Effect of Muramyl Dipeptides on Postsynaptic GABA, NMDA, and AMPA Receptors and Presynaptic NMDA Receptors in Rat Brain

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We studied the effect of muramyl dipeptides on postsynaptic GABA, NMDA, and AMPA receptors and presynaptic NMDA receptors. L,D-Dipeptide more potently than L,L-dipeptide inhibited postsynaptic NMDA receptors, potentiated GABA and AMPA receptors, and inhibited $^{45}Ca^{2+}$ uptake in synaptosomes from of rat brain cortex. Our results indicate that muramyl dipeptides modulate function of glutamate and GABA receptors.

Key Words: *muramyl dipeptides; receptors; synaptosomes; calcium*

Muramyl dipeptides (MDP), products of degradation of bacterial peptidoglycan, trigger the multicomponent system of the protective immune response in mammals and activation of proinflammatory cytokines [11,13]. They have a toxic effect on epithelial cells, cause dysfunction of the kidneys and liver [6], and induce fever and loss of appetite [5]. Previous studies were performed to evaluate the direct effect of MDP on some neuroreceptors, including serotonin receptors [9]. MDP stimulate the production of interleukin-1β [7], which has various effects on the central nervous system (CNS) and modulates activity of presynaptic NMDA receptors in rat brain cortex [2]. MDP stereoisomers have various toxic effects on liver cells. L,D-MDP is 100-fold more toxic than L,L-MDP and D,D-MDP [8,14].

Here we studied the effect of MDP on postsynaptic GABA, NMDA, and AMPA receptors and presynaptic NMDA receptors.

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MATERIALS AND METHODS

Electrophysiological studies were performed on freshly isolated cerebellar Purkinje cells and cortical neurons from rats aging 12-17 and 7-9 days, respectively. These neurons were obtained by the enzymatic-and-mechanical method. Transmembrane currents in response to application of GABA, NMDA, or kainic acid (KA) were recorded electrophysiologically by the whole-cell patch-clamp technique on an EPC-9 device (HEKA). The substances were applied by the method of rapid superfusion. Since AMPA causes strong and rapid desensitization of AMPA receptors, activation of these receptors was induced by KK [4].

Cerebral cortical synaptosomes (CCS) were isolated from newborn Wistar rats (9-10 days age) by the standard method. The brain was homogenized (glass-Teflon) in a 10-fold volume of cold sucrose (0.32 M, 900 rpm). The homogenate was centrifuged at 1500*g* for 10 min. The supernatant was centrifuged at 10,000*g* for 20 min. For accumulation of the radioactive label, the P_2 fraction of CCS was suspended in incubation buffer A containing 132 mM NaCl, 5 mM KCl, and 5 mM HEPES

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(pH 7.4, protein concentration 1.5-2.0 mg/ml). Calcium concentration in the final volume was 1.25 mM (1.4 μCi/ml). NMDA (200 mM NMDA and 5 μM glycine) was used to stimulate $^{45}Ca^{2+}$ uptake into CCS. After 3-min incubation with NMDA at 37°C, $^{45}Ca^{2+}$ uptake was stopped by filtration through GF/B fiberglass filters (Whatman) with 3-fold washing with cold buffer B containing 145 mM HEPES, 10 mM Tris, and 54 mM Trilon B (pH 7.4). All measurements were performed in 4 parallel samples (4-5 independent experiments). Radioactivity was measured on a liquid scintillation β-counter. $^{45}Ca^{2+}$ uptake into CCS was evaluated as the difference between the amounts of the label upon stimulation with NMDA and in the absence of stimulation. This parameter was expressed as percent of the control (100%). Specific uptake of $^{45}Ca^{2+}$ was calculated as follows:

$$K_{(43/21)}=[(Ca_4-Ca_3)/(Ca_2-Ca_1)]\times 100\%,$$

where Ca_1 is $^{45}Ca^{2+}$ uptake in the control (without NMDA and MDP); Ca_2 is $^{45}Ca^{2+}$ uptake in the presence of NMDA (without MDP); Ca_3 is $^{45}Ca^{2+}$ uptake in the presence of MDP (without NMDA); and Ca_4 is $^{45}Ca^{2+}$ uptake in the presence of NMDA and MDP.

The results were analyzed by Student's *t* test.

RESULTS

L,D-MDP in various concentrations increased the amplitude of GABA-induced currents in cerebellar Purkinje cells. GABA-induced currents increased most significantly under the influence of L,D-MDP in concentrations of 10^{-10}-10^{-8} M ($p \leq 0.05$). Increasing the concentration of L,D-MDP was accompanied by an increase in the potentiating effect on GABA-induced currents. However, these changes were not monotonous (Fig. 1). L,D-MDP in concentrations of 10^{-13}-10^{-8} M decreased the amplitude of NMDA-induced currents in cortical neurons. Under the influence of L,D-MDP in concentrations of 10^{-11}-10^{-10} M, the response was 60-70% of the control ($p \leq 0.05$, Fig. 2).

MDP stereoisomers inhibited the glutamate-induced uptake of $^{45}Ca^{2+}$ into rat CCS. L,D-MDP (10^{-10} M) and L,L-MDP (10^{-6} M) inhibited $^{45}Ca^{2+}$ uptake into CCS by 20%. Hence, MDP have a modulatory effect on presynaptic glutamate receptors. Then, we identified the type of receptors affected by MDP.

$^{45}Ca^{2+}$ uptake into CCS upon stimulation with NMDA is related to activation of glutamate NMDA receptors. NMDA-induced $^{45}Ca^{2+}$ uptake into CCS

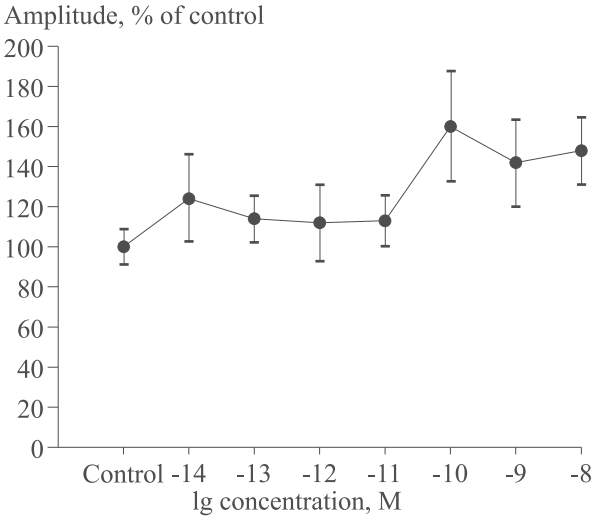

Fig. 1. Effect of L,D-MDP on GABA-induced currents in rat cerebellar Purkinje cells.

decreased after treatment with the following antagonists of NMDA receptors: MK-801 (IC_{50} ~1 μM), CPP (IC_{50} ~100 μM), memantine (IC_{50} ~0.4 μM), and Mg^{2+} (IC_{50} ~100 μM). Our results are consistent with published data that NMDA activates inotropic NMDA receptors in the P_2 fraction of rat CCS [2]. During stimulation of $^{45}Ca^{2+}$ uptake by 200 μM NMDA (+5 μM glycine), L,D-MDP in concentrations of 10^{-12}-10^{-8} M had an inhibitory effect on NMDA-induced $^{45}Ca^{2+}$ uptake into CCS. The inhibition of $^{45}Ca^{2+}$ uptake into CCS was most significant (100%) under the influence of L,D-MDP in a concentration of 10^{-10} M ($IC_{50}=5\times10^{-12}$ M). The dependence of NMDA-induced $^{45}Ca^{2+}$ uptake into CCS on the concentration of L,D-MDP was described by a bell-shaped curve (Fig. 3). L,L-MDP

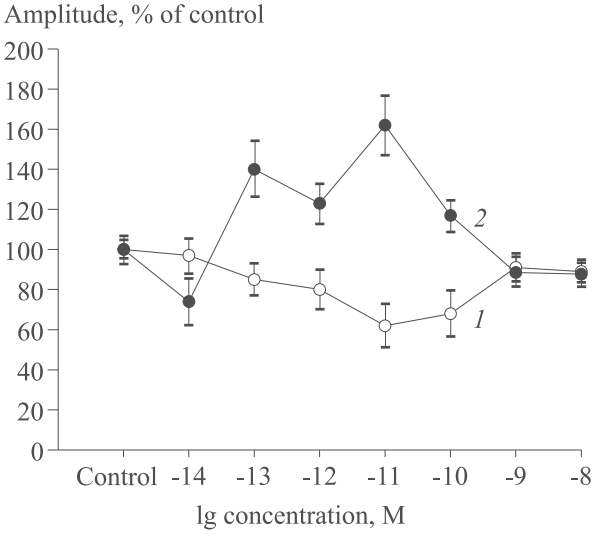

Fig. 2. Effect of L,D-MDP on NMDA-induced (*1*) and KA-induced currents in rat cerebral cortical neurons (*2*).

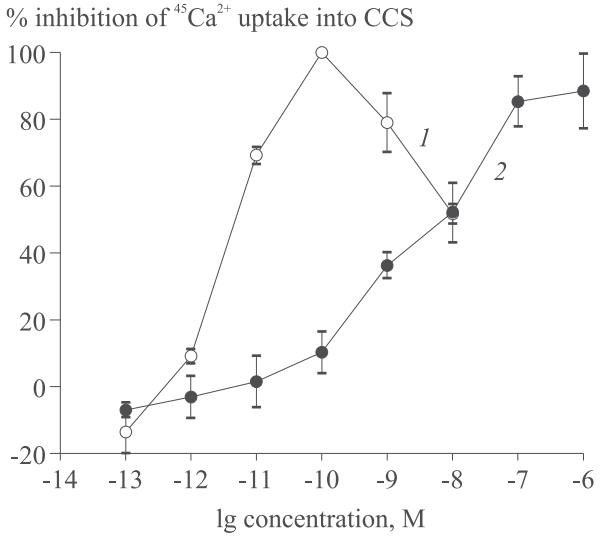

Fig. 3. Effects of L,D-MDP (*1*) and L,L-MDP (*2*) on $^{45}Ca^{2+}$ uptake into rat CCS upon stimulation with NMDA.

in concentrations of 10^{-11}-10^{-6} M had an inhibitory effect on $^{45}Ca^{2+}$ uptake into CCS during stimulation with NMDA. The inhibition of $^{45}Ca^{2+}$ uptake into CCS depended on the concentration of L,L-MDP. L,L-MDP in concentrations of 10^{-7}-10^{-6} M was most potent in inhibiting $^{45}Ca^{2+}$ uptake into CCS ($IC_{50}=6\times10^{-9}$ M). Therefore, MDP affect presynaptic NMDA receptors.

L,D-MDP in a concentration of 10^{-14} M most significantly inhibited KA-induced currents in hippocampal neurons. However, administration of L,D-MDP in concentrations of 10^{-13}-10^{-9} M was followed by an increase in KA-induced currents. L,D-MDP in a concentration of 10^{-8} M had an inhibitory effect on KA-induced currents (Fig. 2).

Our results indicate that L,D-MDP has a modulatory effect on postsynaptic GABA, NMDA, and AMPA receptors and presynaptic NMDA receptors. L,D-MDP potentiates GABA receptors, which probably contributes to the activating effect on slow-wave sleep [3]. MDP inhibit NMDA receptors, but potentiate AMPA receptors. These receptors are involved not only in signal transduction in CNS, but also in the mechanisms of memory and realization of cognitive functions in the brain [10, 12]. It should be emphasized that L,D-MDP has a similar effect on presynaptic and postsynaptic NMDA receptors. Stereoisomers differ in the ability to modulate presynaptic NMDA receptors. L,D-MDP was 800-fold more potent than L,L-MDP in inhibiting the uptake of $^{45}Ca^{2+}$ in CCS.

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