentration returned to values close to those of normothermia, but noradrenalin secretion remained high. During this period the α-tocopherol concentration and SOD activity in the myocardium rose (Fig. 2). However, the preservation of a high noradrenalin concentration, oxidized when present in excess into a semiquinone, generating the superoxide radical and activating LPO, at this stage of DH is evidently the cause of the increased MDA concentration in the heart muscle (Fig. 2).

Superoxide radical generation is inhibited by SOD. The enzyme molecule consists of two identical subunits, each of which has a disulfide bridge within the protein chain, and 1 SH-group. The free sulfhydryl group, which is a hydrogen donor, can directly reduce oxygen radicals and (or) intermediate products of their metabolism [10]. The disulfide group, when reduced, is the source of two active SH-groups. This protective antiradical system in the myocardium preserves high activity during 30 min of exposure to DH (Fig. 2). After this period, exposure to DH again increased activity of the adrenergic system, the α-tocopherol concentration in the myocardium fell, SOD activity decreased, and the MDA concentration rose.

In order to identify substances preserving the activity of the myocardial antioxidative system during long-term DH, we tested the action of panthetin, of the dipotassium salt of D,L-α-tocopheryl phosphate, and of a combination of both. The dipotassium salt of D,L-α-tocopheryl phosphate before cooling had no effect on SOD activity either during initial cooling or during long-term DH (Table 1).

Panthetin (N-pantothenyl-β-aminoethyl disulfide) increased SOD activity during normothermia and during cooling from 30 to 20°C in the myocardium. The combined administration of these compounds during long-term DH increased myocardial SOD activity by 1.5 times (Table 1), probably due to restoration of the active SH-groups of the enzyme.

TABLE 1. Action of Dipotassium Salt of D,L-α-Tocopheryl Phosphate (a) and Panthetin (b) on SOD Activity in Myocardium during Long-Term DH (M ± m)

Experimental conditions	SOD activity, conventional units/mg protein			
	control	a	b	a + b
Normothermia	11,49±1,05	12,98±1,68	16,12±1,04	—
DH, min				
0	10,90±1,07	11,76±1,02	13,10±0,92	—
30	13,88±1,27	13,88±0,8	8,77±1,0	10,86±0,49
40	10,98±0,72	9,80±1,05	12,04±1,16	9,34±0,78
50	10,52±0,99	9,34±1,04	7,51±0,62	12,98±0,33
60	12,34±1,37	12,05±1,25	11,36±0,77	18,86±1,06

Legend. n = 12 In all groups.

Consequently, panthetin exerts its restorative action on SOD activity when membrane permeability is unchanged, under conditions of normothermia and moderate hypothermia To maintain SOD activity during long-term deep hypothermia, when permeability of the myocyte membranes is considerably increased [14], panthetin should be used simultaneously with an antioxidant, stabilizing the myocyte membrane.

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