Binary Mechanism of Action of Cognition Enhancer NT1505 on Glutamate Receptors

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 153, No. 3, pp. 272-275, March, 2012
Original article submitted December 13, 2010

Compound NT1505 potentiates AMPA receptors in rat brain neurons and simultaneously non-competitively blocks NMDA receptors via two different mechanisms. Considering previously obtained data on strong cognition-enhancing properties of this compound we can conclude that NT1505 is a novel cognition stimulator exhibiting properties of a positive modulator of AMPA receptors and a blocker NMDA receptor.

Key Words: *AMPA receptors; NMDA receptors; derivatives of alkyl isothiourea*

We have recently found that a series of isothiourea derivatives representing open chain structural analogs of MK-801 (a high-affinity blocker of the intrachannel binding site of the NMDA receptor) improve learning and memory from a toxin-induced animal model of Alzheimer-type dementia [4]. Compounds of this series are patented as efficient stimulators of memory and cognitive functions [2].

Here we studied the mechanisms underlying the effects of compound NT1505 (S-ethyl-N-allyl-N',N'-dibenzylisothiourea hydroiodide), the hit compound of this series, on NMDA and AMPA glutamate receptors in rat brain neurons, which are known to play an important role in the mechanisms of memory [11].

MATERIALS AND METHODS

We studied the effects of compound NT1505 on AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid) and NMDA (N-methyl-D-aspartate) receptors. The study was carried out using whole-cell patch-clamp electrophysiological technique on Purkinje neurons freshly isolated from the cerebella of 12-15-day-old rats and on neurons of cerebral cortex of 7-9-day-old rats according to a modified method [6]. Brain slices (400-600 μ) were placed in a 10-ml thermostatic chamber. The isolation medium contained (in mM): 150 NaCl, 5 KCl, 2 $CaCl_2$, 2 $MgSO_4 \times 7H_2O$, 10 HEPES, and 15 glucose (pH 7.42). The sections were incubated in this solution for 60 min and then in the same solution containing pronase (2 mg/ml) and collagenase (1 mg/ml) and for 70 min. After 20-min washout with the initial solution, the sections were placed in a Petri dish and mechanically disintegrated with a Pasteur pipette. The solutions were constantly aerated with 100% oxygen at 34°C. Purkinje neurons were placed in a 0.6-ml chamber. Working solution contained (in mM): 150 NaCl, 5 KC1, 2.6 $CaCl_2$, 2 $MgSO_4 \times 7H_2O$, 10 HEPES, and 15 glucose (pH 7.36).

Transmembrane currents in individual cells were induced by application of kainic acid or NMDA (in this case, Mg^{2+}-saline was used and NMDA solution contained 7 μM glycine) using the method of rapid superfusion. The currents were recorded by borosilicate microelectrodes (resistance 2.5-4.5 MΩ) filled with the following solution (in mM): 100 KCl, 11 EGTA, 1 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, and 5 ATP (pH 7.2).

Currents were recorded using Pulse software (HEKA). The data were processed using Pulsefit soft (HEKA).

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RESULTS

Compound NT1505 blocked currents evoked by activation of NMDA receptor in the rat cortical neurons. By the nature of the blocking effect, the neurons can be divided into two groups. In most neurons (n=13), compound NT1505 blocked NMDA-activated currents, but in much higher concentrations (IC_{50}~23 μM) than MK-801 (IC_{50}~0.078 μM), a high-affinity blocker of intrachannel site of NMDA receptor. The magnitude of the blocking effect in these neurons depended on not the amplitude of NMDA-activated currents, but only NT1505 concentration. Both compounds required long-term washout (15-20 min) to restore the amplitude of NMDA-activated currents.

At the same time, a group of neurons was found among cortical neurons, in which NT1505-blocking effect on NMDA-activated currents had a completely different character. Blockade of NMDA-activated currents was caused by much lower concentrations of NT1505. An important feature of NT1505 in these neurons (n=5) was more effective blockade of high-amplitude currents. For maximum currents evoked by 1 mM NMDA application, IC_{50} value was 0.40±0.15 μM, for the minimum currents evoked by 20-50 μM NMDA application, IC_{50} value was ~5-fold higher (2.1±0.4 μM).

We can assume that NT1505 blocks NMDA receptors in the neurons of both groups via different mechanisms. In neurons of the first group, NT1505 binds apparently only with MK-801 binding site in NMDA receptor ion channel, but in much higher concentrations than MK-801 itself.

Comparative analysis of NT1505 effects on neurons of the second group and published data showed that its blocking effects are similar to those of ifenprodil (Fig. 1). It was previously found that ifenprodil is more effective in blocking NMDA-evoked high-amplitude currents than currents of lower amplitude [7]. The difference in IC_{50} at ifenprodil-produced block of high- and low-amplitude currents was equal to 5 as in the case of the NT1505 (0.17 and 0.85 μM respectively). This suggests that compound NT1505 in neurons of the second group blocks the binding site for ifenprodil located on the NR2B-subunit of NMDA receptor.

The study of the effect of compound NT1505 on AMPA receptors showed that unlike the action on the NMDA receptor, NT1505 does not block, but significantly potentiates the responses of AMPA receptors. We used kainic acid as a partial agonist of AMPA receptors leading to their incomplete desensitization while AMPA and glutamate cause a very strong and rapid desensitization of AMPA receptors [12]. It was found that NT1505 reversibly potentiated AMPA receptor currents in a dose-dependent manner with their activation in Purkinje neurons by kainic acid (Fig. 2). The maximum potentiation reached 1050±55% from the control for currents evoked by 50 μM kainic acid application and 30 μM NT1505. Thus, NT1505 acts as a positive modulator of AMPA receptor like the previously described ampakines, which potentiating effect is mediated by reduction of AMPA receptor desensitization [9].

Among positive modulators of AMPA receptors there are effective memory enhancers. This allows us to consider them as potential medications for the

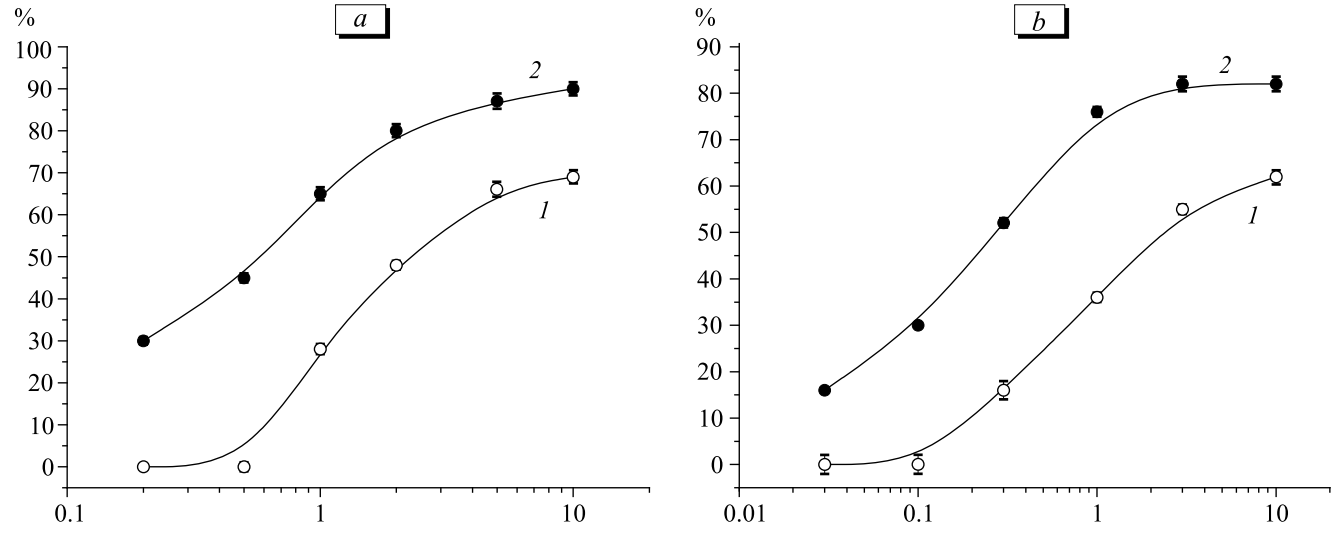

Fig. 1. Comparison of the blocking effect of compound NT1505 (*a*) on NMDA receptor currents in the neurons of the second group and ifenprodil (*b*) on currents of NR2B-containing NMDA receptors [7]. Horizontal axis: logarithm of the concentrations (μM). Vertical axis: magnitude of the blocking effect; complete blockade, 100%. *1*) low-amplitude currents (evoked by application of 50 μM (*a*) and 10 μM (*b*) NMDA); *2*) high-amplitude currents (evoked by application of 10 μM (*a*) and 100 μM (*b*) NMDA).

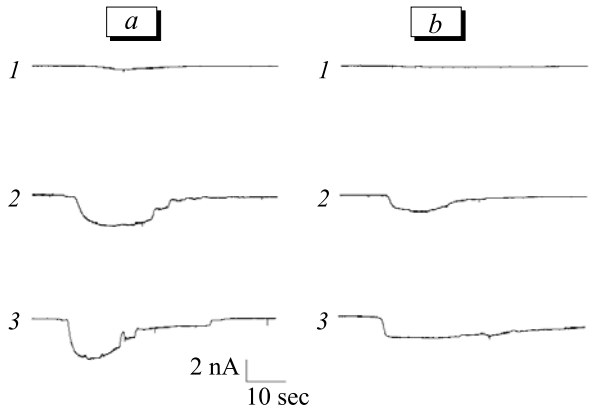

Fig. 2. Effect of compound NT1505 on AMPA receptor currents in Purkinje neurons. *1*) control currents evoked by application of 0.2 μM kainic acid (*a*) and glutamate (*b*); *2*) corresponding currents in the presence of 5 μM (2) и 30 μM (3) NT1505.

treatment of cognitive disorders [1,3,9]. At the same time, activation of AMPA receptor precedes and determines activation of NMDA receptor, because the latter requires neuronal depolarization with activation of AMPA receptor and magnesium unblock of NMDA receptor. Coordinated function of AMPA and NMDA receptors in induction of LTP and LTD was recently demonstrated with different roles of NR2A- and NR2B-subunits of NMDA receptors in synaptic transmission critically important in memory [8,10]. Behavioral experiments revealed that the blockers of NR2B-subunit do not impair [11], but improve memory in experimental animals [5] unlike competitive and noncompetitive antagonists of NMDA receptors.

Compound NT1505 is unique because it both potentiates AMPA receptors and blocks NMDA receptors containing NR2B-subunits. Thus, this compound exerts so-called multitarget effects simultaneously on both subtypes of ionotropic glutamate receptors playing a key role in memory consolidation and maintenance of cognitive function.

As was earlier established in our institute, compound NT1505 at doses of 1-5 mg/kg improves learning and memory in mice subjected to active avoidance test and Morris water maze test after intracerebral injection of cholinotoxin AF64A causing degeneration of cholinergic neurons [4]. Strong cognition-enhancing properties of NT1505 combined with low toxicity of this preparation (LD_{50} more than 4 g/kg intramuscularly) and the absence of MK-801-like psychotomimetic side effects [2] permit us to regard NT1505 as a possible candidate drug for the treatment of numerous neurodegenerative pathologies related to impaired functioning of the glutamatergic system, and the binary mechanism of the effect on ionotropic glutamate receptors implemented by the example of this compound, as a promising approach for the aimed construction of next-generation multitarget cognitive promoters.

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