The response of the adrenals to cooling in hypophysectomized rats may therefore take place in the absence of the adenohypophysis, but when the blood VP level is sufficiently high. The state of the thyroid gland and its response to cold in hypophysectomized rats are independent of the blood VP concentration.

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PREVENTION OF DISTURBANCES OF ACTIVITY OF THE MONOOXYGENASE SYSTEM AND OF HEPATOCYTE ULTRASTRUCTURE AFTER ACUTE HEPATIC ISCHEMIA BY α-TOCOPHEROL AND LIDOCAINE

O. R. Grek, V. I. Sharapov,
I. A. Sokirchenko, and V. A. Shkurupii

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Prevention of postischemic disturbances of the detoxication function of the liver arising as a result of certain surgical procedures or pathological states is an urgent problem in medical practice. Intensification of peroxidation processes [3, 6] and activation of endogenous phospholipases [5] are the leading factors in the development of postischemic disturbances of hepatic structure and function.

The aim of the present investigation was to study activity of microsomal monooxygenases and hepatocyte ultrastructure in rats at different stages of the postischemic period, and the effect of combined prophylactic administration of the antioxidant α-tocopherol (TP) and the phospholipase inhibitor lidocaine (L) on them.

EXPERIMENTAL METHOD

Experiments were carried out on 200 male Wistar rats weighing 150-240 g. Total ischemia of the liver for 30 and 60 min was produced by the method described previously [2]. The effectiveness of combined administration of TP and L was assessed by noting the survival rate of the animals after total hepatic ischemia for 60 min. All biochemical investigations were

Department of Pharmacology, Central Research Laboratory, Novosibirsk Medical Institute.

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TABLE 1. Effect of Combined Administration of TP and L on Microsomal Monooxygenase Activity in the Postischemic Period (M ± m)

Parameter	Experimental conditions	Postischemic period, days					Intact animals
		1	3	7	14	21	
N-demethylation of aminopyrine	I	1,61±0,06[a]	1,05±0,07[a]	1,23±0,07[a]	1,41±0,03[a]	2,04±0,26	2,00±0,13
	II	1,92±0,25	1,78±0,13[b]	1,88±0,07[b]	1,96±0,09[b]	1,93±0,09	
p-Hydroxylation of aniline	I	0,28±0,01[a]	0,19±0,02[a]	0,17±0,02[a]	0,20±0,01[a]	0,36±0,02[a]	0,49±0,03
	II	0,32±0,03[a]	0,31±0,03[a,b]	0,29±0,02[a,b]	0,37±0,02[a,b]	0,37±0,01[a]	
Cytochrome P-450	I	0,90±0,06	0,65±0,02[a]	0,62±0,04[a]	0,68±0,05[a]	1,01±0,10	0,99±0,06
	II	0,82±0,06	0,99±0,09[b]	0,90±0,09[b]	0,91±0,08[b]	1,15±0,15	
b_5	I	0,59±0,02[a]	0,48±0,02[a]	0,44±0,01[a]	0,65±0,01	0,65±0,05	0,68±0,03
	II	0,68±0,06	0,77±0,06[b]	0,74±0,04[b]	0,73±0,03[b]	0,79±0,05	

Legend. Number of animals in groups was 8-14. Here and in Table 2: velocity of N-demethylation of aminopyrine shown in nmoles HCHO/min/mg protein, velocity of p-hydroxylation in nmoles p-aminophenol/min/mg protein; cytochrome concentrations shown in nmoles/mg protein; I) control (ischemia), II) TP + L + ischemia; significance of differences: a) compared with initial value (intact animals), b) with control.

TABLE 2. Effect of Prophylactic Injection of TP and L on Monooxygenase Activity Induced by Phenobarbital in the Postischemic Period (M ± m, n = 8)

Parameter	Experimental conditions	Postischemic period, days			Initial level
		7	14	21	
N-demethylation of aminopyrine	I	3,39±0,21[a]	4,34±0,20[a]	4,92±0,21	5,14±0,25
	II	5,44±0,13[b]	5,10±0,25[b]	—	
p-Hydroxylation of aniline	I	0,68±0,04[a]	0,76±0,04[a]	0,72±0,02[a]	1,09±0,06
	II	1,00±0,06[b]	1,06±0,06[b]	—	
Cytochrome P-450	I	2,06±0,15[a]	2,54±0,13[a]	3,47±0,28	3,80±0,12
	II	3,01±0,20[a,b]	2,91±0,10[a,b]	—	
b_5	I	0,65±0,04[a]	0,76±0,03[a]	0,84±0,03[a]	0,97±0,05
	II	0,96±0,06[b]	1,01±0,05[b]	—	

Legend. Initial level – intact animals + induction by phenobarbital.

conducted after hepatic ischemia lasting 30 min. Concentrations of cytochromes P-450 and b_5 [12], and the velocity of the aminopyrine-N-demethylase [13] and aniline-p-hydroxylase [10] reactions were determined on the 1st, 3rd, 7th, 14th, and 21st days of the postischemic periodic. Changes in activity of the monooxygenase also were estimated on the 7th, 14th, and 21st days of the postischemic period after administration of phenobarbital to the animals in a dose of 50 mg/kg once daily for 4 days before decapitation. The concentration of microsomal cytochromes was determined on a Hitachi-356 differential spectrophotometer (Japan). The protein concentration in the microsomes was determined by Lowry's method [11].

For morphological investigation of the effectiveness of prevention of the postischemic damage to the hepatocytes by TP and L, ischemia of the central and left lobes of the liver was produced for 2 h by ligation of the vascular pedicle after preliminary isolation of the bile duct, so that the course of necrotic changes in the liver parenchyma could be observed. The operation was performed under pentobarbital anesthesia (40 mg/kg, intraperitoneally). The animals were decapitated after 2 and 24 h and 7 days of recirculation. The liver of intact animals served as the control. Samples of liver for light microscopy were fixed in 10% neutral formalin solution and embedded in paraffin wax. Paraffin sections were stained with hematoxylin and eosin and used for morphometric determination of the volume of zones of necrotic changes in the liver. Pieces of the left lobe of the liver for electron microscopy were fixed in 1% OsO_4 solution in phosphate buffer and embedded in Epon. Ultrathin sections were stained with a saturated aqueous solution of uranyl acetate and lead citrate and studied in the JEM-100S electron microscope. Quantitative investigations of the structural organization of the endoplasmic reticulum and of the volume of the necrotic tissues were undertaken with the aid of a square lattice test system [14].

In all experiments TP acetate (100 mg/kg, intraperitoneally) and L (10 mg/kg, intraperitoneally) were injected 12 and 1 h, respectively, before ligation of the hepatic vessels.

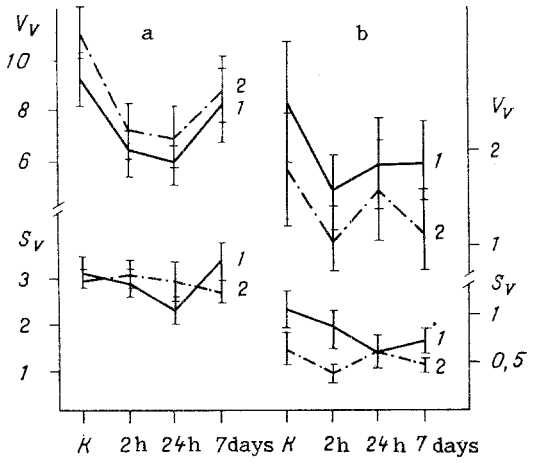

Fig. 1. Results of investigation of bulk (V_V, % of volume of cytoplasm) and surface (S_V, μ^2/μ^3 of cytoplasm) density of cisterns of RER (a) and SER (b) of rat hepatocytes after hepatic ischemia lasting 2 h without correction (1), and after preliminary combined administration of TP and L (2). Abscissa, periods of postischemic recirculation. K) Control.

The results were subjected to statistical analysis by Student's t test. Differences between the mean values were considered to be significant at the $p < 0.05$ level.

EXPERIMENTAL RESULTS

During prophylactic administration of TP and L the survival rate of the animals increased to 87.1% compared with the control (ischemia – 45.2%).

Prophylactic injection of TP and L completely prevented the fall in activity of the aminopyrine-N-demethylase reaction. This effect was linked with the ability of the test preparations to prevent a fall in concentration of cytochromes P-450 and b_5 in the microsomal fraction of the liver in the recovery period. The level of aniline metabolism in the postischemic period in animals treated with a combination of these preparations was 24.4, 24.5, and 34.7%, respectively, higher than in the control group on the 3rd, 7th, and 14th days of the recovery period (Table 1).

In the late periods after ischemia of the liver the velocity of N-demethylation of aminopyrine and of p-hydroxylation of aniline after administration of phenobarbital remained lower than initially [7]. The levels of microsomal cytochromes P-450 and b_5 in the postischemic period in the induced animals also were lower than initially. The degree of induction of cytochrome P-450 and of aminopyrine-N-demethylase was restored on the 21st day of the postischemic period, but the cytochrome b_5 concentration and aniline-p-hydroxylase activity remained lower by 13.4 and 33.9%, respectively, at this period.

Prophylactic admininstration of TP and L, by preventing inactivation of monooxygenases, guaranteed preservation of an adequate induced increase in the velocity of N-demethylation of aminopyrine, of p-hydroxylation of aniline, and of the cytochrome b_5 concentration 7 and 14 days after ischemia. The induced increase in the cytochrome P-450 concentration exceeded the value of this parameter in the control group by 25.0 and 9.8% 7 and 14 days, respectively, after ischemia (Table 2).

The formation of necrotic changes in the liver after irreversible ischemia takes place after 24 h of recirculation [8, 9]. In the animals not receiving TP and L, hepatic ischemia for 2 h led to death of 24.9 ± 1.8% of the liver tissue. The process of necrosis began in the center of the lobule and spread peripherally through it.

After preliminary combined administration of TP and L the volume of necrotic tissue in the organ was 7.4 ± 9.0% after 24 h of recirculation, or 3.3 times less than in the experiment without administration of the preparations. Between the necrotically changed and undamaged zones of the liver parenchyma a zone of hepatocytes with vacuolar degeneration was observed, accounting for 6.9 ± 0.7% of the volume of the liver, which was not observed in animals not receiving these preparations. According to existing views on intracellular repair processes [4], these degeneratively changed cells can recover spontaneously or they are regarded as amenable to therapeutic measures.

After 2 and 24 h of recirculation the bulk density of the rough endoplasmic reticulum (RER) was reduced by 31-38%; its surface area 24 h after restoration of the blood flow was reduced by 25% (Fig. 1a). In the postischemic period (except after 2 h of recirculation) a lasting decrease was observed in the bulk and surface densities of the smooth endoplasmic reticulum (SER) (Fig. 1b).

Preliminary (before ischemia for 2 h) injection of TP and L caused constriction of the cisterns of the RER, as a result of which the bulk density of RER was reduced by 31-39% compared with the control. The surface area of the RER membranes remained unchanged in this case (Fig. 1a). Prophylactic injection of TP and L led to preservation of the structural organization of the SER, except during the initial period of recirculation (after restoration of the blood flow for 2 h), when the bulk and surface densities of SER were reduced (Fig. 1b).

Prophylactic combined administration of the antioxidant TP and the phospholipase inhibitor L thus had a marked anti-ischemic effect, and reduced the severity of the postischemic structural and functional changes in the liver.

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EFFECT OF ALCOHOL POISONING ON LEVELS OF ANTIBODIES TO CATECHOLAMINES AND SEROTONIN IN ANIMALS DIFFERING IN PREDISPOSITION TO EXPERIMENTAL ALCOHOLISM

T. V. Davydova and V. G. Fomina

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It was shown previously [3] that chronic alcohol poisoning in animals predisposed to develop experimental alcoholism is characterized by strengthening of the immune response to sheep's red blood cells (SRBC) and by hypoactivity of SRBC-induced T suppressor cells, whereas in animals rejecting alcohol, on the other hand, depression of antibody formation and hyperactivity of antigen-specific and concanavalin A-induced T suppressor cells are observed. Evidence of the formation of antibodies to catecholamines and serotonin (5-HT) in pathological

Laboratory of Pathological Physiology of the Immune System, Research Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow.

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