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INHIBITION OF 4-AMINOPYRIDINE-INDUCED SEIZURES IN MICE BY A NOVEL 3-SUBSTITUTED 1,4-BENZODIAZEPINE

Key words: propoxazepam, 4-aminopyridine, anticonvulsive action, potassium channels

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ГАЛЬМУВАННЯ СУДОМ, СПРИЧИНЕНИХ 4-АМІНОПІРИДИНОМ У МИШЕЙ, НОВИМ 3-ЗАМІЩЕНИМ 1,4-БЕНЗОДІАЗЕПІНОМ

Ключові слова: пропоксазепам, 4-амінопіридин, протисудомна дія, калієві канали

The epilepsies constitute a family of disorders characterized by spontaneous disturbances in the normal electrical activity of the brain associated with changes in behavior (seizures). Both the electrical and the behavioral aspect of seizures can be quite variable and complex, even in a single patient. Seizures can be induced by a variety of pathologic conditions, including acquired injuries and genetic abnormalities [1].

Therapy with antiepileptic drugs (AEDs) remains the mainstay of treatment of patients with epilepsy. Most clinically significant AEDs have their principal actions on four classes of ion channels, the central currency of membrane excitability: these include chloride channels associated with inhibitory GABA_A receptors, ligand-gated sodium and calcium channels associated with inotropic excitatory glutamate receptors (NMDA, N-methyl-D-aspartate; AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid), kainate; voltage-dependent sodium channels and voltage-dependent calcium channels). More recently, AED development also has targeted both pre- and postsynaptic membrane-bound receptors and enzymes involved in neurotransmitter metabolism [2].

A novel 3-substituted 1,4-benzodiazepines, 7-bromo-5-(*o*-chlorophenyl)-3-propoxy-1,2-dihydro-3H-1,4-benzodiazepin-2-one (propoxazepam), has been found to have a potent anticonvulsant effect in models of chemically (picrotoxin, pentylenetetrazol, strychnine, bemegride) and maximal electroshock-induced seizures [3–5].

Propoxazepam had shown high activity on the model of GABA-deficient thiosemicarbazide-induced convulsions [6]. On the basis of dose–effect curves, using comparative quantile analysis for chemoconvulsants with different action mechanisms, we showed different stages of interaction of propoxazepam with GABA and glycine receptors under *in vivo* conditions.

A radio-ligand binding assay [7] on rat membranes to determine the target receptor of propoxazepam showed that substance had a strong affinity for the central benzodiazepine binding site of GABA_A – receptor (GABA-R). Calculations have shown that GABA-shift for propoxazepam is 1.9, which allows it to regard it as full GABA-R agonist.

Using the method of molecular docking we showed [8] that there are several binding sites of propoxazepam to the part of GABA-R with the complexes formation energy from -78.64 to -85.29 kcal/mol. The largest contribution to the complex formation is carried out by the remnants of polar amino acids (serine, asparagine, methionine and arginine), which create a polar binding subcenter). For individual conformers, aromatic amino acids, preferably phenylalanine (Phe-31 and Ala-135 – hydrophobic binding subcenter), play a significant role.

Therefore, the aim of this study was to investigate the anticonvulsant effects of propoxazepam on 4-aminopyridine-induced seizures in mice for better understanding the propoxazepam`s antiepileptic profile and action mechanisms.

Methods

All experimental procedures with animals (white outbred mice of both sexes) 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.

To determine the propoxazepam anticonvulsive action on the model of 4-aminopyridine (4-AP) – induced seizures the chemoconvulsant was injected subcutaneously (at dose 10.3 mg/kg, LD₉₇ for mice) 30 min followed by intraperitoneal propoxazepam administrations (at doses 20, 28, 40, 60 and 80 mg/kg). The anticonvulsive action value was estimated as relative quantity of animals survived 2 hours after chemoconvulsant injection). The following indices have also been recorded – time and the quantity of myoclonic and tonic convulsions as well as total time to the lethal effect. The data are represented as first-third quartile, median (maximal, minimal value). Significance level of differences was made on the base of non-parametric Mann-Whitney U-criteria [9].

Results and Discussion

Aminopyridines have been used as standard reference compounds in a variety of studies involving the functions and properties of K1 channels (Kv1). These compounds have been classically used as blockers of Kv1 efflux and conductance in a number of physiological preparations from both central and peripheral tissues [10]. The stimulation effects of 4-AP on neurotransmitter release have been reported for norepinephrine, dopamine, gamma-aminobutyric acid and glutamate.

Paying attention to the fact diazepam (as well as propoxazepam being the 1.4-benzodiazepine derivative) inhibits the onset of tonic-clonic seizures caused by 4-aminopyridine (4-AP) and death of mice [11] it is expedient to investigate its protective effect on this model. This makes it possible to characterize the possible role of propoxazepam in the modulation of the function of voltage dependent potassium channels.

In our studies, the «dose–effect» curve of the protective effect of propoxazepam on the 4-AP-induced model of convulsion has a S-shaped shape, but even at high propoxazepam doses (80 mg/kg) 100% effect is not reached. This is similar to propoxazepam anticonvulsive action in the strychnine-induced seizures model [4] and may also indicate that the anticonvulsant effect of the test compound is not receptor-agonistic, but through the different mediatory systems, the effectiveness of the interaction between which determines the maximal effect achieved. However, the slope of the curve (s) is 1.15, which corresponds to the receptor mechanism of interaction with the effect increasing within wide doses range (approximately 1.0 per log scale). The propoxazepam average effective dose for this test

was 37.3 ± 7.9 mg/kg, which is almost twice that of 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-R picrotoxin, this value is 1.67 ± 0.09 mg/kg [4]. Based on the anticonvulsive effect value 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, different indicators of 4-AP-induced seizures against the background of propoxazepam administration (Fig. 1, *A*) demonstrate slight antagonistic interactions between these compounds. In the whole range of doses used, the latency time of myoclonic seizures development (an indicator of the beginning of the destabilization development in the central nervous system) does not show statistically significant differences from the control values (Mann-Whitney U-criteria) and is within 5–7 minutes (only at a dose of 60 mg/kg taking a high value).

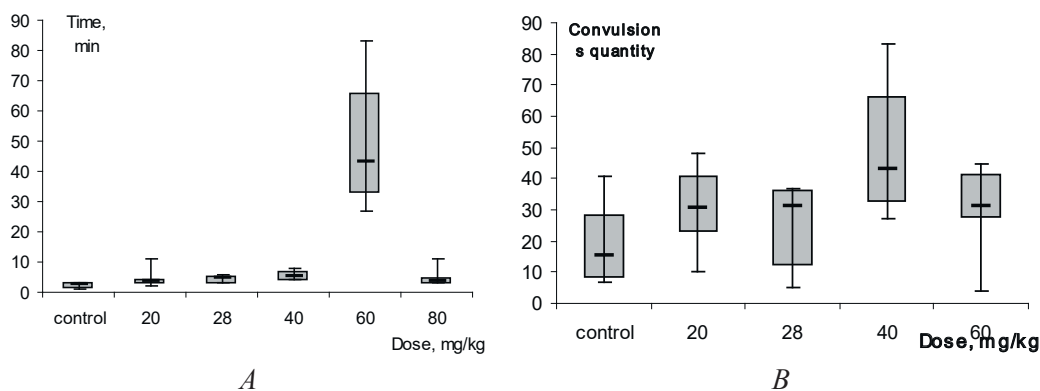


Fig. 1. Change of latency time of myoclonic seizures (*A*) and their number (*B*) development in mice after 4-AP injection (10.3 mg/kg) after previous propoxazepam administration in different doses (first–third quartile, median (minimum, maximum value))

Also there is has statistically significant difference in the number of episodes of myoclonic seizures (Fig. 1, *B*), 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, at almost all doses administered the latent time of myoclonic seizures development (as an indicator of the beginning of the development of uncontrolled destabilization in the central nervous system) did not undergo statistically significant differences. A similar process is observed in the analysis of paroxysmal activity parameters, which is manifested in a partial increase in the time of development of tonic seizures (Fig. 2, *A*) and a significant increase in their number (Fig. 2, *B*).

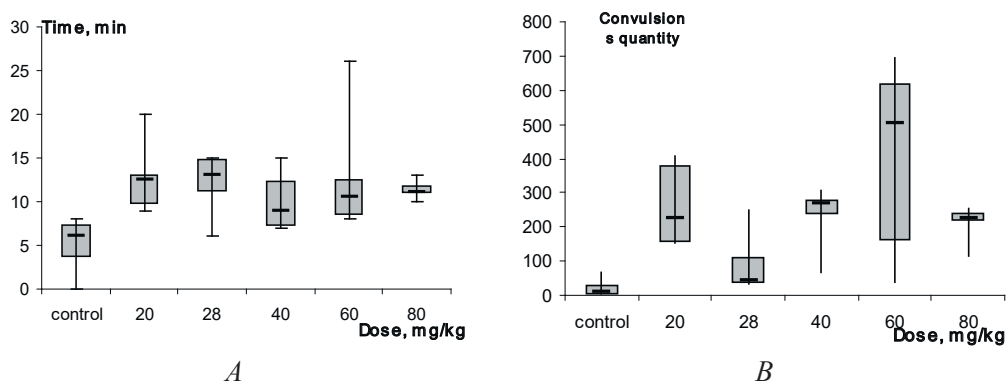


Fig. 2. Change in tonic seizures latency time (A) and their quantity (B) in mice after 4-AP injection (10.3 mg/kg) after previous propoxazepam administration in different doses (first–third quartile, median (minimum, maximum value))

In animals of the control group the tonic seizures development quickly leads to a lethal effect by blocking respiratory muscles, while with propoxazepam increasing dose the CNS ability to control these processes increases. As a result not only the seizures onset time widens (with simultaneous increase of tonic seizures latency time and their quantity) but also their quantity, since the specific frequency of the paroxysmal activity foci remains unchanged with the longer period of their manifestations.

However, as previously suggested, since propoxazepam does not exhibit direct antagonism with 4-AP at the level of potassium channels, its protective effect is manifested at doses that are significantly higher than those in tests using GABA-R antagonists (pen-tylenetetrazol and picrotoxin). Also, as a result, the increase of total lifetime of animals (the onset of a lethal effect after the administration of a seizure-inducing agent) has a hyperbolic shape – reaches the maximum value at a dose increase (~ 30–35 min) with the simultaneous narrowing of the experimental values range (Fig 3, A). The possible explanation is that during this time there is a 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 propagation of uncontrolled excitation with subsequent lethal effect.

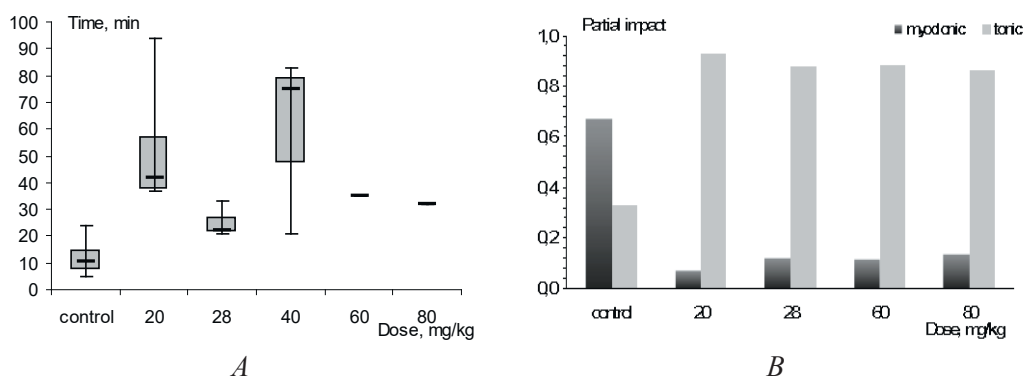


Fig. 3. Change of life duration of mice (A), and the contribution of individual components of the seizures to the general structure of convulsive attack (B) after the 4-AP injection(10.3 mg/kg) after previous propoxazepam administration at different doses

Conclusion

Thus, on the models of 4-AP-induced convulsions (a blocker of fast potentially dependent potassium channels), propoxazepam shows moderate activity ($ED_{50} = 37.3 \pm 7.9$ 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 myoclonic/tonic convulsions development latency time 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 carried out through GABA-ergic mechanisms. The total lifetime of animals (the onset of a lethal effect after the administration of the seizure-inducing agent) has a hyperbolically reaches the maximum values in 30–35 minutes in a dose-increasing condition.

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ГАЛЬМУВАННЯ СУДОМ, СПРИЧИНЕНИХ 4-АМІНОПІРИДИНОМ У МИШЕЙ,
НОВИМ 3-ЗАМЩЕНИМ 1,4-БЕНЗОДІАЗЕПІНОМ

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А Н О Т А Ц І Я

Поліmodalність фармакологічної дії притаманна деяким з вискоєфективних протіепілептичних засобів та зумовлює можливість їх застосування при патогенетично споріднених станах. Вплив на гальмівні медіаторні системи у мозку визначає можливість поєднання у їхньому фармакологічному спектрі дії протіепілептичної, анальгетичної (антинейропатичної) та інших видів активності.

Метою роботи була оцінка протисудомної дії 7-бром-5-(о-хлорфеніл)-3-пропокси-1,2-дигідро-3Н-1,4-бензодіазепін-2-ону (пропоксазепам) на моделі судом, спричинених 4-амінопіридином (4-АП), та характеристика його можливої участі в модуляції функції потенціал-залежних калієвих каналів.

4-АП (10,3 мг/кг, підшкірно) вводили через 30 хв після внутрішньоочеревинного ведення різних доз пропоксазепаму (20, 28, 40, 60 та 80 мг/кг) і оцінювали кількість і час настання різних типів судом.

Встановлено, що на цій моделі пропоксазепам виявляв помірну активність ($ED_{50} = 37,3 \pm 7,9$ мг/кг). Навіть за високих доз (80 мг/кг) досліджуваної сполуки протисудомна дія не досягала 100%. Кількість міоклонічних судом і латентний час їх розвитку не зазнають статистично значущих відмінностей порівняно з показниками тварин контрольної групи. Навпаки, підвищується кількість (і процентна репрезентація) тонічних судом у загальному судомному нападі, що зумовлено можливою гальмівною дією пропоксазепаму, яка реалізується переважно через ГАМК-ергічні механізми.

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ИНГИБИРОВАНИЕ СУДОРОВ, ВЫЗВАННЫХ 4-АМИНОПИРИМИДИНОМ У
МЫШЕЙ, НОВЫМ 3-ЗАМЕЩЕННЫМ 1,4-БЕНЗОДИАЗЕПИНОМ

Ключевые слова: пропоксазепам, 4-аминопиридин, протисудорожное действие,
калиевые каналы

А Н Н О Т А Ц І Я

Полиmodalность фармакологического действия свойственна некоторым высокоэффективным противосудорожным средствам и обуславливает возможность их использования при патогенетически родственных состояниях. Влияние на тормозные медиаторные системы в мозге определяет возможность объединения в фармакологическом спектре противосудорожной, анальгетической (антинейропатической) и других видов активности.

Целью работы была оценка противосудорожного действия 7-бром-5-(о-хлорфенил)-3-пропокси-1,2-дигидро-3Н-1,4-бензодиазепин-2-он (пропоксазепам) на модели острых миоклонических судорог, вызванных 4-аминопиридином (4-АП), и характеристика его возможного участия в модуляции функции потенциал-зависимых калиевых каналов.

4-АП (10,3 мг/кг, подкожно) вводили через 30 мин после внутривентриального введения разных доз пропоксазепам и оценивали количество и время наступления различных типов судорог.

Установлено, что на этой модели пропоксазепам проявлял умеренную активность ($ED_{50} = 37,3 \pm 7,9$ мг/кг). Даже при высоких дозах (80 мг/кг) исследуемого соединения противосудорожное действие не достигало 100%. Количество миоклонических судорог и латентное время их развития не претерпевают статистически значимых отличий по сравнению с животными контрольной группы. Наоборот, повышается количество (и процентная репрезентация) тонических судорог в общем судорожном приступе, что обусловлено возможным тормозным действием пропоксазепам, реализующегося преимущественно через ГАМК-эргические механизмы.

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INHIBITION OF 4-AMINOPYRIDINE-INDUCED SEIZURES IN MICE BY A NOVEL 3-SUBSTITUTED 1,4-BENZODIAZEPINE

Key words: propoxazepam, 4-aminopyridine, anticonvulsive action, potassium channels
A B S T R A C T

Some of highly effective antiepileptic substances share the polymodal pharmacological action which determines the possibility of their use for treatment of pathogenetically similar diseases. Inhibitory mediator systems influence for example, suggests the combination in the pharmacological spectrum such actions as antiepileptic, analgesic (antineuropathic) and other actions.

The aim of the study was evaluation of anticonvulsant effect of 7-bromo-5-(o-chlorophenyl)-3-propoxy-1,2-dihydro-3H-1,4-benzodiazepin-2-one (propoxazepam) on the model of 4-aminopyridine (4-AP) – induced myoclonic seizures and characterization of its possible participation in modulation of the function of voltage-dependent potassium channels.

4-AP (10.3 mg/kg, subcutaneously) was administered 30 minutes after intraperitoneal administration of propoxazepam different doses (20, 28, 40, 60 and 80 mg/kg) and the time and quantity of myoclonic and tonic convulsions as well as total time to the lethal effect were evaluated.

It was found that in this model, propoxazepam possess moderate activity ($ED_{50} = 37,3 \pm 7.9$ mg/kg) Even at high doses (80 mg/kg) of the test compound, anticonvulsive action did not reach 100%. The quantity of myoclonic seizures and the latency time of their onset have no statistically significant differences in comparison with the data of animals of the control group. On the contrary, the number (and percentage representation) of tonic convulsions in the common seizure episode increased, which is due to the possible inhibitory effect of propoxazepam, which is carried out primarily through GABA-ergic mechanisms.

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