

Antidepressant-Like Effects of Substances with Cerebroprotective Activity

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Abstract

Studies of hippocampal slices of rats were show that non-competitive blocker of the NMDA glutamate receptor Ketamine (10 μ M) and a selective inhibitor of COX-2 Nimesulide (10 μ M) decrease a brain damages caused by excitotoxic action of NMDA (50 μ M) and procedures of anoxia/aglycaemia on hippocampal slices. The brain damages were appered by disturbance of transmission in Schaffer collaterals synapses and dendrites of pyramidal neurons in the CA1 area of the hippocampus. Behavioral studies have shown that systemically administered Ketamine (5 mg/kg) and Nimesulide (5 mg/kg) reduced the period of immobilization of rats in the forced swimming test, but they increased the residence time of rats in the open sleeves of the elevated X-shaped maze. Social isolation of rats leads to depressive behavior. The manifestations of behavior depression of rats were decreased and it indicates antidepressant-like activity of testing substances.

Keywords: Ketamine, Nimesulide, cerebroprotective action, antidepressant-like activity

Introduction

Depression is a serious disease in men which leads to formation of disability. This disease affects on 6% - 17% in the human population of North America and Europe and has strong tendency to expansion. For the treatment of the disease it has developed several groups of antidepressants during the 50 past years. Activity of such antidepressants results in changes of metabolism of monoamines in a brain. The currently used antidepressants have two major drawbacks: a) slow development of the therapeutic effects (after 2 - 4 weeks), b) the therapeutic efficacy is not more than 50 - 70% [1, 2]. Therefore, an intensive search of new substances for a faster and more intensive treatment of the depressive syndrome is going on.

Intravital studies of a brain by using the methods of NMR- and PET-spectroscopy were revealed reduction of the gray matter volume in several limbic structures in patients with mono- and bipolar depression. So patients with unipolar depression it was found increasing of blood flow from the left ventrolateral prefrontal cortex to the medial prefrontal cortex [3], reduction of activity of the prefrontal cortex (that situated ventrally from genu of corpus callosum), reduction of volume of gray matter in this area reached 48 % [4, 5]. Depressive syndrome in women, untreated with psychopharmacological drugs, it was revealed a reduction of the volume of ventral part of the anterior callosal cortex in comparison with mentally healthy ones [6]. It was also established a reduction of one of the central limbic structures (the hippocampus) in patients with different forms of the depressive syndrome [7, 8]. Such facts were indicated that development of the depressive syndrome is attended by death of neurons and glial cells in the limbic structures, due, apparently, to the action of glucocorticoids, antiinflammatory cytokines and other neuroactive polypeptides.

On the other hand, it was found that a widely used now for the treatment of mono- and bipolar depression various classes of antidepressant drugs (lithium, valproic acid) have cerebroprotective activity. This activity manifests by prevention the neuronal death caused by anoxia, aglikemia, excitotoxicity [9 - 12].

Here we tried to find out whether randomly selected substances with cerebroprotective action [13, 14] have the antidepressant-like activity. It were general anesthetic Ketamin and anti-inflammatory agent - selective inhibitor COX-2 Nimesulide.

Materials and methods

Animals

The studies were carried out on white inbred rats of both sexes, weighting 150 - 250 g, which were kept in cages for 4 - 6 individuals, under 12 hour cycle of light/dark time. The light was switched on at 7.00. The rats had free access to food and water. The studies were performed in accordance with requ-

rements of Bioethics Commission of the M. Gorky Donetsk National Medical University.

Electrophysiological experimental protocols

Electrophysiological studies were performed on slices of the dorsal hippocampus. Details of the method are described previously [15]. Briefly: the rats were anesthetized by intraperitoneal administration of Ketamine 50 mg/kg. After reaching anesthesia the animals were decapitated, the brain was removed from the skull, it was immediately cooled by 4-6 °C solution for the preparation. Dorsal hippocampus was isolated from the posterior pole of the brain. Slices of a thickness of 400 µm were prepared by vibratome. Further cross slices of the hippocampus were isolated; slices were placed in the incubation chamber, where they were superfused by Krebs solution of the following ionic composition in µM: NaCl -124, KCl -3; KH₂PO₄ -1.25; NaHCO₃ -26, CaCl₂ -2, MgSO₄ -1, glucose-10. Krebs solution in the incubation chamber was saturated with carbogen, temperature was maintained 25 °C, flow rate 2 ml/min. After 90 min incubation, one of the slices was placed in the working chamber volume of 0.5 ml, where it was superfused by carbogen-saturated Krebs solution at a temperature 28 ± 0.5 °C; flow rate was - 2 ml/min. There were recorded threshold field EPSPs pyramidal neurons CA1 area in hippocampal slices, that caused by electrical stimulation of Schaffer collaterals. Stimulation of synaptic inputs was performed by bipolar nichrome electrode with a rectangular electric current impulses duration 0.1 msec. After fEPSP amplitude was stabilized, there was constructed curve dependence of amplitude fEPSP from the intensity of presynaptic stimulation.

NMDA excitotoxic action was investigated by the method proposed by Liu Y., et al. [16]. For that hippocampal slices were exposed to 50 µM NMDA in the presence of 1 µM Glycine for 15 min. After that, slices were transferred to an incubation chamber, where to the test group with the Krebs solution was added Ketamine or Nimesulide in concentration 10 µM, and they were remained for at least 1 hour. Slices were taken in 1 hour after the termination of the action of NMDA in electrophysiological studies. Anoxia and aglycemia were simulated by method Tian G. And Baker A. J. [17]. Slices were placed in a chamber with atmosphere of nitrogen in Krebs solution where Glucose was replaced by an equivalent amount of Mannitol to 5 minutes at a temperature 32 °C. Then the slices were transferred to an incubation chamber in to the aerated Krebs solution contain Ketamine or Nimesulide for test groups. The electrophysiological studies of slices were taken in 1 hour after the termination of the procedure aglycaemia and anoxia.

Simulation of behavioral depression

In behavioral studies, the level of depression of animals was determined by the usual method [18]. Rats were placed in aquaria of 50 cm height, filled with water 2/3 of height; water temperature of water — 22 - 25 °C. It was recorded duration of the immobilization of rats. The immobilization appears by passive

swimming without limb movement, the front legs were pressed to his chest, back stretched, during 300 seconds forced swimming session. The level of anxiety of the rats were determined in elevated X-shaped maze [19]. Animals were placed into the central area facing to the opening sleeve. During 5 min it was registered time of the animals residence in the open sleeves with a quantity of exits in to the open sleeves, the number of looks out from open spaces.

Behavioral depression was simulated by social isolation of animals. Social isolation was performed by moving rats out the family cage in to the experimental one. The animals were placed in to the cage 25 x 15 x 10 cm, which limited the locomotor activity and they stayed there from 19.00 until 7.00. The experimental animal had free access to food and water. At ~ 1/3 animals that were subjected to this procedure for 5 days, developed a depressive syndrome. The rats show an increase of time of immobility in the forced-swim test, an increase of the level of anxiety, an increase of aggression. The test drugs Ketamine (5 mg/kg) and Nimesulide (5 mg/kg) were administered intraperitoneally for 4 days (from 2 until 5 days of social isolation) at 10 p.m. Next day after the termination of social isolation animals took part in behavioral studies.

Statistic analysis

The results were analyzed using analysis of variance by the licensed statistical program «Medstat». Average error and standard error were determined for each series. The significance of differences compared values were evaluated by using of the Student's t-test. Differences were considered as statistically significant at a value of $p < 0.05$.

Results

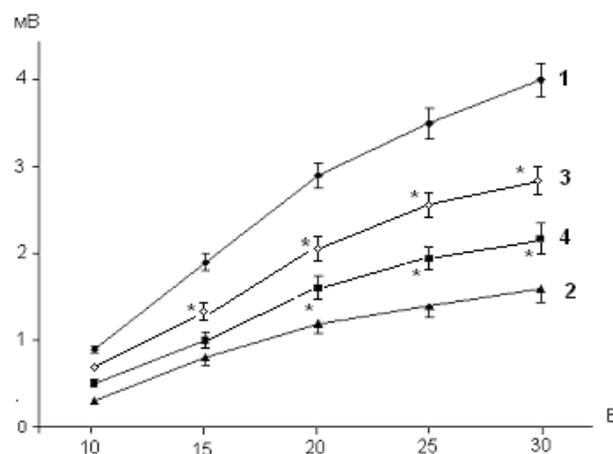


Fig. 1. Effects of Ketamine and Nimesulide used in concentration 10 μ M for excitotoxic inhibition of synaptic reactivity pyramidal neurons area CA1, induced by action 50 μ M NMDA on hippocampal slices

1 - the dependence of the amplitudes fEPSPs pyramidal neurons in mV against intensity of the presynaptic stimulation of V (the initial synaptic reactivity); 2 - inhibition of the synaptic reactivity after 1 hour the termination of the action on the slices NMDA; 3 - the same in association with Ketamine; 4 - the same in association with Nimesulide.

*- if $P < 0.05$, differences are statistically significant relative to the curve 2.

Influence on hippocampal slices by 50 μM NMDA + 1 μM Glycine for 15 min induced excitotoxic damage of pyramidal neurons in the CA1 area. It manifested by decrease of the amplitudes fEPSP, therefore it decrease synaptic reactivity of pyramidal neurons, almost three times (Fig. 1, curve 2). However, the effect on hippocampal slices NMDA together with Ketamine (Fig. 1, Curve 3) and to a less extent with Nimesulide (Fig. 1, curve 4) caused less severe excitotoxic damage of synaptic reactivity of the pyramidal neurons. Therefore, attenuation of excitotoxicity may be one of the components of cerebroprotective action of Ketamine and Nimesulide.

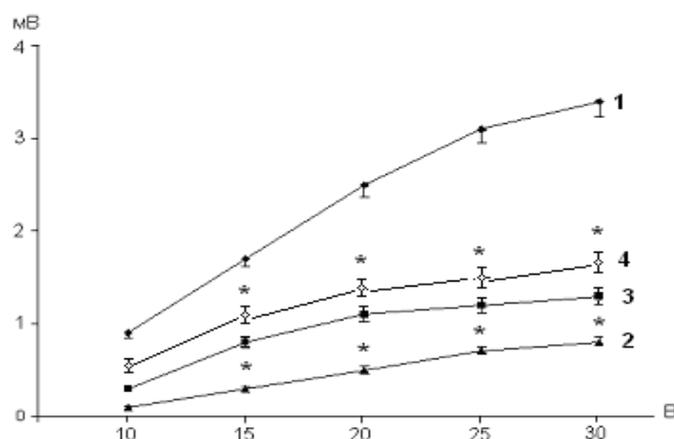


Fig. 2. Effect of Ketamine and Nimesulide in concentration 10 μM used for inhibition of synaptic reactivity CA1 area of the pyramidal neurons, it induced by action on hippocampal slices anoxic procedure and aglycaemia for 7.5 min. Indications like on Fig. 1

If hippocampal slices exposed of Oxygen and Glucose deprivation for 7.5 minutes, it lead to irreversible damage of the brain tissue. That manifests by reduction of amplitude fEPSP pyramidal neurons almost ten times (Fig. 2, curve 2). Affecting on hippocampal slices by Ketamine and Nimesulide simultaneously with procedure of aglycaemia and anoxia in the ensuing hour after cessation decreased damage of pyramidal neurons. It confirm some increase of fEPSP amplitude against to curve 2 (Fig. 2, curves 3 and 4). Nimesulide had a greater cerebroprotective activity than Ketamine. Thus, the spectrum of cerebroprotective

activity of Ketamine and Nimesulide in addition to antiexcitotoxic action is complemented by antihypoxic and antihypoglycaemic action.

If, as noted previously, the development of a depressive syndrome is accompanied by neurodegenerative processes of various origins, then a priori we can assume that substances with cerebroprotective activity in some measure can decrease symptoms of depression. As a model we have chosen stress-induced behavioral depression caused by long-term isolation from the family ambience of rats. As can be seen from Fig. 3 and 4 social isolation of rats for 5 days resulted in an increase of the level of depression and anxiety, which manifested by increase of the time of immobilization of rats in the forced-swim test and decrease time of residence of the animals in the open sleeves of the elevated X-shaped maze. It is interesting because in a clinic there are many patients who have comorbidity of the depressive and anxious syndromes [20].

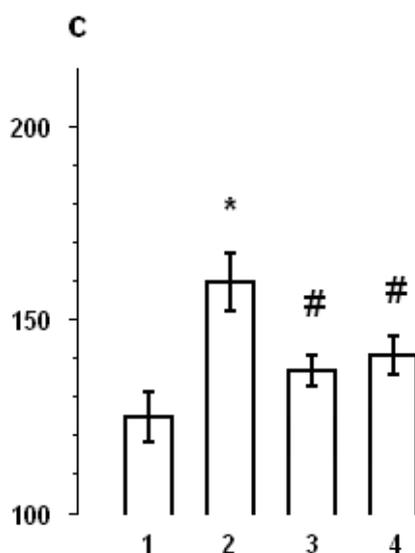


Fig. 3. Time of immobilization of rats in the forced swimming test

Column 1 - time of immobilization of control rats; column 2 - the same after the termination of social isolation; columns 3 and 4 - time of immobilization after social isolation in association with Ketamine and Nimesulide.

The vertical scale - time in seconds.

* - The differences were statistically significant at $P < 0.05$ relative to the control; # - the same against to the experimental data (column 2).

In control group of rats time of immobility in the swim test was averaged 125.3 ± 6.2 sec with ($n = 24$). In the part of the population of rats that had been subjected to social isolation only ($n = 6$) the mean time of immobilization was increased to 160.4 ± 6.7 sec, so significantly differ from controls ($P = 0.0021$). However, administration of Ketamine and Nimesulide for 2 - 5 days isolation (Figure 3, columns 3 and 4) were reduced time of immobilization of animals up to

135,5 ± 5,7 sec. ($P = 0.037$) and 141,2 ± 6,1 sec. ($P = 0.043$). Therefore, Ketamine and Nimesulide reduce the level of depression of animals in an experimental model of depression, when they are administered systemically. So they have antidepressant-like effects.

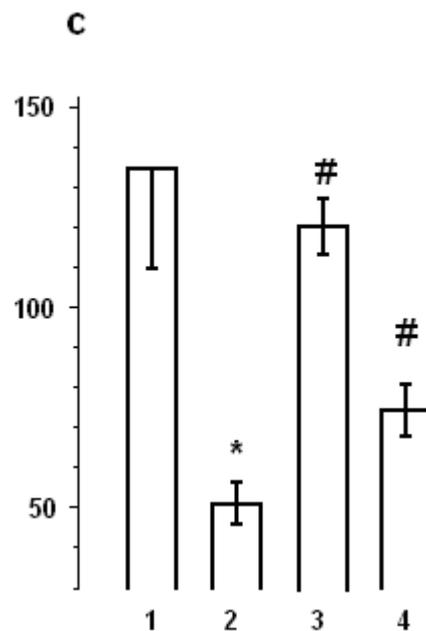


Fig. 4. The time of residence of rats in the open sleeves of the elevated X-shaped maze

Indications like on Fig. 3.

The average time of residence of the control rats ($n = 24$) in open sleeves of elevated X-shaped maze (it constitute a threat to animals) is equal to 133,3 ± 25,2 sec. Long social isolation led to a significant ($P = 0,0013$) reduce of this parameter till 52,0 ± 4,9 sec (Fig. 4, column 2). It indicate a significant increase of the level of anxiety of the testing animals. Introduction to rats of Ketamine and Nimesulide from 2 to 5 days significantly increased the duration of their stay in the open sleeves of maze up to 120,3 ± 14,9 sec and 74,2 ± 4,1 sec, corresponding to (Fig. 4, s columns 3 and 4). Thus, the chronic administration of Ketamine and Nimesulide reduced the level of anxiety. It is a component of the continuum of subsyndroms of the depressive syndrome.

Conclusions

The present study allow to think, that substances with cerebroprotective activity have revealed antidepressant-like effects in experiment, due to decrease of excitotoxicity and/or increase the tolerance of the brain to hypoxia or hypoglycaemia.

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Received: November 15, 2015; Published: December 2, 2015