LIVER MICROSOMAL CYTOCHROME: P-450p AND P-450h ACTIVITY AND BLOOD CORTICOSTEROID LEVELS OF EXPERIMENTAL ANIMALS EXPOSED TO PHYSICAL FACTORS

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The microsomal monooxygenase system of the liver is one of the functional systems of homeostasis, for it is responsible for metabolism and inactivation not only of xenobiotics, but also of endogenous compounds, such as hormones, that are important for maintaining the constancy of the internal milieu [9]. The wide substrates specificity of the microsomal monooxygenases is maintained by the multiplicity of forms of the key enzyme of the system, namely cytochrome P-450 [11]. Oxidation of steroids in the liver microsomes takes place through the participation of several forms of cytochrome P-450: P-450a, P-450h, P-450i, P-450p [7, 12]. It has been shown that inducers of microsomal enzymes stimulate metabolism of steroid hormones [5, 6], although, according to the opinion of the authors cited, the physiological consequences of such induction are not clear. On the other hand, exposure of the body to therapeutic physical factors also leads to hormonal changes [4]. Physical factors, as has recently been shown, may, however, affect the activity of enzymes of the liver microsomal system [1, 2].

The aim of this investigation was to determine the action of physical factors used for therapeutic purposes, mainly low-intensity laser radiation with a wavelength of 0.89 μ, microwave radiation with a frequency of 2450 MHz in the centimeter band (CMW), and low-intensity ultrasound with a frequency of 880 kHz (US), on the catalytic activity of steroid-metabolizing cytochromes P-450h and P-450p in liver microsomes and on the blood corticosteroid levels in mice.

EXPERIMENTAL METHOD

Experiments were carried out on 80 male Wistar rats weighing 200-250 g. The region of the animals' liver was exposed to the action of the physical factors by a contact method. One group of rats was exposed to ultrasound (five daily procedures with an intensity of 0.2 W/cm^2 and a duration of 5 min), the 2nd group was exposed to five sessions of CMW on alternate days (power 2.5 W, exposure 2 min), and the 3rd group was exposed to radiation of a Ga–As semiconductor laser (five daily procedures, frequency 300 Hz, exposure 256 sec). The control group of rats was not exposed to any of these physical factors. Biochemical tests were carried out 24 h after the last procedure. The liver microsomal fraction was isolated by the usual method of differential centrifugation. Functional activity of cytochromes P-450h and P-450p was assessed by determining the rate of formation of 16α- and 6β-OH metabolites of androstenedione respectively [13]. The total blood level of the protein-bound hormone corticosterone was determined by competitive protein binding of tritium-labeled and native corticosterone, extracted with methylene chloride

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TABLE 1. Changes in Functional Activity of Liver Microsomal Cytochromes P-450h and P-450p and in Blood Corticosteroid Levels of Rats Exposed to Physical Factors (M ± m, n = 5)

Series of experiments	Androstenedione hydroxylase, nmoles·min^{-1}·mg^{-1}		Corticosterone concentration, nmoles·liter^{-1}	11-OHCS concentration, mmoles·liter^{-1}
	16α-OH	6β-OH		
Control	0,682±0,121	0.924±0.066	132±8,4	430±11,0
US	0,424±0,110***	0,662±0,077*	140±26,9	883±3,6*
CMw	0,862±0,100***	1,178±0,093**	115±7,3	562±10,0*
Laser	0,990±0,260***	1,750±0,230*	119±28,0	540±8,0*

Legend. *$p < 0.001$, **$p < 0.01$, ***$p < 0.05$.

[10]. The 11-hydroxycorticosteroid (11-OHCS) content was determined by a fluorometric method [3]. The experimental results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

On the basis of the results in Table 1 it is possible to compare functional parameters of the liver microsomes and the blood glucocorticoid level of the control and experimental rats. The physical factors studied were found to act differently on the catalytic activity of cytochromes involved in steroid metabolism. Under the influence of US the velocity of hydroxylation of androstenedione in positions 6β (the marker reaction for cytochrome P-450p) and 16α (the marker reaction for cytochrome P-450h) in the liver microsomes was reduced by 1.4 times. Exposure to CMW and the Ga–As laser, however, was accompanied by increased activity of these forms of cytochrome P-450. Under these circumstances both marker reactions were increased by 1.3 times by the action of CMW, whereas irradiation with the Ga–As laser increased the catalytic activity of cytochrome P-450h by 1.5 times and of cytochrome P-450p by 1.9 times. Thus exposure of the hepatic region to low-intensity ultrasound leads to weakening of activity of two microsomal enzymes hydroxylating steroid hormones, whereas CMW and the Ga–As laser stimulate their activity.

The action of physical therapeutic factors was not reflected in the blood level of the physiologically inactive transport form of corticosterone (Table 1). The level of the biologically active hormone (11-OHCS), however, was significantly increased (almost doubled) under the influence of US. Exposure to CMW and to the Ga–As laser also was accompanied by a rise of this parameter, although it was smaller (by 1.3 times). Consequently, weakening of activity of steroid-metabolizing enzymes in the liver microsomes during exposure to ultrasound was accompanied by an increase in blood levels of the biologically active, unmetabolized form of corticosterone in the rats. Meanwhile increased activity of the steroid-metabolizing enzymes of the liver microsomes under the influence of CMW and the Ga–As laser did not lower the blood level of the biologically active form of corticosterone in the animals. It can be tentatively suggested that the increase in the rate of corticosterone metabolism is compensated by increased secretion of ACTH and glucocorticoids through a negative feedback mechanism [8]. In this respect there are some interesting data showing that exposure of animals to CMW is accompanied by potentiation of the glucocorticoid activity of the adrenals and an increase in the blood level of free forms of corticosterone [4]. Ultrasound also has the property of stimulating the pituitary-adrenal system [4], but in that case this effect is combined with weakening of activity of the steroid-metabolizing hepatic monooxygenases, which may be connected with slowing of corticosterone metabolism and a marked increase in the blood level of the biologically active hormone in the rats.

Data on changes in activity of the steroid-metabolizing cytochromes P-450 in the liver microsomes and in the blood level of the free, unmetabolized form of corticosterone, due to the action of physical factors on the hepatic region of rats, suggest the existence of correlation between these parameters. The absence of changes in the content of the protein-binding form of the hormone under these conditions, a unique depot of corticosterone and with a compensating function under unfavorable conditions, and the abrupt changes in concentration of the biologically active form may be evidence in support of the physiological character of the action of physical factors, with the parameters studied, on liver function.

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CHANGES IN BRAIN LIPID PEROXIDATION IN THE FETAL ALCOHOL SYNDROME

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Since the clinical picture of the so-called fetal alcohol syndrome (FAS) was described [4] in 1968, it has been the subject of many clinical and experimental investigations. However, the picture of the neuropathological processes determining the central nervous correlates of the cognitive defects developing after perinatal exposure to ethanol, is still far from a full explanation. We know that ethanol passes readily through biological membranes and can thus affect the enzyme systems which maintain the ionic gradients through neuronal membranes. One factor which lies at the basis of the damaging action of alcohol is intensification of liquid peroxidation (LPO), induced by alcohol [8, 9], which leads to quantitative and qualitative changes in the lipid composition of the membranes. We accordingly decided to study to what degree perinatal exposure to alcohol is reflected in LPO processes in different regions of the animal brain.

EXPERIMENTAL METHOD

Experiments were carried out on male rats aged 12 weeks, divided into three groups, with 10 animals in each group. The 1st group consisted of young rats whose mothers had received a 6% aqueous solution of ethanol as the

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