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THE USE OF PROPOXAZEPAM FOR TREATMENT A SPECIFIC EPILEPTIC SYNDROME (PAROXYSMAL MANIFESTATIONS), WHICH IS ACHIEVED BY POLYMODAL MECHANISM OF ANTICONVULSANT ACTION: LITERATURE REVIEW OF OWN PRECLINICAL RESEARCH

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INTRODUCTION

Epilepsy refers to chronic polyethyologic diseases of the brain characterized by recurrent seizures that occur as a result of excessive neuronal discharges and accompanied by various clinical and paraclinical symptoms. Anticonvulsant therapy remains the basis for treating patients with epilepsy, which involves inhibition or a significant reduction in the number of attacks[1]. Currently, the term antiepileptic are synonymous with anticonvulsant agents as they all selectively suppress seizure and their use is determined predominantly by the nature of paroxysmal manifestations or its equivalents. Depending on the clinical manifestations of epilepsy, different anticonvulsants can be prescribed. Often, for the treatment of epilepsy, combined use of several medicines is rational (simultaneously or sequentially). Therefore, the success of the treatment of epilepsy is on the way to finding new anticonvulsants, which would have had an effect on different pathogenetic links in the formation of all variability of seizure states.

Antiepileptic drugs act on different molecular targets, selectively changing the excitability of neurons in such a way that the neuronal activity associated with attacks is blocked without disturbing the normal activity required to transmit signals between neurons. Various mechanisms can lead to reducing the excitability of the neurons of the epileptogenic cell. Basically they consist either in inhibiting activating neurons, or in activating depressing nerve cells, i.e., they are reduced to three major pharmaco-neurophysiological effects[2]: relief of GABA or glycine-dependent transmission, reduction of excitatory (glutamate or aspartate) transmission

[1] Muro VM, Connolly MB. Classifying epileptic seizures and the epilepsies. In: Epilepsy. ed. Miller JW, Good-kin HP, 2014. Wiley, Chichester, UK. P. 10–14.

[2] John G.R. Jefferys**.** Advances in understanding basic mechanisms of epilepsy and seizures. *Seizure*. 2010. N 19. P. 638–646. DOI 10.1016/j.seizure.2010.10.026.

and non-specific modification of ion currents (sodium, calcium and potassium channels). At the same time it is believed that the most effective control of seizure readiness is realized precisely because of GABA-ergic mechanisms. In addition, it is possible to increase the level of GABA in the neuron, not due to inhibition of its metabolism, but due to the increased synthesis of GABA. Despite the fact that these medicines take maximum account of the mechanisms of epileptogenesis, the issue of improving their effectiveness is still relevant. Antiepileptic drugs have a number of side effects, among which a special place is taken by muscular tone and neurological and cognitive deficits[3].

At present, specialists allocate up to forty different types and forms of epilepsy, and in this regard, for each form, physicians appoint a separate therapeutic scheme. Most animal tests used in epilepsy studies are models of epileptic seizures, not epilepsy models. Since epilepsy is characterized by spontaneous recurrent convulsions, even a test as a maximal ejector (MES), in which acute attacks are induced electrically in normal non-epileptic animals, can not be considered as a model of epilepsy. Given the fact that the choice of antiepileptic drugs depends on the type of seizures (specific epileptic syndrome) that in most forms of epilepsy have the same neurophysiological nature (partial, tonic-clonic, absentees, myoclonic, atonic-tonic) in the experimental pharmacology, the search for antiepileptic drugs is concentrated on their anticonvulsant actions. Drugs should have the ability to warn in animal experiment seizures caused by specific electrical or chemical convulsants. In this case, the convulsants should interact with the appropriate biomass and cause certain attacks that are characteristic of the course of human epilepsy. The selectivity of this effect in some representatives of anticonvulsants is expressed in different ways. For some substances, approximately the same activity in relation to the experimental seizures of one or another origin is characteristic, for others it is absent.

The aim of this work was the 7-bromo-3-propoxy-5 (2-chlorophenyl) – 1,3-dihydrobenzo [e] [1; 4] diazepin-2-one (Propoxazepam) pharmacological profile analysis on the models of different seizures (chemically and electrically induced) and the determination of the propoxazepam influence on manifestations and redistribution on different seizures for pharmacological indices calculation.

[3] Panayiotopoulos C.P. The epilepsy: seizures, syndromes, and management. Chipping Norton: Bladon Medical Publishing; 2005.

1. Materials and methods

Propoxazepam was synthesized according to the method described earlier[4]. The structure of the substance was determined and approved by a complex of physicochemical methods (IR and mass spectroscopy, as well as X-ray diffraction analysis). Chemical purity was confirmed by elemental analysis (99%).

Experiments were performed on males and females of outbred white mice weighing 20–22 g. All experimental procedures were conducted in accordance with the rules of the "European Convention for the Protection of Vertebrate Animals, Used for Experimental and Other Scientific Purposes" in accordance with the Directive of the Council of the European Union 86/609 of the EU of November 24 1986. During experiments, the animals were kept under standard conditions (12 h lightshade mode and with access to water and food *ad libitum*). The use of differect seizures inducers (picrotoxin, penthylenetetrazole, strychnine, thiosemicarbazide, bemegrid, 4-aminopyridine and maximal electroshock allowed us to model different paroxyzmal manifestations and to suggest the Propoxazepam mechanisms of antiseizure action. The test compound was administered intraperitoneally (in a tween emulsion) at doses, whose choice of boundaries was based on previous pilot studies and the requirements of statistical and calculation methods. Solutions of chemoconvulsants (at doses causing the lethal effect in 95% of animals) were administered subcutaneously to animals (6–8 mice in each experimental group) 30 min after Propoxazepam. The counting time of the experiment started from the moment of seizure agent administration. During the observation period, the number of myoclonic tremors and generalized attacks in the form of tonic extensia, as well as the time before the onset of the lethal effect, were recorded. To characterize the representativeness of each seizure types, the experimental data are presented in the relative form ($M \pm m$) of the total number of recorded seizure episodes. Since the mechanism of convulsions development under the action of thiosemicarbazide involves a long exhaustion of endogenous GABA, the observation of animals was carried out within 3 h, indicating the number of individual components of convulsive attack (myoclonic tremor, generalized attacks in the form of tonic extension, the total number of cases of these types, and the time of their manifestation) and total time to the lethal effect, marking the time periods since the chemoconvulsant administration. During the next 24 h, the endlethal effect was recorded in each group of animals, which gave an opportunity to evaluate the possible timedependent effects of propoxazepam and the general characteristic of its protective effect.

[4] Reder A., Larionov V., Golovenko N., Andronati S. Influence of particle size on the anticonvulsant activity of propoxazepam. *To Chemistry Journal*. 2019. 2. P. 132–141.

The analysis of the lethal effect was carried out in an alternative form, by the number of animals that survived in experimental groups. Evaluation of the lethal effect was carried out in an alternative form (presence or absence of effect). The protective effect of the substance (the value of ED_{50}) was estimated by the number of animals (frequency of effect) that survived in each individual group. The calculation of ED_{50} values was carried out using the probability of developing the effect by the Kerber method (corrected by the Barren method) and probit analysis[5]. The statistical significance of the differences between the control and experimental groups for the individual parameters of convulsive activity (after the previous analysis for compliance with the normal distribution law), as well as the final experimental data, were assessed on the basis of the Student's criteria or nonparametric statistics methods Wilcoxon–Mann–Whitney Criteria[6].

2. Analysis of the interaction of Propoxazepam with antagonists of GABA and glycine receptors

In order to study the interaction of Propoxazepam with receptors, we used the pharmacological analyzers picrotoxin, penthylenetetrazol and strychnine, which allowed to simulate various paroxysmal manifestations and, with a certain degree of reliability, judge its mechanisms of anticonvulsant action.

Picrotoxin is a non-competitive antagonist since it acts within the ion channel of the GABA-receptor complex (GABA-RC) and reduces the seizure of picrotoxin as a manifestation of the inhibitory effect of the test substance that is realized through the mechanisms of interaction of the compound with pointed biotarget.

Strychnine is a classical competitive blocker of the glycine receptor, low doses of which promote excitation, and large causes generalized tonic seizures with severe pain syndrome, mainly of central origin.

As a chemoconvulsant, penthylenetetrazole is most commonly used. In this procedure, convulsions simulate primary-generalized seizures in so-called abscess seizures. In moderate doses, the administration of penthylenetetrazole leads to the development of clonic, and in high tonic-clonic and generalized seizures (epistatus) and even the death of an animal. Penthylenetetrazole is a ligand of both GABA-RC and glycine-erosive system, although its binding sites are identified in the chloro-ionophore

[5] Urbach V.U. Statistical analysis in biological and medical research. M. Medicine, 1975. 297 p.

[6] Van der -Varden B. Mathematical statistics. M. Publishing Foreign Literature 1960. 436 p.

channel of GABA-RC, which is why its effect is often characterized as "noncompetitive" with respect to derivatives of 1.4-benzodiazepines, which exhibit anticonvulsant effect.

For conducting an effector analysis of the mechanism of anticonvulsant activity of Propoxazepam on models of chemically induced convulsions, its values of mean weight effective doses (ED_{50}) were determined. Solutions of chemoconvulsants (at doses causing a lethal effect in 95% of animals) were: picrotoxin-6,5 mg/kg; pentylenetetrazol – 120 mg/kg; strychnine-2 mg/kg. The main point index of the pharmacological activity of the Propoxazepams the dose at which the probability of developing the effect in 50% of the animals will be maximum (ED_{50}). However, not always the initial experimental data correspond to such a distribution, being a generalized characteristic of the body's response. Thus, the initial indices of anticonvulsant activity of Propoxazepam for antagonism with picrotoxin and penthylenetetrazole are characterized by an achievement of 100% effect, while the antagonism with strychnine administration of even high doses of Propoxazepam (up to 30 mg/kg) does not allow to achieve such an indicator. On the hand, whith using high doses of Propoxazepam, even a "reverse", paradoxical effect is recorded. The ED_{50} values of the protective effect of Propoxazepam upon administration of chemoconvulsants were determined after correction of the experimental data by the Barrence method and subsequent representation in semilogarithmic coordinates. Only for the GABA-RC ligand antagonists the shape of the curves is closer to the classical sigmoid, whereas for an antagonism with strychnine, the maximum achievable protective effect is recorded at the level of 70–80. The values of average weighted effective doses (ED_{50}) of Propoxazepam for antagonism with picrotoxin 1.67 ± 0.09 mg/kg, penthylenetetrazole 0.9 ± 0.04 mg/kg and strychnine, 14.24 ± 0.47 mg/kg, which indicate to the high activity of the substance on the basis of data of "dose-effect" curves[7]. Indicators of ED_{50} are statistically significant ($p < 0,03$, Student's criteria) differ from each other, even in the case of picrotoxin and penthylenetetrazole. Since the antagonism with the penthylenetetrazole compound and exhibits a higher activity, then GABA-ergic and partially glycine-ergic system are likely to be involved. On the model of strychnine seizure, the activity of Propoxazepam is almost an order of magnitude lower and is 40.33 ± 14.91 mg/kg, which indicates a small participation of glycine – an ergic system in the

[7] Golovenko N.Ya., Larionov, V.B., Reder, A.S., & Valivodz' I.P. An effector analysis of the interaction of propoxazepam with antagonists of GABA and glycine receptors. Neurochemical Journal. 2017. 11(4). P. 302–308. DOI 10.1134/S1819712417040043.

implementation of anticonvulsant activity of the compound. This is confirmed by the higher value of the angle of inclination of the dose-effect curve (1,368 for the Strychnine seizure model – in this model, the protective action of Propoxazepam is recorded in a wider range of doses, whereas the sloping nature of the inclination the corresponding curves for picrotoxin (0.789) and penthylenetetrazole (0.851) predict the concentration-dependent nature of the development of the effect. The ratio of the curvature values of the "picrotoxin/strychnine" and "penthylenetetrazole/strychnine" curves (0.57 and 0.6, respectively) are close to the magnitude of the maximum reported anticonvulsant effect of Propoxazepam on the pattern of strychnine convulsions.

On the basis of the obtained data using the comparative nonparametric analysis of the paired curves "dose-effect" we can conclude that the protective (anticonvulsant) effect of Propoxazepam on the model of strychnine convulsions in low doses (to ED_{50}) also involves mechanisms that are implemented and with its antagonism from picrotoxin. This allows us to assume a significant contribution of GABA-system in this dose range. Glycine is an ergic component of arresting strychnine convulsions involved in the administration of the compound and in doses exceeding ED_{50}. Despite the close nature of the interaction with GABA-RC picrotoxin and penthylenetetrazole, the protective effect of Propoxazepam on antagonism with these agents is different. This is due to differences in the mechanisms of action of these chemoconvulsants (picrotoxin is direct and penthylenetetrazole is an indirect antagonist). An analysis of the experimental data structure of the generalization of the excitation of animals showed that they do not correspond to the normal distribution law and are characterized by large differences in the parameters of asymmetry and excess. Only the values of the lifetime of animals exhibit dose-dependent effects, while the indicators of time and number of development of myoclonic and tonic components of convulsions are parabolic. However, against the background of the administration of various doses of Propoxazepam there is a statistically significant ($p \leq 0,02$) increase in lifetime. In general, Propoxazepam significantly increased (2.5–3,0 times) the lifetime of animals after the administration of seizure agents, and at high doses (3 mg/kg for penthylenetetrazole and 8 mg/kg for picrotoxin) had a protective effect.

Normalized indices of the separate contributions of myoclonic and tonic components (as the ratio of the number of each type to the total amount of the seizure) differ in the structure of the convulsive attack caused by various chemoconvulsants[7]. Thus, with the administration of low doses of Propoxazepam in the structure of animal convulsions, the tonic component

intensity of which (after the administration of picrotoxin or penthylenetetrazole) is reduced in proportion to the increase in the administered dose of Propoxazepam, reflecting a preferential increase in the intensity of the inhibitory processes that occur in the central nervous system and reducing the formation of paroxysmal cells activity.

3. Model of GABA-deficient thiosemicarbazide-induced convulsions

Pathological action of semicarbazide is carried out by blocking the key enzyme for the synthesis of isomer GAD65 GABA – glutamate decarboxylase, which catalyzes the formation of GABA for neurotransmitter needs, as a result of which its reserves in the nervous tissue are not replenished, which causes insufficient restriction of excitatory processes[8]. Typically, this test is used for biological screening of compounds whose anticonvulsant activity is due to affinity for GABA-RC under conditions of GABA deficiency. This model simulates primary generalized seizures. The test compound was administered intraperitoneally at rising doses (0.01–20 mg/kg) for 0.5 hours prior to the administration of the seizure agent (thiosemicarbazide, 20 mg/kg, subcutaneously). Since the mechanism of excitation development under the action of thiosemicarbazide involves a long exhaustion of endogenous GABA, observation of animals was carried out within 3 hours, indicating the number of individual components of convulsive attack (myoclonic tremor, generalized attacks in the form of tonic extension, the total number of cases of these types and the time of their manifestation) and total time to the lethal effect, marking the time periods since the chemoconvulsant administration. Also during the next 24 hours, the end-lethal effect was recorded in each group of animals, which gave an opportunity to evaluate the possible time-dependent effects of Propoxazepam and the general characteristic of its protective effect. The analysis of the lethal effect was carried out in an alternative form, by the number of animals that survived in experimental groups.

For the overall protective effect (decrease in mortality in the groups), during the three hours of observation, Propoxazepam proved to be quite active: up to 90% of the animals were exposed to protective effects when administered at a dose close to 0.2 mg/kg. However, mortality in animal groups receiving lower doses of Propoxazepam was higher with observation over 24 hours due to not only the duration of toxic effects of thiosemicarbazide, but also the effect on the binding of GABA to the corresponding receptor complex, and not just the

[8] Panosyan E. H., Lin H.J., Koster J., Lasky J. L. In the search for druggable targets for GBM amino acid metabolism. BMC Cancer. 2017. 17. P. 162–171. DOI 10.1186/s12885-017-3148-1.

synthesis of GABA in the brain tissue[9]. It should be noted that the protective effect of Propoxazepam develops rather quickly, and the quantitative characteristic of the rate of development of the process (slope of the dose-effect curve) confirms the concentration of the antagonism of Propoxazepam and thiosemicarbazide in this model, indicating the possible receptor nature of the implementation effect.

Taking into account the fact that the average effective dose of Propoxazepam (ED_{50}) is not statistically different during the observation of 3 and 24 hours, it can be concluded that the protective effect of the compound for a long time is less than in the acute period of development of the convulsive state under the conditions of GABA -deficiency Such a partial increase in the calculated ED_{50} may be due to several reasons, of which both neurochemical (exhaustion of GABA stock in the corresponding synapses and dissociation of the GABA receptor complex) and pharmacokinetics (decrease in the concentration of Propoxazepam in the biophase of action due to metabolism and elimination, because of compounds is ~ 0.02 h^{-1}, which corresponds to a half hour of elimination for 36 hours)[10].

The first manifestations of convulsive activity in animals begin to appear at the first minute after the administration of thiosemicarbazide (control group), whereas administration of Propoxazepam in a dose of 0.01 mg/kg increases this time to ~ 70 minutes. As the dose increases, this figure is gradually increased, but for animals of each experimental group a small distribution of the values obtained is noted (individual variations of this index do not exceed 1-2 minutes), which is explained by the high rate of absorption of thiosemicarbazide after its subcutaneous administration. It is unusual, at first glance, to reduce the time of development of myoclonic seizures and a certain increase in their number in conditions of increasing dose of Propoxazepam. However, latent time of manifestation of tonic seizures is increased and decreases (and at high doses at all disappears) their number in the experimental groups of animals. Such a redistribution of representations of various severity by the seizures is a consequence of increasing the effectiveness of the inhibitory processes in the central nervous system.

[9] Golovenko M. Ya., Reder A. S., Larionov V. B., Balivodz' I. P. Propoxazepam influence on thiosemicarbazide-induced GABA-deficient seizures development in mice. Клінічна фармація. 2017. 21, 2. С. 34–40. DOI 10.24959/cphj.17.1419.

[10] Andronati S. A., Pavlovsky V. I., Golovenko M. Ya., Reder A. S.,. Larionov V. B., Valivodz' I. P. Synthesis and extraction efficiency from biological fluids of [2^{14}C]Propoxazepam: a potent analgesic with multifunctional mechanism of action. JCBPS, Section A; 2019. 9, 4, P. 323–333. DOI 10.24214/jcbps.A.9.4.32333.

The life duration of animals after the administration of thiosemicarbazide is quite indicative in this regard. In animals of the control group, it is at a level of 80 minutes with a small spread of values. In most cases, 62 ± 2 minutes after the administration of the chemoconvulsant, animals developed tonic seizures, and after 83 ± 2 min, due to respiratory paralysis, a lethal effect was observed. Against the background of the administration of Propoxazepam (0.1 mg/kg), an increase in animal life was observed at 128 ± 16 min, and at doses greater than 0.3 mg/kg, animals experienced a period of three hours of observation. Thus, on the model of GABA-deficient sequester induced by thiosemicarbazide, Propoxazepam showed a fairly high activity and, in the form of a dose-effect curve, has antagonistic interaction with thiosemic arbazide.

Thus, the obtained results indicate that the first manifestations of convulsive activity in animals begin to appear at the first minute after the administration of thiosemicarbazide (control group), whereas administration of Propoxazepam (0.01 mg/kg) increases this time to ~70 minutes. Against the background of the administration of Propoxazepam (0.1 mg/kg), an increase in animal life was observed at 128 ± 16 min, and at doses greater than 0.3 mg/kg, animals experienced a period of three hours of observation. Increasing the dose of the administered Propoxazepam leads to a certain redistribution between the manifestation of cloning and tonic judgment. In experimental groups, there is a reduction in the time of myoclonic convulsions, along with an increase in their number, along with a decrease in the number of tonic seizures, which reflects an increase in the effectiveness of inhibitory processes in the central nervous system. The average effective dose of the protective effect of Propoxazepam on the model of thiosemicarbaside-induced convulsions is 0.18 ± 0.10 mg/kg with the angle of inclination of the dose-effect curve of 0.6, which corresponds to the rapid development of the protective effect and antagonistic interaction at the receptor level.

4. Valuation of anticonvulsant action of Propoxazepam on pentylenetetrazole-kindling model of seizure in mice

The analysis of anticonvulsant activity of Propoxazepam on the model of PTZ induced kindling in mice (equivalent to absence form of epilepsy) was performed, and the dynamics of convulsions redistribution of various severity degrees was characterized. The model of the PTZ kindling reproduces the GABA-RC dependent epileptogenesis. The kindling model in the development of chronic epileptogenesis is also considered as a pharmacologically resistant form of the epileptic syndrome[11].

[11] Sharma A.K., Reams R.Y., Jordan W.H., Miller M.A., Thacker H.L., Snyder P.W. Mesial temporal lobe epilepsy: pathogenesis, induced rodent models and lesions. Toxicol Pathol. 2007. 35. P. 984–999. DOI 10.1080/01926230701748305.

It should be noted[12] that seizures of the fifth (highest) severity degree were not observed in any experimental animal group, and seizures of the fourth severity were observed only in single animals and only on the terminal days of the experiment. For seizures of the first to third severity degree the calculated values of the difference significant level do not show an adequate level ($p > 0,2–0,6$), which does not allow us to conclude that the effects of the administered doses of Propoxazepam on the development of convulsions during the chronic PTZ administration have not been demonstrated. For indicators of the number of second-degree severity seizures in the group of animals receiving Propoxazepam at a dose of 2.0 mg/kg the calculated significance levels were less than 0.05 (eighth and ninth administration). For animals of control and experimental groups, there was a decrease in the latent time of seizures manifestation of the first severity degree. In the control group of animals and in the group receiving Propoxazepam at a dose of 0.2 mg/kg, the minimum value of the latent time of the first degree of severity convulsion appeared on the 8^{th}–11^{th} day of PTZ administration, what can be regarded as the absence of significant influence of the compound on development prolonged neurodegenerative changes.

In the control group of animals before the 8^{th} PTZ injection, there is also the occurring of convulsions of the third and fourth degree of severity, however, these indicators begin to stabilize only after the 11^{th} administration. In the experimental group of animals (0.2 mg/kg of Propoxazepam), seizures of the third and fourth degree of severity occur later (13^{th} administration). In addition, convulsions of the third type of severity are observed even when high doses of Propoxazepam were administrated (1.0 and 2.0 mg/kg). Obviously, the effect of Propoxazepam even in high doses on GABA-RC completely does not compensate the functional changes in other mediator systems (NMDA-, aspartate and glutamate), which are involved in the processes of kindling development. In terms of severity of kindling-epilepsy pathological process development the partial contribution of each of convulsions types to general seizure spectra is indicative. To relatively assess the changes in qualitative composition of seizures attack, the participation of each type of convulsion was determined in relation to the total amount of seizures. This method of data transformation not only reflects the individual contribution of each type of convulsion, but also gives an idea of the depth of influence of the long-term administration of PTZ on the balance of inhibitory and excitation systems. In animals of the control group during the development of the kindling-state a gradual decrease in the

[12] Golovenko N.Ya., Larionov V.B., Reder A.S., Andronati S.A., Valivodz' I.P. Evaluation of anticonvulsant action of propoxazepam on pentylenetetrazole-kindling model of seizure in mice. American Scientific Journal. 2018. 21, 2. P. 23–31.

number of first-degree convulsions with a simultaneous increase in the representation of the higher-severity seizure arising from functional changes in the central nervous system is observed. The fourth administration leads not only to increase of third group convulsions contribution increase, but also the appearance (to 3–6%) of more severe components. After the sixth administration, their presence in the seizure spectrum becomes constant, although the total contribution does not exceed 6%. In general, the assessment of the partial contribution is more indicative for the effect of the Propoxazepam administered doses on the spectrum of seizure attack. In the spectrum of convulsive activity of animals that received Propoxazepam in a dose of 2.0 mg/kg, practically there is no manifestation of attacks of even a second degree of severity, indicating a direct effect on GABA-RC. One of the classical parameters that characterizes the development of the kindling-state is the index of convulsions, which is both normalized and the average indicator of the contribution of each convulsion type to the general state of the kindling, which is registered. Calculated values of the seizure index do not undergo significant changes and do not exceed the value of 2.0. Both in the control group and in the experimental groups of animals receiving low doses of Propoxazepam (0.2 and 0.6 mg/kg), this indicator does not show statistically significant differences, and only in the group of animals receiving Propoxazepam at dose of 2.0 mg/kg, its tendency to a significant increase is not observed. In general, the analysis of primary data (latent time of convulsive attacks development, their number and degree of severity) does not allow to make conclusion about the amount of contribution of Propoxazepam to inhibit the development of the kindling state in experimental animals. However, the variance analysis of these data from control and experimental groups characterizes the effect of the total factor of the Propoxazepam administered doses (0.2–2.0 mg/kg) at 23% and leaves about 77% of other factors. At first glance, this is a low value (despite a statistically significant result with $p = 0.002$) for a compound that has shown a high anticonvulsant effect in acute PTZ experiments, especially given the statistically significant differences in severity of convulsions. However, this may be due to the lack of exposure to low doses of Propoxazepam, the excessive variability of control values, or a combination of mutually exclusive factors (for example, the lack of efficacy of Propoxazepam in the terminal stages of the kindling state). Separation of a complex factor (a combination of Propoxazepam doses) into separate subgroups and an assessment of the effect of each dose administered (relative to the control group) can reveal an increase in the dose effect of the Propoxazepam (from 11.8% to 51.9% for doses 0.2–2.0 mg/kg). On the basis of the calculated values of the contribution of these factors, it is possible not only statistically significantly (for each individual group, the probability level was < 0.001) to detect the existence of dose-dependent intermittent effects of

Propoxazepam, but also to explain the impossibility of detecting this effect by other methods of data analysis. It has been found that administration of low doses (0.2–0.6 mg/kg) of Propoxazepam during the period of formation of the kindling does not have a significant anticonvulsant effect, although it inhibits the manifestation of the third and fourth severity degree convulsions. Propoxazepam high doses (1.0–2.0 mg/kg) inhibit the development of high severity degree seizures; at the administration of high doses (2.0 mg/kg), with practically no manifestation of attacks even a second degree of severity. According to the results of the dispersion analysis, the contribution to the factor of the Propoxazepam dose on the model of kindling-epilepsy ranges from 11.8% to 51.9% for doses 0.2–2.0 mg/kg). The presented data also indicate that there is no reduction in the response to Propoxazepam at doses of 0.2–2.0 mg/kg under repeated and continuous administration schemes, which indicates that there is no experimental pharmacodynamic resistance to the antiepileptic action of the substance. According to the literature 12 valproate – one of the mostly used antiepileptic drug – in high doses (100–200 mg/kg) in chronic oral prophylactic administration maximally suppresses the development of generalized clonical-tonic convulsions of a 4^{th} severity degree and only in a small number of mice prevents the development of local clonic convulsions with severity of $1\text{-}2^{th}$ degree. The deficiency of valproate can also be attributed to the weakening of its anticonvulsant effect by 8–11% to 21 days of kindling formation, which may indicate the development of tolerance to valproate in high doses with prolonged use. PTZ-induced kindling may be related to permanent attenuation of inhibitory function of GABAergic system in the brain. Repetitive single dose application ends up with decreased GABA ergic activity[13]. According to this suggestion the functioning of the GABA-RC in the CNS can be examined in vivo by estimation of the competitive effects of benzodiazepines and seizure-inducing agents (pentylenetetrazol, strychnine, and picrotoxin). On the basis of dose–effect curves, using comparative quantile analysis for chemoconvulsants with different mechanisms of action, we showed different stages of Propoxazepam interaction with GABA and glycine receptors under in vivo conditions[14].

[13] Corda M.G., Orlandi M., Lecca D., Carboni G., Frau V., Giorgi O. Pentylenetetrazol-induced kindling in rats: effect of GABA function inhibitors. Pharmacol Biochem Behav. 1991. 40, 2 P.329-33. DOI 10.1016/0091-3057(91)90562-g.

[14] Golovenko N.Ya., Larionov, V.B., Reder, A.S., & Valivodz' I.P. An effector analysis of the interaction of propoxazepam with antagonists of GABA and glycine receptors. Neurochemical Journal. 2017. 11(4). P. 302–308. DOI 10.1134/S1819712417040043.

5. Effects of propoxazepam on maximal electroshock-induced convulsions in mice

The technique of maximum maximal electroshock seizure (MES) models primary-generalized seizures (epileptic status) and partial paroxysms and is a basic test for the evaluation of the action of substances with anticonvulsant activity. It is believed that the MES test is a model for proticulic drugs that act on Na^+ channels (eg carbamazepine, phenytoin). At the same time, some anticonvulsants are effective in the MES model, but they interact with other targets: GABA-RC and glutamate receptors[15].

Our studies12 indicate that in control animals, electrostimulation did not cause tonic-clonic seizures, and the structure of the epileptic attack was represented only by a tonic component associated with irreversible processes of propagation of excitation, which caused a high level of lethality in this group (100%, n = 6). The average duration of the tonic component in animals was 14.9 ± 2.6 s, and this figure did not change due to the effects of various doses of Propoxazepam. Pre-administration of mice in increasing doses (0.2-20 mg/kg) of Propoxazepam leads to an increase in the protective effect in the MES test, with a maximum protective effect (100%) at a dose of 20 mg/kg. However, attention is drawn to the fact that the dose-effect relationship in this test does not correspond to the normal distribution in the range of doses used. Thus, doses in the range of 10-90% of the protective effect cause almost linear dependence, whereas the part of the curve within the maximum frequency of protective action has a more decreasing inclination. According to the protective effect in the MES test, Propoxazepam exhibits a significant protective (antiepileptic) effect (ED_{50} 0.57 ± 0.23 mg/kg). The inclination of the curve, equal to 0.51, indicates a slow increase in the protective effect under conditions of dose increase (within the value of ED_{50}). Also, for the selected separate concomitant states of convulsive attack, the average effective dose and the angle of inclination of the dose-effect curve were calculated. For all individual parameters of convulsive attack in this pharmacological model, the rate of change in effect within the median effective dose (as the angle of inclination of the curve) is rather small (s values in absolute value less than 1). Probable explanation for this fact is the changes in the functional state of the central nervous system, which under the influence of MES are so significant that compound I in the used dose interval does not provide

[15] Castel-Branco M.M., Alves G.L., Figueiredo I.V., Falcão A.C., Caramona M.M. The maximal electroshock seizure (MES) model in the preclinical evaluation of potential new antiepileptic drugs. Methods Find Exp Clin Pharmacol. 2009. 31, 2. P. 101–106. DOI 10.1358/mf.2009.31.2.1338414.

complete blockage of all components of convulsive attack. It is noteworthy that the shape of these curves is not symmetric in relation to the median effective dose, so the data are presented in the form of ED_{50} (ED_{82}÷ED_{18}), that is, the estimated doses that cause 50%, 82% and 18% of the effect, respectively. The obtained results[16] indicate that the achievement of the average effect frequency occurs at lower doses of Propoxazepam, whereas the increase in the frequency of the effect requires a disproportionate increase in dose (the median dose of ED_{50} has a shift to lower values of ED_{18} than to ED_{82}. This may be the result of the compound and inhibitory processes in the central nervous system. There are also changes in the manifestation of certain states of convulsive attack, in particular, dose-dependent decrease in the frequency of tonic and clonic-tonic seizures in animals with a decrease of up to ~ 10% of the total number of animals. However, there is also a decrease in the frequency of the manifestation of the refractory phase, indicating a predominant activation of the central nervous system in the period prior to the use of MES.

Increasing the dose of the compound also causes the redistribution of individual components of convulsive attack. Under the influence of MES in animals of the control group there are tonic attacks, which have high lethal consequences. When administering Propoxazepam, even in a dose of 0.2 mg/kg, the structure of the caused epileptic seizure is represented by a clonic component (up to 15%), and further increase of the dose not only increases the partial contribution of this condition, but also increases the manifestation of refractory time associated with the protective effect.

The results obtained regarding the protective effect of Propoxazepam (ED_{50} 0,57 ± 0,23 mg/kg) in the MES model are somewhat unusual, not only for 1,4-benzodiazepine derivatives, but also for most antiepileptic drugs. The calculated ED_{50} value for Propoxazepam protective effect in the PTZ-induced convulsions test was 0,92 ± 0,38 mg/kg. Taking into account the errors of experiments, it is possible to recognize the indices obtained for MES and PTZ as similar. At the same time, from literary sources[17] is aware of the lack of similarity between the noted indicators. So diazepam has the following ratio MES/PTZ (mg/kg, mouse), – 3,5/0,52; clonazepam (7,5/0,06); nitrazepam (4,5/0,27); phenazepam (10,2/0,037); gidazepam (41,2/0,36). Barbituric acid derivatives (phenobarbital, primidon) have

[16] Larionov V.B., Reder A.S., Golovenko N.Ya. Differential effects of propoxazepam on pentylenetetrazole – and maximal electroshock-induced convulsions in mice. The Scientific Heritage. 2018. No 28. P. 7–13.

[17] Koella W.P. Animal Experimental Methods in the Study of Antiepileptic Drugs. In: Antiepileptic drugs. Berlin, Heidelberg: Springer-Verlag, 1985, P. 283–340.

respectively the following indices: 21,8/13,2; 11,4/58,6, and diphenylhydantoin (12,8/149,8). Some anticonvulsants (phenytoin, carbamazepine) do not affect seizures caused by PTZ, but are active in relation to MES (9,5 and 8,8, respectively). Only for metsuximide (76,3/68,3) and valproic acid (271,7/148,6) there are approximately similar indica-tors. Obviously, the protective properties of the drugs in these models depend, at least, on two components: the effect of seizure agents on various anatomical and morphological structures of the central nervous system11 and molecular mechanisms of seizures.

With regard to the significant difference in the ratios of MES/PTZ of related compounds (1,4-benzodiazepines) having similar mechanisms of action, their explanation may be data on the structure of GABAergic system, for which the effect of PTZ is often characterized as "noncompetitive" in relation to anticonvulsant derivatives of 1,4-benzodiazepine. It should be noted that a part of GABA-RC is represented by a number of subtypes, among which are responsible for the seizure effect of chemoconvulsants. Moreover, each benzodiazepine prefers a specific receptor subtype[18]. So diazepam, clonazepam and nitrazepam are high-affinity compounds of a2 and a3 receptors.

6. Inhibition of 4-aminopyridine-induced seizures in mice by Propoxazepam

In view of the fact that Propoxazepam refers to derivatives of 1,4-benzodiazepine, and diazepam inhibits the onset of tonic-clonic seizures caused by 4-aminopyridine (4-AP) and death of mice[19] it is legitimate to investigate its protective effect on this model, which makes it possible to characterize the role of the Propoxazepam in the modulation of the function of potential dependent potassium channels.

In our studies[20], the "dose-effect" curve of the protective effect of Propoxazepam on the 4-AP model of convulsion has a S-shaped shape, but even with its high doses (80 mg/kg), 100% effect is not observed. The registered effect is similar to the profile the anticonvulsant effect of

[18] Clayton T., Chen J., Ernst M. et al. An up-dated, unified pharmacophore model of the benzodiazepine binding site is γ-aminobutyric acid receptors: correlation with comparative models. Current Medicinal Chemistry. 2007. V.14. P. 2755–2775. DOI 10.2174/092986707782360097.

[19] Brian S. Meldrum, Michael A. Rogawski. Molecular Targets for Antiepileptic Drug Development. Neurotherapeutics. 2007. 4, 1, P. 18–61. DOI 10.1016/j.nurt.2006.11.010.

[20] Golovenko N.Ya., Larionov V.B., Reder A.S., Valivodz' I.P. Inhibition of 4-aminopyridine-induced seizures in mice by a novel 3-substituted 1,4-benzodiazepine. Фармацевтичний журнал. 2018. 4-5. P. 90-96. DOI 10.32352/0367-3057.5-6.18.7.

Propoxyzepam on the judgmental model caused by strychnine[7] and may also indicate that the anticonvulsant effect of the test Propoxazepams realized not so much on the receptor (antagonistic), but at the level of individual receptor systems, the effectiveness of the interaction between which causes maximum value achievable protective effect. However, the slope of the curve (s) is 1.15, which corresponds to the receptor mechanism of interaction with the effect implementation within one order of magnitude. (Approximately 1.0 per log scale). The average effective dose of Propoxazepam for this test was 37.3 ± 7.9 mg/kg, which is almost twice the same for strychnine (16.4 ± 6.1 mg/kg), and also indicates that it has no significant effect directly to this type of receptor. For a real antagonist of GABA-RC picrotoxin, this value is 1.67 ± 0.09 mg/kg. Based on the magnitude of the protective effect in this test, it can be concluded that Propoxazepam does not exhibit direct and pronounced action on potassium channels that are blocked by the 4-AP.

However, indicators of seizure attack induced by 4-AP against the background of the administration of Propoxazepam indicate slight antagonistic interactions between these compounds[21]. In the whole range of doses used, the latent development time of myoclonic seizures (an indicator of the beginning of the development of destabilization of the central nervous system) does not show statistically significant differences from the control values Wilcoxon-Mann-Whitney Criteria[22] and is within 5–7 minutes (only at a dose of 60 mg/kg , taking a high value).

Also, there is no statistically significant difference in the number of episodes of myoclonic seizures, although there is a tendency for a small increase in their number with an increase in the dose of Propoxazepam. It should be noted that the balance between episodes of myoclonic and tonic seizures reflects the rate and intensity of development and generalization of the excitatory process in the CNS. Rapid suppression of inhibitory processes leads to the fact that the myoclonic component is practically not registered in the structure of convulsive attack (for example, when strychnine is used as a seizure agent) and a partial increase in the number of myoclonic seizures with an increase in the dose of Propoxazepam may be due to activation of the actual GABA-ergic system. The absence of direct antagonism between Propoxazepam and 4-AP at the level of potassium channels is also indicated by the development of the tonic component of convulsive attack. Thus, almost all doses of the latent time of development of myoclonic seizures (as an indicator of the beginning of the development of uncontrolled

[21] Koella W.P. Animal Experimental Methods in the Study of Antiepileptic Drugs. In: Antiepileptic drugs. Berlin, Heidelberg: Springer-Verlag, 1985, P. 283–340.

[22] Van der -Varden B. Mathematical statistics. M. Publishing Foreign Literature. 1960. 436 p.

destabilization of the central nervous system) did not undergo statistically significant differences. A similar process is observed in the analysis of parameters of paroxysmal activity, which is manifested in a partial increase in the time of development of tonic seizures and a significant increase in their number[23]. In animals in the control group, the development of tonic seizures quickly leads to a lethal effect by blocking respiratory muscles, and with increasing dose, and the ability to control these processes from the CNS increases. As a result, not only is the "blurring" of the time of the actual seizure (with an increase in the latent time of development of myoclonic and tonic seizures), but also an increase in their absolute number, that is, the specific frequency of the formation of cells of paroxysmal activity remains virtually unchanged, and their absolute magnitude increases due to reduction of the severity of individual episodes of convulsive attack. However, as previously suggested, since Propoxazepam does not exhibit direct antagonism with 4-AP at the level of potassium channels, its protective effect is realized at doses that are significantly higher than those in tests using GABA-RC antagonists (penthylenetetrazole and picrotoxin). Also, as a result, the total lifetime of animals (the onset of a lethal effect after the administration of a seizure agent) has a hyperbolic character – reaches the maximum value at a dose increase (~ 30-35 min) with the simultaneous narrowing of the spread of experimental values. The possible explanation is that during this time there is complete absorption of the seizure agent from the injection site, the maximum blockade of the inhibitory processes in the central nervous system and, accordingly, the proliferation of uncontrolled excitation with subsequent lethal effect.

Thus, on the models of convulsions induced by 4-AP (a blocker of fast potentially dependent potassium channels), Propoxazepam exhibits moderate activity (ED50 = 37.3 ± 7.9 mg/kg). Even at high doses (80 mg/kg), its anticonvulsant effect did not reach 100%, indicating no possible component of the antagonistic interaction with 4-AP at the receptor level.

The number of myoclonic seizures and the latent time of their development do not show statistically significant differences in comparison with the control animals. On the contrary, the number (and percentage representation) of tonic seizures in the general paroxysmal attack increases. The possible explanation for this is the inhibitory effect of Propoxazepam, which is mainly realized through GABA-ergic mechanisms. The total lifetime of animals (the onset of a lethal effect after the administration of the seizure agent) has a hyperbolic character and reaches the maximum values in 30–35 minutes in a dose-increasing condition.

[23] Koella W.P. Animal Experimental Methods in the Study of Antiepileptic Drugs. In: Antiepileptic drugs. Berlin, Heidelberg : Springer-Verlag, 1985, P. 283–340.

CONCLUSIONS

The results presented indicate a high protective activity of Propoxazepam based on the data of the dose-effect curves: picrotoxin 1.67 ± 0.09, penthylenetetrazole 0.9 ± 0.04, strychnine 14.24 ± 0.47, MES 0.57 ± 0.23, thiosemicarbazide 0.18 ± 0.09, 4-aminopyridine 37.3 ± 7.9 mg/kg, and a penthylenetetrazole -kindling model. Changing the time of onset seizures and their redistribution in the course of an epileptic syndrome makes the compound promising in epilepsy treating.

Comparison[24] of the spectrum of anticonvulsant activity of Propoxazepam with the majority of medicines used to suppress specific epileptic syndrome (Table 1) showed its preference. Only valproate, as in Propoxazepam, is characterized by inhibition of all variability of convulsive states.

Table 1

The effectiveness of antiepileptic medicines for different types of seizures

Medicines \ Seizures type	**partial**	**tonic-clonic**	**absences**	**myoclonic**	**atonic-tonic**
Phenobarbital	+	+	+	0	?
Phenytoin	+	+	-	-	0
Carbamazepine	+	+	+	+	0
Valproate	+	+	+	+	+
Tomiramat	+	+	?	+	+
Gabapentin	+	+	-	-	0
Lamotrigine	+	+	+	+	+
Ethoxumide	0	0	+	0	0
Levetyratsytam	+	+	+	+	?
Felbamat	+	+	?	+	+
Vigabatrin	+	+	-	-	?
Propoxazepam	+	+	+	+	+

Note: “+” – effective; “? + “ – maybe effective; “0” ineffective; “-“ – increased attacks; “?” – unknown.

According to the main hypothesis, valproate stimulates GABA-ergic mechanisms by inhibiting the GABA-transferase enzyme, which leads to an increase in the content of GABA in the central nervous system. Another

[24] Golovenko N.Ya., Andronati S.A., Larionov V.B., Reder À.S.. A pharmacological profile of propoxazepam – a new antiepileptic substance. Доповіді НАН України. 2018. 12. С. 93–100. DOI 0.15407/dopovidi2018.12.093.

mechanism for increasing GABA-ergic activity is the direct influence of valproate on postsynaptic receptors (especially GABA-RC), which simulates or enhances the effect of GABA. In addition, the drug has a direct effect on the potential-dependent sodium channels and can affect the activity of the membranes, changing the conductivity for potassium ions[25]. It is noticeable that, according to the values of ED_{50}, Propoxazepam predominates valproat. Propoxazepam (0,92 ± 0,38/0,57 ± 0,23 mg/kg) and valproat (271,7/148,6).

Propoxazepam, is considered a promising drug and is undergoing preclinical trials. Similar to gabapentin and pregabalin, which are well-known drugs used in general medical practice in the treatment of neuropathic pain[26], propoxazepam also has an anticonvulsant effect wich explains the analgesic component of the pharmacological spectrum[27].

SUMMARY

The analysis of the dose effect pharmacological action for propoxazepam on the models of chemically and electrically induced seizures is carried out. The peculiarities of the compound influence on the different genesis seizures manifestations and redistribution are determined. Pharmacological indices have been calculated, which characterize the efficacy and safety of propoxazepam. The results of the study prove the high safety of propoxazepam use for the treatment of GABA associated pathologies and primarygeneralized seizures (status epilepticus) in the tests of thiosemicarbazide- and MES-induced seizures, as well as MES-induced partial paroxizmas. The data presented suggest that the future use of propoxazepam as an antiepileptic agent is more secure in those pathologies that are more relevant to the GABA and glycineergic mechanisms.

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