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ACTIVITY OF THE MONOOXYGENASE SYSTEM AND RATE OF LIPID PEROXIDATION IN RAT LIVER MICROSOMES DURING REINDUCTION BY POLYCHLORINATED BIPHENYLS

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One of the mechanisms of protection of the body against the action of many foreign substances is induction of the microsomal monooxygenases of the liver; this process develops rapidly and a high level of monooxygenases is maintained for a long time, depending on the chemical nature of the inducer and the conditions of its introduction [1, 7, 9]. The possibility of periodic entry of foreign substances into the body accounts for the great interest in the study of their reinducing effects, which have so far received little investigation, especially after complete partial restoration of the original level of enzyme activity [4, 15]. In previous investigations the writers discovered some features of induction of the monooxygenase system (MOS) of the endoplasmic reticulum of the liver during acute exposure to polychlorinated biphenyls (PCB), which are widespread pollutants of the biosphere [5, 6].

The aim of this investigation was to study the functional state of MOS and also the velocity of lipid peroxidation (LPO) in rat liver microsomes during repeated administration of PCB at different times after primary induction.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing initially 200-220 g. The animals were kept on the normal animal house diet and were given food and water *ad lib*. Soval (a mixture of PCB of Soviet manufacture) was dissolved in corn oil and administered to the rats by the intragastric route (2 ml/kg body weight) in a dose of 500 mg/kg. In experimental

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TABLE 1. Weight of Liver, Content of Hemoproteins, and Rate of Oxidation of Certain Substrates in Rat Liver Microsomes after a Single Dose of Sovol (M ± m, n = 7-8)

Parameter	Time after administration of Sovol, days					
	4		70		150	
	Control	Experiment	Control	Experiment	Control	Experiment
Weight of liver, g/100 g	4,04±0,12	6,23±0,29*	3,02±0,05	3,67±0,12*	2,54±0,08	2,84±0,11
Cytochrome P-450, nmoles/g	13,30±0,39	53,50±1,26*	11,30±1,02	32,70±2,84*	8,73±0,96	18,60±1,73*
Cytochrome b_5, nmoles/g	9,58±0,20	14,50±0,40*	9,87±0,74	16,5±0,7*	9,17±0,60	14,70±0,55*
N-demethylation of DMA, nmoles/g	115,4±5,23	233,0±9,1*	131,4±16,0	276,4±15,7*	99,8±5,7	214,0±8,0*
N-demethylation of AP, nmoles/min/g	114,4±5,0	219,2±15,6*	91,4±5,3	205,3±12,6*	104,1±6,8	170,0±11,0*
p-hydroxylation of AN, nmoles/min/g	7,91±0,42	11,3±1,3*	7,31±0,75	8,97±0,55	7,66±0,30	11,0±0,84*

Legend. *P < 0.05 compared with control.

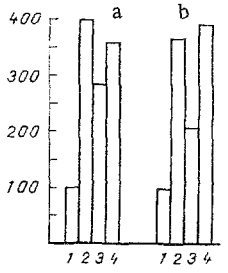

Fig. 1. Cytochrome P-450 concentration in rat liver microsomes at various times after single or repeated administration of Sovol. Ordinate, cytochrome P-450 concentration (in per cent of control). 1) control, 2) single dose 4 days before sacrifice, 3) single dose 70 (150) days before sacrifice, 4) two doses, 70 (150) days and 4 days before sacrifice.

group 1 Sovol was given in a single dose and the animals were killed 4, 70, and 150 days later. In experimental group 2 Sovol was injected twice (the second injections were given on the 66th or 146th day of the experiment) and the rats were killed 4 days after the last dose (70th and 150th days). Sovol was given in a single dose to a separate group of rats on the 66th or 146th day, and these animals also were killed 4 days later. Control animals received equal volumes of corn oil. The rats were deprived of food for 12 h before sacrifice. The microsomal fraction was sedimented from the postmitochondrial supernatant after a single washing on the L-5 centrifuge (from Beckman, USA). The content of cytochromes P-450 and b_5 [14], the rate of p-hydroxylation of aniline (AN), of N-demethylation of aminopyrine (AP), and of dimethylaniline (DMA) [3], activity of NADPH- and ascorbate-dependent LPO [2], and the protein content were determined in isolated microsomes. The concentrations of hemoproteins and velocity of the enzyme reactions were calculated per milligram protein, per gram tissue, per weight of the liver, and per 100 g body weight. The distribution of lipids was studied in histological sections stained by Goldman's method and with Sudan black, and Shabadeash's reaction for glycogen and Brachet's reaction were carried out. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

A single dose of Sovol administered to rats caused an increase in the relative weight of the liver (by 1.5 times), in the content of cytochromes P-450 (by 4 times) and b_5, and also in the rate of oxidation of substrates of the first and second types compared with the control after 4 days (Table 1). Histological changes characteristic of PCB were observed in the liver [8, 11] and, in particular, diffuse fatty infiltration, centilobular foci of proliferation of Kupffer cells, and an increase in volume of the hepatocytes.

The level of hemoproteins and activity of MOS in the liver 70 days after administration of Sovol were considerably higher than the control values. By the 150th day after the

beginning of the experiment activity of the system was not yet restored completely to normal. For instance, whereas the cytochrome P-450 concentration was lower, it was still 2.1 times higher than the control, and the cytochrome b_5 concentration and rate of oxidation of the test substrates were virtually unchanged. By this time the degree of fatty infiltration was reduced in the experimental rats, and foci of micronecrosis appeared in the center of the hepatic lobules.

The results are evidence of the long duration of the inducing effect of Sovol on MOS of the rat liver after administration of a single dose, which distinguishes the PCB from classical inducers such as phenobarbital and methylcholanthrene [1, 10, 15]. The prologed effect is evidently linked with accumulation of these compounds, especially the highly chlorinated derivatives of biphenyls, in the tissues and their slow elimination from the body [8, 13]. The level of response of the liver MOS to acute poisoning with a mixture of PCB (on the 66th and 146th days) was virtually the same, although the basal level of hemoproteins and the rate of oxidation of the test substrates showed a tendency to decline with increasing age of the animals.

It will be clear from Fig. 1 that repeated administration of the PCB mixture had an inducing action on cytochrome P-450. Incidentally, despite the different cytochrome P-450 levels in the animals' liver by the time of reinduction, the response of the MOS in both cases (after 70 and 150 days) reached the effect of acute poisoning with Sovol, i.e., the lower the cytochrome level, the higher the index of reinduction. So far as cytochrome b_5 and the rate of oxidation of substrates of the first and second types in the liver microsomes are concerned these parameters remained at the characteristic level for acute poisoning with Sovol. The picture of the morphological changes in the liver of the reinduced animals were similar to the changes in acute Sovol poisoning, but there were some differences in the degree of fatty infiltration, in the distribution of large fat drops inside the lobule, and in the size of the nuclei, and other parameters.

The velocity of LPO in liver microsomes of the experimental animals was significantly increased (by 1.4-1.5 times), but no definite correlation could be found between changes in the cytochrome P-450 level and the intensity of LPO in the microsomes.

MOS of the rat liver in the late stages can thus respond to the repeated action of a mixture of PCB. The degree of response does not exceed that observed in primary induction. Some differences will be noted in the picture of the morphological changes in response to single and repeated administration of a PCB mixture.

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