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Pharmacodynamics of Interaction between Propoxazepam and a GABA-Benzodiazepine Receptor-Ionofor Complex

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Effects of a propyloxy derivative of 1.4-benzodiazepine, propoxazepam, on GABA-benzodiazepine receptor complexes (GABA RCs) were examined *in vitro* and *in vivo*. The parameters of propoxazepam binding to synaptosomes from the rat brain were estimated *in vitro*. The K_i constant for inhibition of [^{3}H] flumazenil binding by this agent was 3.5 ± 0.3 nM, on average. Considering the value of the GABA shift (1.9), propoxazepam can be considered as GABA-RC full agonist. On the model of picrotoxin-induced seizures *in vivo*, the propoxazepam average effective dose was estimated as 4.1 ± ± 0.21 mmol/kg. It was found that the parameters of myoclonic components (latent period of the onset of myoclonic seizures and their number), as well as death time of the tested animals, characterize adequately an anticonvulsant action of propoxazepam of picrotoxin-induced seizures in mice. Competitive interaction with picrotoxin is the possible mechanism of these effects at the level of $GABA_A$RCs. Significant deviations from a competitive model of monomolecular and cooperative binding of the agent at the receptor level have been found.

Keywords: propoxazepam, picrotoxin, GABA-benzodiazepine receptor complex, affinity, anticonvulsant effect.

INTRODUCTION

Gamma-aminobutyric acid (GABA) is the main inhibitory transmitter in the brain. GABA-mediated transmition has been identified in about 30% of the total amount of CNS synapses; it is mostly realized via GABA ionotropic receptors [1, 2]. Among the latter, $GABA_A$ receptors have been studied in more detail. These receptors are sensitive to muscimol, benzodiazepines, bicyclophosphates, barbiturates, picrotoxin, and other agents. As is believed, such GABA receptors are provided with chloride ion channels and sites of binding with benzodiazepines and barbiturates; this circumstance confirms the prospects for using receptors of this type at therapeutic targets in the case of a few pathologies [3]. Such terms as a GABA-benzodiazepine receptor-ionofor complex/ensemble, GABA-benzodiazepine ionofor, and others are frequently used in the literature. These terms reflect not only a complicate structure of the above complex, but also close contacts between its components [4]. Both GABA agonists (benzodiazepines, barbiturates) and antagonists (picrotoxin, muscimol, pentylenetetrazole) are frequently used in neurophysiological studies as probes for identification of definite structural and functional aspects of the GABA-receptor complexes (GABA RCs). From the pharmacological point of view, detailed information on the mechanism of interaction between GABA RC agonists and antagonists will considerably promote preclinical examination of the anxiolytic, amnestic, myorelaxant, sedative, soporific, and anticonvulsant actions of novel medications [5].

A number of 3-substituted derivatives of 1,4-benzodiazepine were synthetized in the Department of Medical Chemistry of the Physico-Chemical Institute of the NAS of Ukraine; their pharmacological effects were found to be somewhat unusual from a few aspects. Unlike most drugs of this class, the above agents manifested a rather

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significant analgesic activity [6, 7]. One of these compounds called propoxazepam (7-bromo-5-(o-chlorphenyl)-3-propoxy-1,2-dihydro-3H-1,4-benzodiazepine-2-one) effectively inhibited neuropathic pain (more intensely than nociceptive pain). Such curative means as gabapentine and pregabaline are extensively used in the clinical practice for inhibition of neuropathic pain of different geneses; simultaneously, these agents demonstrate antiepileptic properties [8, 9]. Considering this, we examined the analogous properties of propoxazepam in an experimental mode.

In contrast to a competitive GABA antagonist, bicuculline, picrotoxin is a noncompetitive GABA antagonist; the latter acts inside the ion channel and not on the site of GABA recognition. Benzodiazepines are directly bound to the GABA RCs; this leads to changes in their conformation followed by increases in the frequency and duration of opening of the related chloride channels [3, 4]. Considering this, we examined the process of binding of propoxazepam to synaptosomes obtained from the rat brain, the effect of this agent on the GABA shift in GABA RCs, and also interaction between the effects of propoxazepam and picrotoxin (i.e., the GABA RC antagonist) in *in vivo* experiments.

METHODS

In vivo experiments were carried out on mongrel albino mice of both sexes. The animals were kept on the standard laboratory diet, at a natural light/darkness cycle, with free access to water and food.

The tested agent (propoxazepam) was administered intraperitoneally (i.p.); this excluded the effects of absorption, a possible effect of the primary transient via the liver, and other factors affecting the dynamics of formation of the maximum concentration of the agent in the organisms' internal medium. The anticonvulsant action of propoxazepam was estimated according to its protective effect (number of survived animals in the group within a 2-h-long period after subcutaneous administration of a high dose of the seizure-inducing agent, 6.5 mg/kg picrotoxin). Within the above time interval, the latency of appearance of myoclonic tremor, generalized attacks looking as tonic extension, number of the above-type seizures, and time of death of the animal (total time of survival after administration of the seizure-inducing agent) were measured. The estimate of the lethal effect was presented in a binary form (the presence or absence of the effect). A ED_{50}, at which the probability of manifestation of the protective effect in 50% of the animals was the greatest, was selected as the quantitative criterion of the protective action of the agent. This protective effect was estimated according to primary indices within the groups (number of survived animals in separate groups). The ED_{50}value was calculated using corrected indices of the probability of the development of the effect according to the Kerber's method [10, 11].

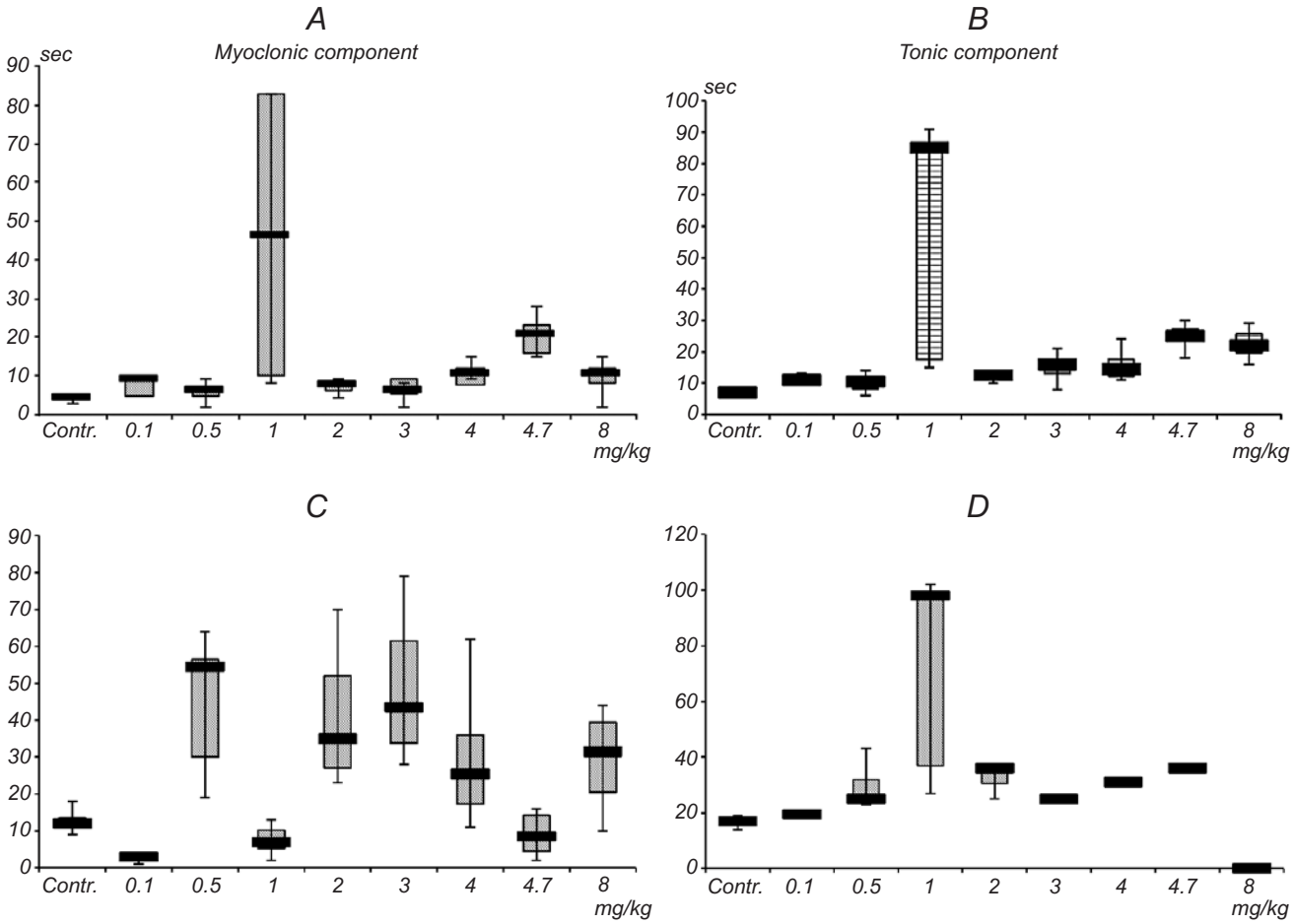
The affinity of propoxazepam with respect to GABA RCs was measured *in vitro* in a synaptosomal fraction of the membranes obtained from the rat brain (the technique of preparation was described earlier [12]); the radioligand analysis was used (a scintillation counter, Rackbeta 1219 Spectral, LKB, Switzerland).

Binding of the tested compound to the GABA RCs was estimated according to the displacement of [^{3}H]flumazenil (Ro 15-1788) from sites of its specific binding in the synaptosomal fraction of the cerebral membranes. For this purpose, the samples were incubated in the presence of 10^{-6} M nonradioactive flumazenil; the specific binding was measured as a difference between the total and nonspecific binding. For estimation of the IC_{50}, eight concentrations of the compound within a 10^{-12}–10^{-6} M range were used. The value of nonspecific binding of the radioligand (SB_0) did not exceed 10% of the total one.

The IC_{50}values were calculated by linearization of an S-like curve in the Klotz coordinates. The constant of inhibition K_i was calculated using the Cheng–Prusoff formula [13].

$$K_i = \frac{IC_{50}}{1 + \frac{[L]}{K_d}},$$

where IC_{50} is the concentration of the tested compound at which 50% of the radioligand are displaced from the sites of its specific binding with the receptors, [L] is the initial concentration of the radioligand, and K_d is the dissociation constant for a radioligand-receptor complex.



F i g. 1. Characteristics of myoclonic (A) and tonic (B) seizures in mice after picrotoxin (6.5 mg/kg) subcutaneous injection in the control (Contr.) and against the background of different doses of propoxazepam, mg/kg, administered i.p. 30 min prior to picrotoxin injection. A, B) Latencies, sec, and C, D) number of seizures, respectively.

RESULTS AND DISCUSSION

When resolving the task of our study, we should first develop methods of modeling and analysis and search for experimental pharmacological models allowing one to correlate the kinetics of the processes of formation of receptor-ligand complexes in the effector compartments of the organism with the dynamics of "postreceptor" consequences of the processes, namely changes in the parameters of functioning of an integral biological system (i.e., pharmacological effects).

According to modern concepts on realization of pharmacological effects on a molecular level, receptor macromolecules can exist in a few functionally active states (or active conformations) [14]. The effects of direct and reverse agonists or antagonists are determined by the affinity of ligands with respect to the corresponding receptor conformation. Biding of the compounds with a definite receptor form increases the probability of existence of the latter in this state and, consequently, induces definite effects on the cellular (depolarization/hyperpolarization of the membrane, synthesis of secondary messengers, etc.) or systemic (transmission of the excitatory signal or its inhibition) levels. In addition, some ligands (modulators) can induce conformation states of the receptors, which manifest their existence not in a mode of an immediate physiological response, but in some other mode. Agonists, due to their selective affinity with respect to different conformations, provide redistribution of these conformations. In particular, it was mentioned that the GABA receptor possesses agonist- and antagonist-affine conformations at biding [15, 16].

Our radioreceptor studies demonstrated that a value of the K_i in inhibition of specific binding of [^{3}H]flumazenil with synaptic membranes from the rat brain by propoxazepam is, on average, 3.5 ± ± 0.3 nM. As compared with the respective values for other benzodiazepine agents, it is a rather significant value. In particular, the analogous indices for diazepam, chlordiazepoxide, nitrazepam, and oxazepam are 6.3, 220, 6.4, and 14.0, respectively [17].

Estimation of the integral activity of the compound is a rather important moment in our study. This index can be estimated according to the value of a GABA shift in the graph of displacement of the radioligand by the examined ligand in the absence and presence of 10^{-4} M GABA. The respective calculations showed that the GABA shift for propoxazepam is equal to 1.9. This fact allows us to consider the examined compound a full agonist of GABA RCs [13]. The corresponding indices for such full agonists of the above-mentioned receptor

T a b l e 1. Statistical Parameters of Distributions for Seizures Obzerved in Mice after Picrotoxin (6.5 mg/kg) Injection against the Background of Different Doses of Propoxazepam (Pr) Administered i.p. 30 min prior to Picrotoxin (Means and s.e.m. are Shown; *n* is the Number of Animals in the Group).

Indices	Myoclonic component		Tonic component		Time to death, min
	latency, min	seizure number	latency, min	seizure number	
			Control		
Asymmetry	0.00	1.13	0.00	–0.94	–0.71
Exsess	1.50	2.23	–6.00	1.5	1.79
$M \pm m$ ($n = 4$)	4.0 ± 0.41	12.8 ± 1.89	7.0 ± 0.58	37 ± 1.47	16.8 ± 1.03
			Pr, 0.1 mg/kg		
Asymmetry	–	–1.73	0.37	0.56	–0.85
Exsess	–	–	–3.90	0.928	–1.29
$M \pm m$ ($n = 4$)	9	2.3 ± 0.7	11.3 ± 0.8	17.8 ± 1.3	19.3 ± 0.5
			0.5 mg/kg		
Asymmetry	0.94	–0.83	0.37	0.56	–0.85
Exsess	1.69	–1.76	–3.90	0.93	–1.29
$M \pm m$ ($n = 4$)	5.8 ± 0.9	45.2 ± 7.9	11.3 ± 0.8	17.8 ± 1.3	19.3 ± 0.5
			1.0 mg/kg		
Asymmetry	0.001	0.11	–2.73	0.16	–0.64
Exsess	–3.33	–0.92	7.60	–0.58	–3.08
$M \pm m$ ($n = 4$)	46.3 ± 16.4	7.5 ± 1.7	56.9 ± 14.2	11.7 ± 3.2	72.4 ± 16.6
			2.0 mg/kg		
Asymmetry	0.84	0.86	–0.38	0.36	–1.69
Exsess	–0.06	–0.81	–1.48	–0.55	–
$M \pm m$ ($n = 4$)	7.8 ± 0.8	40.8 ± 7.7	12.3 ± 0.7	24.2 ± 6.8	32.7 ± 3.8
			3.0 mg/kg		
Asymmetry	0.60	0.65	1.11	–0.14	–
Exsess	–1.74	–1.19	0.81	–1.53	–
$M \pm m$ ($n = 4$)	6.8 ± 1.1	48.7 ± 8.2	15.7 ± 1.9	15.3 ± 1.9	31
			4.0 mg/kg		
Asymmetry	–0.70	1.10	–0.55	0.04	–
Exsess	–1.62	0.92	0.06	–3.20	–
$M \pm m$ ($n = 4$)	9.7 ± 1.1	29.7 ± 7.7	15.2 ± 1.9	29.3 ± 9.5	25 ± 1
			4.7 mg/kg		
Asymmetry	–0.3	0.1	–0.8	2.2	–
Exsess	–1.4	–1.8	1.5	4.7	–
$M \pm m$ ($n = 4$)	19.6 ± 1.8	9.0 ± 1.9	25.0 ± 1.7	9.7 ± 6.7	36
			8.0 mg/kg		
Asymmetry	1.1	–0.5	0.13	0.67	–
Exsess	1.5	–1.3	–1.01	–0.45	–
$M \pm m$ ($n = 4$)	11.0 ± 1.9	29.3 ± 5.4	22.5 ± 1.9	2.2 ± 0.5	–

as diazepam and flunitrazepam are equal to 2.89 and 2.73, respectively.

To great regret, it should be taken into account that experimental data obtained in *in vitro* experiments are confirmed under *in vivo* conditions not in all cases. Rapidly reversible effects of agonists of benzodiazepines against the background of administration of the antagonists are the most prospective direction in the studies of interaction between the ligand and the receptor. The measured minimum effective doses of convulsion-inducing agents can be related in this case to a certain definite time interval of the experiment. The respective data can be presented in both binary and gradual forms [18].

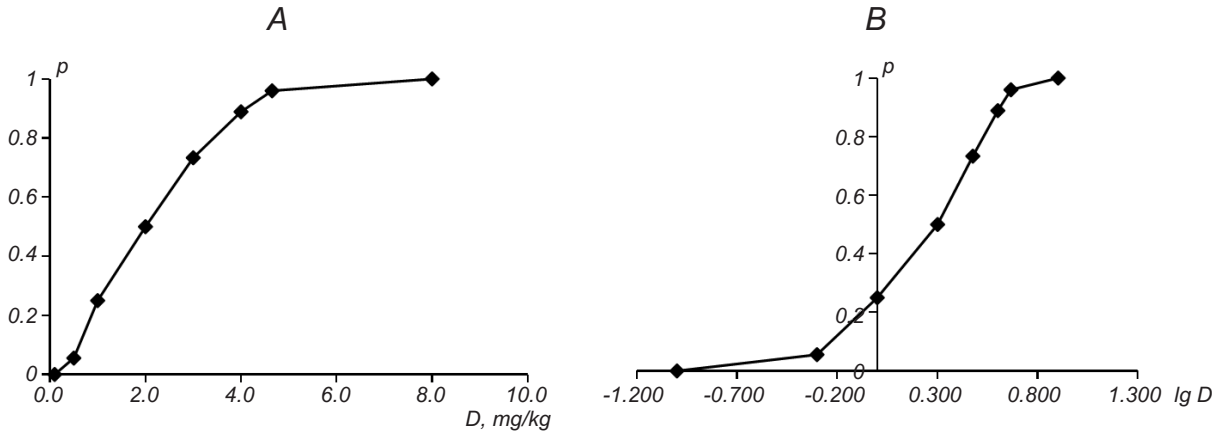
Within the framework of such approach, the functioning of GABA RCs in the CNS can be examined *in vivo* by estimation of the competitive effects of benzodiazepines and seizure-inducing agents, picrotoxin in particular. The picrotoxin binding sites are localized in the molecules of GABA RCs; this is why a decrease in the seizure-inducing efficiency of picrotoxin is interpreted as manifestation of the inhibitory action of the examined compound realized via the mechanisms of GABA binding.

The dose-effect dependence for the protective action of the propoxazepam in semilogarithmic coordinates demonstrated a classic S-like shape (Fig. 2). The mean value of ED_{50} (1.67 ± 0.09 mg/kg) for this compound, considering its molecular mass (407.7 g/mol) is 4.1 ± 0.21 μmol/kg (Table 2). The ED_{50}value calculated by a probit-method was 2.72 ± 0.70 mg/kg (6.67 ± 1.72 μmol/kg. These values differ somewhat from the above-mentioned ones; such a discrepancy is probably related to different approaches in mathematical treatment of the experimental data. In the probit technique, data corresponding to a central part of the dose-effect curve are mostly used; thus, in the case of a flat pattern of the dependence at high doses, a "shift" of the calculated ED_{50} value toward greater figures is observed (similarity to what was found in our case). The Kerber's technique that takes into consideration all data of the curve and averages this data toward a symmetric form gives values corresponding to a more "symmetrical" development of the effect. Nonetheless, the data obtained by both methods of calculation differ from each other statistically insignificantly ($P > 0.05$). The above-mentioned form of the dose-effect curve is similar to that obtained in *in vitro* experiments where parameters of receptor binding were estimated; this also confirms a concentration-dependent pattern of the protective effect of propoxazepam in the case of its antagonism with picrotoxin

Using an antagonistic pharmacological model, however, allows researchers to characterize the competitive mode of the process also under *in vivo* conditions. Thus, the recorded effect is an integrative characteristic of the process of binding with the respective conformational forms of the R_{GABA} receptor with propoxazepam (Pr) and picrotoxin (PT)

$$\left[R^{Pr.}_{GABA}Pr\right] \underset{{}^{Pr.}k_{+1}}{\overset{{}^{Pr.}k_{-1}}{\rightleftarrows}} \left[Pr.\right] + \left[R^{Pr.}_{GABA}\right] \rightleftarrows R^{0}_{GABA} \rightleftarrows$$

$$\rightleftarrows \left[R^{PT}_{GABA}\right] + \left[PT\right] \underset{{}^{PT}k_{-1}}{\overset{{}^{PT}k_{+1}}{\rightleftarrows}} \left[R^{PT}_{GABA}PT\right]$$



F i g. 2. Dose-effect curves for the action of propoxazepam on picrotoxin-induced seizures in the linear (A) and semilogariphmic (B) scales. Abscissa) Dose of propoxazepam, mg/kg (A) and logarithm of the dose, LGD (B); ordinate) frequency of the effect in the binary form.

T a b l e 2. Estimation of the Mean Propoxazepam Effective Dose (ED_{50}) in the Test of Picrotoxin-Induced (6.5 mg/kg) Seizures Using the Kerber Method

Dose		Number of animals in the group	Presence or absence of the effect		Corrected number of the presence/ absence		Number of animals for the corrected effect	Frequency of the effect	Area under the dose-effect curve	
mg/kg	lg D		«–»	«+»	«–»	«+»	*N*	«+»	*S*	m_s
10	1	4	0	4	0	34	34	1	–	–
8.0	0.903	6	0	6	0	30	30	1	0.289	–
4.7	0.667	9	1	8	1	24	25	0.96	0.169	$3.625 \cdot 10^{-5}$
4.0	0.602	6	1	5	2	16	18	0.889	0.221	$5.262 \cdot 10^{-5}$
3.0	0.477	6	2	4	4	11	15	0.733	0.239	0.0003164
2.0	0.301	6	3	3	7	7	14	0.5	0.151	0.0010944
1.0	0.000	8	5	3	12	4	16	0.25	0.056	0.0011327
0.5	–0.301	6	5	1	17	1	18	0.056		
0.1	–1.000	4	4	0	21	0	21	0		
								lg D	0.223	0.051
								D, mg/kg	1.67	0.09

Level of significance	**Dose range**	
	minimal	**maximal**
0.1	1.531	1.815
0.05	1.503	1.843
0.01	1.449	1.897

At a fixed dose of the injected picrotoxin (that evoking the effect in 95% of the cases), the antocinvulsive effect is a function of the ratio between the numbers of receptors occupied by propoxazepam $\left[R^{\text{Pr.}}_{GABA}\,\text{Pr.}\right]$ and by picrotoxin and is proportional to an equilibrium concentration of propoxazepam with a definite proportionality coefficient. The latter is a ratio between the constants of binding of picrotoxin ($K^{PT}_{bound} = \dfrac{\left[R^{PT}_{GABA}PT\right]}{\left[R^{PT}_{GABA}\right][PT]}$) and propoxazepam ($K^{\text{Pr.}}_{bound} = \dfrac{\left[R^{\text{Pr.}}_{GABA}\right]}{[\text{Pr.}]\left[R^{\text{Pr.}}_{GABA}\right]}$) with the corresponding conformational forms $\left[R^{\text{Pr.}}_{GABA}\right]$ and $\left[R^{PT}_{GABA}\right]$:

$$\frac{\left[R^{\text{Pr.}}_{GABA}\right]}{\left[R^{PT}_{GABA}\right]} = \frac{K^{PT}_{bound}}{K^{\text{Pr.}}_{bound}}[\text{Pr.}]$$

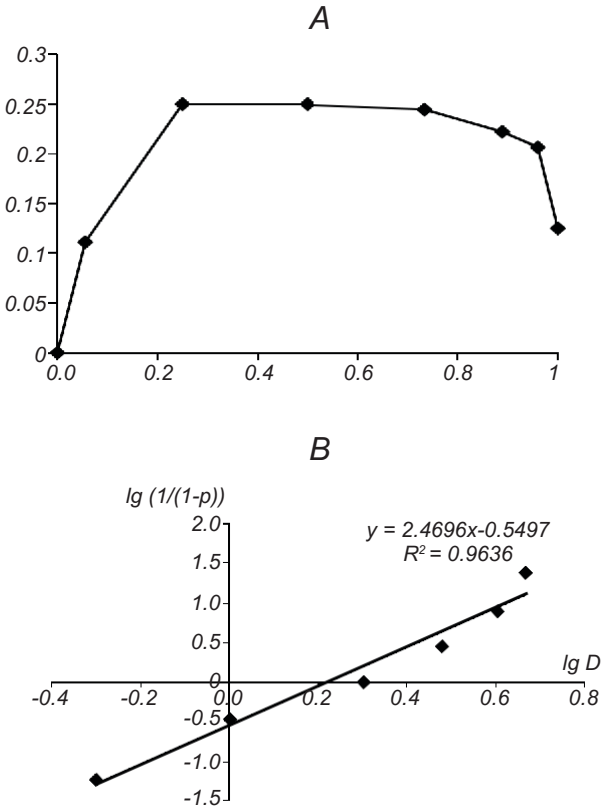
Thus, under *in vivo* conditions and, in particular, in the used model of competitive receptor interaction, there is a possibility to characterize the relative efficiency of the compound and the comparative mechanism of receptor interaction using only an integral pharmacological effect.

In a simplified form, the number of receptors occupied by propoxazepam and probability of the effect development, which are dependent on the introduced dose of the examined compound, can be presented in a form similar to the Michaelis–Menten equation (it should be supposed that the processes of the mass transition of the compound to the biophase of its action are linear):

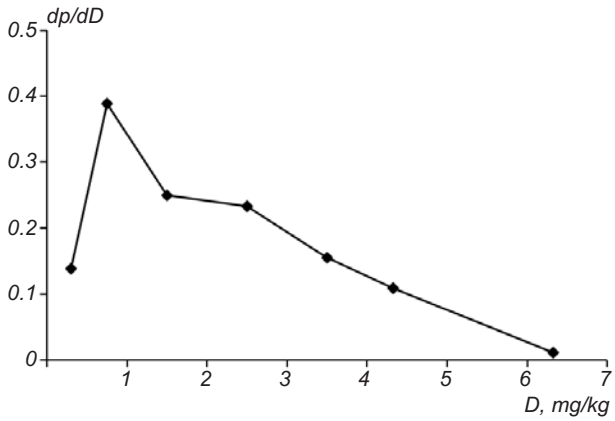
$$\left[R^{\text{Pr.}}_{GABA}\,\text{Pr.}\right] = \frac{[\text{Pr.}]}{\dfrac{K^{PT}_{bound}}{K^{\text{Pr.}}_{bound}} + [\text{Pr.}]}$$

Direct estimation of the equilibrium concentration of propoxazepam in the brain under *in vivo* conditions is a rather difficult task. At the same time, pharmacokinetic studies showed that the propoxazepam concentration depends linearly on the administered dose. Due to this, a formally administered dose of the compound D can be used instead of the equilibrium concentration of the agent, and the competitive process of interaction of propoxazepam with picrotoxin can be characterized on a receptor level. In particular, a graph of the function in the coordinates $\left(\left[R^{\text{Pr.}}_{GABA}\,\text{Pr.}\right]\Big/[\text{Pr.}];[\text{Pr.}]\right)$, which corresponds

to the Sketchard coordinates, demonstrates a significant deviation from a simple monomolecular competitive model (Fig. 3A). In the Hill coordinates $\left(\log\left(p/1-p\right); \log D\right)$, the slope of the graph equal to 2.47 (Fig. 3B) shows that there is positive cooperative interaction within the complex "picrotoxin–propoxazepam–$GABA_A$ receptor." Because a rapidly reversible process of competition with picrotoxin is analyzed, and not the process of binding of propoxazepam with the $GABA_A$ receptor, the value of crossing of the graph with the abscissa corresponds to the ratio of the binding constants. This relation shows that the affinity of propoxazepam corresponds to only 59% of the affinity of picrotoxin to the receptor. When we take into account that the $GABA_A$ receptor has two sites of binding for benzodiazepines on subunits α and γ, the fact of cooperativity of binding of these compounds with the $GABA_A$ receptor can result from localization of binding sites for picrotoxin within the cavity of the chloride channel [19]. These sites become more accessible after preliminary interaction of the receptor with propoxazepam and at preservation of the open conformation of the receptor for a long time. This supposition is confirmed by the analysis of a differential graph for the dose-effect relation (*dp*/*dpD*; *D*), where a noticeable maximum of the rate of change of the effect is observed at introduction of small propoxazepam doses (Fig. 4). The presence of the maximum within this dose range and an asymmetric pattern of the dependence correspond to the equilibrium in the competitive interaction picrotoxin/propoxazepam at the doses close to ED_{50} (Fig. 4).



F i g. 3. Dose-effect curves for the action of propoxazepam on picrotoxin-induced seizures in the Sketchard (*p*/*D*; *p*) and Hill (lg (*p*/(1-*p*); lg *D*) coordinates (A and B, respectively).



F i g. 4. Dependence of the rate of change (*dp*/*dD*) of the anticonvulsive effect of propxazepam on the dose of the later.

Generalization of excitation in the brain with the development of a lethal effect is the final stage in a sequence of the stages of blocking of the inhibitory function of the GABA-ergic system. Within this period, however, intermediate stages of competitive interaction between propoxazepam and picrotoxin are reflected at a physiological level as patterns of involuntary muscle contractions with definite values of the frequency and intensity; these patterns characterize the rate of development and severity of an epileptiform attack. This is why we recorded separate components of picrotoxin-induced seizure attacks corresponding to different levels of generalization of excitation in the brain (developing due to suppression of the inhibitory effect on the $GABA_A$-ergic system). Such separate phenomena, namely myoclonic tremor (head, limb, or body twitches), clonic seizures looking as alternative episodes of high-amplitude tremor, tonic seizure attacks (unexpected muscle spasms and short body movements) evoked by simultaneous contractions of flexor and extensor muscles, constrained body position, epileptic attacks in the full sense, etc.,

were recorded. We also recorded the time points of initiation of separate components and time of the death of the animal. The latter index is especially important considering that it reflects generalized spreading of abnormal processes of excitation across the brain cortex and other brain structures. This spreading is not limited by the development of pathological reflex excitation of the autonomic nervous system and the effect on the respiratory center in particular. Nearly in all cases, animals perished because of spastic paralysis of the respiratory musculature and mechanical asphyxia.

Analysis of the structure of numerical experimental data for each index showed that the respective distributions were usually characterized by significant values of the coefficients of asymmetry and excess (Table 1). Thus, these distributions differed in most cases from Gaussian ones (Table 1), which makes necessary using nonparametric criteria (Wilcoxon–Mann–Whitney criterion in particular) for the comparison of the data.

The asymmetry coefficient close to that typical of the normal distribution (within a –0.7 to +0.7 range) was found only for the times of development of separate seizure components. This fact allows us to conclude that the mean time of manifestation of the epileptic components is a value dependent only on the rate of coming of the convulsive agent from the site of introduction to the brain; it is also determined by individual peculiarities of the animals. Among the analyzed indices, only the latencies of myoclonic seizures at most administered doses of the agent demonstrated symmetric distributions of the experimental data. *Vice versa*, the asymmetry coefficient for such an index as the number of clonico-tonic seizures was much more variable (especially in the control animal group and at administration of small doses of the examined compound). The structure of primary values of this index demonstrated bidirectional variations; in the control group, most animals showed a greater, as compared with the mean, number of clonico-tonic seizures (asymmetry coefficient 1.13). At the same time, this trend was weaker against the background of preliminary administration of propoxazepam. The asymmetry coefficient for groups of animals injected with 0.1 and 0.5 mg/kg propoxazepam was within a 1.73 to –0.83 range. Probably, such small doses of the agent suppressed initial processes of excitation under the influence of picrotoxin, but did not affect their generalization within later time intervals. At the same time, administrations of greater doses resulted in competitive interaction of the convulsive and anticonvulsive agents, which was realized in nearly equal probabilities of initiation of tonic seizures. The absence of a symmetric pattern of the data may result from the heterogeneity of animal samplings in the groups. The animals could belong to different genetic strains, and this can determine significant variations in the number of the respective receptors, affinity (capability of binding the compound), and physiological peculiarities of neuronal connections in separate animals.

It looks expedient to present the above indices in a mode reflecting more adequately the structure of their distributions, i.e., to show their central values (medians) and values of the first and third quartiles [11]. Among the presented data (Fig. 1), only indices of the number of tonic seizures and time to the animal's death demonstrated a dose-dependent pattern and, respectively, can be considered related to the mechanism of the development of the effect, namely that related to competitive interaction of the effects of picrotoxin and benzodiazepine at the $GABA_A$ receptor complex. Values of the latency of mioclonic seizures at the action of 8 mg/kg of the agent demonstrated an analogous pattern. At the same time, the number of tonic seizures, i.e., the index most directly reflecting the process of spreading of excitation within the brain, at doses of 2.0, 3.0, 4.0, 4.7, and 8.0 mg/kg, demonstrated differences from the control group with $P \leq 0.26$, $P \leq 0.02$, $P \leq 0.28$, $P \leq 0.33$, and $P \leq 0.67$, respectively. On the contrast, other indices (duration and numbers of myoclonic components of the epileptic attack, total time until animal's death, etc., demonstrated significant deviations from those in animals of the control group. Therefore, myoclonic seizures reflect the development of excitatory processes due to the action of picrotoxin and their inhibition against the background of action of propoxazepam to a greater extent. The myoclonic component is probably closer to the equilibrium state in the competitive process of interaction between picrotoxin and propoxazepam at a neuronal level; because of this, it looks as the most significant index characterizing the antiseizure activity of propoxazepam. At the same time, in the case of using of other convulsive agents with sites of binding on the $GABA_A$ RC and with interaction with other transmitter systems differing from those of picrotoxin, other components of the epileptic attack will predominate.

Thus, considering both data of radioligand binding ([^{3}H]flumazenil inhibition binding constant Ki = 3,5 ± 0,3 nM) and the GABA-shift value (1.9), propoxazepam can be regarded as the GABA-RC full agonist. This agent possesses a significant anticonvulsive action *in vivo* (in a picrotoxin-induced seizure model, its ED_{50} is 4.1 ± 0.21 mmol/kg). On the base of analysis of the dose-effect curve, the possible competitive mechanism of picrotoxine and propoxazepam has been suggested. Certain deviations from the monomolecular competitive model and indications for cooperative binding on the receptor level have been found.

Experiments on animals were conducted in compliance with the Helsinki declaration 1975, revised and supplemented in 2000, as well as Directives of National Committees for Ethics in Scientific Researches. Conducting of the experiments was agreed with the Ethics Committee of the Bogatsky Physico-Chemical Institute of the NAS of Ukraine. The work complies with modern rules for the maintenance and use of laboratory animals that comply with the principles of the European Convention for the Protection of Vertebrate Animals used for experiments and other scientific purposes (86/609/EEC, Strasbourg, 1985).

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