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CORRELATION BETWEEN CHANGES IN CONTENT AND ACTIVITY OF MICROSOMAL CYTOCHROME P-450 IN RAT LIVER WITH INTENSIFICATION OF LIPID PEROXIDATION DURING STRESS

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KEY WORDS: stress; cytochromes P-450 and b_5; demethylase activity; lipid peroxidation.

It has been suggested that changes in the cytochrome P-450 content and demethylase activity in the microsomal fraction of the liver after hypothermia in rats are connected with intensification of lipid peroxidation (LPO) in the tissues of the animals [2].

In view of data on intensification of LPO in other forms of stress [4, 5, 6], it was decided to study the effect of immobilization, severe physical exertion, and injection of adrenalin on demethylase activity in rat liver microsomes and in their content of cytochromes P-450 and b_5.

EXPERIMENTAL METHOD

Noninbred male rats weighing 150-180 g were used. As stressors the animals were subjected to prolonged immobilization [6] and physical exertion (swimming for 3 h at 25°C). Adrenalin was injected intramuscularly in a dose of 800 μg/kg. The microsomal fraction of the liver was isolated by gel-filtration on Sepharose 2B [14]. Protein was determined by the method in [12]. The content of cytochromes P-450 and b_5 was determined spectrophotometrically [13], using extinction coefficients of $E_{450-500} = 91\ mM^{-1} \cdot cm^{-1}$ for cytochrome P-450 and $E_{424-409} = 185\ mM^{-1} \cdot cm^{-1}$ for b_5.

Aminopyrine demethylase activity of the microsomal fraction was estimated from the rate of formaldehyde formation during demethylation of aminopyrine [2].

Lipids were extracted from the liver by Folch's method. The content of hydroperoxides in the lipids was determined by a ferroredoximetric method [1].

EXPERIMENTAL RESULTS

After the action of the stressors and injection of adrenalin, to simulate exposure to stress [7], the cytochrome P-450 level in the rat liver microsomes was lowered. After swimming for 3 h and immobilization of the animals for 4 h the cytochrome P-450 content was reduced by 19 and 24%, respectively (Table 1). The fall in the cytochrome P-450 level after swimming and immobilization was of short duration: By the 25th hour of immobilization the cy-

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TABLE 1. Content of Cytochromes P-450 and b_5 (in nmoles/mg protein) in Rat Liver Microsomes after Swimming for 3 h and after Immobilization of Animals (M ± m)

Time after beginning of procedure, h	Swimming		Immobilization	
	P-450	b_5	P-450	b_5
Control	0,839±0,018 (6)	0,451±0,004(6)	0,839±0,018 (6)	0,451±0,004(6)
3	0,685±0,045*(4)	0,419±0,014(4)	0,642±0,035† (6)	0,450±0,027(6)
24	1,093±0,069*(4)	0,489±0,020/4)	0,910±0,063(6)	0,435±0,038(6)

Legend. Number of experiments in parentheses. *P < 0.05, † P < 0.01 compared with control.

TABLE 2. Aminopyrine Demethylase Activity (in nmole/min/mg protein) of Rat Liver Microsomes after Injection of Adrenalin and Immobilization of the Animals (M ± m)

Time after beginning of procedure, h	Immobilization	Adrenalin
Control	7,36±0,50 (6)	7,07±0,48 (5)
3	5,70±0,11*(6)	8,01±0,51 (5)
24	5,05±0,16*(6)	4,08±0,91 (5)

Legend. Number of experiments in parentheses. *P < 0.01 compared with control.

tochrome P-450 concentration in the microsomes of the experimental rats was completely back to normal, whereas in the animals which swam the cytochrome P-450 level 24 h after the beginning of the experiment was even higher than in the control.

The concentration of microsomal cytochrome b_5 showed virtually no change after swimming or immobilization of the animals.

During the first 3 h after injection of adrenalin the cytochrome P-450 level in the microsomes remained unchanged but the content of cytochrome b_5 actually increased a little (Fig. 1). This was followed by a phase of prolonged (over 48 h) decline in the content of both forms of cytochrome followed by restoration of their normal level toward the end of the 3rd day of the experiment.

Changes in the content of microsomal cytochromes found after injection of adrenalin resemble those after hypothermia in rats [2], and just as in hypothermia adrenalin caused marked intensification of LPO, which preceded the phase of decline of the cytochrome content. The quantity of intermediate products of LPO — hydroperoxides of unsaturated fatty acids — in liver lipids of the experimental animals was almost doubled (increased from 6.1 ± 1.6 to 12.0 ± 2.4 nmoles/mg lipids) 1 h after injection of adrenalin.

During the period of decline of the cytochrome P-450 content after swimming and immobilization, the microsomal aminopyrine demethylase activity also was depressed (Table 2). At other times of investigation of the action of stressors (3 h after injection of adrenalin, immobilization for 24 h) changes in microsomal aminopyrine demethylase activity did not correspond to changes in their cytochrome P-450 content (Table 2).

Previously published results indicate that different forms of stress (immobilization, severe physical exertion, adrenalin, hypothermia [2], acute nociceptive stimulation [9]) — cause changes in the cytochrome P-450 content in the endoplasmic reticulum of the rat liver. One of two effects is observed under these circumstances: 1) a relatively deep and prolonged fall in the cytochrome P-450 level, accompanied by a parallel change in the content of cytochrome b_5; 2) a more rapid, short, and less deep fall in the content of cytochrome P-450 accompanied by no change in the cytochrome b_5 level.

Intensification of LPO, found by ourselves [3] and other workers [5, 6, 8] after various forms of stress, precedes changes in the cytochrome content in the endoplasmic reticulum. It has been suggested that accumulation of LPO products in the tissues of stressed animals leads

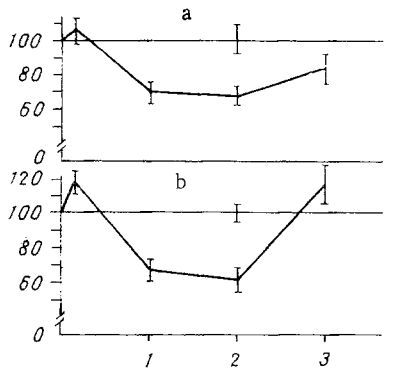

Fig. 1. Change in content of cytochromes P-450 (a) and b_5 (b) in rat liver microsomes after injection of adrenalin. Abscissa, time after injection of adrenalin (in days); ordinate, cytochrome content (in % of control).

to labilization of lysosomes, liberation of proteolytic enzymes and, consequently, to injury to biomembranes and the enzymes located in them [4]. If it is recalled that direct destruction of cytochrome P-450 by LPO products — peroxides and hydroperoxides of unsaturated fatty acids [15] — also is possible, there are grounds for concluding that changes in the cytochrome P-450 level after stress may evidently be connected with preceding intensification of LPO. This hypothesis is supported by the fact that changes in the content of cytochrome b_5, which is relatively resistant to the destructive action of LPO products [10], in the liver microsomes of stressed rats are smaller than changes in the cytochrome P-450 content.

Besides a decrease in the cytochrome concentrations, the forms of stress studied also caused other disturbances in the microsomal enzyme system for xenobiotic metabolism. This is shown by differences in the time course of changes in aminopyrine demethylase activity of the microsomes and their content of cytochrome P-450 during immobilization and after injection of adrenalin into the animals.

The possibility cannot be ruled out that the short-term increase in aminopyrine demethylase activity after injection of adrenalin, just as after hypothermia in animals [2], is connected with changes in the phospholipid composition of the biomembranes [3], as a result of which the enzyme activity of cytochrome P-450 changed. According to Meerson [4], this kind of regulation, independent of biosynthesis, of the activity of lipid-dependent enzymes can be regarded as an adaptive response of the organism in the stage of urgent adaptation.

One probable cause of the low aminopyrine demethylase activity of the liver microsomes at the end of the 1st day of immobilization of the animals may be a decrease in the proportion of the isozyme with highest enzyme activity, and the one most sensitive to LPO products, in the composition of the microsomal cytochrome P-450 pool [2, 11].

The facts described above suggest that the decrease in cytochrome P-450 content in the endoplasmic reticulum of the liver is a nonspecific reaction of the body to stress connected with generalized intensification of LPO in the animals' tissues. Differences in the types of time course of changes in the cytochrome P-450 level after stress described above may evidently be determined, along with other factors, by the relationship between processes of direct and indirect action of LPO products on membranes of the endoplasmic reticulum of the liver cells and hemoproteins located in them.

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CREATINE PHOSPHOKINASE ACTIVITY IN MEMBRANES OF THE MYOCARDIAL SARCOPLASMIC RETICULUM IN EXPERIMENTAL CORONARY INSUFFICIENCY

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Recently developed views on the creatine kinase mechanism of energy transport in myocardial cells [6, 11, 14] have compelled investigators not only to continue the search for new ways of solving complex and largely unexplained problems of energy supply for the contractile function of the heart, but also to reassess results already obtained. According to a current hypothesis [6, 8, 10], the creatine kinase reaction is reversible within the limits of one cardiomyocyte. For instance, in the intermembranous space of the mitochondria the direct reaction of transfer of phosphate from ATP to creatine is effected by means of the mitochondrial isozyme creatine phosphokinase (CPK), whereas in myofibrils the reverse reaction of phosphorylation of ADP with the formation of ATP takes place with the participation of the MM isozyme. CPK isozymes have now been found in all subcellular structures producing or utilizing energy: in the mitochondrial membrane, myofibrils, membrane of the sarcoplasmic reticulum (SPR), plasma cell membrane, etc. [3, 14].

According to one view [15], CPK isozymes, which are in close structural connection with cell membranes and provide the cellular apparatus with energy, are in close functional association with other enzymes responsible for the transfer of materials and ions through the membrane. For instance, CPK in mitochondria functions in close association with ATP-ADP translocase, and in myofibrils, the SPR membrane, and the sarcolemma, it is associated with Mg-dependent, Ca-dependent, and Na,K-dependent ATPase.

The study of the role of membrane formations in energy transport and utilization by the myocardial cells assumes particular importance in myocardial ischemia, when significant changes are observed in the structure and properties of the protein–lipid layer of the membranes [7, 12], and, in particular, in their phospholipid composition. This is a particularly important matter in connection with information in the literature that certain CPK isozymes are most probably bound with the membrane by means of phospholipids [13].

Changes in activity of the mitochondrial CPK isozyme during measured limitation of the coronary blood flow were found previously by the present writers [1]. Meanwhile the study of yet another unsolved problem is extremely important – that is energy supply for Ca^{++} transport through the SPR membrane of myocardial cells and the link between this process and certain other parameters determining the functional state of the membrane apparatus of the SPR.

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