Effect of 3-Orthocresylphosphate on the Toxicity of GABA-lytics for Mice

A. I. Golovko, G. A. Sofronov, T. V. Nikolaeva, N. N. Pluzhnikov, and T. V. Klyuntina

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Carboxylesterases (CE) (alyesterases) play an important role in the detoxication of several xenobiotics, including organophosphorous compounds (OPC), by their ability to bind with them [9]. Inhibition and induction of CE alter the toxicity of parathion, paraoxon, and other anticholinesterase substances [3,4,6]. The role of CE in the detoxication of GABA-lytics is still unclear. The aim of the present study was to determine the toxicity of picrotoxin, bicuculline, and 3-mercaptopropionic acid (3-MPA) for mice preliminarily injected with 3-orthocresylphosphate (3-OCP). At the same time, the effect of 3-OCP on the specific binding of ^{3}H-GABA and ^{3}H-t-butylbicycloorthobenzoate (TBOB) with the synaptic membranes of the intact animal's brain was estimated. The CE activity was measured in the mouse blood serum after injection of 3-OCP.

MATERIALS AND METHODS

The experiments were carried out on male gray mice (CBA/C57Bl)$\times F_1$ weighing 21-23 g. Picrotoxin and bicuculline were suspended in physiological solution with the aid of Tween-80. 3-OCP (125 mg/kg) was dissolved in olive oil, 3-MPA in physiological saline. All the reagents are manufactured by Sigma (USA). The solutions were injected intraperitoneally. A minimum of 6 animals and not less than 5 doses were tested when estimating the toxicity. LD_{50} was calculated by regression analysis using the method of least squares. The effect of 3-OCP on the specific binding of ^{3}H-GABA (Izotop, Russia; 1.4 TBq/mM; 20-150 nM) and ^{3}H-TBOB (Amersham, England; 1.09 TBq/mM; 5 nM) with synaptic membranes of the intact mouse brain was investigated. The preparation of the membranes and the radioligand analy-

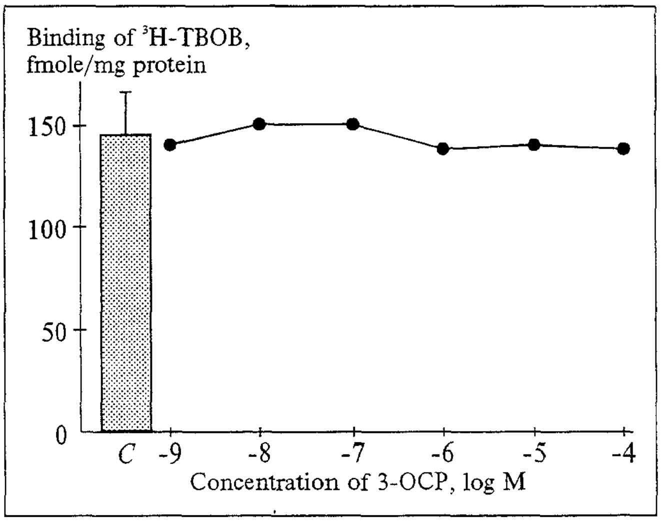

Fig. 1. Effect of 3−OCP ($10^{-9}-10^{-4}$ M) on binding of ^{3}H−TBOB (5 nM) with brain membranes of intact mouse. Ligand binding in control (C) was 138±22 fmole/mg protein. 3−OCP was dissolved in dimethylsulfoxide.

S. M. Kirov Military−Medical Academy, St. Petersburg

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TABLE 1. Toxicity of GABA-Lytics for Mice Preliminarily Injected with 3-OCP ($M \pm m$)

Reagent	LD_{50}, mg/kg		
	picrotoxin	bicuculline	3-MPA
Physiological saline	7.00±0.33	10.24±0.88	32.72±2.51
3-OCP, 125 mg/kg, 2 h before injection of GABA-lytics	4.94±0.33*	7.91±0.40*	33.11±0.84

Note. Here and in Table 2 the data with $p<0.05$ are designated by an asterisk.

sis have already been described [1,2]. The unbound label was applied on GF/B filters (Whatman). Radioactivity was estimated on a 1217 802 Rackbeta Meter. Scatchard analysis of the data was performed using regression analysis by the method of least squares. All the data were collected from 4 independent experiments carried out in triplicate. The CE activity of the mouse blood was estimated by a method previously described [11]. The protein content was determined after Lowry [7].

RESULTS

Table 1 presents data concerning the toxicity of picrotoxin, bicuculline, and 3-MPA injected 2 h after 3-OCP (125 mg/kg). The toxicity of picrotoxin and bicuculline, measured after the injection of CE inhibitor, increased by 29 and 23%, respectively. The sensitivity of the mice to 3-MPA was unchanged. The CE activity of the blood serum decreased by 89.1±4.5% 2 h after 3-OCP injection. Figure 1 presents results of the estimation of the effect of OCP (10^{-9}-10^{-4} M) on the specific binding of ^{3}H-TBOB (5 nM) with the synaptic membranes of the intact mouse brain. Under such conditions the parameters of radioligand binding were not changed. On the other hand, 3-OCP (10 μM) decreased the affinity of the GABA receptors of intact mice to ^{3}H-GABA, which was expressed in a 39% increase of K_d (Table 2). The density of the receptors remained unchanged.

3-OCP is a specific inhibitor of CE activity in the mammalian organism [6]. Preliminary injection of 3-OCP caused an increase of the toxicity of OPC [4,8]. Apparently, this phenomenon is connected with the inhibition of CE, enzymes that bind OPC [9]. The esterase activity is thought to be inhibited not by 3-OCP itself, but by its metabolites, such as phenylallylgeninphosphate [13].

TABLE 2. Effect of 3-OCP (10 μM) on the Binding of ^{3}H-GABA with Brain Membranes of Intact Mice ($M \pm m$)

Conditions	^{3}H-GABA binding	
	K_d, nM	B_{max}, fmole/mg protein
Control	43.5±3.4	840.1±67.4
3-OCP, 10 μM	60.5±5.8*	860.4±75.2

Note. 3-OCP was dissolved in dimethylsulfoxide.

The present study shows that the inhibition of the CE activity of the mouse serum by 3-OCP is attended by an increase of bicuculline and picrotoxin toxicity. We can assume that these GABA-lytics are inactivated in the organism by alyesterases. On the other hand, this mechanism is hardly likely with respect to 3-MPA, for its toxicity was not changed at all after injection of 3-OCP.

It was established that 3-OCP did not affect ^{3}H-TBOB binding with the synaptic membranes of the intact mouse brain. Thus, the increase of the picrotoxin toxicity is not connected with the alteration of the functional state of the Cl^- channel belonging to the GABA-benzodiazepine complex. Picrotoxin and ^{3}H-TBOB bind with the ionophore of the $GABA_A$ receptor.

On the other hand, 3-OCP decreased the affinity of the receptors to ^{3}H-GABA. the density of the specific binding sites of the radioligand did not change. Probably, the decreased affinity can be explained by the influence of 3-OCP on the lipid environment of the receptors. it has been established that lipotropic agents such as alcohol alter the functional state of the GABA receptors [5].

Inhibition of neurotoxic esterase of the mammalian brain is known to take place during 3-OCP intoxication [12]. Perhaps the increase of GABA-lytic toxicity can be connected with that effect of 3-OCP. However, the role of neurotoxic esterase in the realization of the effects of GABA-lytics is still unclear,

Thus, preliminary injection of 3-OCP to mice results in an increase of bicuculline and picrotoxin toxicity, but not of the toxicity of 3-MPA. 3-OCP decreased the affinity of the membrane receptors of the intact animal's brain to ^{3}H-GABA and did not influence the binding of the Cl^- ionophore ^{3}H-TBOB ligand. Potentiation of the GABA-lytic toxicity is apparently related to the inhibition of the blood CE. However, a direct effect of 3-OCP and its metabolites on the GABA-benzodiazepine receptor complex cannot be excluded.

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Effect of Amiridine and Tacrine on Potassium Currents in the Nerve Fiber

Yu. V. Burov, E. M. Peganov, and L. M. Shapovalova

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The mechanism of the positive therapeutic effect of derivatives of aminoacridine and aminoquinoline, tacrine (T) [10] and amiridine (A) [3], in dementia of different genesis and in Alzheimer's disease is still to be clarified. Analysis of the spectrum of pharmacological activity of A and T and their derivatives, as well as of a known blocker of K^+ channels, 4-AP, has cast doubt on both the traditional explanation of the action of these preparations (by their anti-cholinesterase activity) and the possibility of the action potential (AP) being markedly affected by them [1]. Despite the fact that a blocking effect of T on Na^+ and different types of K^+ channels has been discovered for various objects in a number of studies [5,7,9], the efficacy of blocking has proved to be quite low: IC_{50} for potassium currents reportedly varies from 50 to 500 μM, whereas a clinical effect has been observed for a concentration in the blood serum of just 0.02 μM.

The aim of the present study was to analyze in detail the previously discovered [2] effects of A and T on steady-state K^+ currents over the range of membrane potentials (MP) near the resting potential (RP) in connection with the possible effect of these agents on RP.

MATERIALS AND METHODS

The experiments were carried out on isolated nerve fibers of *Rana ridibunda* frogs by recording the ionic currents under MP clamp conditions after Dodge and Frankenhaueser [4] using Sigworth's modification of this method [8], which makes it

All-Russia Scientific Center for Safety of Biologically Active Compounds, Kupavna, Moscow Region. (Presented by P. V. Sergeev, Member of the Russian Academy of Medical Sciences)

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