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Effect of Phenobarbital Pretreatment on the Toxicity of GABA-lytic Agents in Mice

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Barbiturates are known to increase the resistance of animals to toxic effect of phosphoroorganic compounds (POS) [5, 10]. The effect is connected with a modulation of the activity of the monooxygenase system, blood and liver carboxyl esterases, and other detoxication systems [4, 6, 7, 11].

It may be speculated that the advance administration of inducers of the biological defense systems could change the sensitivity of animals to GABA-lytic agents.

In order to elucidate this problem, in the present study we assessed the toxicity of picrotoxin, bicuculline, and 3-mercaptopropionic acid (3-MPA) in male white mice pretreated three times with phenobarbital and benzonal. We also studied the antidote efficiency of diazepam in picrotoxin and bicuculline toxicity under conditions of phenobarbital-mediated modulation of detoxication systems.

MATERIALS AND METHODS

Mature male white mice weighing 26-30 g were injected intraperitoneally with sodium phenobarbital or benzonal (40 mg per kg body weight) daily for 3 days. The toxicity of picrotoxin, bicuculline, and 3-MPA was assayed 24 hours after the last injection. Picrotoxin and bicuculline were suspended in saline with Tween 80. 3-MPA was dissolved in saline. The agents were introduced via the intraperitoneal route. Diazepam was administered intraperitoneally in a dose of 5 mg per kg 10, 30, 90, and 180 min before the GABA-lytic agents. All substances used in the study were purchased from Sigma. At least six animals per dose were used and at least five doses were tested in the course of the determination of toxicity. LD_{50} was calculated using regression analysis with the method of least squares. The reliability of the differences was evaluated using Student's *t* test.

RESULTS

Table 1 presents the data regarding the toxicity of picrotoxin, bicuculline, and 3-MPA in the mice 24 hours after they had received the third injection of phenobarbital and benzonal. Under these conditions the resistance of the animals to picrotoxin and bicuculline reliably increased. For instance, in the mice pretreated with benzonal, the resistance rose by

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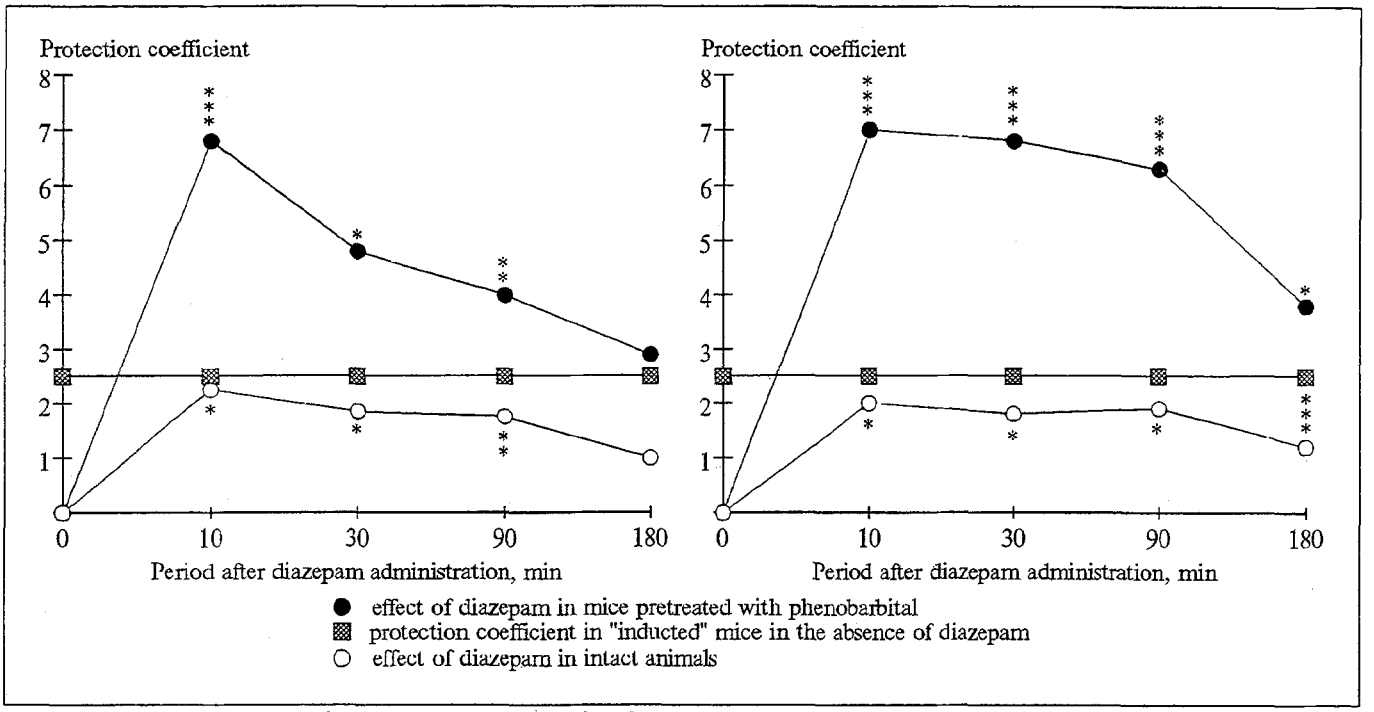

Fig. 1. Antidote efficiency of diazepam in mice treated with picrotoxin (*a*) and bicuculline (*b*). * – $p<0.05$, ** – $p<0.01$, *** – $p<0.001$ as compared to protection coefficient in "induced" mice in the absence of diazepam.

114% and 102%, respectively. After pretreatment with phenobarbital the increase of resistance was more pronounced: by 135 and 160%, respectively. However, 3-MPA toxicity remained unchanged.

In the next series of experiments we evaluated the antidote efficiency of diazepam (5 mg per kg) on GABA-lytic activity in the mice pretreated with phenobarbital (Fig. 1) Diazepam was injected 10, 30, 90, and 180 min before the toxins. As can be seen in Fig. 1, *a*, when picrotoxin was administered to intact animals, the protective effect of the antidote lasted only 90 min. The coefficient of protection (CP) at the 10th, 30th, and 90th min was equal to 2.2, 1.7, and 1.4, respectively. At these times the efficiency of diazepam was substantially enhanced by pretreatment with phenobarbital, and CP rose to 5.6, 4.7, and 4.1, respectively. In the experiments with bicuculline administration to the intact animals CP was 1.9, 1.8, and 1.8 after 10, 30, and 90 min, respectively (Fig. 1, *b*). "Induced" animals exhibited an increased level of diazepam effect at the same time points (CP was equal to 7.0, 6.9, and 6.4, respectively). These results prove the enhanced antidote activity of diazepam in the mice pretreated with phenobarbital.

TABLE 1. Toxicity of GABA–Lytic Agents in Mice Pretreated with Phenobarbital and Benzoate (40 mg per kg, Three Times at 24–Hour Intervals)

Compound	LD_{50}, mg/kg		
	control	benzonal	phenobarbital
Picrotoxin	4.56±0.66	9.78±1.19*	10.71±1.75*
Bicuculline	8,57±0.49	17.30±1.09**	22.31±2.37**
3–MPA	33.6±3.1	33.3±3.3	30.3±4.1

Note. asterisks indicate the reliability of differences: * – $p<0.01$; ** – $p<0.001$.

Barbiturates are known to modulate a number of biochemical systems related to the detoxication of xenobiotics in the organism. For instance, repeated administration of phenobarbital to rodents was reported to be accompanied by an increase in the activity of the liver monooxygenase systems [6, 7] and of carboxyl esterases [5, 6]. Induction of the detoxication systems resulted in a rise of the organism's resistance to the toxic effect of POC [5, 10, 11]. In this case, according to the opinion of several authors, increased resistance to POC results mostly from a rise of the concentration of plasma proteins, including carboxyl esterases [6, 7, 10, 11]. These enzymes are known to bind the mentioned toxins [5, 6, 10, 13]. Such a mechanism is also likely to occur in the case of GABA-lytic agents. In our laboratory it was shown that the toxicity of picrotoxin and bicuculline reliably rose in the mice against the background of a preliminary administration of triorthocresylphosphate, a substance which selectively inhibits carboxyl esterases [13].

The rise of the antidote efficiency of diazepam in our experiments is not to be explained solely by the modulation of the detoxication systems. One can assume that repeated administration of phenobarbital is accompanied by a change in the functional state of the GABA/benzodiazepin receptor complex in the animal brain. For instance, chronic treatment with barbiturates

has been reported to induce shifts of the parameters of specific binding of[^{3}H]-flunitrazepam,[^{3}H]-muscinol, and [^{35}S]-TBPS [1, 3, 8, 9, 12]. This mechanism may be involved in the potentiation of the antidote activity of diazepam. At the same time, one should also take into account the possibility of the modulation of other, non-GABA-ergic, neurotransmitter systems [2, 9].

Thus, pretreatment with phenobarbital and benzonal increased the resistance of white mice to the toxic effect of picrotoxin and bicuculline but not of 3-MPA. The antidote efficiency of diazepam increased reliably under these conditions. The effects revealed may be related to a modulation of the detoxication systems as well as to a change of the functional state of the GABA(benzodiazepin)chloroionophore-receptor complex in the animal brain.

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