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ANTIEPILEPTIC PROPERTIES OF L-ARGININE ON THE MODELS OF SIMPLE AND COMPLEX PARTIAL SEIZURES
АНТИЭПИЛЕПТИЧЕСКИЕ ЭФФЕКТЫ L-АРГИНИНА В УСЛОВИЯХ ЭКСПЕРИМЕНТАЛЬНЫХ МОДЕЛЕЙ ПРОСТЫХ И КОМПЛЕКСНЫХ ПАРЦИАЛЬНЫХ СУДОРОГ

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Summary. On the model of amygdalar kindling in rats (complex partial seizures) it was shown that L-arginine (500,0 mg/kg, i.p.) caused the suppression of behavioral seizures. The effect was similar to that one induced via diphenylhydantoin (DPhG, 100 mg/kg, i.p.) administration. On the model of simple partial seizures induced in the brain cortex via penicillin solution application L-arginine (500,0 mg/kg, i.p.) elicited more pronounced decreasing of spike frequency in comparison with phenobarbital (10,0 mg/kg, i.p.), while DPhG (100 mg/kg, i.p.) did not affect focal epileptogenesis. It is concluded that L-arginine possessed efficacy both against simple and complex partial seizures.
Key words: L-arginine, kindling, epileptic focus, penicillin, diphenylhydantoin, phenobarbital.

Резюме. На модели киндлинга миндалины у крыс (комплексная парциальная эпилепсия) показано подавление поведенческих судорог под влиянием L-аргинина (500,0 мг/кг, в/бр). Выраженность эффекта была подобна таковой, наблюдавшейся после применения дифенилгидантоина (ДФГ, 100 мг/кг, в/бр.). На модели простых парциальных судорог, индуцированных в коре головного мозга аппликацией раствора пенициллина, L-аргинин (500,0 мг/кг, в/бр) способствовал более выраженному снижению частоты разрядов, чем фенобарбитал (10,0 мг/кг, в/бр), в то время как ДФГ (100 мг/кг, в/бр) не влиял на фокальный эпилептогенез. Сделан вывод, что L-аргинин является эффективным в отношении простых и комплексных парциальных судорог.
Ключевые слова: L-аргинин, киндлинг, эпилептический очаг, пенициллин, дифенилгидантоин, фенобарбитал.

ПРОТИЕПІЛЕПТИЧНІ ЕФЕКТИ L-АРГІНІНА ЗА УМОВ ЕКСПЕРИМЕНТАЛЬНИХ МОДЕЛЕЙ ПРОСТИХ ТА КОМПЛЕКСНИХ ПАРЦІАЛЬНИХ СУДОМ. На моделі кіндлінгу

мигдалику у щурів (комплексна парціальна епілепсія) показано пригнічення поведінкових судом під впливом L-аргініну (500,0 мг/кг, в/очер). Цей ефект за виразністю був подібним до такого, який спостерігався після застосування дифенілгідантоїну (ДФГ, 100 мг/кг, в/очер). На моделі простих парціальних судом, які індукували в корі мозку шляхом аплікації бензилпеніциліну натрію, L-аргінін (500,0 мг/кг, в/очер) спричиняв більш виразне зниження частоти спайкових потенціалів порівняно з таким ефектом фенобарбіталу (10,0 мг/кг, в/очер), в той час як ДФГ (100 мг/кг, в/очер.) не впливав на осередковий епілептогенез. Зроблено висновок, що L-аргінін є ефективним у відношенні щодо простих та комплексних парціальних судом.

Ключові слова: **L-аргінін, кіндлінг, епілептичний осередок, пеніцилін, діфенілгідантоїн, фенобарбітал.**

It was established that L-arginine, as a precursor of NO administered i.p. (500 mg/kg) increased the latency and attenuated the severity of penicillin-induced generalized convulsions in rats whereas the administration D-arginine (500 mg/kg), inactive enantiomer, did not influence either the frequency or amplitude of epileptiform ECoG activity [1]. Several data are in favor for L-arginine antiepileptic effectiveness in the different model of experimental epilepsy [1, 2, 11]. In contrast, L-arginine (500 mg/kg) did not affect the convulsive threshold for the clonic phase of PTZ-induced seizures [7].

Hence, further investigations of L-arginine antiepileptic action in different models of epilepsy are actual. That's why we have explored two different models for L-arginine effects testing. The first – behavioral manifestations of amygdalar electrostimulative kindling, which is regarded as an experimental equivalent of complex partial epilepsy. The second– electrographic manifestations of penicillin induced foci, which are regarded as model of simple partial epilepsy [9].

The **aim** of the present work was to study whether L-arginine is able to display seizure- protective effects under conditions complex and simple partial models of experimental epilepsy. Besides, L-arginine activity was compared with the effects of classical antiepileptic compounds - diphenylhydantoin (DPhG) and phenobarbital (PhB). These AEDs were chosen as far as DPhG prevalently affected seizures spreading, while PhB more actively suppressed process of seizures origination [9].

Material and methods. Male Wistar rats with initial weight of 180-250 g were used. Not less than 6 rats were used in each group. Animals were kept at standard conditions (room temperature, 12 hrs dark/light cycles, standard diet and tap water given ad libitum).

Animals were anaesthetized with Nembutal (Ceva, France, 35 mg/kg, i.p.) and the stainless guide cannulas (ext. diameter 0,6 mm) were implanted stereotaxically into the left lateral brain ventricle (AP=0,8; L=1,5; H=3,5) according to the rat brain atlas [8]. Cannulas were fixed to the skull by dental cement (SPOFA). Post-operative period lasted for 7-10 days.

Studies on kindling seizure syndrome. Animals were anesthetized with i.p. Nembutal (35,0 mg/kg) and bipolar constantan electrodes (distance between electrodes 0,3 mm) were implanted stereotaxically into the left basolateral amygdala according the coordinates of rat brain atlas (AP=-2.2;

L=4.7; H=8.5) [8]. Electrodes were fixed to the skull by dental cement.

Kindling was started 10-14 days after the surgery. Electrical stimulation (ES) of the amygdala was performed by means of universal electrostimulator ESU-2 (FSU). Rectungle electrical stimuli (one-phase) with the frequency 60 Hz, duration 1 ms were applied. The duration of ES was 1 s. For kindling procedure those intensities were used for each animals individually which were followed by the appearance of afterdischarges in the EEG. In average the index mentioned was 20-50 mcA. Animals received 15-42 daily (once a day) trials to reach stage 4-5 of the convulsions (see below).

Severity of convulsions was evaluated according the scale of [9] immediately after ESs: stage 0 - behavioral arrest: animal stops normal exploratory or grooming behavior for a few seconds poststimulation. Stage 1 - facial and jaw clonus: rhythmic mouth movements or facial twitches. Stage 2 - head nodding: clonic contractions of neck and shoulder muscles; rearing without forelimb movements. Stage 3 - unilateral forelimb clonus: rhythmic ipsilateral or contralateral forelimb movements. Stage 4 - bilateral forelimb clonus: rhythmic movements of both forelimbs with rearing. Stage 5 -rearing and falling seizure: animal loses control of postural reflexes, e.g. righting reflex

For the observation of AEDs effects the only animals were used which responded with 3-5 stage convulsions on two subsequent ES. 2-4 hrs after the last kindling trial animals were treated with the DPhG (Sigma-Aldrich Co., USA; 100,0 mg/kg, i.p). DPhG was dissolved ex tempore in methylcellulose (5,0 %). Control animals were treated with methylcellulose (5,0 %). L-arginine (Sigma-Aldrich Co., USA) was dissolved ex tempore in 0,9% NaCl and administered i.p. in dosage of 500,0 mg/kg in a volume of 5,0 ml/kg. Control animals were treated with the equal amount of the saline. 45 min after the administration of the compounds ES of the amygdala was performed and seizure severity was evaluated.

Studies with focal cortical epilepsy. Experimental procedure was carried out according the method described in literature [6]. Skull trepanation was performed on Nembutal anaesthetized rats (35 mg/kg, i.p.) fixed in stereotaxic device. After a hole (3,0-4,0 mm in diameter) was drilled in the frontal parts of the skull the dura mater was dissected and through blotting paper (2,0 x 2,0 mm) soaked in sodium salt of benzylpenicillin solution (20000 IU/ml) application the epileptic foci were created. All wound edges and pressure points were infiltrated with a local anaesthetic (procaine, 2,0%). Infiltration was repeated every 30 min during experimental session. Electrographic recordings were performed using computer EEG system (DX- 5000, Kharkov, Ukraine). Reference electrode was localized in nasal bones. Body temperature was maintained between 34-37 ^{o}C.

For epileptic activity evaluation the frequency of discharges, their amplitude and duration of epileptic foci existence (time from the first to the last spike discharge) were estimated [6].

PhB, (Sigma-Aldrich Co., USA; 10,0 mg/kg) and DPhG, (Sigma-Aldrich Co., USA; 100,0 mg/kg)

were dissolved ex tempore in methylcellulose (5%) and injected i.p. L-arginine (500,0 mg/kg) was administered i.p. in a volume of 5,0 ml/kg. Control animals were treated with i.p. methylcellulose (5%) or saline i.p. All injections were performed at the level of steady epileptic discharges generation (10-20 min from the moment of the beginning of penicillin application).

The data were analyzed by means of one-way ANOVA or repeated measure ANOVA in proper cases, followed by Newman-Keuls test and Kruscall-Wallis test where it was appropriate. $P<0.05$ was chosen as an minimal statistic criteria.

Results and discussion.

1. Effects of L-arginine DPhG and PhB on electrical kindling behavioral manifestations.

The control amygdalar ES induced seizure of 4 stage in 2 out of 10 experimental animals, while the rest (8 rats) demonstrated 5th stage of seizure severity (Fig.1). After i.p. administration of L-arginine in investigated dose the ES of the amygdala was followed by facial/jaw clonuses, clonic contractions of neck and shoulder muscles, rearings without forelimb movements or rhythmic ipsilateral or contralateral forelimb contractions; the behavioral manifestations were thus equal to the 1-3 stages of kindling convulsions. The mean behavioral severity of seizures decreased up to 31,9% compared to control ($P<0,05$, Fig.1).

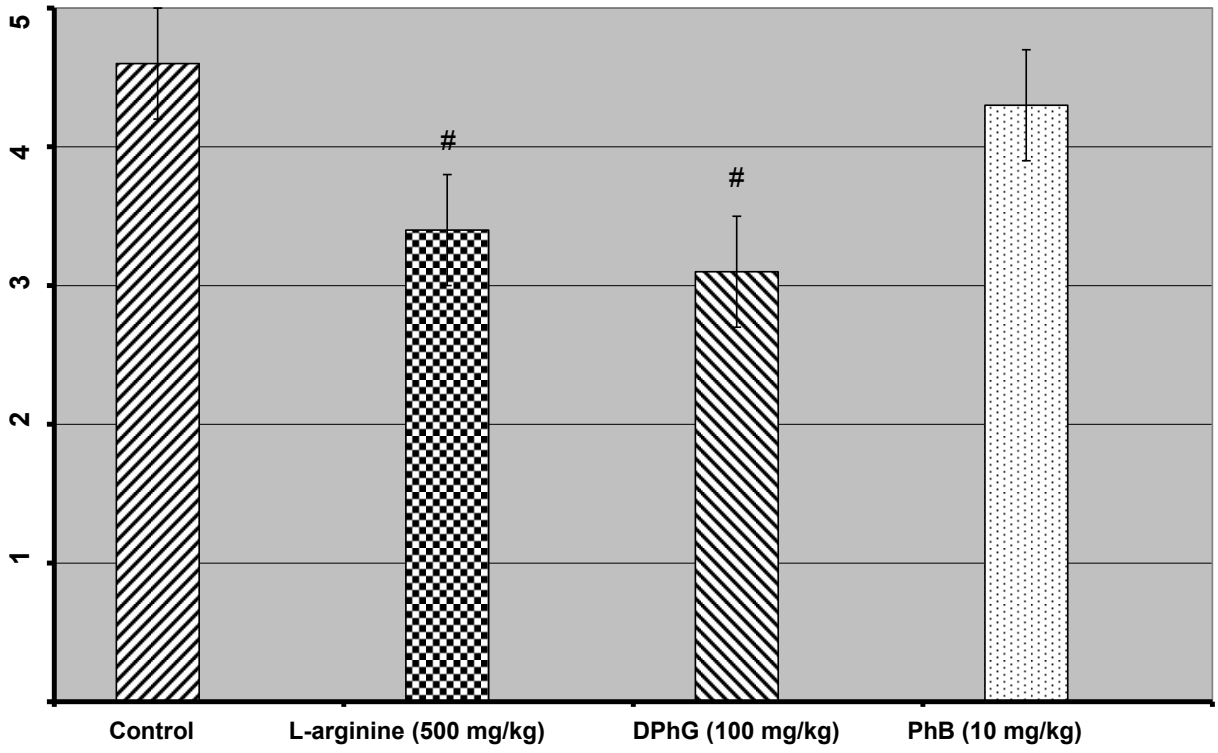

Fig.1. Effects of L-arginine, DPhG and PhB on amygdalar kindled seizures.
Abscissa- compounds used; ordinate - seizure severity (points).
- $P<0,05$ – statistical differences of the indexes investigated compared with the same in control observation (Kruscall-Wallis test)

Similar behavioral effect was observed for DPhG (100 mg/kg) - seizure severity decreased up to 37,6% compared with the index in control group ($P<0,05$, Fig. 1). Severity of kindled seizures induced after PhB administration was followed be seizures, which were reduced by 16,7% in comparison with the control ones ($P>0,05$) (Fig. 1).

2. Effects of L-arginine, DPhG and PHB on penicillin cortical foci activity.

Application of penicillin upon the cortex of control animals (methocel i.p.) was followed by the appearance of spike discharges in 3-11 min from the moment of application and in 5 min from the appearance of focal activity the average frequency was 16,67$\pm$ 2,25/per min and amplitude of discharges- 0,6$\pm$0,02 mV. Next 10 min the increasing of frequency up to 19,2$\pm$2,03 /min, and amplitude of discharges to 0,69$\pm$0,01 mV was observed. Further registration of epileptic discharges revealed continuous decreasing of investigated characteristics and general duration of foci existence was 114$\pm$12 min.

Under condition of pharmacons application (L-arginine, 500 mg/kg, DPhG, 100 mg/kg, and PHB, 10 mg/kg, all i.p.) the slight decreasing of the magnitude of spike discharges was observed in groups treated with DPhG and PhB, while L- arginine caused significant reduction starting from the 40^{th} min from the moment of compound administration (Fig. 2, A). Significantly different discharge frequency when compared with the control group of animals was achieved already on the 20-th min from the moment of L-arginine administration ($P<0,05$; Fig. 2, B). Pronounced reduction of frequency of discharges maintained till the end of observation, and on 80^{th} min significant decreasing of frequency of epileptic discharges was observed in rats treated with PhB ($P<0,05$; Fig. 2, B).

The total lifespan under condition of L-arginine administration was 61,8$\pm$10,75 min ($P<0.01$ in comparison with the control group). Both DPhG and PHB did not affect foci lifespan ($P>0,05$).

The obtained data permit to conclude that L-arginine possessed antiepileptic activity under conditions of both complex (kindling) and simple (penicillin foci) partial epileptic syndromes. The potency of the compound was comparable to that one for DPhG applied to kindled animals and was greater on the model of focal activity in comparison with the efficacy of both tested AEDs.

The fact of seizure protective effect of L-arginine upon kindled convulsions supposes that the drug is able to restrict the seizure propagation process which is simulated by kindling model [9]. On the other hand, blocking of behavioral seizures under the conditions of kindling does not mean the complete elimination of seizure manifestations: even in case of highly effective antiepileptic treatment the epileptiform afterdischarge could be recorded in the site of ES [6, 9].

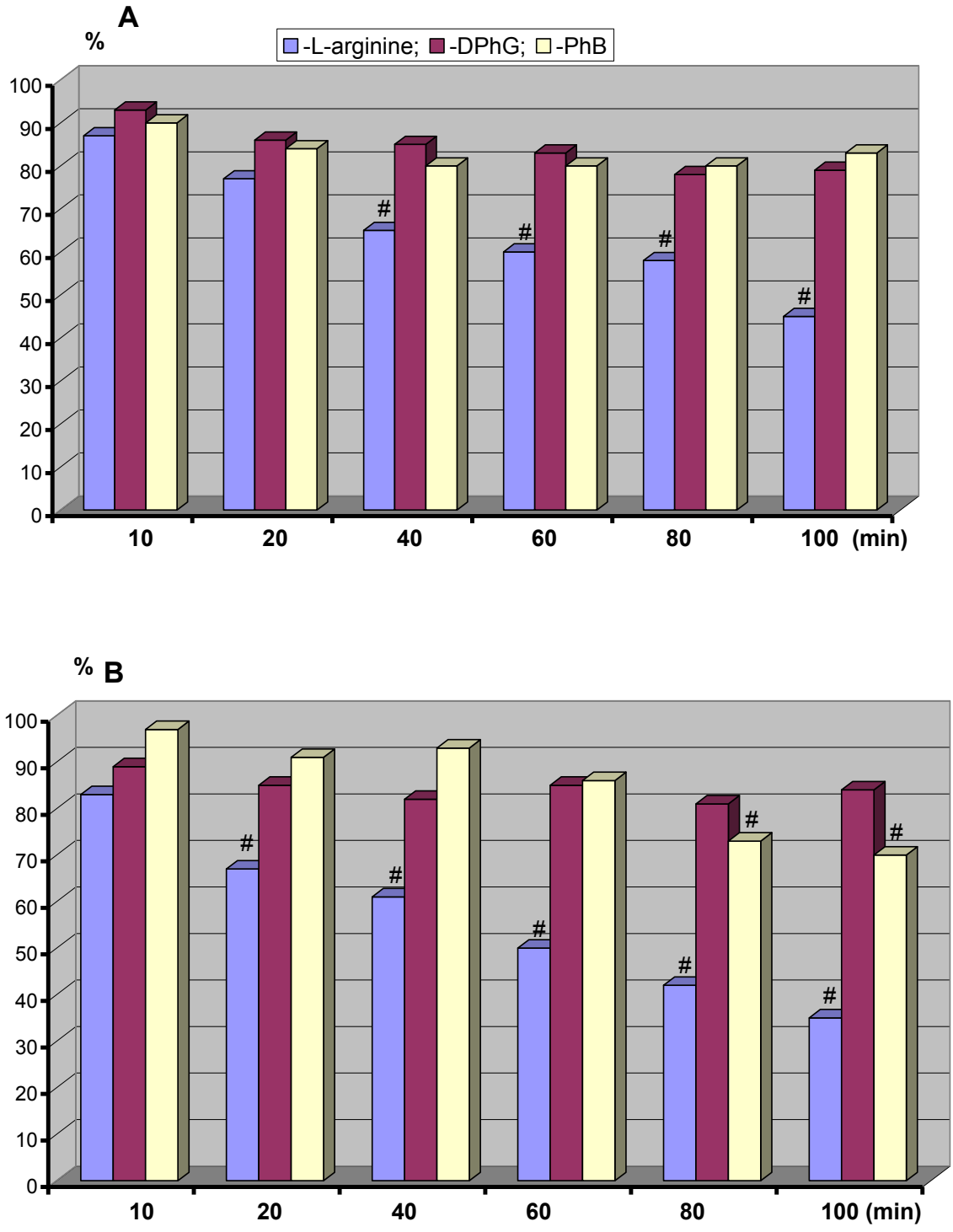

Fig.2. Effects of L-arginine, DPhG and PhB upon magnitude (A) and frequency (B) of penicillin-induced focal epileptic discharges.

Abscissa (both fragments) - time (min) from the moment of compounds administration; Ordinate - frequency (A) and magnitude (B) of epileptic discharges in experimental groups pertaining to the correspondent characteristics in control group (100%).

- $P<0,05$ – statistical differences of the indexes investigated compared with the same in control observation (ANOVA test)

This standpoint made us to study the effects of L-arginine on the model of cortical penicillin-induced foci in motionless animals. It was shown that i.p. applied L-arginine caused suppressive action upon different characteristics of epileptogenesis. Hence, both frequency and magnitude of discharges were diminished and lifespan of epileptic foci was shortened. Comparatively, PhB was less potent in the last respect and it's antiepileptic action was confined to suppression of the epileptic discharges frequency observed at the late stage of PhB action (after 80^{th} min from the moment of PhB administration). Thus, obtained data testified for efficacy of L-arginine as anticonvulsant limited propagation and effectively increasing seizure threshold.

Concerning possible mechanism of antiepileptic activity of L-arginine it could be assumed that strengthening of GABAergic inhibition is expected. This suggestion is supported by mode of epileptogenic action of penicillin - compromotation of GABA-gated chloride influx [3], and recently shown ability of NO to elaborate GABA shown within the nucleus tractus solitarii should also be mentioned [5].

It is also of interest to note that NO elaboration (L-arginine-depended process) underplays antiepileptic action of different compounds including ascorbic acid [11], ghrelin (agonist of cannabinoid receptors) [10] and some antiepileptic drugs as well [4]. Such unspecificity resembles that one observed after activation of kappa- opioid receptors [6, 9]. Hence, functional interlink between NO and endogenous opioid system is quite possible to exist.

Conclusions:

1. L-arginine (500,0 mg/kg, i.p.) caused antiseizure action both in complex partial (amygdalar ES-induced kindling) and simple partial (penicillin induced cortical foci) forms of experimental epilepsy.

2. Pronouncement of antiepileptic effects of L-arginine (500,0 mg/kg, i.p.) is comparable with that one observed after DPhG administration (100,0 mg/kg i.p.) in case of complex partial model of epilepsy, and is more pronounced in case of simple partial epilepsy than antiepileptic action of PhB (10,0 mg/kg, i.p.).

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