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.

Materials 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 ED50 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
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-3H-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. 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).

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 semicarbazide-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-alglycine, 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.
However, except GABA-like compounds, barbiturates and benzodiazepines, 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 $\alpha$-ketoglyutarate (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 $\sim$0.019 h$^{-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
(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.

Table

<table>
<thead>
<tr>
<th>Dose, mg/kg</th>
<th>LogD</th>
<th>The effect, frequency</th>
<th>The mean effective dose, $ED_{50}$, mg/kg ($\mu$mol/kg)</th>
<th>The &quot;dose–effect&quot; curve slope, s</th>
<th>The effect, frequency</th>
<th>The mean effective dose, $ED_{50}$, mg/kg ($\mu$mol/kg)</th>
<th>The &quot;dose–effect&quot; curve slope, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>-2</td>
<td>0.08</td>
<td>0.18 ± 0.10 mg/kg (0.31 ± 0.05 $\mu$mol/kg)</td>
<td>0.60</td>
<td>0</td>
<td>0.28 ± 0.18 mg/kg (0.68 ± 0.44 $\mu$mol/kg)</td>
<td>0.15</td>
</tr>
<tr>
<td>0.1</td>
<td>-1</td>
<td>0.27</td>
<td>0.178</td>
<td>0.8</td>
<td>0.15</td>
<td>0.33</td>
<td>0.15</td>
</tr>
<tr>
<td>0.237</td>
<td>0.63</td>
<td>0.93</td>
<td>0.316</td>
<td>1.0</td>
<td>0.90</td>
<td>0.93</td>
<td>0.15</td>
</tr>
<tr>
<td>0.56</td>
<td>-0.25</td>
<td>1.0</td>
<td>0.56</td>
<td>1.0</td>
<td>0.94</td>
<td>0.95</td>
<td>0.15</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>1.0</td>
<td>1</td>
<td>1.0</td>
<td>0.96</td>
<td>1</td>
<td>0.15</td>
</tr>
<tr>
<td>5</td>
<td>0.70</td>
<td>1.0</td>
<td>5</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>1.0</td>
<td>10</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>1.30</td>
<td>1.0</td>
<td>20</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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))
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-
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.

References


References


Відомості про авторів / Information about authors / Информация об авторах
Головенко М. Я., академік НАМН України, доктор біологічних наук, професор, завідувач відділу фізико-хімічної фармакології, Фізико-хімічний інститут ім. О. В. Богатського НАН України

Golovenko M. Ya., academician of NAMS of Ukraine, doctor of sciences in biology, professor, Head of Department of A. V. Bogatsky Physical-Chemical Pharmacology, Physical-Chemical Institute of NAS of Ukraine

Головенко Н. Я., академик НАМН Украины, доктор биологических наук, профессор, заведующий отделом физико-химической фармакологии, Физико-химический институт им. А. В. Богатского НАН Украины

Larionov V. B., доктор біологічних наук, старший науковий співробітник відділу фізико-хімічної фармакології, Фізико-хімічний інститут ім. О. В. Богатського НАН України

Ларіонов В. Б., доктор биологических наук, старший научный сотрудник отдела физико-химической фармакологии, Физико-химический институт им. А. В. Богатского НАН Украины

Редер А. С., кандидат хімічних наук, старший науковий співробітник, Фізико-хімічний інститут ім. О. В. Богатського НАН України

Reder A. S., PhD in chemistry, Senior Researcher of A. V. Bogatsky Physical-Chemical Institute of NAS of Ukraine

Редер А. С., кандидат химических наук, старший научный сотрудник, Физико-химический институт им. А. В. Богатского НАН Украины

Валіводзь І. П., аспірант, Фізико-хімічний інститут ім. О. В. Богатського НАН України

Valivodz` I. P., PhD student of A.V. Bogatsky Physical-Chemical Institute of NAS of Ukraine

Валиводзь И. П., аспирант, Физико-химический институт им. А. В. Богатского НАН Украины

Адреса для листування: 65080, м. Одеса, Люстдорфська дор., 86, відділ фізико-хімічної фармакології ФХІ ім. О. В. Богатського НАН України. Тел. (048) 765 94 02. E-mail: vitaliy.larionov@gmail.com, lvb_78@ukr.net

Mailing address: 65080, Odessa, Lyustdorfskaya road, 86, A. V. Bogatsky Physical-Chemical Institute of NAS of Ukraine. Tel. (048) 765 94 02. E-mail: vitaliy.larionov@gmail.com, lvb_78@ukr.net

Адрес для переписки: 65080, г. Одесса, Люстдорфская дор., 86, отдел физико-химической фармакологии ФХИ им. А. В. Богатского НАН Украины. Тел. (048) 765 94 02. E-mail: vitaliy.larionov@gmail.com, lvb_78@ukr.net

Надійшла до редакції 10.04.2017 р.