impairment of the results of surgical treatment. The increase in the transcapillary flow of albumin in the early postoperative period, as it must also be pointed out, is evidently connected with hemodilution during the period of artificial circulation.

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ROLE OF LIPID PEROXIDATION IN REGULATION OF LIVER MICROSOMAL MONO-OXYGENASE ACTIVITY OF HOMOIOTHERMIC ANIMALS EXPOSED TO COLD

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The study of the specific manifestations of long-term cold stress has shown that the pattern of function of the microsomal mono-oxygenase system, responsible not only for metabolic transformation of xenobiotics (drugs, toxins), but also for synthesis and oxidation of certain important endogenous biologically active compounds (steroid hormones, catecholamines, protaglandins), is the least studied component in the formation of the general adaptation syndrome. In investigations devoted to this problem no attempt has been made to analyze the mechanisms of regulation of the liver mono-oxygenase activity in the adapted organism [8]. Yet we know that an inseparable component against whose background adaptive reactions take place is activation of lipid peroxidation (LPO) in different organs and tissues [1, 3, 5].

The object of this investigation was to study the character of the mutual influence of two processes coupled in the system of microsomal oxidation, namely LPO and biotransformation of xenobiotics, during prolonged exposure to severe cold.

EXPERIMENTAL METHOD

Experiments were carried out on 40 male Wistar rats weighing 150-200 g. The animals were exposed to continuous cooling (excluding during meals) in a thermal chamber at −7°C for eight days. Liver microsomes were isolated by differential centrifugation. Aniline, 3,4-benzypyrene, and aminopyrine were used as substrates for microsomal mono-oxygenases. The rate of p-hydroxylation of aniline and of N-demethylation of aminopyrine [6] and the maximal velocity of the 3,4-benzypyrene-hydroxylase reaction [13] in a modification of the

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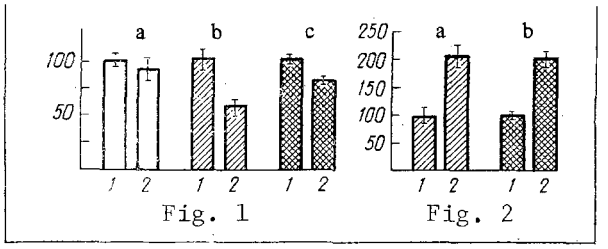

Fig. 1 Fig. 2

Fig. 1. Velocity of N-demthylation of aminopyrine and of hydroxylation of aniline and 3,4-benzapyrene in liver microsomes of control (1) and experimental (2) rats. a) Velocity of N-demethylation of aminopyrine (control 8.1 ± 0.51 nmoles formaldehyde/min/mg); b) velocity of p-hydroxylation of aniline (control 0.41 ± 0.04 nmole p-aminophenol/min/mg); c) velocity of hydroxylation of 3,4-benzypyrene (control 145.0 ± 7.4 nmoles of 3-hydroxybenzypurene/min/mg). Ordinate, reaction velocity (in percent of control).

Fig. 2. Activity of NADPH- and ascorbate-dependent LPO in liver microsomes of control (1) and experimental (2) rats. a) Activity of NADPH-dependent LPO (control 1.13 ± 0.17 nmole/min/mg; b) activity of ascorbate-dependent LPO (control 2.96 ± 0.24 nmoles MDA/min/mg). Ordinate, LPO activity (in percent of control).

method in [7], and the concentration of cytochrome P-450 in the microsomal fraction [12] were determined. NADPH- and ascorbate-dependent LPO of the microsomal membranes were studied by measuring accumulation of malonic dialdehyde (MDA), and the initial level of this product was determined in the liver [11]. Accumulation of hydroperoxides was estimated by the UV absorption spectrum of a solution of lipids in methanol—hexane (5:1), characteristic of conjugated dienes [4]. The concentration of reduced glutathione in the homogenate was determined [10]. The concentration of tocopherol in the microsomes was recorded fluorometrically [15]. Protein was determined by Lowry's method. Both light and electron microscopy were used for morphological investigation of the liver.

EXPERIMENTAL RESULTS

During analysis of hydroxylation processes (Fig. 1) the fact was noted that cold led to a marked reduction in activity of aniline and 3,4-benzypyrene hydroxylases, reflecting the concentration and catalytic activity of cytochromes P-450 and P-448. In fact, in the experimental group the concentration of cytochrome P-450 in the microsomal fraction was considerably lower than in the control (0.25 ± 0.048 and 0.74 ± 0.086 nmole/mg protein respectively; $P < 0.001$). Meanwhile aminopyrine demethylase activity, mainly dependent on the rate of reduction of the cytochrome P-450—substrate complex in the oxidation chain of NADH, did not differ significantly from the control. The changes in mono-oxygenase activity observed may evidently be based, in the opinion of several workers [9, 2], on conformational changes in the microsomal membrane, an essential role in the regulation of which is played by peroxidation of polyunsaturated acyls of membrane phospholipids.

Examination of this class of oxidative reactions in the microsomes revealed a marked increase in the activity of enzymic and ascorbate-dependent LPO systems in the experimental animals (Fig. 2). This increase in oxidizability of microsomal lipids as a result of cooling of the body is probably evidence of an increase in the content of LPO substrate (polyunsaturated acyls of membrane phospholipids) and also of an increase in accessibility of the substrate for membrane radical-forming systems. In the presence of a sufficient quantity of catalytic factors (ascorbate, NADPH) and in the presence of oxygen, in turn this increases the risk of uncontrollable hydroperoxide formation in lipids of the microsomal membranes *in vivo* followed by a decrease in the catalytic activity of cytochrome P-450

and its conversion into the inactive form. The suggestion that the initial level of radical formation may be increased is confirmed by the small increase in the content of one of the chief LPO products (MDA) in the liver (1.57 ± 0.13 and 1.23 ± 0.084 nmoles/mg protein; $P < 0.05$) in animals exposed to cold, and also the increased concentration of hydroperoxides in the microsomal lipids of these animals (14.3 ± 1.37 and 8.9 ± 0.94 nmoles/mg lipids; $P < 0.01$).

Besides the structural orderliness of membranes of the endoplasmic reticulum, water- and lipid-soluble antioxidants also have a substantial influence on the development of chain radical reactions in the microsomal mono-oxygenase system. It has been shown that the content of the basic structural antioxidant α-tocopherol in the microsomes is unchanged by cold. By contrast to this, a considerable decrease in the content of reduced glutathione in the liver of experimental animals (1.5 ± 0.084 and 2.8 ± 0.21 mg/g tissue; $P < 0.001$) can significantly affect the activity of the principal enxymes of antiperoxide protection — glutathione peroxidase and glutathione S-transferase.

The results of the biochemical investigation are confirmed by those of morphological analysis. Electron-microscopic investigation revealed considerable reduction of the rough endoplasmic reticulum in the liver of animals exposed to cold, together with a decrease in the number of ribosomes and polysomes in the hepatocytes. A decrease in the number of glycogen granules compared with the control was found histochemically in the cytoplasm of the liver cells.

Prolonged exposure of homoiothermic animals to severe cold thus leads to inhibition of microsomal oxidation of various substrates. An increase in the oxidizability of polyunsaturated acyls of microsomal phospholipids probably plays the role in this situation of a factor leading to changes in conformation of the membranes, and it thus introduces qualitative differences in the manifestation of activity of membrane-bound mono-oxygenases (aminopyrine demethylase). Another factor of definite importance is evidently the direct effect of hydroperoxides on cytochrome P-450 under conditions of increased formation of LPO products, uncompensated by the system of water- and lipid-soluble antioxidants.

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