Effects of Phenobarbital on Activity of Mitochondrial Enzymes in Peripheral Blood Lymphocytes and Oxidative Phosphorylation in Liver Mitochondria

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Treatment with phenobarbital (30 mg/kg) for 2 weeks increased succinate dehydrogenase activity in peripheral blood lymphocytes of male rats. In 38% phenobarbital-treated rats succinate-cytochrome *c* oxidoreductase activity was lower than in the control due to accumulation of cells exhibiting no enzyme activity; in 44% animals this parameter was higher than in the control. The rates of state 3 respiration (oxidation substrate succinate), phosphorylation, and uncoupled respiration in liver mitochondria (oxidation substrate glutamate/malate mixture) increased after 35-day treatment with phenobarbital. The respiratory control and ADP/O ratio for these substrates did not differ from the control.

Key Words: *phenobarbital; oxidative phosphorylation; liver; mitochondrial enzymes*

Phenobarbital is an antiepileptic drug used for the therapy of *grand mal* [4]. Treatment with phenobarbital even in single anticonvulsive doses leads to uncoupling in cerebral mitochondria and decreases the rates of oxidation-reduction processes and phosphorylation [2]. Long-term treatment with phenobarbital produces similar, but more pronounced changes in bioenergetic processes in various brain regions [2,3], which contributes to the development of neurological disorders. It should be emphasized that phenobarbital activates microsomal enzymes (*e.g.*, cytochrome P-450 [13]), stimulates glucose phosphorylation and NADPH production, induces expression of more than 50 genes [6], and promotes synthesis of DNA [11] and ubiquinone [8]. It remains unclear why long-term treatment with phenobarbital leads to liver atrophy and changes in leukopoiesis [12]. Here we studied the effects of long-term treatment with phenobarbital on *in situ* activity of mitochondrial enzymes in lymphocytes and oxidative phosphorylation in liver mitochondria (LMC).

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MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 170-200 g. The animals were perorally administered with 30 mg/kg phenobarbital for 2 weeks. This treatment leads to changes in cell metabolism, which are similar to those caused by chronic administration of phenobarbital in a dose 10-fold exceeding the maximum daily dose for humans. On day 15, the blood from the caudal vein was taken for cytochemical assay. Then the dose of phenobarbital was increased by 5 mg/kg during each successive treatment, which was related to the necessity of maintaining effects of this preparation and rapid adaptation of rats to exogenous toxic factors. The final maximum dose of phenobarbital was 85 mg/kg. Control animals received an equivalent volume of distilled water (injection volume did not exceed 0.5 ml). The last dose was given 1 day before euthanasia. The animals were decapitated 35 days after the start of treatment. Liver and blood smears were subjected to cytochemical assays. Succinate dehydrogenase (SDG) and succinate-cytochrome *c* oxidoreductase (SCO) activities in peripheral blood lymphocytes (PBL) were measured cytochemically [1,14].

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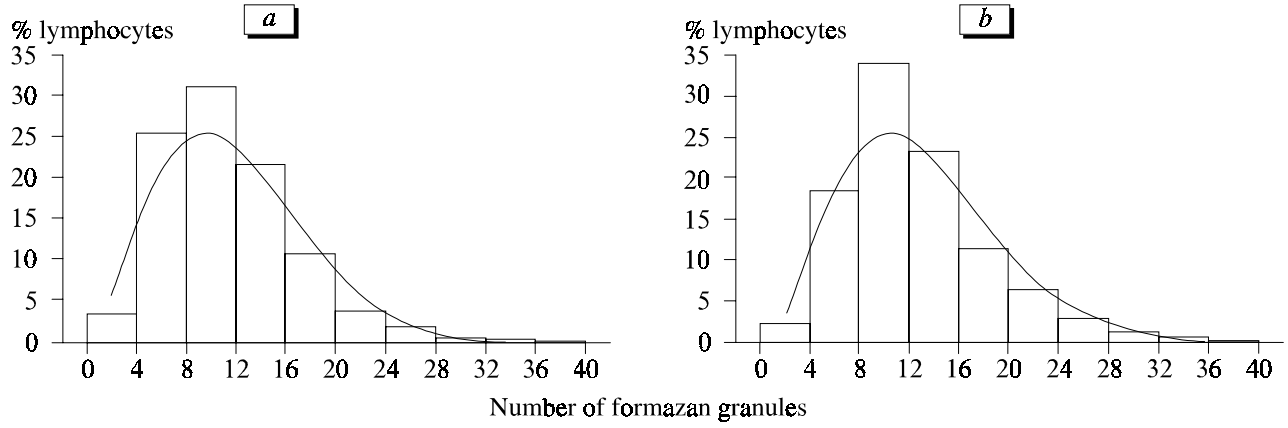

Fig. 1. Distribution of cells with different SDG activities in the control (*a*) and after 15-day phenobarbital treatment (*b*). Curves show smoothed approximation by the least square method.

SDG activity was estimated by the content of formazan granules (FG) per cell (50 cells in each smear). SCO activity was evaluated by the Keplow index (100 cells in each smear). Mitochondria were isolated from liver homogenates by differential centrifugation in a medium containing 0.32 M sucrose and 1 mM EDTA (pH 7.4). Respiration was recorded polarographically using a Clarke platinum electrode with a Teflon membrane. The incubation medium contained 200 mM sucrose, 20 mM KH_2PO_4, and 5 mM $MgCl_2$ (27°C). Succinate (6 mM), β-hydroxybutyrate (7 mM), or glutamate-malate mixture (3 mM each) were used as the oxidation substrate to estimate the respiratory rate. State 3 was induced by addition of 200 μM ADP. 2,4-Dinitrophenol (40 μM) was used as an uncoupling agent. Protein content in LMC was estimated by the biuret method [7].

RESULTS

Treatment with phenobarbital for 15 days increased SDG activity in PBL (12.1±0.2 FG, n=800, p<0.01) compared to the control (11.0±0.2 FG, n=650, Fig. 1), which resulted from accumulation of cells with high SDG activity and a decrease in the content of cells with low enzyme activity.

Figure 2 shows SCO activity in PBL of rats treated with phenobarbital for 15 days. SCO activity in 18% animals receiving phenobarbital did not differ from the control. In 44% rats SCO activity increased, while in 38% animals this parameter decreased (in these rats, the number of cells characterized by negative cytochemical reaction increased). After 35 days SCO activity in all rats treated with phenobarbital was lower than in the control.

Long-term treatment with phenobarbital increased the rates of state 3 respiration and uncoupled respiration (Table 1). After long-term treatment with phenobarbital, both single and repeated administration of ADP produced more pronounced stimulatory effects on respiration and phosphorylation. In the experimental group acceleration of state 3 respiration, increase in phosphorylation rate, and stimulation of respiration were accompanied by increased ADP/O ratio and respiratory control only after repeated administration of ADP. The mean protein content in mitochondrial samples did not differ between phenobarbital-treated and control animals.

Thus, changes in SDG and SCO activities in PBL after long-term phenobarbital treatment were similar to barbiturate-induced shifts in cerebral mitochondria [2]. Since reduced SCO activity indicates impaired electron transport between enzyme complexes II and III in the mitochondrial respiratory chain (which is usually accompanied by inhibition of ATP synthesis), hematological and immunological changes observed

TABLE 1. Mitochondrial Respiration Rate in Rats Treated with Phenobarbital for 35 Days (M±m, n=6)

Substrate	Respiratory rate, natom O/sec/mg protein			
	state 3		2,4-dinitrophenol addition	
	control	phenobarbital	control	phenobarbital
Succinate	2.16±0.06	2.64±0.11*	2.08±0.34	2.26±0.19
Glutamate-malate	1.49±0.09	1.48±0.04	1.57±0.04	1.83±0.08*

Note. *p<0.05 compared to the control.

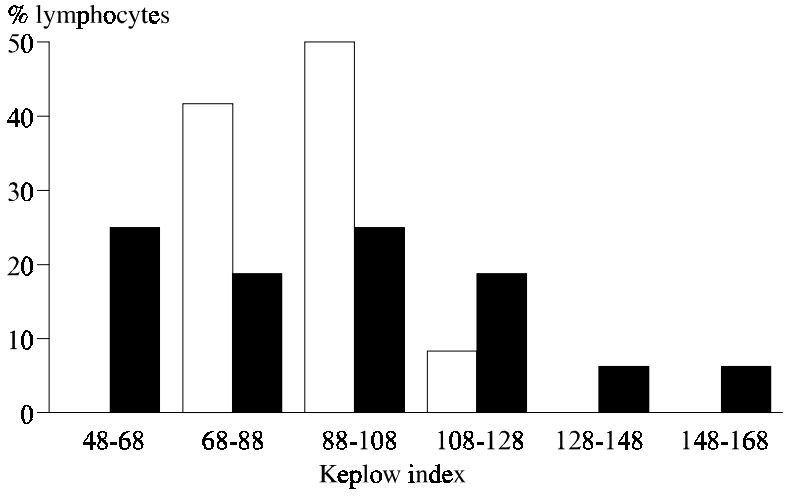

Fig. 2. Percentage of cells with different succinate-cytochrome *c* oxidoreductase activities in the control (light bars) and after 2-week treatment with phenobarbital (dark bars).

during epilepsy probably result from phenobarbital-induced impairment of bioenergetic processes in mitochondria of myeloid and lymphoid tissues. On the other hand, changes in enzyme profile observed in PBL during phenobarbital treatment can be useful in evaluating treatment efficiency and will help to understand molecular changes underlying side effects of this drug.

Our experiments with LMC and published data [10,15] suggest that phenobarbital induces not only microsomal and cytosolic enzymes, but also stimulates mitochondrial enzymes involved in oxidation-reduction processes.

Acceleration of state 3 respiration and phosphorylation and elevation of respiratory control in rats receiving phenobarbital for 35 days indicate that long-term treatment with this preparation stimulates bioenergetic processes and, therefore, increases functional activity of tissues. These changes provide a molecular basis for physical dependence.

Our results and published data [5,9] indicate that *in vivo* long-term treatment with phenobarbital initially activates, but then inhibits oxidation-reduction enzymes and suppresses functions of the mitochondrial respiratory chain. The development of stable suppression and atrophic changes probably depends on cell sensitivity to toxic factors.

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