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Nootropic Dipeptide Noopept Enhances Inhibitory Synaptic Transmission in the Hippocampus

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Application of nootropic agent Noopept on hippocampal slices from Wistar rats enhanced the inhibitory component of total current induced by stimulation of Shaffer collaterals in CA1 pyramidal neurons, but did not affect the excitatory component. A direct correlation between the increase in the amplitude of inhibitory current and agent concentration was found. The substance did not affect the release of inhibitory transmitters from terminals in the pyramidal neurons, which indicated changes in GABAergic interneurons.

Key Words: *hippocampal slices; patch-clamp; Shaffer collaterals; Noopept; inhibitory interneurons*

Behavioral tests on animals have demonstrated that proline-containing dipeptide Noopept (NP) can restore spatial memory disrupted by β-amyloid peptide, the main pathogenic factors during Alzheimer's disease [1,2,12]. NP also normalizes avoidance reaction conditioning during changed spatial configuration of surroundings [8].

NP is structurally similar to endogenous peptide cyclopropyl glycine that is present in many brain structures; it maximum concentration was found in the hippocampus [4,7] that participates in the processes of space mapping [14,15] and serves as one of the key structures and neurobiological basis for spatial memory [11]. It is known that interactions between the neocortex and hippocampus, which are the basis of consolidation processes, depend on activity of internal neuronal network of the hippocampus [5]. These data indicate that the hippocampus is the most convenient object for studying the neurophysiological effects of nootropic substances, *e.g.* NP.

We have previously observed an increase in spontaneous inhibitory transmission in CA1 area of hippocampal sections after NP treatment and found interneurons with increased activity under these conditions [3,10]. These results are consistent with the effects of other nootropic agents tested on similar experimental model [13].

Here we studied changes in ion currents in the pyramidal neurons induced by stimulation of Shaffer collaterals, the main synaptic inputs to hippocampal CA1 area.

MATERIALS AND METHODS

Experiments were performed on Wistar rats aging 14-17 days. The brain isolated after decapitation was placed into a chamber for slice sectioning containing cold incubation solution saturated with carbogen (2.5 mM $CaCl_2$, 1.3 mM $MgSO_4$, 2.5 mM KCl, 119 mM NaCl, 1 mM NaH_2PO_4, 26.2 mM $NaHCO_3$, and 11 mM D-glucose). Sections (300 μ) containing hippocampal tissue taken from each brain hemisphere were incubated at room temperature for 2 h.

After 2 h, the sections were put into a chamber for registration of electrophysiological parameters using the patch-clamp technique. Composition of the solution used in the experiment was similar to the composition of the incubation solution. Shaffer collaterals stimulation was performed over 5 min with 1-msec

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rectangular impulse (1 pulse per 15 sec) via a bipolar electrode placed at *str. radiatum* of CA1 area (100 μ behind the pyramidal layer). Current intensity was chosen individually in each experiment, the amplitude of current on the membrane of pyramidal neuron was not less than the half of maximal value.

Pyramidal neurons placed at the distance of about 1000 μ from application point were chosen for registration of response to Shaffer collaterals stimulation. Pyramidal neurons, which did not spontaneously discharged, with membrane potential not less than -55 mV, and access resistance no more than 30 MΩ, were used in the experiment. If access resistance of a neuron changed by more than 30% during the experiment, this neuron was not analyzed. Registration of ion currents through the membranes of pyramidal neurons was performed using whole-cell micropipette made from borosilicate glass and filled with a solution containing 130 mM calcium gluconate, 1 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM EGTA, 10 mM HEPES H, and 5 mM NaCl. Microelectrode resistance was 2-5 MΩ. Membrane potential was clumped at -65 mV; 20 μM of picrotoxin, NP in doses of 2, 4, 6, and 10 μM, and 1 μM NBQX were added to the experimental solution.

The data were registered and analyzed by WinWCP (University of Strathclyde) and MiniAnalysis (Synaptosoft Inc.) softwares. The amplitude of peak of ion current after each single stimulation was quantitatively measured. Parameters of each neuron were normalized (%) to mean amplitude under control conditions (without addition of the substance). Statistical treatment and comparison of relative data of each measurement were performed using Student's *t* test.

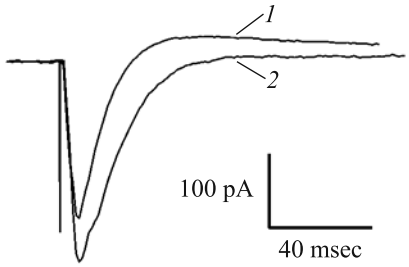

Fig. 1. Changes in total current induced by stimulation of Shaffer collaterals after addition of picrotoxin (20 μM). Each curve is a mean of 20 responses over 5 min. Clamping potential -65 mV. *1*) Control; *2*) picrotoxin.

RESULTS

Stimulation of Shaffer collaterals induced the appearance of negative current in hippocampal CA1 pyramidal neurons, which mostly consisted of the activation component, because clamping potential was similar to reversal potential for Cl^- ions (-65 mV).

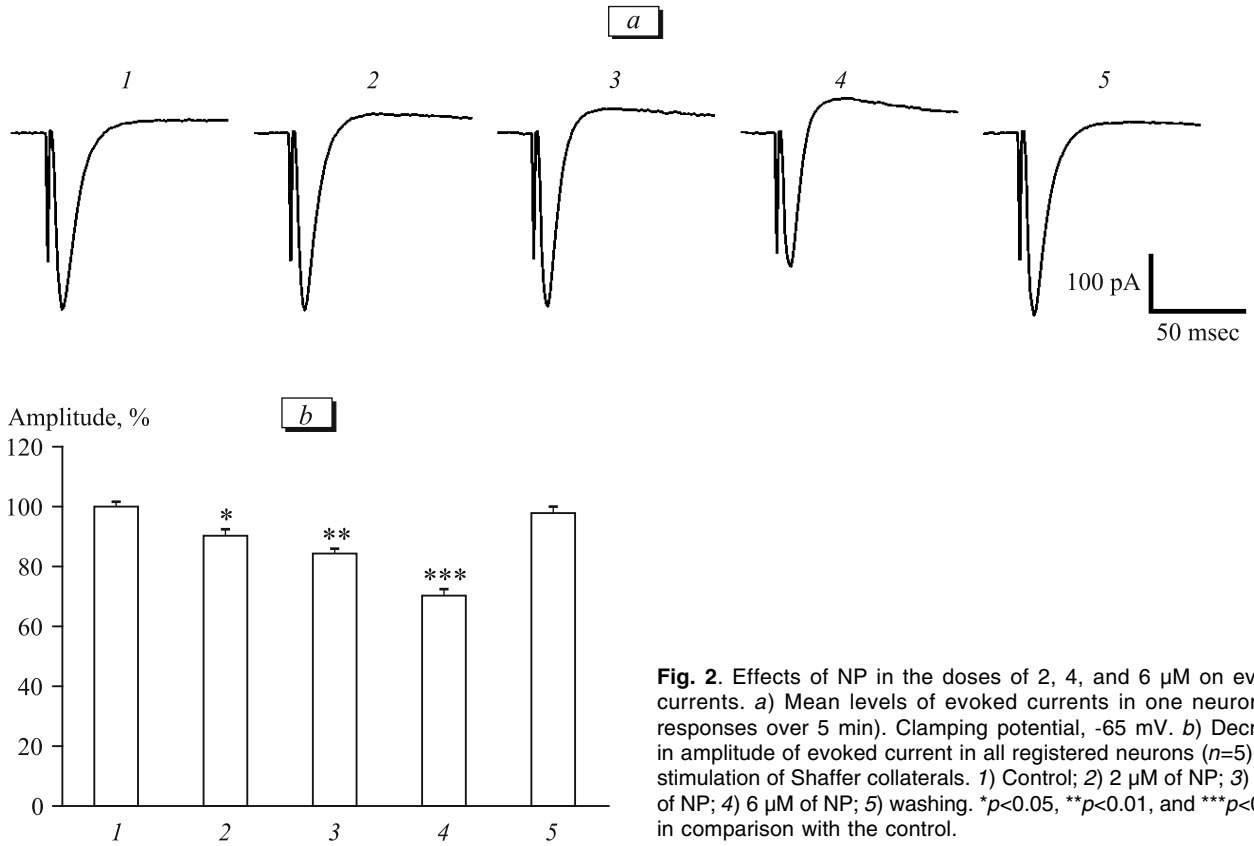

Fig. 2. Effects of NP in the doses of 2, 4, and 6 μM on evoked currents. *a*) Mean levels of evoked currents in one neuron (20 responses over 5 min). Clamping potential, -65 mV. *b*) Decrease in amplitude of evoked current in all registered neurons (n=5) after stimulation of Shaffer collaterals. *1*) Control; *2*) 2 μM of NP; *3*) 4 μM of NP; *4*) 6 μM of NP; *5*) washing. *p<0.05, **p<0.01, and ***p<0.001 in comparison with the control.

Application of picrotoxin (20 μM) blocking the inhibitory current to flowing fluid was followed by a slight increase in total response. These data suggest that the excitatory and inhibitory currents are oppositely directed (Fig. 1).

Direct application of NP in doses of 2, 4, and 6 μM induced a dose-dependent decrease in the amplitude of total current. This parameter was 90.29±2.08% ($p<0.05$), 84.31±1.79% ($p<0.01$), and 70.10±2.11% ($p<0.001$) from the control level (100%) in all registered pyramidal neurons ($n=5$), respectively (Fig. 2, *b*). The decrease in the amplitude of total current after NP treatment reflects activation of the inhibitory component of this current [9]. This inhibitory current in the form of a positive component was preceded by a negative component (Fig. 2, *a*) and increased after NP administration simultaneously with a decrease in the negative component. Similar pattern was found after inhibition of this positive component with picrotoxin (Fig. 1). Effects of NP were completely reversible and the current amplitude practically returned to control level (97.82±1.96%; Fig. 2, *a*, *b*) during washing that lasted twice longer than the application.

Changes in the total current were not observed after application of NP in doses of 2, 4, 6, and 10 μM ($n=5$) after addition of picrotoxin in a concentration of 20 μM. These data suggest that NP does not affect the excitatory current.

Monosynaptic inhibitory current with low latency observed after addition of 1 μM NBQX (blocker of AMPA-receptors) during potential clamping at -45 mV (for best visualization) was a result of direct stimulation of fibers of the inhibitory interneurons and did not change after applications of NP in doses of 2, 4, 6, and 10 μM ($n=5$). NBQX blocks the excitatory (glutamatergic) inputs to inhibitory interneurons. Absence of changes in inhibitory current after NP treatment reflects that NP affects not the release of inhibitory transmitter from terminals, but the activity of inhibitory interneurons.

Thus, changes in the amplitude of total response induced by NP did not depend on variations in the excitatory transmission. However, NP had no direct effects on fibers from the inhibitory interneurons and these interneurons exposed to Shaffer collaterals stimulation can be a target for NP [6].

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