Recommended by Doctor of Medicine, Professor I. A. Zupanets

UDC 615.213 DOI: 10.24959/cphj.17.1419

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The effect of propoxazepam on development of thiosemicarbazide-induced GABA-deficient seizures in mice

Aim. To study the mechanisms of action for propoxazepam, a new compound with the analgesic action, on the model of thiosemicarbazide-induced GABA-deficient seizures.

Matherials and methods. A chemoconvulsive agent was injected subcutaneously (20 mg/kg) 0.5 hours after intraperitoneal introduction of propoxazepam. The number and the time of appearance of different types of convulsions, as well as the relative number of survived animals (for ED_{50} calculation) were registered.

Results. The first seizure manifestations in animals began to appear at the first minute after thiosemicarbazide introduction (control), while introduction of propoxazepam already in the dose of 0.01 mg/kg increased this time up to 70 min. Against the background of propoxazepam introduction (0.1 mg/kg) there was an increase in the animals' life duration up to 128 ± 16 min, with the doses above 0.3 mg/kg the survival was longer than 3-hour period of observation. The increase of the propoxazepam dose led to redistribution between the clonic and tonic convulsions. In the experimental groups there was a decrease in the time of occurrence of myoclonic convulsions and an increase in their number along with a reduction in the number of tonic convulsions. It indicates the increase in efficiency of inhibitory processes in the CNS.

Conclusions. The mean effective dose of propoxazepam as a protective effect on the model of thiosemicarbazide-induced seizures is 0.18 ± 0.10 mg/kg (0.31 ± 0.05 μmol/kg) with the "dose–effect" curve slope of 0.6 corresponding to the rapid development of the protective effect and antagonistic interactions at the receptor level.

Key words: propoxazepam; thiosemicarbazide; convulsions; GABA-deficiency

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Вплив пропоксазепаму на розвиток ГАМК-дефіцитних судом у мишей, викликаних тіосемікарбазидом

Мета роботи – визначення механізмів дії нової сполуки з аналгетичною дією пропоксазепаму на моделі ГАМК-дефіцитних судом, викликаних тіосемікарбазидом.

Матеріали та методи. Хемоконвульсант вводили підшкірно (20 мг/кг) через 0,5 год після внутрішньоочеревинного введення пропоксазепаму. Реєстрували кількість та час виникнення окремих типів судом, а також відносну кількість тварин, що вижили, для розрахунку $ЕД_{50}$.

Результати. Перші прояви судомної активності у тварин починають проявлятись уже на першій хвилині після введення тіосемікарбазиду (контрольна група), тоді як введення пропоксазепаму вже у дозі 0,01 мг/кг підвищує цей час до ~70 хв. На тлі введення пропоксазепаму (0,1 мг/кг) спостерігалось підвищення тривалості життя тварин до 128 ± 16 хв, а в дозах понад 0,3 мг/кг тварини переживали період у три години спостереження. Підвищення дози пропоксазепаму, що вводиться, приводить до певного перерозподілу між проявом клонічних та тонічних судом. В експериментальних групах відмічається скорочення часу виникнення міоклонічних судом разом із підвищенням їх кількості поряд із зменшенням виникнення кількості тонічних судом, що відображає підвищення ефективності гальмівних процесів у ЦНС.

Висновки. Середня ефективна доза захисної дії пропоксазепаму на моделі тіосемікарбазид-індукованих судом складає 0,18 ± 0,10 мг/кг (0,31 ± 0,05 мкмоль/кг) із кутом нахилу кривої «доза-ефект» 0,6, що відповідає швидкому розвитку захисного ефекту та антагоністичній взаємодії на рецепторному рівні.

Ключові слова: пропоксазепам; тіосемікарбазид; судоми; ГАМК-дефіцит

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Влияние пропоксазепама на развитие вызванных тиосемикарбазидом ГАМК-дефицитных судорог у мышей

Цель работы – изучение механизмов действия нового соединения с анальгетическим действием пропоксазепама на модели ГАМК-дефицитных судорог, вызванных тиосемикарбазидом.

Материалы и методы. Хемоконвульсант вводили подкожно (20 мг/кг) через 0,5 часа после внутрибрюшинного введения пропоксазепама. Регистрировали количество и время возникновения отдельных типов судорог, а также относительное количество выживших животных для расчета $ЕД_{50}$.

Результаты. Первые проявления судорожной активности у животных проявляются уже на первой минуте после введения тиосемикарбазида (контрольная группа), тогда как введение пропоксазепама уже в дозе 0,01 мг/кг

увеличивает это время до ~70 мин. На фоне введения пропоксазепама (0,1 мг/кг) наблюдается увеличение длительности жизни животных до 128 ± 16 мин, в дозах выше 0,3 мг/кг животные переживали период 3 часа наблюдения. Повышение дозы пропоксазепама приводит к перераспределению между проявлением клонических и тонических судорог. В экспериментальных группах отмечается уменьшение времени возникновения миоклонических судорог вместе с увеличением их количества наряду с уменьшением количества тонических судорог, что отражает повышение эффективности тормозных процессов в ЦНС.

Выводы. Средняя эффективная доза по защитному действию пропоксазепама на модели индуцированных тиосемикарбазидом судорог составляет 0,18 ± 0,10 мг/кг (0,31 ± 0,05 мкмоль/кг) с углом наклона кривой «доза-эффект» 0,6, что соответствует быстрому развитию защитного эффекта и антагонистическим взаимодействиям на рецепторном уровне.

Ключевые слова: пропоксазепам; тиосемикарбазид; судороги; ГАМК-дефицит

Neuropathic pain, which appears as a result of organic injuries or dysfunctions of different divisions of the central nervous system (CNS) is both medical and social economic problem. And if the proper treatment is absent, it tends to become chronic with delayed patient's recovery [1].

Despite the achievements of the last decade in the use of analgesics with the central component of pain inhibition the real progress in the neuropathic pain treatment is very modest. Each of its clinical manifestations (pain syndromes of different etiology) is characterized with different mechanisms of development, and it determines the individual approach in choosing pharmacotherapy for patients. Atypical anticonvulsants – gabapentin and pregabalin are of particular interest. In addition to nociceptive structures inhibition (due to calcium channels blocking) they also activate the antinociceptive system by increasing GABA levels and inhibiting the glutamate synthesis [2].

The new substance – 7-bromo-5-(o-chlorophenyl)-3-propoxy-1,2-dihydro-3-H-1,4-benzodiazepine-2-one (under the name of propoxazepam) is now extensively studied in A. V. Bogatskiy Physical-Chemical Institute of the NAS of Ukraine (Odessa). It exhibits the analgesic properties on the experimental models of nociceptive and neuropathic pain. It also possesses the anticonvulsive action, which is similar to gabapentin and pregabalin, and it can explain the observed analgesic component of the pharmacological spectrum [3-5].

The aim of the study was to determine the mechanisms of action for propoxazepam on the model of thiosemicarbazide-induced GABA-deficient seizures.

Materials and methods

The substance studied was introduced intraperitoneally in the doses of 0.01-20 mg/kg 0.5 hour prior to the subcutaneous injection of the convulsive agent (20 mg/kg) [6]. Since the mechanism of thiosemicarbazide-induced convulsions development assumes a long period of endogenous GABA depletion, animals were observed for 3 hours with registration of the number of different types of convulsions (myoclonic tremor, generalized seizures as tonic extension, the total number of the convulsions mentioned, as well as the time of their onset) and the time of the lethal effect onset. The time count was started from the moment of the chemoconvulsant introduction. Within the following 24 hours the final – lethal – effect in each experimental group was determined to assess the possible time-dependent influence and the total characteristics of the propoxazepam protective action. The lethal effect was estimated in an alternative form by the number of survived animals. The primary experimental data were corrected in accordance to Barrens procedure and calculated with the probit-method [7]. Taking into account that data variance did not meet normal distribution (asymmetry and excess values) these data were presented as "the first–third quartile, median (minimal÷maximal values)".

Results and discussion

It is well-known that the leading role in the anti-epileptic action of drugs belongs to the ability of GABA-gated ion channel since this mediator is the major inhibitory transmitter in the CNS, and activation of this system leads to inhibition of pathologic excitement distribution. A wide choice of chemoconvulsants allows determining the main mechanism of action of the substance studied on certain models. However, among the pharmacological approaches of epilepsy models the GABA-deficient states are rarely used. It is mostly determined by the fact that widely-used chemoconvulsive agents cause the concentration-dependent effect due to their concurrency antagonism on inhibition systems, thus, their effect can be represented as rapidly-reversible and dose-dependent. However, the protective effect of the substances studied after the previous introduction of semicarbazide can not be always represented in an alternative form (as the number of animals without the lethal effect).

Pathogenesis of semicarbazide (and substances with the similar mechanism of action, such as 4-deoxypyridoxine, isoniazid, L-allylglycine, or consumption deprivation of pyridoxine) evolves through blocking the GABA synthesis key enzyme – glutamate decarboxylase, EC 4.1.1.15, mostly GAD65 isoform synthesizing GABA for neurotransmitter needs) [8]; it leads to GABA insufficiency and further inability to inhibit excitement processes. Usually this mechanism determines the model choice for screening of compounds with the expected GABA-mimetic effect.

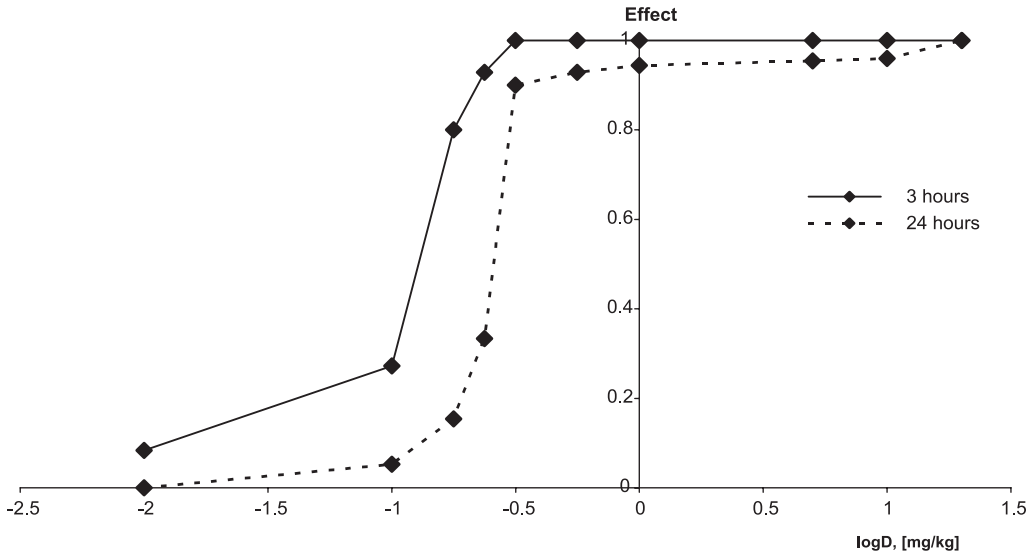

Fig. 1. The "dose – effect" dependence for propoxazepam on the model of thiosemicarbazide-induced seizures (the period of observation – 3-24 h)

However, except GABA-like compounds, barbiturates and bensodiazepines, which actually have no GABA structural similarity, also appear to have protective properties under these conditions; it means that there are another ways of their influence. The most possible explanation of their protective action is that acting as GABA-receptor complex allosteric modulators they increase affinity of binding sites to GABA, as a result, the receptor-ligand complex can exist for a longer time (increasing the hyperpolarization state duration) in contrast to structures with native properties. The increase of the characteristic time of the GABA-receptor complex also causes reduction of GABA degradation. The main metabolic route of GABA transformation is transamination with α-ketoglytarate (GABA-transaminase). This enzyme inhibition is the main mechanism of action for vigabatrin [9], which is used as antiepileptic drug. From this point of view the pharmacological model of the GABA-deficient state formed by thiosemicarbazide introduction can give a holistic understanding of the propoxazepam influence on the GABA ionic receptor – both as having the intrinsic activity (a direct effect on GABA-binding sites) and as possible allosteric regulation of receptor-ligand interactions with endogenous GABA.

In our experiments by the total value of the protective action (reduction of lethality in the experimental groups) propoxazepam showed the high activity within three hours of the observation period – about 90 % of the animals survived when introducing them the dose of 0.2 mg/kg. However, in the experimental groups with lower doses mortality was higher within 24 hours of observation (Fig. 1). Presumably, it is determined not only with the longer period of thiosemicarbazide toxic effect development, but also with the impact on GABA binding (but not on the GABA synthesis in the brain tissue).

It should be noted that the protective effect of propoxazepam developed very rapidly, and its quantitative characteristics (the "dose – effect" curve slope, s, Tab.) confirmed the concentration-dependent manner of antagonism of propoxazepam and thiosemicarbazide in the model used (indicating the receptor-based mechanism of the effect).

Taking into account the fact that propoxazepam in the mean effective dose (ED_{50}) has no statistically significant difference within 3 and 24 h of observation the conclusion can be made that the protective effect of the substance during the longer period is lower than in the acute period of the GABA-deficiency state. This partial increase of the calculated ED_{50} value can be explained by some causes – neurochemical (depletion of GABA in the synapses and GABA-receptor dissociation) and pharmacokinetic ones (concentration reduction in the biophase due to metabolism and elimination since k_{el} of the substance is ~0.019 h^{-1} corresponding to 36 h of the elimination half-life time) [10].

The first seizure manifestations in animals began to appear at the first minute after thiosemicarbazide introduction (control), while introduction of propoxazepam already in the dose of 0.01 mg/kg increased this time up to 70 min (Fig. 2). With the dose increase this indicator also increased continuously; however, the narrow data variation was recorded for the animals of every experimental group (interindividual variations did not exceed 1-2 min). It is explained by the rapid thiosemicarbazide absorption after the subcutaneous injection.

Unusual was the fact of the time reduction of myoclonic convulsions (Fig. 2) and increase of their number (Fig. 3) under conditions of the propoxazepam dose increase. In addition, in the experimental groups the latent time of tonic convulsions also increased (Fig. 4), while their number decreased

Table

The protective action of propoxazepam on the model of thiosemicarbazide-induced seizures

3 hours					24 hours		
Dose, mg/kg	LogD	The effect, frequency	The mean effective dose, ED_{50}, mg/kg (μmol/kg)	The "dose–effect" curve slope, s	The effect, frequency	The mean effective dose, ED_{50}, mg/kg (μmol/kg)	The "dose – effect" curve slope, s
0.01	-2	0.08	0.18 ± 0.10 mg/kg (0.31 ± 0.05 μmol/kg)	0.60	0	0.28 ± 0.18 mg/kg (0.68 ± 0.44 μmol/kg)	0.15
0.1	-1	0.27			0.05		
0.178	-0.75	0.8			0.15		
0.237	-0.63	0.93			0.33		
0.316	-0.50	1.0			0.90		
0.56	-0.25	1.0			0.93		
1	0	1.0			0.94		
5	0.70	1.0			0.95		
10	1	1.0			0.96		
20	1.30	1.0			1		

(with the complete disappearance at high doses) (Fig. 5). Such redistribution of representation of convulsions with different severity was the result of higher efficacy of inhibition processes in the CNS.

In this regard, the life duration of experimental animals after thiosemicarbazide injection was rather demonstrative (Fig. 6). It was 80 min with negligible variations in animals of the control group.

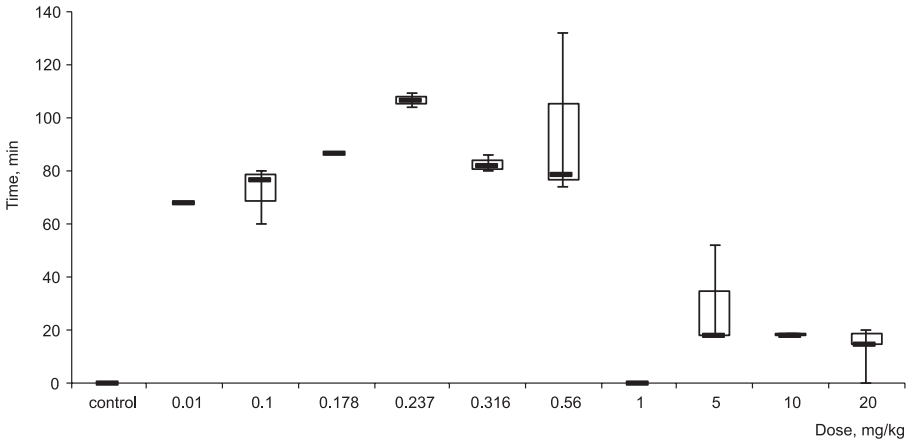

Fig. 2. The change in the latent time of myoclonic convulsions after thiosemicarbazide injection in mice with the previous introduction of different doses of propoxazepam (the first–third quartile, median (minimal÷maximal values))

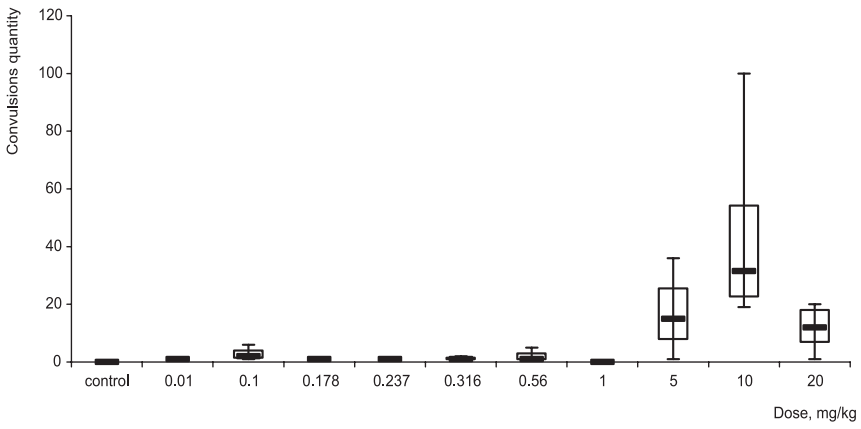

Fig. 3. The change in the number of myoclonic convulsions after thiosemicarbazide injection in mice with the previous introduction of different doses of propoxazepam (the first–third quartile, median (minimal÷maximal values))

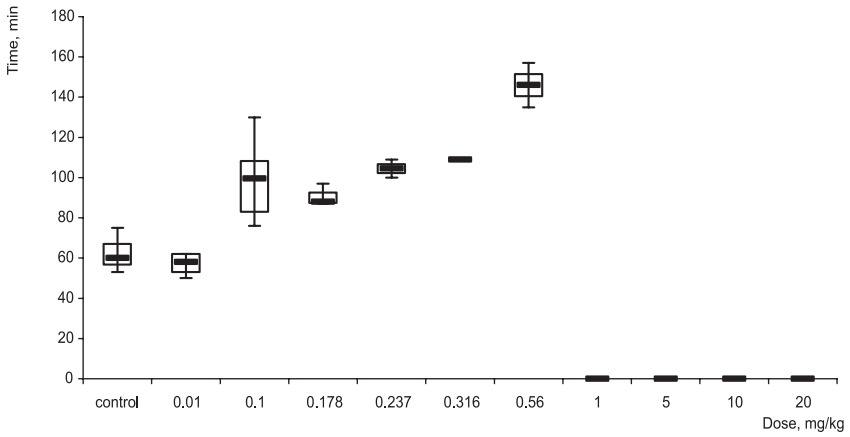

Fig. 4. The change in the latent time of tonic convulsions after thiosemicarbazide injection in mice with the previous introduction of different doses of propoxazepam (the first–third quartile, median (minimal÷maximal values))

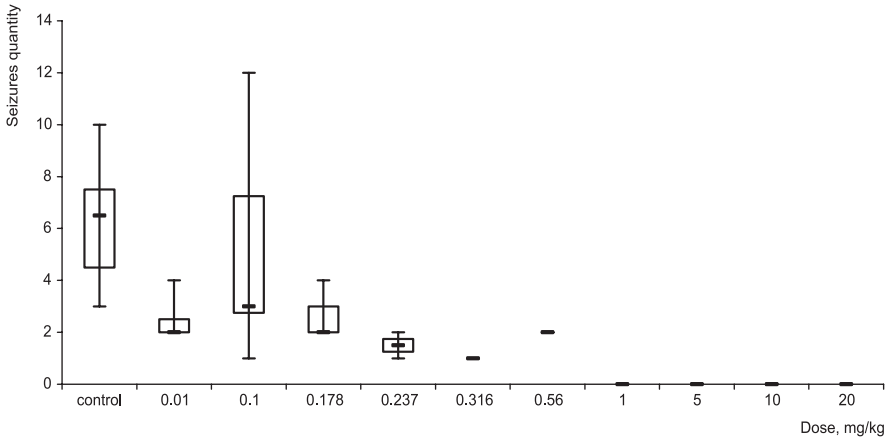

Fig. 5. The change in the number of tonic convulsions after thiosemicarbazide injection in mice with the previous introduction of different doses of propoxazepam (the first–third quartile, median (minimal÷maximal values))

In most cases tonic convulsions developed in 62 ± ± 2 min after introduction of the chemoconvulsive agent, and the lethal effect was observed within 83 ± ± 2 min because of the respiratory musculature paralysis. Against the background of propoxazepam introduction (0.1 mg/kg) there was an increase in the animals' life duration up to 128 ± 16 min, with the doses above 0.3 mg/kg the survival was longer than 3-hour period of observation.

Thus, propoxazepam showed the high activity on the model of GABA-deficient thiosemicarbazide-induced seizures. It had the antagonistic interaction with thiosemicarbazide by the shape of the "dose-effect" curve. The mean effective doses for prop-

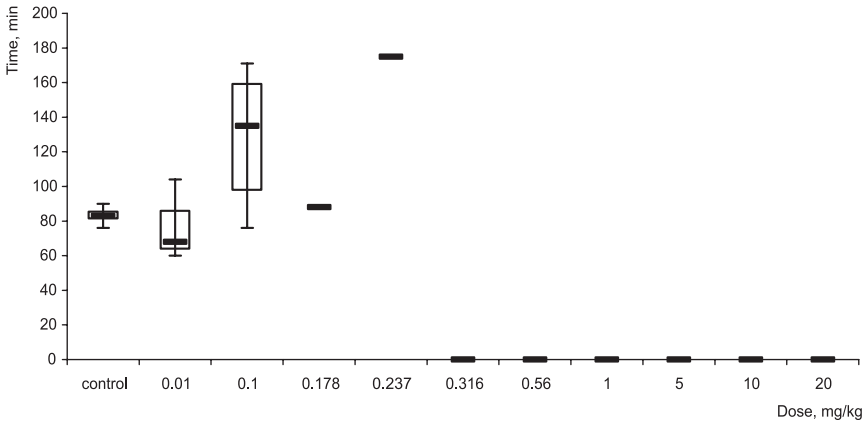

Fig. 6. The change in the survival time of mice after thiosemicarbazide injection with the previous introduction of different doses of propoxazepam (the first–third quartile, median (minimal÷maximal values))

oxazepam in acute (3 hours) and remote (24 hours) periods of observation had no statistically significant differences.

CONCLUSIONS

1. The mean effective dose of the protective action for propoxazepam on the model of GABA-deficient thiosemicarbazide-induced seizures is 0.18 ± ± 0.10 mg/kg (0.31 ± 0.05 μmol/kg). The "dose-effect" curve slope equals 0.6 corresponding to the rapid development of the protective effect and antagonistic interactions at the receptor level.

2. The increase of the propoxazepam dose leads to certain redistribution between the clonic and tonic convulsions. In the experimental groups there is a decrease in the time of occurrence of myoclonic convulsions and an increase in their number along with a reduction in the number of tonic convulsions. It indicates the increase in efficiency of inhibitory processes in the CNS; as a result, blocking of tonic seizures is more effective.

3. The increase of the propoxazepam dose increases the survival time of animals – in the dose of 1.0 mg/kg on this experimental model the effect is almost 100 %.

Conflicts of Interests: authors have no conflict of interests to declare.

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Надійшла до редакції 10.04.2017 р.