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STABILIZING ACTION OF α-TOCOPHEROL IN POSTISCHEMIC DAMAGE TO THE MEMBRANE HYDROXYLATING SYSTEM OF THE ENDOPLASMIC RETICULUM OF RAT LIVER

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KEY WORDS: ischemia of the liver; hydroxylation of xenobiotics; microsomes; lipid peroxidation; α-tocopherol.

One cause of injury to membrane structures and cell enzyme systems connected with them during the period of reoxygenation of organs after ischemia may be activation of free-radical lipid peroxidation [2].

Accordingly, in the investigation described below, the effect of the antioxidant α-tocopherol on activity of the hydroxylating system of enzymes in the endoplasmic reticulum of the liver was studied in the postischemic period.

EXPERIMENT METHOD

Experiments were carried out on 80 noninbred male albino rats weighing 180-210 g, kept on the standard animal house diet. α-Tocopherol was injected into the stomach of the animals of one group in the form of an oily emulsion in a dose of 50 mg/kg every 12 h for 48 h before and 3 days after ischemia of the liver. The dose, therapeutic form, and interval between injections were chosen on the basis of data on its optimal action as antioxidant in the corresponding doses [15], its better resorption from the intestine in emulsion form [10], and its maximal tissue level 12 h after peroral administration [5].

Rats of the other group also received the oily emulsion for the same period of time, but without α-tocopherol.

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TABLE 1. Effect of Ischemia of Rat Liver on Activity of Xenobiotic-Metabolizing Endoplasmic Reticulum Membrane Enzymes of Hepatocytes and Protective Action of α-Tocopherol (M ± m)

Parameter	Control	Ischemia	Control	Ischemia + α-tocopherol
Amidopyrine N-demethylation, nmoles HCHO/sec	76,5±4,33	45,0±3,82 (58,8)*	74,3±2,33	69,8±2,67 (93,9)
Aniline-p-hydroxylation, nmoles aminophenol/sec	10,3±1,12	8,0±0,45 (77,4)*	11,0±0,82	11,5±0,6 (104,9)
Cytochrome P-450, μmoles	0,81±0,1	0,33±0,04 (40,7)*	0,88±0,04	0,69±0,05 (78,4)*
Cytochrome b_5, μmoles	0,46±0,047	0,40±0,04 (86,7)	0,39±0,02	0,35±0,01 (89,7)
NADPH-ferricyanide reductase, μmoles/sec	3,7±0,32	3,65±0,17 (98,6)	3,49±0,23	3,07±0,23 (87,9)
NADPH-neotetrazolium reductase, μmoles/sec	0,37±0,04	0,25±0,02 (67,6)*	0,38±0,021	0,36±0,02 (94,7)
NADPH oxidase, nmoles/sec	109,8±6,33	55,7±4,83 (50,7)*	126,7±13,6	147,7±9,21 (116,6)*

Legend. Figures in parentheses indicate percent of control. *P < 0.05 compared with control. All parameters expressed per milligram microsomal protein.

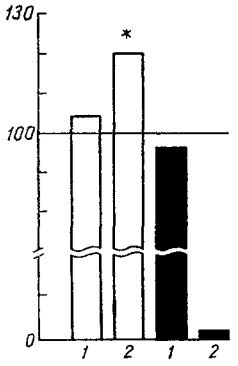

Fig. 1. Rate of MDA formation in rat liver microsomes 72 h after ischemia for 40 min (unshaded columns) and after preliminary injection α-tocopherol (black columns). 1 and 2) NADPH-dependent and ascorbate-dependent LPO, respectively. *P < 0.05. Ordinate, rate of MDA formation (in percent of control).

Ischemia of the liver was produced in both groups of rats under ether anesthesia by applying a small clamp to the vascular pedicle of the left lateral and middle lobes of the liver for 40 min. The animals were decapitated 72 h after resumption of the circulation. The liver was perfused through the inferior vena cava with cold 1.15% KCl solution [1]. The liver tissue was minced and homogenized in the same solution in the ratio 1:3. Microsomes were sedimented from the postmitochondrial fraction on a VAC-602 centrifuge (East Germany) at 105,000g for 1 h. All procedures were conducted at 4°C. The rate of N-demethylation of amidopyrine (substrate for type I hydroxylation) [12] and p-hydroxylation of aniline (type II substrate) [9], the content of cytochromes P-450 and b_5 [13], activity of NADPH-oxidase [8], the fraction of NADPH-specific flavoprotein (NADPH-ferricytochrome c oxidoreductase), and the middle component of the NADPH-specific electron-transport chain, estimated by NADPH-ferricyanide-potassium- and neotetrazolium-reductase reactions respectively [6, 14], were determined in microsomes resuspended in 100 mM Tris-HCl buffer (pH 7.4). The content of cytochrome and oxidoreductase activity in preparations of the microsomes were recorded on a Spe-

cord UV-Vis spectrophotometer (East Germany). The protein concentration in the microsomes was determined by Lowry's method [11].

Lipid peroxidation (LPO) activity was determined from the quantity of malonic dialdehyde (MDA) formed [2].

The control for each group of animals consisted of rats undergoing mock operations (laparotomy under general anesthesia).

EXPERIMENTAL RESULTS

The rate of N-demethylation of amidopyrine and of p-hydroxylation of aniline, activity of NADPH-neotetrazolium reductase and NADPH-oxidase, and the content of cytochrome P-450 in membranes of the endoplasmic reticulum of the hepatocytes 72 h after ischemia of the liver for 40 min in untreated rats were lowered by 41.2, 22.6, 32.4, 49.3, and 59.3%, respectively (Table 1). There was no significant change in the cytochrome b_5 level or NADPH-ferricyanide reductase activity.

Injection of α-tocopherol prevented the above-mentioned disturbances of xenobiotic metabolism and inhibition of NADPH-neotetrazolium reductase and NADPH-oxidase reactions (Table 1). The cytochrome P-450 content remained twice as high as in the untreated ischemic rats, although it did not reach the control level (animals undergoing the mock operation).

After 72 h of the postischemic period the rate of ascorbate-dependent LPO of the endoplasmic reticulum membranes in the untreated rats was 20% higher than the control value, but in the treated animals the ascorbate-dependent peroxidation reaction was depressed to such a degree that it was almost undetectable (Fig. 1). No significant changes were found in activity of NADPH-dependent peroxidation by this time compared with the control.

The results suggest an important role of disturbance of LPO specificity in postischemic repression of the hydroxylating system of the endoplasmic reticulum of the liver.

Activation of LPO is known [3] to lead to damage to the hydrophobic region of biomembranes and, in particular, to hydrolysis of phospholipids and, consequently, to disturbance of the function of membrane-bound enzymes. The possibility cannot be ruled out that endogenous phospholipases may play a role in the postischemic destruction of membranes of the endoplasmic reticulum, for a connection has been found between LPO and phospholipase damage to biomembranes [4].

On the basis of recent data in the literature it is difficult to explain unambiguously the protective action of α-tocopherol. Most probably this effect may be linked not only with the antioxidant action of the compound, which, in particular, is responsible for blocking peroxide radicals and lowering the LPO level, but also with its membrane-stablizing properties. It has been claimed that stabilization of biomembranes through the action of α-tocopherol takes place as a result of its binding with sulfide-containing proteins and polyunsaturated phospholipids [7]. This leads to inhibition of oxidative destruction of unsaturated fatty acids, prevention of hydrolysis of phospholipids by endogenous phospholipases, and to a decrease in membrane permeability.

Preliminary injection of α-tocopherol thus prevents postischemic repression of enzymes of the hydroxylating system and activation of LPO in membranes of the endoplasmic reticulum of rat liver. The protective effect is evidently due to its antioxidant and membrane-stabilizing properties.

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ROLE OF EMOTIONAL STRESS IN DISTURBANCES OF CARBOHYDRATE TOLERANCE

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One of the tests used to determine the state of carbohydrate metabolism is the glucose tolerance test; even if the initial blood sugar level is normal, this test can reveal latent periods of disturbances of carbohydrate metabolism. It is widely used in clinical practice for the diagnosis of diencephalic pathology [4, 5, 7] and also of latent diabetes [1]. Despite evidence that one cause of the transition of latent diabetes in adults and children into its manifest form is psychic trauma [1, 2], we have been unable to find any systematic experimental investigations demonstrating the role of strong or prolonged emotional strain as a primary factor in the onset of lasting disturbances of carbohydrate metabolism. Meanwhile such information would be important when studying the pathogenesis not only of diabetes, but also of ischemic heart disease, for we know that a disturbance of carbohydrate tolerance is one of the risk factors in the development of this disease, especially when combined with hypercholesterolemia and arterial hypertension [9, 10, 15, 16].

The aim of this investigation was to determine whether strong and prolonged emotional strain of negative character can effect changes in the blood sugar level and glucose tolerance. A model of "collisions" between food and nociceptive stimulation, and also stimulation of negative emotiogenic zones of the hypothalamus, were used for this purpose.

EXPERIMENTAL METHOD

Two series of experiments were carried out on 21 rabbits weighing 2.5-3.5 kg. The experiments of series I were performed on 10 animals — five experimental and five control. For 4 months twice a day the experimental rabbits were subjected to "collisions" between food and nociceptive stimuli. All the rabbits were accustomed to receive carrot juice daily from a small (5 ml) syringe, the end of which was connected to a rubber tube introduced painlessly into the mouth between the teeth. After a certain period during which the rabbits became accustomed to this procedure, twice or three times a week a "collision" was created between food and nociceptive stimulation: Simultaneously with receiving carrot juice, the rabbit also received an electric shock (20 V, 60 Hz, 0.5 msec) from an ÉSA-2 stimulator through a wire running alongside the rubber tube. Control rabbits continued to receive only carrot juice as before during this period.

After 1 year the experimental and control rabbits in this series of experiments were subjected to a glucose tolerance test in a dose corresponding to that used in clinical practice. Glucose solution (40%) was injected slowly in a volume of 2 ml/kg body weight into the marginal vein of the ear of the fasting rabbits. Blood samples were taken before and 15, 30, 60, and 120 min after injection of glucose.

In the experiments of series II glucose tolerance was tested after relatively prolonged (10 days) chronic emotional stress caused by stimulation of the negative emotiogenic zones of the hypothalamus, into which bipolar nichrome electrodes were implanted in accordance with

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