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ENHANCEMENT OF THE ANTICONVULSANT EFFECT OF THE H_3 HISTAMINE RECEPTOR BLOCKER THIOPERAMID AGAINST THE USE OF PIOGLITAZONE

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Abstract

The purpose of the study was to study the dynamics of foci of epileptic activity, which were reproduced in the cerebral cortex of kindled rats using the sodium salt of benzylpenicillin, under the conditions of individual and combined use of pioglitazone and thioperamid. Under conditions of acute observation in rats kindled with pentylenetetrazole repeated administration (30-35 mg/kg, i.p.), the influence of thioperamid (2,5 and 15,0 mg/kg, i.p.) and pioglitazone(50,0 and 200,0 mg/kg, i.p.) administrations upon penicillin-induced foci in the frontal cortex have been investigated. The data obtained showed that pioglitazone use in a previously ineffective dose (50.0 mg/kg, i.p.) but against the background of the administration of thioperamid (2.5 mg/kg, i.p.) was accompanied by the development of an anticonvulsant effect, which was expressed as inhibition of the amplitude and frequency of epileptic seizures potentials, as well as in reducing the life span of epileptic foci. A similar result indicates the possibility of restoration of sensitivity to the anticonvulsant effects of the

studied drugs when they are used together in kindling provoked resistance to the action of antiepileptic drugs. Thus, the development of the antiseizure effect of thioperamid and pioglitazone, which were used in initially not effective dosages (2,5 and 50,0 mg/kg correspondently), have been described. This fact favors the synergy of the antiepileptic action of investigated compounds.

Keywords: thioperamid; pioglitazone; pentylenetetrazole kindling; focal epilepsy.

Introduction. About 70 million people worldwide have epilepsy today [1]. In Ukraine, the prevalence of epilepsy is about 2.5-4.5 per 1000 [2, 3]. Despite the emergence of new, more effective antiepileptic drugs, up to 40% of patients are insensitive to their actions [1, 4]. Pharmacological resistance is defined as the inability to prescribe (sequential or combined) two antiepileptic drugs in adequate doses to eliminate the manifestations of epilepsy [5]. The problem of solving pharmacological resistance requires the urgent development of new antiepileptic drugs and the definition of new strategic approaches to its treatment.

Earlier, it was established that under the condition of blocking the H3 histamine receptor, there is a decrease in the convulsive readiness of the brain of experimental animals [6]. Thus, thioperamid, an antagonist of H3 receptors, slowed down the development of PTZ-induced kindling in rats [7].

Accordingly, the anticonvulsant effect of non-imidazole drugs - antagonists of H3 histamine receptors dose-dependently prevented generalized tonic-clonic seizures in models of maximal electric shock seizures, as well as acute seizures caused by the use of pentylenetetrazole (PTZ) [6, 8]. Pitolisant, both an antagonist and an inverse agonist of H3 receptors, has been identified as an effective agent in patients with photosensitive epilepsy both when used alone and in combination with antiepileptic pharmacological agents [9].

Agonists of γ-receptors that activate peroxisomal proliferator (PPARγ) also show anticonvulsant properties, including in models of PTZ - induced kindling, pilocarpine-induced and febrile seizures [10, 11, 12]. In our studies, it was determined that blocking PPARγ using BADGE ensured a reduction in the expressiveness of the anticonvulsant effects of non-invasive irritations of cerebellar structures [13]. However, until recently, the effectiveness of the combined use of H3 histamine receptor blockers and PPARγ agonists in relation to the manifestations of chronic epileptic syndrome - PTZ-induced kindling, which allows to reproduce resistance to the effects of antiepileptic drugs, has not been studied [5, 14].

The aim of the work was to study the dynamics of foci of epileptic activity, which were reproduced in the cerebral cortex of kindled rats using the sodium salt of benzylpenicillin, under the conditions of individual and combined use of pioglitazone and thioperamid.

Materials and methods

Observations were carried out under the conditions of an acute experiment on male Wistar rats weighing 180-250 g. Kindling in rats was induced by daily injections of PTZ in a subthreshold (30.0-35.0 mg/kg, in/ocher) dose. A total of 21 injections of the epileptogen were carried out, after which only those animals were used for observation; during the last three injections, the formation of generalized clonic-tonic convulsive attacks was noted. At the same time, these animals were studied after a three-week period of absence of PTZ, which allows for modeling a pharmacologically resistant epileptic syndrome [7, 14].

Rats under ketamine (100.0 mg/kg, i.p.) anesthesia and the conditions of fixation in the stereotaxic device SEZh-5 were exposed to the frontal sections of the cerebral cortex of both hemispheres, where the active electrode was placed. The indifferent electrode was fixed in the nasal bones of the skull. After 30 min, the animals were injected with d-tubocurarine (Orion, Finland, 0.15 mg/kg, intravenously) and put on artificial respiration. The sites of head tissue dissection and compression points were infiltrated with a solution of novocaine (0.25%).

The electrical activity of the brain structures was recorded 1.5 hrs after the surgical intervention using the DX-5000 computer system (Kharkiv, Ukraine). At the same time, the polling frequency of the channels was 256 per sec and the data was visualized on the screen and recorded on the hard disk for further processing, which was carried out using the Matlab 7.0 program. The frequency range of the signals was 0.5-40 Hz.

Foci of epileptic activity (EpA) were created by applying to the surface of the cerebral cortex pieces of filter paper soaked in a freshly prepared solution of the sodium salt of benzylpenicillin (30,000 IU/ml) [14]. Thioperamid (Sigma-Aldrich, USA) was used in doses of 2.5 and 15.0 mg/kg, i.p. Pioglitazone (Lilly S.A., Spain) was used in doses of 50.0 and 200.0 mg/kg, i.p. Animals of the control group were treated with 0.9% physiological solution of NaCL under similar conditions. Injections of the drugs were performed against the background of generation in the cells of spiking activity stable in terms of frequency and amplitude.

The power of focal EpA was assessed by the frequency-amplitude characteristics of spike potentials, as well as the total duration of the life span of the foci [14]. Statistical processing of research results (latent period and power of bioelectric activity) was performed using the one-way ANOVA method and the Newman-Keuls test. Seizure severity was assessed using the Kruskal-Wallis test.

Results. The formation of foci by application of penicillin solution to the cerebral cortex of rats in the control group was accompanied by the formation of interictal potentials, the latent period of which was from 2.5 to 5.5 min with a value of 0.6-1.1 mV. Within 5-10 min from the moment of their occurrence, an increase in the frequency and amplitude of discharges was noted, corresponding to 25-45 min and 1.5-2.0 mV. A steady state in the frequency and amplitude of EpA in the cell was recorded for 15-25 min, after which a gradual decrease in the value and frequency of spikes occurred within 30-50 min. The duration of foci of the control group ranged from 57 to 89 min (on average 71.6$\pm$0.5 min).

After 30 minutes from the moment of administration of thioperamid at a dose of 2.5 mg/kg, the frequency of generation of discharges in the cells was 18.7$\pm$2.4 min and was 19.3% less than that in the control group ($P<0.05$; Fig. 1, A). Significant differences between groups were maintained during the next 30 min of observation (Fig. 1, A). In addition, after 50 min from the moment of application of thioperamid (15.0 mg/kg, i.p.), the amplitude of the discharges was smaller than in control by 18.4% and was 1.42$\pm$0.30 mV ($P<0.05$) (Fig. 1, B). The duration of foci created under the conditions of application of thioperamid at a dose of 15.0 mg/kg ranged from 45 to 80 min (on average 65.3$\pm$8.4 min) and did not differ from the data of the control group ($P>0.05$).

In 30 min from the moment of pioglitazone injection at a dose of 50.0 mg/kg, i.p., there was a significant decrease in the frequency of generation of spike discharges in the cells - by 27.8% compared to the corresponding data in the control group ($P<0.05$; Fig. 2, A).

Further continuous observation for 30 min revealed the preservation of significant differences between groups, which at 60 min was 37.9% ($P<0.05$; Fig. 2, A). Under the influence of pioglitazone (200.0 mg/kg, i.p.) for 40 min, a decrease in the amplitude of discharges was noted in comparison with that in the control group by 21.5% ($P<0.05$; Fig. 2, B). Significant differences between the groups remained until the end of observation, and at the 60th min, the differences increased to 24.8% ($P<0.05$; Fig. 2, B). The use of pioglitazone did not change the life span of penicillin-induced foci, which was shorter than the corresponding

indicator in the control group by 7.5% and by 12.1% when the drug was administered in doses of 50.0 and 200.0 mg/kg (P>0.05).

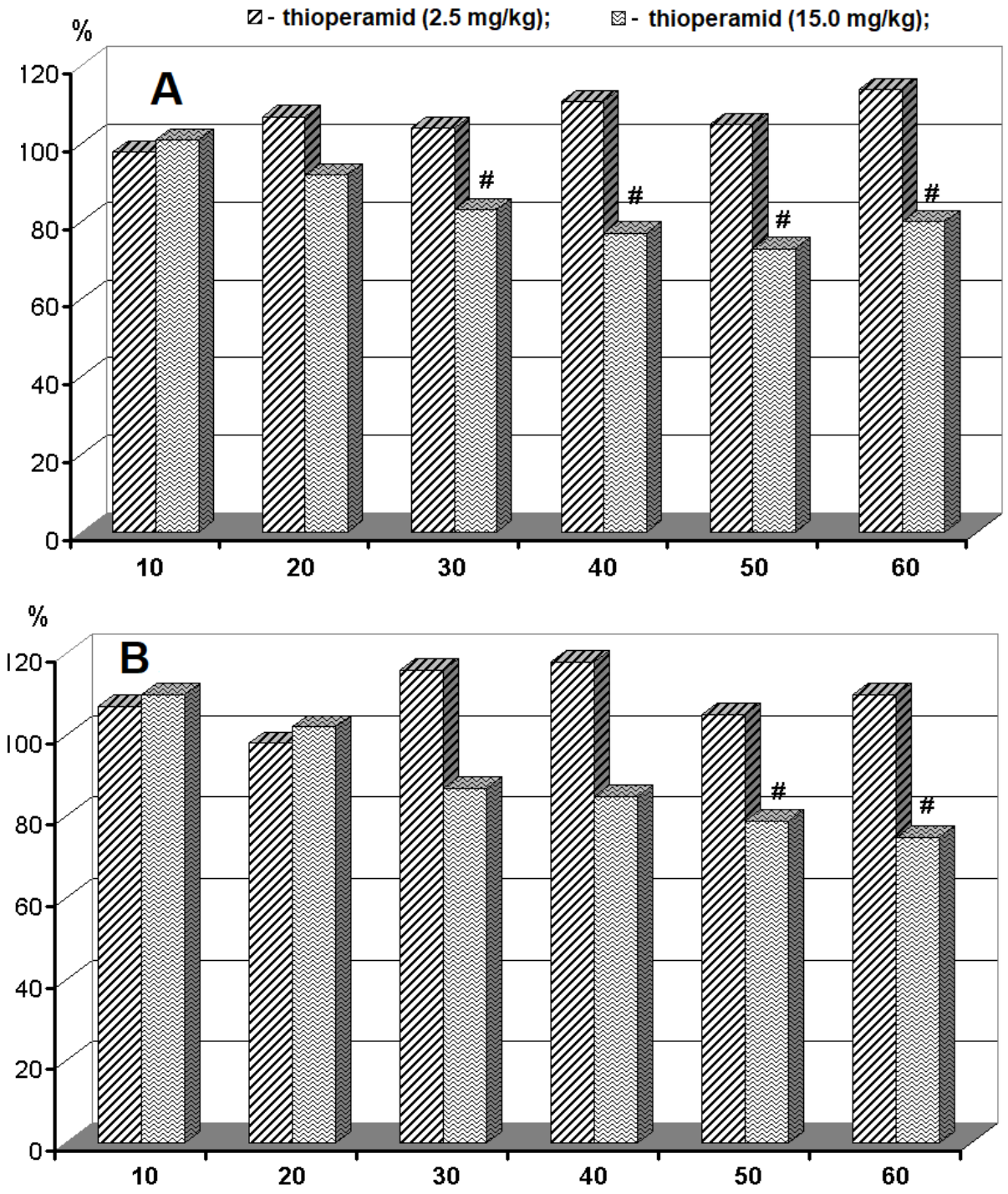

Fig. 1. Dynamics of the frequency (A) and amplitude (B) of penicillin-induced foci of epileptic activity in the cerebral cortex of kindled rats under the conditions of thioperamid administration.

Symbols: along the abscissa is the time from the moment of injection of thioperamid (minutes); on the ordinate axis - the studied indices (in %) are related to those in the control group, taken as 100%.

#- P<0.05 compared to the corresponding data in the control group.

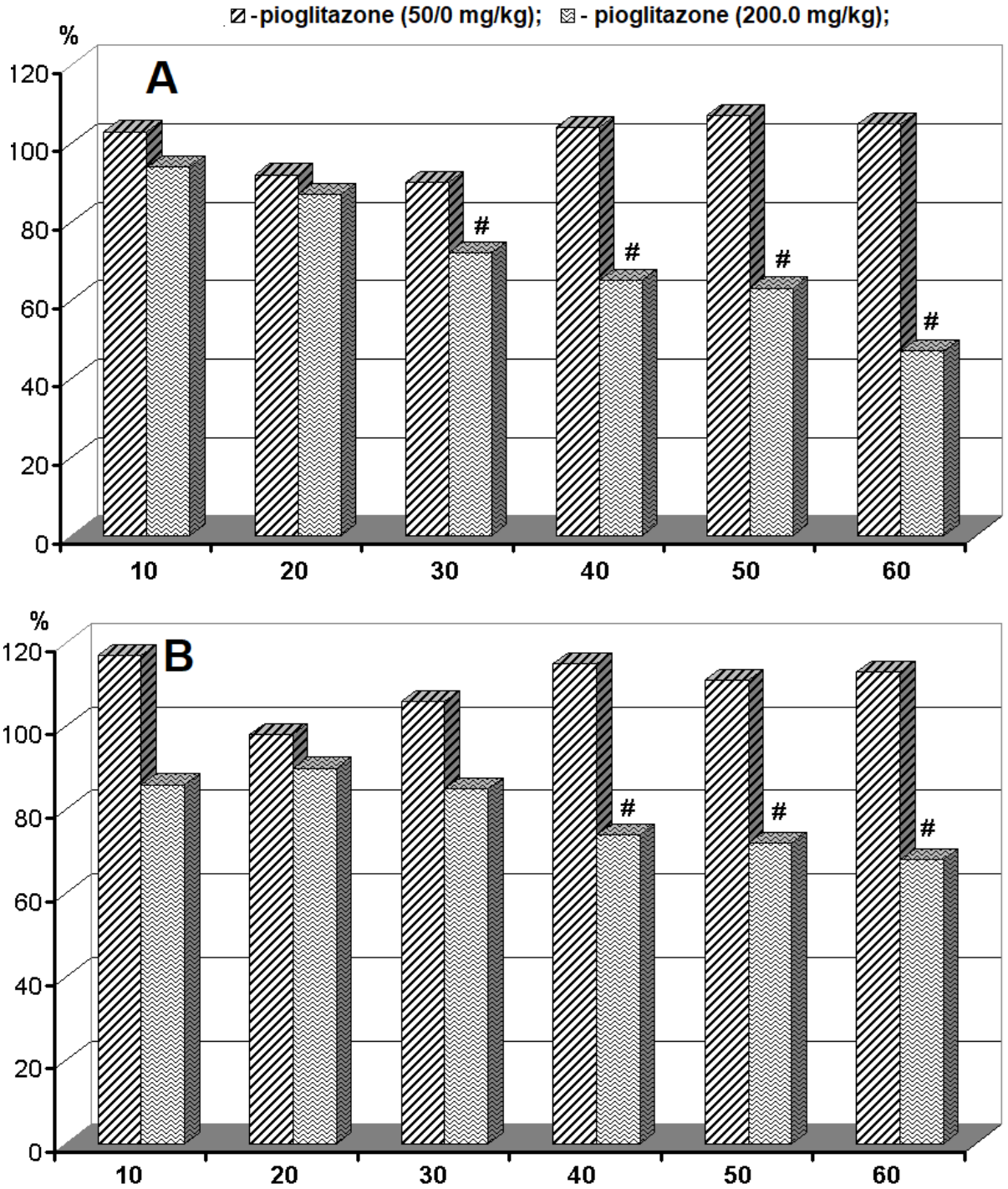

Fig. 2. Dynamics of the frequency (A) and amplitude (B) of penicillin-induced foci of epileptic activity in the cerebral cortex of kindled rats under the conditions of pioglitazone administration.

Symbols: on the abscissa is the time from the moment of pioglitazone administration (minutes); on the ordinate axis - the studied indices (in %) are related to those in the control group, taken as 100%.

#- P<0.05 compared to the corresponding data in the control group.

Under the influence of thioperamid and pioglitazone in independently ineffective doses (respectively, 50.0 and 200.0 mg/kg), already after 20 min, the frequency of generation of spike potentials decreased by 22.8% compared to the corresponding indicator in the control group. It comprised 16.3±1.5 discharges per minute (P<0.05; Fig. 3).

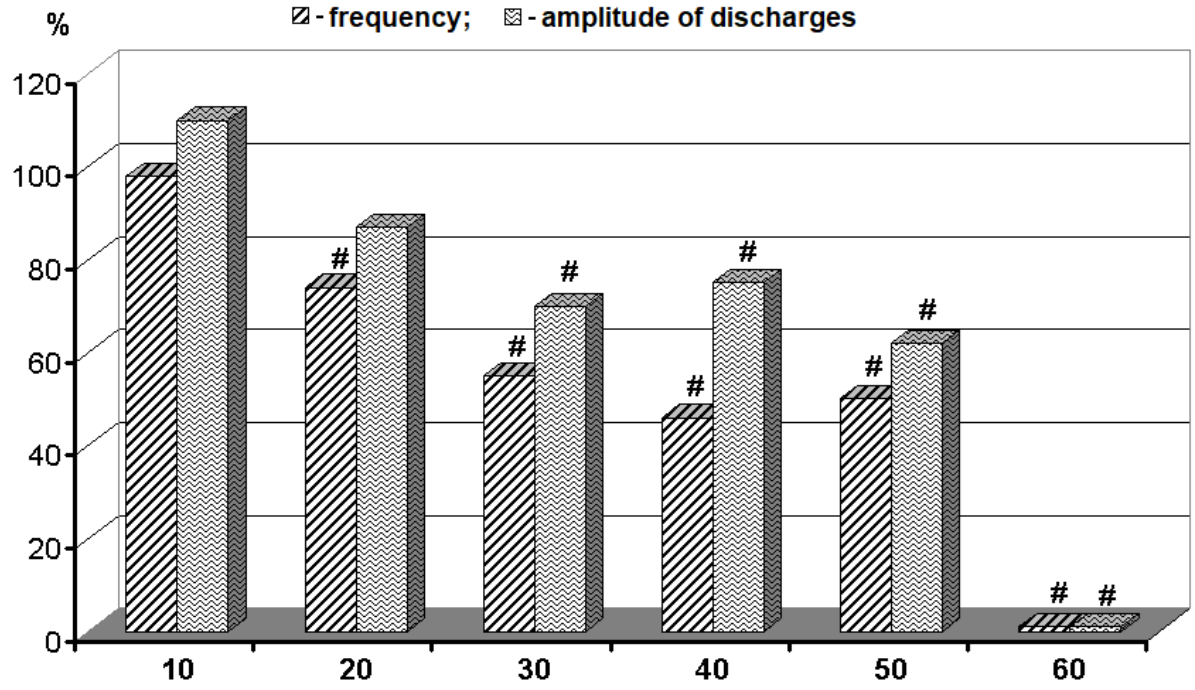

Fig. 3. Dynamics of the frequency (A) and amplitude (B) of penicillin-induced foci of epileptic activity in the cerebral cortex of kindled rats under the conditions of thioperamid (2.5 mg/kg) and pioglitazone (50.0 mg/kg).

Symbols: along the abscissa axis is the time from the moment of drug administration (minutes); on the ordinate axis - the studied indices (in %) are related to those in the control group, taken as 100%.

#- P<0.05 compared to the corresponding indices in the control group.

A significant decrease in the amplitude of discharges - by 26.0% was noted at the 30th min (P<0.05). At the same time, significant differences between the groups remained until the end of observation, and the duration of foci was reduced to 47.9 min (with fluctuations from 27.5 to 62.0 min, P<0.05).

Discussion

Thus, the presented results showed that the creation of a penicillin solution with the help of an application to the frontal sections of the cerebral cortex of rats with a chronic form of epileptic syndrome, which is carried out against the background of the previous use of thioperamid in a dose of 15.0 mg/kg, i.p., is accompanied by a significant decrease dynamics of amplitude-frequency characteristics of spike potentials. At the same time, the use of thioperamid at a dose of 2.5 mg/kg and pioglitazone at a dose of 50.0 mg/kg was ineffective under the conditions of the formation of penicillin-induced focal epileptogenesis, which indicates a relatively high resistance of the focal epileptic activity that is formed in animals under the conditions of the fully kindled syndrome, which has signs of resistance to the influence of anticonvulsant factors [14].

A higher dose of pioglitazone (200.0 mg/kg, i.p.) caused suppression of the amplitude-frequency characteristics of foci, although it did not lead to a reduction in the total duration of their existence.

The use of pioglitazone in a previously ineffective dose (50.0 mg/kg, i.p.) but against the background of the administration of thioperamid (2.5 mg/kg, i.p.) was accompanied by the development of an anticonvulsant effect, which was expressed as inhibition of the amplitude and frequency of epileptic seizures potentials, as well as in reducing the life span of epileptic foci. A similar result indicates the possibility of restoration of sensitivity to the anticonvulsant effects of the studied drugs when they are used together in kindling provoked resistance to the action of antiepileptic drugs. Probably, such a synergy is due to more significant inhibition of the main mechanisms of the neuroimmune inflammatory process due to the blocking of histaminergic and cytokine components [5].

It is also important to note that penicillin exerts an epileptogenic effect by breaking down GABAergic inhibition [1]. Therefore, one of the possible mechanisms for developing enhanced anticonvulsant effects of thioperamid and pioglitazone may be the elimination of the effect of reducing the expression of GABA-ergic receptors and increasing the activity of GABA-ergic neurons.

Conclusions:

1. Appearances of epileptic activity that form in the cerebral cortex of rats with pentylenetetrazol-induced kindling syndrome differ in resistance to the action of antiepileptic drugs.

2. The H3 histamine receptor antagonist thioperamid and the PPARγ receptor agonist pioglitazone provide a synergistic anticonvulsant effect when used together.

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