

UDC 615.21:616-009.24+577.152.162  
DOI: 10.15587/2519-4852.2023.265308

## PECULIARITIES OF THE EFFECT OF ANTIEPILEPTIC DRUGS ON SEIZURES IN MICE WITH CORNEAL KINDLING AGAINST THE BACKGROUND OF LOW-DOSE PREMEDICATION WITH CARBAMAZEPINE AND SULTHIAME

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**The aim.** The aim of the study was to evaluate the effectiveness of antiepileptic drugs (AED) with a different mechanism of action against the background of prior use of low doses of carbamazepine and sultiam in the model of corneal kindling in mice.

**Materials and methods.** The corneal kindling model in mice was used. The convulsive model was reproduced under the parallel preliminary administration of carbamazepine and sulthiame 30 minutes before electrostimulation. The anticonvulsant activity of AED (sulthiame, levetiracetam, carbamazepine, valproate, lamotrigine and retigabine) was studied under conditions after the formation of a stable syndrome of the generalised convulsive activity.

**Results and discussion.** The administration of carbamazepine and sulthiame drugs modulates the activity of the cytochrome P450 enzyme system. Thus, carbamazepine in the dose of 7 and 12 mg/kg showed no significant anticonvulsant activity (the convulsive intensity –  $4.42 \pm 0.25$  points;  $4.44 \pm 0.32$  points) after its preliminary chronic administration, the same doses of carbamazepine showed a noticeable anticonvulsant effect in the control group of animals ( $3.52 \pm 0.26$  points;  $3.2 \pm 0.6$  points, respectively). The anticonvulsant activity of lamotrigine changed both in the case of the preliminary chronic administration of an inducer (carbamazepine), and an inhibitor (sulthiame) of the cytochrome P450 system.

**Conclusion.** Changes in the pharmacological effects of AED observed against the background of chronic administration of carbamazepine and sulthiame, in our opinion, may be due to both the modulation of the cytochrome P450 system and other groups of enzymes involved in the AED metabolism

**Keywords:** antiepileptic drugs, cytochrome P450, corneal kindling, drug resistance epilepsy, carbamazepine, sulthiame

### How to cite:

Boiko, Y., Tantsura, Y., Boiko, I., Tantsura, L. (2023). Peculiarities of the effect of antiepileptic drugs on seizures in mice with corneal kindling against the background of low-dose premedication with carbamazepine and sulthiame. ScienceRise: Pharmaceutical Science, 3 (43), 67–78. doi: <http://doi.org/10.15587/2519-4852.2023.265308>

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### 1. Introduction

Epilepsy is a chronic nervous system disease; recurrent spontaneous seizures manifest it. About 65 million people worldwide suffer from this disease; a third of them do not achieve remission since they already have or have developed pharmacoresistance to antiepileptic drugs (AED), including new ones [1]. The method of epilepsy therapy remains the pharmacological one, with the need for long-term, sometimes lifelong, treatment with AED, especially in cases of pharmacoresistant forms [2].

The mechanisms of pharmacoresistance development remain insufficiently elucidated and are the subject of close attention of researchers [3, 4]. The pharmacoresistant form of epilepsy, combined with a significant increase in the frequency of severe comorbid disorders [5], increases the cost of treatment [6] and the risk of sudden death [7]. Despite significant advances in understanding the pathophysiological mechanisms of epilepsy and the introduction of new AED, the number of patients who fail to achieve control of seizures remains unchanged. Therefore, the urgent task of experimental and clinical epileptology is to develop more effective pharmacotherapy of pharmacoresistant forms of epilepsy.

The pharmacokinetic theory of the formation of resistance in epilepsy suggests that the increased expression of AED carrier proteins through the blood-brain barrier and the expression of some allelic variants of metabolic enzymes of the cytochrome P450 system modify the concentration of AED in the brain of patients [8]. It has been shown that one of the reasons for the development of pharmacoresistance is an altered individual sensitivity to AED due to polymorphism of genes encoding enzymes of the cytochrome P450 system involved in the metabolism of most AED [9–10]. The enzymes of the cytochrome P450 system metabolize about 90 % of the drugs used. Of all AED that are often used in treating epilepsy in children, only levetiracetam and lamotrigine are not metabolised by cytochrome P450 systems [11]. By the effect on cytochrome P450 enzymes, AED can be divided into inducers and inhibitors. Clinical studies have shown that the combined use of such AED as phenytoin, carbamazepine, phenobarbital, benzodiazines and other AED inducers of the cytochrome P450 enzyme significantly affects their pharmacokinetics [12–13]. On the other hand, such AED as sulthiame and stiripentol are strong inhibitors of cytochrome P450 enzymes [14]. However, their interactions

with other AED have not been studied. Taking into account the fact that patients suffering from pharmacoresistance, as a rule, need combination therapy, any pharmacokinetic interactions among AED or AED and other drugs used for diseases other than epilepsy can significantly affect the effectiveness and safety of the epilepsy therapy.

Unlike most models of acute convulsive syndromes, there are a minimal number of models of pharmacoresistant epilepsy. One of such few models of pharmacoresistant epilepsy is the model of electrostimulation (ES) corneal kindling [15].

This model is characterised by focal seizures with the subsequent secondary generalisation and limited efficiency of AED with elements of pharmacoresistance [16]. In 2015, the screening program for epilepsy therapy at the National Institute of Neurological Diseases and Stroke (USA) proposed to use the corneal kindling model in mice as a model of choice for the initial screening of new anticonvulsant compounds [17]. In addition, this model reproduces behavioural comorbidities and pathophysiological changes similar to those in patients with epilepsy, for example, memory disorders and neuroinflammation [18].

We adapted the approach of Koneval et al. [19] in modelling a pharmacoresistant form of epilepsy, which included ES corneal kindling against the background of the chronic administration of lamotrigine. We used carbamazepine and sulthiame as modulators of CYP450 enzymes.

The aim of this work was to study the effect of inducers and inhibitors of cytochrome P450 enzymes on the severity of the antiepileptic effect of generally accepted AED in the conditions of corneal kindling in mice. A separate task of the work was to study the behavioural changes that occur in animals with the formed corneal kindling syndrome against the background of the introduction of AED.

## 2. Planning(methodology) of the research

The study design is shown in Fig. 1. The experiment consisted of two stages: the formation of a pharmacoresistance epilepsy model and studying the anticonvulsant activity of AED in animals with pharmacoresistance epilepsy.

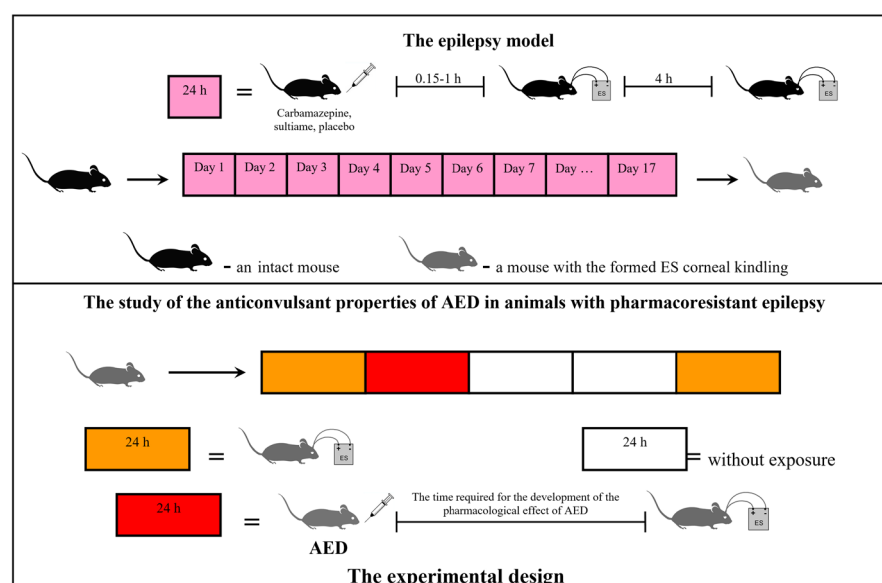


Fig. 1. General plan of the study

## 3. Materials and methods

### Animals.

All studies were conducted in 2021. The studies were performed on white male mice of the BALB/c line weighing 17–19 g of the Experimental Biological Clinic of the Odessa National Medical University; mice were kept in were housed 5 ice/cage in a temperature-controlled vivarium in a 14:10 light/dark cycle vivarium conditions. The experiments were carried out in accordance with the recommendations of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes and approved by the Commission on Bioethics of the Odessa National Medical University (Protocol No. 84 of October 10, 2008). When working with laboratory animals, the “Rules for performing work using experimental animals” were also followed; these rules were approved by order of the Ministry of Health of Ukraine No. 249 of 01.03.2012 and the Law of Ukraine No. 3447-IV “On protection from cruelty to animals” (as amended on 15.12.2009 and 16.10.2012).

### The corneal kindling model.

Kindling was induced using an “Astro-Med/Grass Technologies S48” square pulse stimulator (Artisan Technology Group, USA). ES was performed transcorneally before applying the electrodes, the cornea was irrigated with 2 % aqueous solution of lidocaine (Darnitsa, Ukraine). The ES parameters were as follows: the current frequency – 60 Hz, the current strength – 3 mA, duration – 3 sec [12]. The procedure was performed twice a day, daily, and the interval between the ES test runs was at least 4 hours. Animals were subjected to ES until the development of sustained, repeated seizures with an intensity of at least 5 points. The intensity of seizures in points was assessed as follows: 0 points – the absence of any convulsive manifestations and freezing behaviour; 1 point – myoclonic tremors of the lower jaw; 2 points – myoclonic tremors of the limbs and head; 3 points – clonic convulsions of the forelimbs, 4 points – clonic convulsions of the forelimbs with rearing and falling on the side; 5 points – generalised convulsions with immediate loss

of balance and falling a similar response was considered as a “developed kindling” [20]. Later animals were subjected to electrical stimulation once a day for 5 days. Mice with convulsive reactions with an intensity of less than 5 points were not included in the subsequent analysis [19].

### Experimental groups.

Animals with the developed corneal kindling were divided into 3 groups of 20 mice. To simulate changes in the activity of CYP450 enzymes, the experimental animals were injected with AED, reducing or increasing their activity. The first group of animals received a water-tween emulsion (10 % Tween-80) containing car-

bamazepine (a CYP450 inducer) in the dose of 5 mg/kg, the total volume of the injected emulsion was 0.1 ml, and the injections were performed 15 min before each ES. The second group of animals received a water-tween emulsion (10 % Tween-80) containing sulthiame (a CYP450 inhibitor) in the dose of 50 mg/kg, the total volume of the injected emulsion was 0.1 ml, and the injections were performed 15 min before each ES. The third group was a positive control and received a Tween emulsion (10 % Tween-80) without additives, the volume of the injected emulsion was 0.1 ml, and the injections were performed 15 min before the start of ES. The fourth group (20 mice) was as a negative control; like the positive control group, it received a Tween emulsion (10 % Tween-80) without additives, the volume of the injected emulsion was 0.1 ml, but this group was not subjected to ES. The drugs and placebo were injected intraperitoneally once a day, daily for 17 days. The doses of the drugs used mainly corresponded to the established  $ED_{50}$  [19, 21], the  $ED_{50}$  of sulthiame was established in a preliminary pilot study. In addition, the group of negative control animals was used in the study of the behavioural activity: these animals were not subjected to ES, but during the experiment they were peritoneally injected with a water-tween emulsion.

#### *The study of the anticonvulsant activity.*

The study of the anticonvulsant effect of drugs was started 5 days after the completion of the kindling formation. The following drugs were used: carbamazepine – doses of 7 mg/kg, 12 mg/kg administered 15 min before ES (animals in group – 10); sulthiame – doses of 100 mg/kg, 200 mg/kg, 300 mg/kg administered 1 hour before ES (animals in group – 10); levetiracetam – doses of 30 mg/kg, 60 mg/kg, 120 mg/kg administered 1 hour before ES (animals in group – 8); lamotrigine – doses of 30 mg/kg, 50 mg/kg administered 30 min before ES (animals in group – 10); valproate – doses of 75 mg/kg, 150 mg/kg, 300 mg/kg administered 15 min before ES (animals in group – 10); retigabine – doses of 5 mg/kg, 10 mg/kg, 20 mg/kg administered 30 min before ES (animals in group – 6) [19, 21]. The  $ED_{50}$  of sulthiame was established in a preliminary pilot study. All used agents were pure substances (Sigma-Aldrich). The time of administration of the drugs studied in relation to ES depended on the peculiarities of their pharmacokinetics. All drugs were administered intraperitoneally in a Tween emulsion (water-insoluble) or in a saline solution (water-soluble). The studies of the anticonvulsant effect for each dose were performed on the experimental groups consisting of 6 or more animals. The studies of the anticonvulsant activity were conducted twice; the interval between the experiments was at least 2 days. The results were combined.

#### *The study of the behavioural activity.*

To study the behavioural activity, the “light-dark box” test (LDB) and the “open field” test (OF) were used. The LDB apparatus was a closed box divided into two unequal compartments. The larger compartment had a size of 30×30×20 cm (length×width×height of the walls), and was illuminated from above by an incandescent lamp

with a power of 60 watts raised by 1 m in relation to the floor of the compartment. The smaller compartment had a size of 10×10×20 cm (length×width×height of the walls) and was closed from above with an opaque lid. A 3×3 cm opening in the wall separated the light and dark compartments, which were closed by a movable door.

All studies were conducted in the daytime, from 14.00 to 17.00. The experimental animal was placed in the dark compartment, and the movable door was removed. The total time spent by the animal in the dark compartment of the LDB was recorded.

A plastic square (60×60 cm) with a side height of 20 cm was used as the OF, its floor was marked into 25 equal squares. An animal was placed on peripheral squares. The time spent by the animal in the OF was 180 s. The field was conventionally divided into 3 concentric squares – the inner one consisting of one square, the middle one consisting of 8 squares and the outer one consisting of 16 squares. The number of crossed squares (horizontal activity), upright postures on the hindpaws, and the time spent in each of the three concentric squares were recorded [22].

The behavioural activity of the experimental animals was studied three times: before the onset of the convulsive syndrome, on Day 10 of its formation and after the stable convulsive syndrome was formed (Day 21). The behavioural activity after the convulsive syndrome formation was studied in the morning before the first electrostimulation.

#### *Statistical processing of the data obtained.*

The preliminary distribution of animals into experimental groups was carried out randomly. The data distribution obtained during the studies was checked for normality using Shapiro-Wilk’s test. Since all the data obtained had a normal distribution, ANOVA was performed using the Student’s t-test with the Bonferroni correction. All P values for the corresponding t values are already indicated in the text, considering the Bonferroni correction. Calculations were performed using the MS Office EXCEL software package [23].

## 4. Results

Epilepsy is often accompanied by being overweight due to altered food preferences, decreased physical activity and general mobility. Rigorous exercise can be a factor provoking an attack, so patients consciously reduce them [24]. The increased lipogenesis observed in epilepsy is often a reaction to taking AED. An increase in body weight is recorded both in the case of taking drugs with a sedative effect (phenobarbital) and appetite-enhancing AED – valproate [25], gabapentin [26], and carbamazepine [27]. An additional effect of AED may be an increase in the formation of ketone bodies. Thus, it is interesting to study the dynamics of possible changes in the body weight of the experimental animals under the conditions of corneal kindling against the background of the chronic administration of AED.

Before conducting the research, animals were selected by body weight. The study included mice with a body weight of 17–19 g. Data on changes in the body weight of the experimental animals are shown in Fig. 2. During 18 days of the convulsive syndrome formation, the

average body weight of animals in each experimental group did not change significantly (less than 1 g). At the beginning of the experiment, the average body weight of animals varied from 17.9 g (the control group of animals) to 18.3 g. The highest average body weight was recorded in the group injected with carbamazepine (19.5 g) before ES. Nevertheless, the difference with the other groups observed ( $\Delta m=0.5-0.6$  g) was insignificant ( $P>0.05$ ) and had no fundamental significance. It was most likely due to the deviation of indicators in the sample. The absence of body weight growth was associated with acute stress exposure in the form of double daily ES, and a daily intraperitoneal administration of the anticonvulsant drug. It is also necessary to note the low mortality of animals during the experiment; the number of dead animals was 3.3 %.

The convulsive syndrome was formed using the method of corneal ES kindling. The dynamics of changes in convulsive manifestations in response to the repeated corneal ES are shown in Fig. 3. During the first 6 days, the severity of seizures ranged from 0.5 to 1.5 points; after that a linear increase in the intensity of the convulsive activity was observed in most animals in the group. The maximum indicators of convulsive manifestations were noted by Day 17 of ES and were  $\sim 4.5$  points for each group. The administration of carbamazepine and sulthiame (twice a day, daily) did not significantly affect the dynamics and development of convulsive manifestations (Fig. 3) in the conditions of corneal kindling. It should be noted that both anticonvulsants were used in sub-efficacious doses – 50 mg/kg for sulthiame and 5 mg/kg for carbamazepine, respectively. These doses are close to  $ED_{50}$  in maximum electroshock seizures for carbamazepine [28] and sulthiame. The absence of significant differences in the intensity of manifestations of the convulsive syndrome in different groups of animals may also be due to the exclusion of animals with a high convulsive threshold and a low convulsive response from the analysis.

The severity of the anticonvulsant effects of various AED in the conditions of the formed corneal kindling is presented in Fig. 4.

In the group receiving carbamazepine during the kindling formation, there was no anticonvulsant response when carbamazepine was administered in effective doses (7 and 12 mg/kg), which caused a significant dose-dependent decrease in the intensity of seizures in the sulthiame group ( $2.83\pm 0.41$ ;  $2.7\pm 0.45$  points;  $t=5.3, 5.1$ ;  $P<0.001$ ) and the control group of animals for the dose of 7 mg/kg ( $3.5\pm 0.26$  points;  $t=5.6$ ;  $P<0.001$ ) (Fig. 4, a). Therefore, the preliminary chronic administration of carbamazepine in sub-efficacious doses leads to the fact that carbamazepine loses its ability to have the anticonvulsant effect when administered in effective doses. Such a pharmacoresistant effect may be due to the preliminary induction by the CYP3A4 isoform causing the oxidation of carbamazepine to carbamazepine-10,11-epoxide [29].

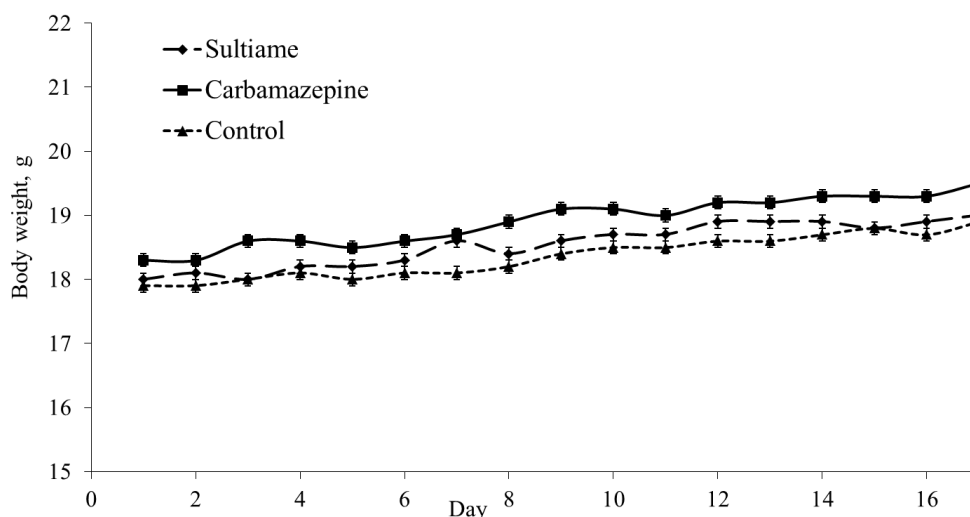


Fig. 2. The dynamics of changes in the body weight of mice under the conditions of the corneal kindling formation and the CYP450 altered activity caused by the administration of anticonvulsants (“Sulthiame” and “Carbamazepine” lines) or the intragastric administration of a water emulsion of Tween-80 (“Control” line)

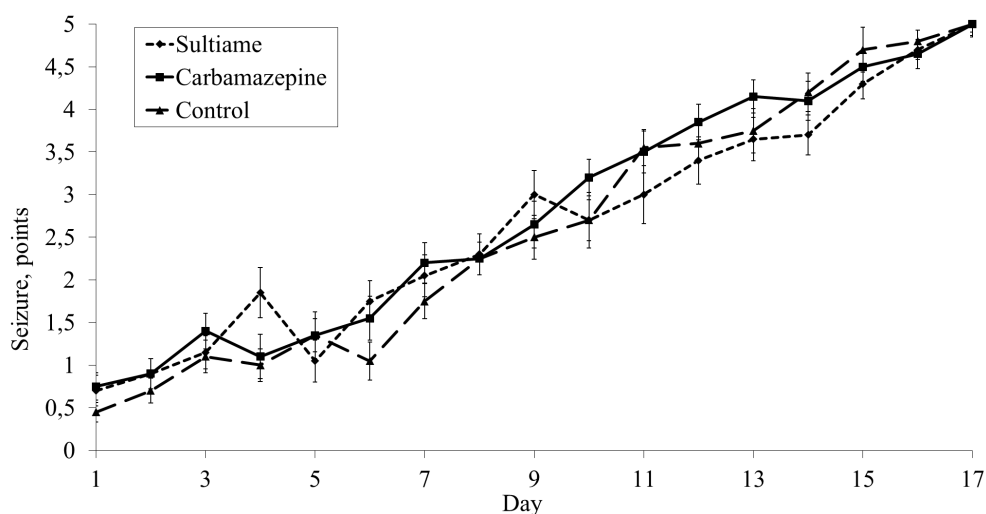


Fig. 3. The severity of a convulsive response caused by the corneal electrical stimulation (3 mA, 60 Hz, 3 s) under conditions of the CYP450 altered activity caused by the administration of anticonvulsants (“Sulthiame” and “Carbamazepine” lines) or intragastric administration of water emulsion of Tween-80 (“Control” line)

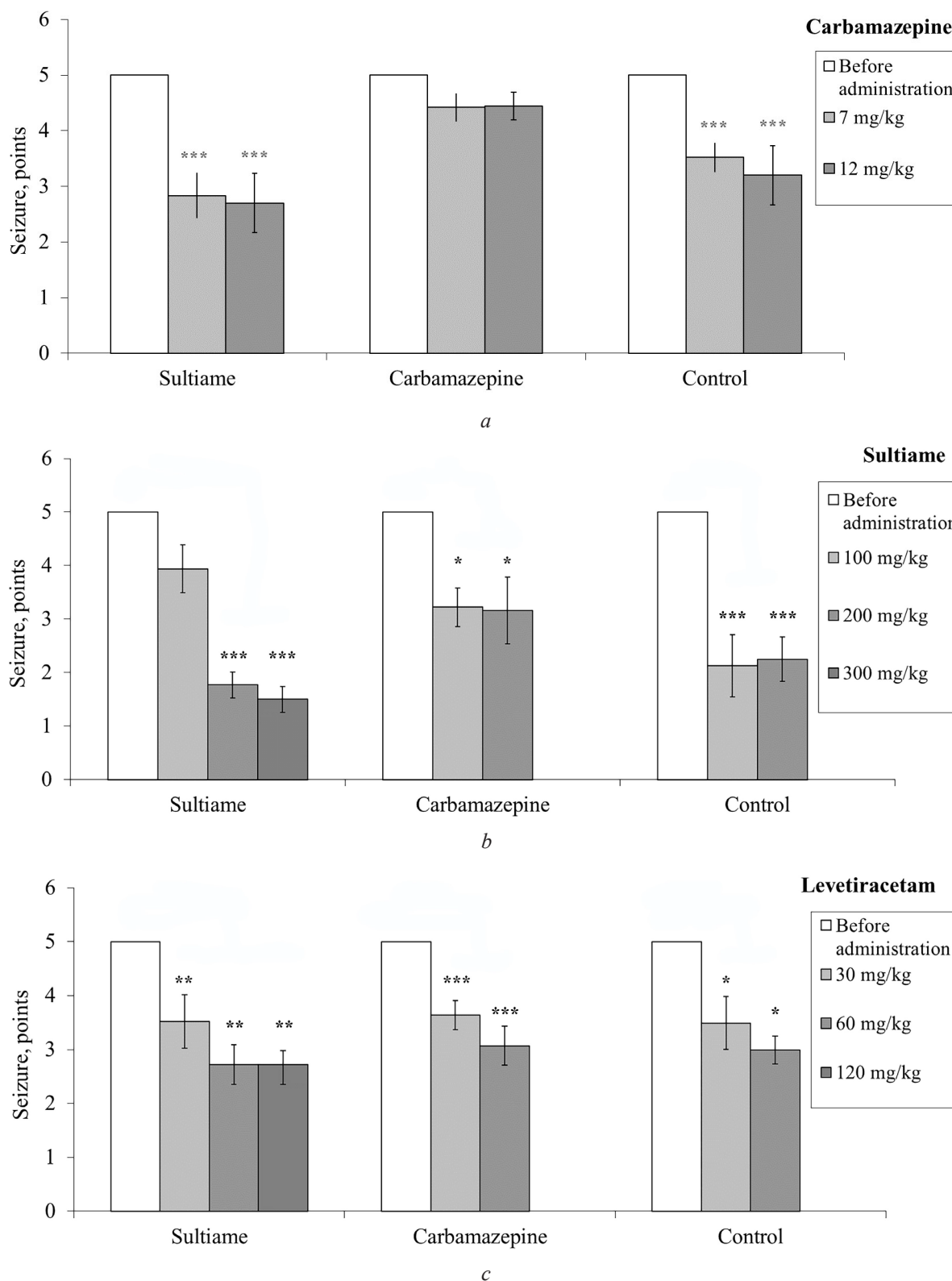


Fig. 4. The assessment of the anticonvulsant effectiveness of various antiepileptic drugs under conditions of the corneal electrical stimulation against the background of the preliminary administration of carbamazepine and sultiame or placebo in animals with the formed convulsive syndrome. Stimulation parameters: 3 mA, 60 Hz, 3 s; \* –  $P < 0.05$ , \*\* – 0.01, \*\*\* – 0,001 between “Before administration” and “Dose of AED”: a – carbamazepine, b – levetiracetam, c – sultiame

Sultiame (Fig. 4, b) did not have the anticonvulsant effect in the 100 mg/kg dose in animals previously received in sub-ineffective doses. At the same time, this dose was sufficient to cause the anticonvulsant effect in the control group of animals ( $2.12 \pm 0.36$  points,  $t = 7.99$ ;

$P < 0.001$ ) and the carbamazepine group of animals ( $3.22 \pm 0.55$  points,  $t = 3.23$ ;  $P < 0.05$ ). An increase in the administered dose of sultiame to 200 and 300 mg/kg was sufficient for the drug to cause the anticonvulsant effect ( $1.77 \pm 0.45$ ;  $1.5 \pm 0.24$  points;  $t = 7.2$ ;  $14.6$ ;  $P < 0.001$ )

in animals previously taken it in sub-*efficacious* doses. Since the metabolism of sulthiame occurs in the liver by unknown enzyme isoforms, it can be assumed that its preliminary administration causes the induction of the

corresponding metabolic systems. This leads to a decrease in the anticonvulsant effectiveness of sulthiame against the background of its chronic administration in the experiment.

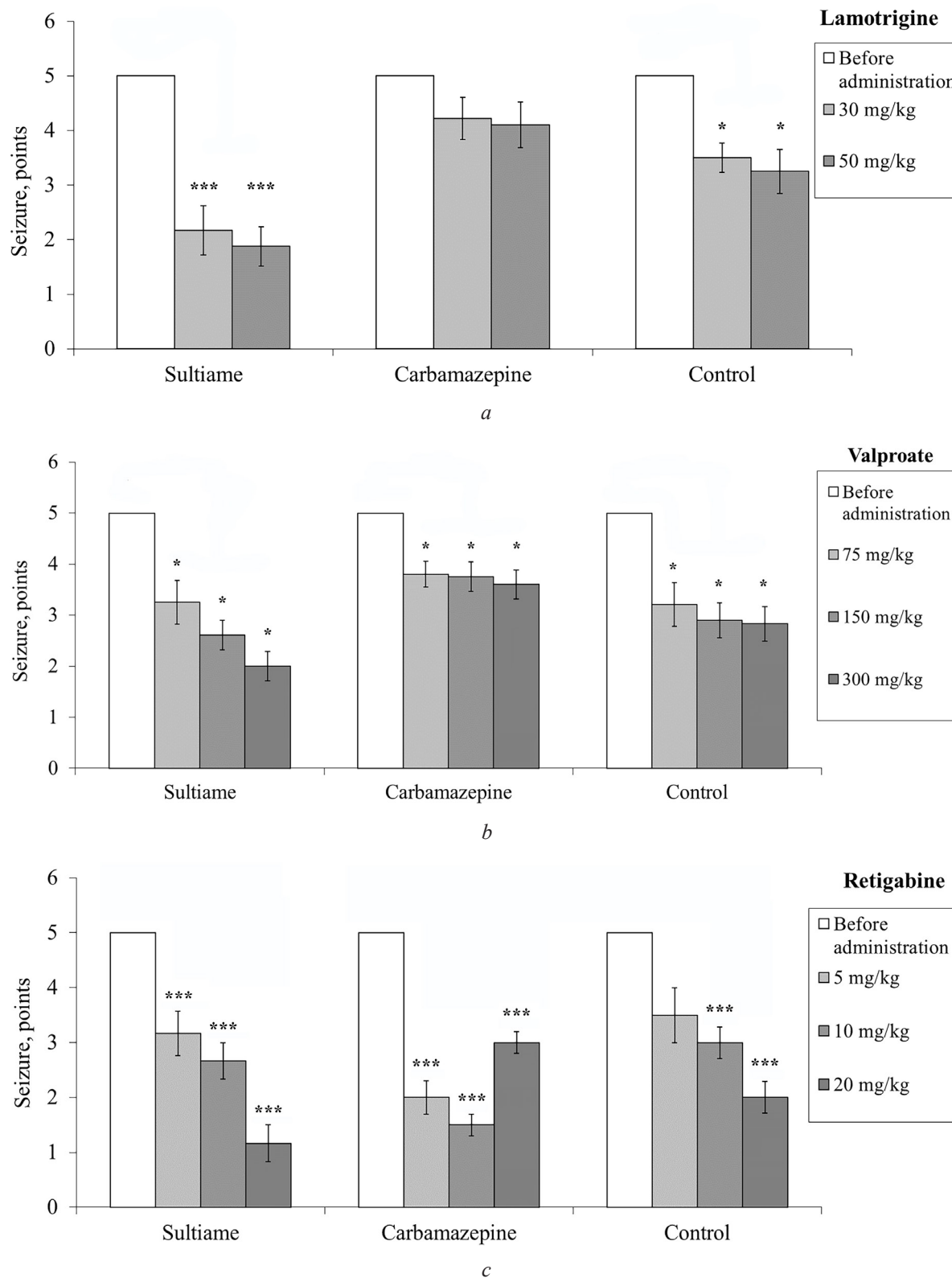


Fig. 4. The assessment of the anticonvulsant effectiveness of various antiepileptic drugs under conditions of the corneal electrical stimulation against the background of the preliminary administration of carbamazepine and sulthiame or placebo in animals with the formed convulsive syndrome. Stimulation parameters: 3 mA, 60 Hz, 3 s; \* –  $P < 0.05$ , \*\* – 0.01, \*\*\* – 0,001 between “Before administration” and “Dose of AED”: *a* – lamotrigine, *b* – valproate, *c* – retigabine

Levetiracetam (Fig. 4, *c*) had an anticonvulsant effect for the sulthiame group ( $3.52 \pm 0.27$ ;  $2.72 \pm 0.49$ ;  $2.72 \pm 0.36$  points,  $t=5.5, 4.6, 6.26$ ;  $P<0.01$ ) and the carbamazepine group ( $3.64 \pm 0.18$ ;  $3.07 \pm 0.27$  points,  $t=7.7, 7.1$ ;  $P<0.001$ ). In the control group of animals, levetiracetam caused changes in the convulsive activity similar to those observed in the carbamazepine and sulthiame groups ( $3.49 \pm 0.41$ ;  $2.99 \pm 0.49$  points,  $t=3.67, 4.1$ ;  $P<0.05$ ). Levetiracetam has extremely limited exposure to hepatic metabolism, mainly excreted by the kidneys in an unchanged form [30]. There is evidence in the literature that carbamazepine can reduce the plasma concentration of levetiracetam by 20–30 % [31]; however, we have not recorded significant differences in anticonvulsant effects between the carbamazepine group and the control group of animals.

The administration of lamotrigine (Fig. 5, *a*) did not have the anticonvulsant effect in the carbamazepine group ( $4.22 \pm 0.3$ ;  $4.1 \pm 0.39$  points). Even though lamotrigine is metabolised by the glucuronosyltransferase family of enzymes (UGT1A4, UGT1A1, UGT2B7) [32], carbamazepine can reduce its plasma concentration [33]. In the control group of animals under the effect of lamotrigine, the intensity of the convulsive syndrome had a dose-dependent significant decrease ( $3.5 \pm 0.25$ ;  $3.25 \pm 0.27$  points;  $t=3.0, 3.37$ ;  $P<0.05$ ) compared to the baseline level of seizures. The most pronounced anticonvulsant effect of lamotrigine was registered in the sulthiame group ( $2.2 \pm 0.2$ ;  $1.9 \pm 0.45$  points;  $t=6.3, 6.9$ ;  $P<0.001$ ). For the 30 mg/kg dose, the anticonvulsant effect was significantly higher than in the control group ( $t=4.16$ ,  $P<0.01$ ).

The administration of valproate (Fig. 5, *b*) caused a 20–60 % decrease in the intensity of seizures compared to the baseline level, and it was a reliable anticonvulsant effect for all experimental groups. Valproic acid undergoes multiple hepatic metabolic changes, including O-glucuronidation,  $\beta$ -oxidation,  $\omega$ -oxidative hydroxylation, and ketone formation, which involve both the cytochrome P450 enzyme family and the glucuronosyltransferase family [34]. The pharmacological interactions of valproic acid with carbamazepine are complex and include pharmacokinetic and pharmacodynamic components. Carbamazepine can enhance valproate metabolism, increasing the concentration of its plasma metabolites to the hepatotoxic level [35]; at the same time, the simultaneous administration of carbamazepine and valproate is accompanied by a noticeable potentiation in the antiepileptic activity [18]. The anticonvulsant effect of valproate observed by us in the carbamazepine group did not significantly differ from a similar effect in the control group. This is, apparently, due to the not simultaneous but preliminary administration of carbamazepine in relation to the administration of valproate. A dose-dependent anticonvulsant effect of valproate was noted for the sulthiame group ( $3.25 \pm 0.23$ ;  $2.6 \pm 0.43$ ;  $2.0 \pm 0.29$  points).

Although the administration of retigabine (Fig. 5, *c*) caused a pronounced anticonvulsant effect in all groups of animals, there were a number of differences

in the severity of the anticonvulsant effect both between groups and within groups. In animals of the carbamazepine group, retigabine had a more pronounced anticonvulsant effect in the dose of 10 mg/kg than in the dose of 20 mg/kg ( $1.5 \pm 0.3$  and  $3 \pm 0.2$  points;  $t=3.6$ ;  $P<0.01$ ). The reduced anticonvulsant effectiveness of retigabine in the dose of 20 mg/kg was noted only in animals of the carbamazepine group, a dose-dependent effect was observed in animals of the sulthiame group when retigabine was administered; retigabine in the dose of 20 mg/kg had a significantly greater anticonvulsant effect in animals of the sulthiame group than in animals of the carbamazepine group ( $1.2 \pm 0.33$ ,  $3 \pm 0.2$  points;  $t=4.7$ ;  $P<0.001$ ). The pharmacokinetics of retigabine includes biotransformation under the action of hepatic glucuronosyltransferases and simultaneous inhibition of cytochrome P450 family enzymes [36]. Probably, these properties can explain the paradoxical decrease in the severity of the anticonvulsant activity of retigabine with an increase in the dose to 20 mg/kg in the case of the carbamazepine group.

Data on the behavioural activity of mice of control and experimental groups in administering AED are presented in Table 1.

All animals that took part in the experiments to study the behavioural activity had a persistent formed convulsive syndrome, except the negative control group, which was not subjected to electrical stimulation.

The most noticeable changes were observed in the duration of the animals' stay in different open field areas (outer, middle and inner squares). Animals of the carbamazepine group and the positive control after forming the convulsive syndrome preferred to be in the outer perimeter of the open field (outer square) ( $155.7 \pm 1.83$ ,  $149.4 \pm 2.37$  s); before the use of ES, the time when animals were in the outer perimeter was significantly lower ( $135.8 \pm 3.6$ ,  $124.6 \pm 3.8$  sec.;  $T=4.93, 4.84$ ;  $P<0.001$ ).

The motor activity of animals in the positive control group and the carbamazepine and sulthiame experimental groups did not change significantly. There were no significant changes in the locomotor behavioural pattern during the kindling formation (on Day 7 of ES). The vertical motor activity (before and after the kindling formation: carbamazepine  $t=1.16$ ,  $P>0.2$ ; sulthiame  $t=0.64$ ,  $P>0.5$ ; control(+)  $t=1.74$ ,  $P>0.1$ ; control(-)  $t=-0.9$ ,  $P>0.2$ ) and the horizontal motor activity (before and after the kindling formation: carbamazepine  $t=0.726$ ,  $P>0.5$ ; sulthiame  $t=0.642$ ,  $P>0.5$ ; control(+)  $t=0.421$ ,  $P>0.5$ ; control (-)  $t=1.788$ ,  $P>0.1$ ) also did not change significantly after the convulsive syndrome formation.

In the LDB test, animals preferred to be in a dark compartment of the box, both before the beginning of ES and after the kindling formation. The average time spent in the dark compartment ranged from 84.5 s up to 114.7 s. The increase in the exploratory activity associated with leaving from the dark compartment of LDB in animals of all the groups studied did not change significantly (before and after the kindling formation: carbamazepine  $t=1.45$ ,  $P>0.2$ ; sulthiame  $t=1.314$ ,  $P>0.2$ ; control(+)  $t=-0.36$ ,  $P>0.5$ ; control(-)  $t=-0.722$ ,  $P>0.5$ ).

Table 1  
Indicators of the behavioural activity of the experimental animals before and after the corneal kindling formation.\*

Experiment. groups	Before electro-stimulation	Ten days after the start of electrostimulation	After the formation of a stable kindling
The horizontal motor activity, crossed squares			
Sulthiame	49.2±4.3	38.4±3.6	44.3±6.3
Carbamazepine	45.7±4.6	33.3±4.8	39.5±7.2
Control +	41.5±6.7	40.9±6.7	38.1±4.5
Control –	48.4±5.1	37.6±5.5	38.0±2.8
The vertical motor activity, rearing			
Sulthiame	18.3±3.4	10.5±2.7	15.6±2.5
Carbamazepine	16.9±2.7	9.7±1.8	12.2±3
Control +	18.4±1.4	13.6±2.3	14.7±1.6
Control –	16.5±1.8	13.2±2.4	19.2±2.4
The time spent in the inner, middle and outer square, s			
Sulthiame	18.4±2.0	14.2±2.5	14.2±2.5
	34.3±3.1	29.6±3.7	24.3±2
	127.3±3.0	136.2±2.5	140.9±2.9
Carbamazepine	13.7±2.5	16.2±3.5	8.8±1.17
	30.5±2.9	25.7±4.7	15.5±1.55
	135.8±3.6	138.1±3.9	155.7±1.83*#
Control +	16.2±4.1	19.6±3.1	11.6±1.63
	39.2±3.3	37±4.2	19.0±2.55
	124.6±3.8	123.4±4.8	149.4±2.37*#
Control –	19.3±2.7	12.8±2.4	16.8±3.5
	24.0±1.8	26.9±3.5	33.2±2.4
	136.7±4.1	140.3±2.6	130±2.8
The exploratory activity, the time spent in the dark compartment of the LDB, s			
Sulthiame	114.7±4.9	105.4±8.0	97.3±12.3
Carbamazepine	103.7±8.2	98.9±6.9	84.5±10.4
Control +	105.3±11.9	101.6±12.7	112.3±15.6
Control –	97.0±10.5	95.4±10.5	106±6.7

Note: The corneal kindling formation was against the background of the administration of sub-efficacious doses of carbamazepine and sulthiame, or a Tween emulsion in the case of the positive control group (control +). Animals of the negative control group (control –) were intact and were not subjected to electrical stimulation.  $M \pm m$  where  $M$  – is the average value,  $m$  – is the error of the average value; \* –  $P < 0.05$ , between “Before electrostimulation” group and “After the formation of a stable kindling” group, # –  $P < 0.05$ , between “Ten days after the start of electrostimulation” group and “After the formation of a stable kindling” group.

## 5. Discussion

The use of repeated corneal ES leads to the appearance of a convulsive reaction and a gradual increase in the intensity of convulsive manifestations to generalised clonic-tonic seizures. These data correspond to [17] and indicate the formation of a stable kindling in the conditions of the model used. Koneval et al. [16] applied the method of lamotrigine administration before the repeated corneal EC, and it led to the formation of a model of pharmacoresistant seizures to some AED. Us-

ing a similar approach, we injected carbamazepine and sulthiame before ES to modulate the activity of the cytochrome P450 family enzymes. At the same time, it is generally believed that various etiological factors take part in the formation of pharmacoresistant epilepsy: changes in the characteristics of the drug biotransformation, changes in the systems of carriers through the blood-brain barrier, changes in neuronal target cells appearing in chronic administration of AED [37].

The studies have shown that the administration of carbamazepine and sulthiame do not prevent the development of convulsive activity in conditions of repeated ES. This is apparently due to their relatively low dose used, which, however, is sufficient to affect enzymatic systems. Further study of the pharmacodynamic profiles of various AED showed the presence of the effect of the preliminary chronic administration of carbamazepine and sulthiame on the level of the anticonvulsant activity in subsequently administered carbamazepine, sulthiame, lamotrigine, retigabine. This confirms our initial assumptions about the existence of a delayed drug interaction.

The ability to induce cytochrome P450 enzymes has long been known for carbamazepine [38]; moreover, carbamazepine stimulates the activity of P-glycoprotein [39]. Both effects underlie the potential mechanisms of pharmacoresistance [35]. We have shown that in the chronic administration of carbamazepine its subsequent anticonvulsant effect, in doses that are effective under normal conditions, is reduced until it completely disappears in the experiment. Such a significant decrease in the pharmacological activity is explained not only by the ability of carbamazepine to induce cytochromes P450 and P-glycoprotein, but also by the fact that subsequently it itself is their target, and it leads to a sharp decrease in its bioavailability for target tissues.

The chronic administration of sulthiame led to the subsequent absence of the anticonvulsant effect in this drug administered in the dose of 100 mg/kg. At the same time, this dose was therapeutically effective in the carbamazepine and control groups. In view of the lack of accurate data on the mechanisms of metabolism of this drug, we can assume that its preliminary administration leads to the activation of enzyme or transport systems involved in the subsequent biotransformation and elimination of this compound.

One of the essential metabolic features of levetiracetam is its extremely limited biotransformation. In view of this, the change in the activity of liver enzymes under the action of the chronic administration of carbamazepine and sulthiame did not have a significant impact on the anticonvulsant effect of levetiracetam.

For lamotrigine, doses sufficient to develop a noticeable anticonvulsant effect in the sulthiame and control groups (30 and 50 mg/kg) did not have the anticonvulsant effect on animals from the carbamazepine group. As mentioned above, lamotrigine is not metabolized by cytochrome P450 enzymes, but is an AED that is carried by P-glycoprotein [40]; it leads to a noticeable decrease in its therapeutic effectiveness when combined with carba-



mazepine. In addition, lamotrigine and carbamazepine act through a common neuronal target – Na<sup>+</sup>-channel. On the contrary, sulthiame potentiated the anticonvulsant effect of lamotrigine, which in the dose of 30 mg/kg was more effective in the sulthiame group than in animals of the control group. Based on this fact, we can cautiously assume the effect of sulthiame on the pharmacokinetic systems involved in the distribution and metabolism of lamotrigine.

When using retigabine the pre-administration of both sulthiame and carbamazepine led to potentiation of the anticonvulsant effect of this drug; this made it possible to achieve the therapeutic effect in doses lower than those required to achieve similar indicators in the control group of animals. As mentioned above, this may be due to the peculiarities of the retigabine biotransformation, which occurs under the action of glucuronosyltransferases and is accompanied by simultaneous inhibition of cytochrome P450 proteins [34].

Summarizing the above, the following new, previously unknown, features of the AED pharmacodynamics with a delayed drug interaction can be distinguished: the repeated administration of the inducer of the cytochrome P450 system and P-glycoprotein, carbamazepine, are accompanied by a subsequent decrease in the anticonvulsant effect of both carbamazepine itself and lamotrigine. A similar, but less pronounced, effect develops in the case of sulthiame – the chronic administration of this drug causes a subsequent decrease in its anticonvulsant effect. On the contrary, the prior use of both carbamazepine and sulthiame potentiated the anticonvulsant effect of levetiracetam in the subsequent administration. It should be noted that cases of potentiation of the anticonvulsant action with carbamazepine were also observed for other AED. For example, the combination of carbamazepine and thiotriazoline leads to a synergistic enhancement of the anticonvulsant effect. The repeated administration of sulthiame also caused an increase in the anticonvulsant activity of lamotrigine. It can be classified as a potentiating effect.

Epilepsy, in addition to convulsive syndromes, can be accompanied by various behavioral disorders [41]. The sub-efficacious doses of AED used could not affect the formation of kindling but could affect the behavioral activity of experimental animals with convulsive syndrome. In addition, changes in behavioral activity may be more subtle indicators than seizures. Therefore, in order to more fully characterize the used seizure model, studies of behavioral activity were carried out.

The changes in the motor activity observed are the result of a complex motor-behavioral reaction. Immediately after placing an animal in the open field the freezing behavior is the most natural, its duration is usually several seconds. In conditions of agoraphobia and isolation from the usual social group the animal is most likely to develop an avoidance response, which may include an exploration component, which intensity will depend on the degree of stress exposure [42]. The development of the persistent convulsive syndrome against the back-

ground of the administration of subthreshold doses of sulthiame and carbamazepine had a selective effect on some behavioral patterns that did not include locomotion. We can postulate that despite the sharply increased convulsive readiness, the overall motor activity has not changed.

The most obvious changes in the behavioral activity concerned the time spent by animals in different parts of the open field. Similar changes were observed by Koneval et al. [16], who noted the hyperactivity of animals after the kindling formation, as well as an increase in the level of anxiety. Due to the peculiarities of the animals' movement between the inner and outer squares of the open field, we came to the conclusion that the formation of the convulsive syndrome led to shifts in the standard behavioral model: it increased the time spent in the peripheral part of the field. The tendency to be located in the outer perimeter of the open field in the presence of tactile contact with the walls of the box was observed in animals from the carbamazepine group and the positive control group. This fact can be regarded as an increase in the anxiety pattern.

The work [43] showed a difference in the behavioural activity in the group of animals with a pharmacoresistant form of epilepsy and epileptic animals susceptible to the action of AED. At the same time, animals with a pharmacoresistant form of epilepsy were in the aversive inner ring for less time. They showed greater anxiety compared to animals with a pharmacosensitive form of epilepsy. In our study, the difference between animals with pharmacoresistant and pharmacosensitive forms of epilepsy was also demonstrated: the time spent by animals of the sulthiame group in the middle ring was significantly higher than in animals of the carbamazepine group (56.7 % higher,  $t=3.48$ ,  $P<0.02$ ).

The LDB test is a method for determining the level of anxiety that allows to assess the balance between research and protective activity. The increase in the level of anxiety in animals with the formed kindling shown in the "open field" test was not accompanied by significant changes in the time spent in the dark compartment of LDB. In the study [44] it was shown that the behavior patterns (for example, the level of anxiety) shown by animals in the LDB test do not match the corresponding behavior patterns shown in the OF test. This may explain the differences we observed in the results of the behavioral activity of the LDB and OF tests.

The data obtained are important for understanding the interaction of AED in conditions of their combined use in pharmacoresistant epilepsy.

**Study limitation.** The used model of pharmacoresistant epilepsy reproduces only one of the possible mechanisms of pharmacoresistance. Changes in the activity of the cytochrome P450 system have not been experimentally studied.

**Prospects of further research.** The results regarding the effect of the prolonged administration of carbamazepine and sulthiame on the anticonvulsant activity of AED as an effective experimental model of

pharmacoresistant epilepsy allow a preclinical assessment of the pharmacological interaction of AED. The theoretical data obtained are also promising in the clinical aspect, allowing us to predict (taking into account the results of other studies, including clinical ones) the effectiveness of the combined use of various AED in treating convulsive syndromes. Further research in this direction will allow us to create a fundamental theoretical basis that can become the basis for the subsequent development of methods of the pharmacoresistant epilepsy therapy.

## 6. Conclusions

The studies have shown that the pre-administration of carbamazepine and sulthiame in sub-efficacious doses changed the anticonvulsant activity of various AED. Carbamazepine at the used doses of 7 and 12 mg/kg, there was no anticonvulsant effect ( $4.42 \pm 0.25$ ,  $4.44 \pm 0.32$  points) in the group of animals taking carbamazepine during the kindling formation. From our point of view, such an effect is explained by the ability of these drugs to modulate the activity of the cytochrome P450 enzyme system, which is responsible for the oxidation of

a number of AED (carbamazepine, valproate, retigabine), as well as the ability to change the activity of P-glycoprotein. The decrease in the anticonvulsant activity of lamotrigine ( $4.22 \pm 0.3$ ;  $4.1 \pm 0.39$  points) observed against the background of prolonged administration of carbamazepine seems to be associated with the activation of P-glycoprotein and a decrease in the bioavailability of this drug for the nervous tissue. The changes in the pharmacodynamics of carbamazepine, sulthiame and levetiracetam after the repeated administration of carbamazepine and sulthiame are also of apparent clinical interest.

## Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this article.

## Funding

The study was performed without financial support.

## Data availability

Data will be made available on reasonable request.

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*Received date 19.04.2023*

*Accepted date 22.06.2023*

*Published date 30.06.2023*

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