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Novel nootropic dipeptide Noopept increases inhibitory synaptic transmission in CA1 pyramidal cells

Rodion V. Kondratenko, Vladimir I. Derevyagin, Vladimir G. Skrebitsky*

Department of Brain Research, Scientific Center of Neurology, Russian Academy of Medical Sciences, by-str. Obukha 5, Moscow 105064, Russia

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ABSTRACT

Effects of newly synthesized nootropic and anxiolytic dipeptide Noopept on inhibitory synaptic transmission in hippocampal CA1 pyramidal cells were investigated using patch-clamp technique in whole-cell configuration. Bath application of Noopept (1 μM) significantly increased the frequency of spike-dependant spontaneous IPSCs whereas spike-independent mIPSCs remained unchanged. It was suggested that Noopept mediates its effect due to the activation of inhibitory interneurons terminating on CA1 pyramidal cells. Results of current clamp recording of inhibitory interneurons residing in stratum radiatum confirmed this suggestion.

The aim of this study was to investigate whether novel proline containing dipeptide Noopept (NP) influenced synaptic transmission in central neurons. NP was synthesized as peptide analog of piracetam which is known as one of the first generation nootropics [4,13]. NP is similar to piracetam in its chemical structure (Fig. 1a) and nootropic activity but displays the effect in much lower concentration. In addition to nootropic activity NP displays anxiolytic effects [5,6,12]. Taking into consideration that hippocampus is related to both of these functions [1,2] we investigated the effects of NP on spontaneous inhibitory transmission in this structure by recording spontaneous IPSCs in CA1 pyramidal cells and firing rate of inhibitory interneurons.

Hippocampal slices (300 μm) were prepared from 14 to 17-day-old Wistar rats essentially as described previously [8]. In short, slices were maintained at room temperature (22–25 °C) in submerged conditions under optical control by an upright microscope. Slices were superfused with artificial cerebrospinal fluid containing (in mM): NaCl – 124; KCl – 3; NaH_2PO_4 – 1.25; $MgCl_2$ – 2.4; $CaCl_2$ – 2.4; $NaHCO_3$ – 26; glucose – 10; pH was buffered to 7.4 by continuously bubbling with a 5% CO_2–95% O_2 mixture. For somatic whole-cell patch-clamp recording borosilicate glass micropipettes (2 MΩ) were filled with (in mM): KCl – 120; $CaCl_2$ – 0.5; $MgCl_2$ – 2; EGTA – 10; HEPES – 10; pH 7.2–7.4. Experiments were performed in the presence of CNQX (1 μM) and APV (1 μM).

Individual neurons (CA1 pyramidal cells and interneurons residing in stratum radiatum) were visually identified using upright microscope with a 40× water immersion objective lens. For the analysis of IPSCs (amplitude, decay time and frequency) we used the Mini Analysis software (Synaptosoft, Decatur, GA, USA) offline. Data are given as mean ± standard error of the mean (SEM) and statistical analysis was performed with the non-parametric Wilcoxon signed-rank test for paired data ($p < 0.05$ was considered as significant and indicated by * in Figs. 1f and 2b).

At a membrane potential of −70 mV, the amplitude of spontaneously occurring currents ranged from 10–15 to 300–400 pA. The amplitude varied with the membrane potential, reversing at 0 mV in symmetrical Cl^-. In 5 cells, bath application of gabazin (10 μM) almost completely blocked detectable synaptic activity. The current can thus be defined as GABA mediated Cl^- currents and will be referred to as inhibitory postsynaptic currents (IPSCs).

The average amplitude of IPSCs varied widely among CA1 pyramidal neurons (range 40.81–72.75 pA, $n = 16$). NP (1 μM) significantly increased IPSC amplitudes in every cell tested ($n = 6$) (Fig. 1b), as was determined by cumulative probability distribution (Fig. 1d; $p < 0.01$, Kolmogorov–Smirnov test), and increased the group mean IPSC amplitude to 82.63 ± 11.65 pA (Fig. 1f). A representative example (Fig. 1c) shows that the average IPSC amplitude is increased whereas kinetic parameters (rise and decay time) remain unchanged. Amplitude histogram (Fig. 1e) clearly demonstrates that increase of the group mean IPSCs amplitude is due mainly to the appearance of relatively small amount of high amplitude (100–350 pA) events whereas the number of low-amplitude IPSCs (around 50 pA) is not changed significantly.

Similar to IPSC amplitude, the baseline rate of IPSCs varied widely among pyramidal neurons (0.49–1.75 Hz; average, 1.03 ± 0.10 Hz; $n = 16$). Bath application of NP caused large, time-

* Corresponding author. Tel.: +7 4959172382; fax: +7 4959172382.
E-mail address: skrebitsky@yahoo.com (V.G. Skrebitsky).

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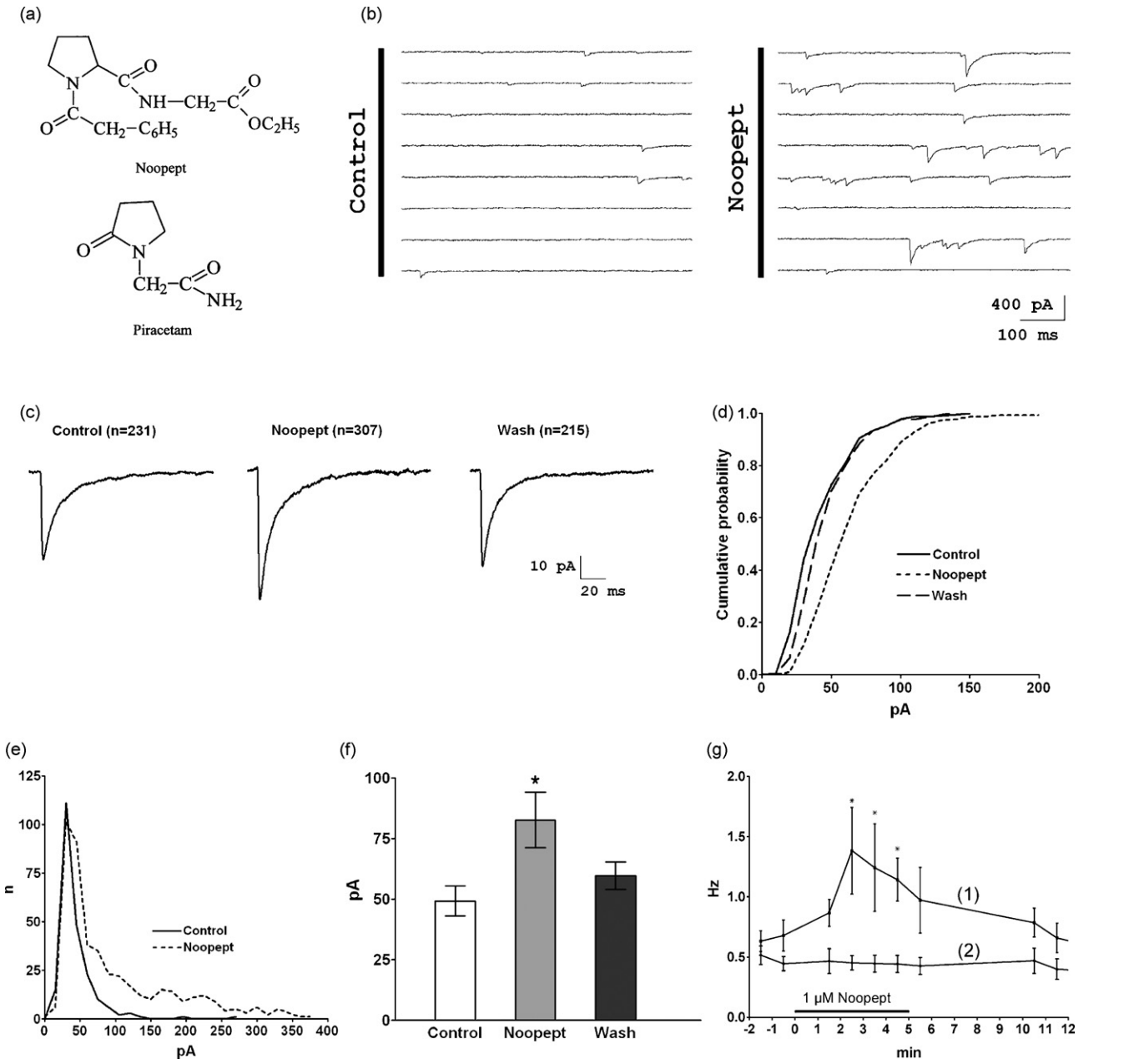

Fig. 1. Effect of Noopept on spontaneous IPSCs measured in CA1 pyramidal neuron. (a) Comparison of chemical structure of Noopept and Piracetam. (b) Consecutive current traces before (Control) and during Noopept (1 μM) superfusion (Noopept). (c) Spontaneous IPSCs averaged during 5 min periods before (control), during (Noopept) and after (wash) Noopept application. Here and in Fig. 2a number of individual IPSCs averaged in each trace is indicated in parenthesis. (d) Cumulative probability distribution of IPSCs amplitude before, during and after Noopept application. (e) Amplitude distribution derived from the same neuron as in "b". (f) Effect of Noopept on IPSCs amplitude for all neurons (mean ± SEM, $n = 6$). (g) Time course of the effect of Noopept on sIPSCs and mIPSCs frequency (curves 1 and 2, correspondingly) averaged (±SEM) across 6 cells.

dependant increase (1.3–2.5 fold) in the frequency of IPSCs in five of six cells (Fig. 1g, curve (1)). During a 5 min NP application, the peak effect was seen during the second and half minute, when the average IPSCs frequency increased approximately two and half fold to 1.40 ± 0.35 Hz. The effect of NP was fully reversed 10 min after termination of peptide superfusion.

To distinguish between spike-dependant and spike-independent IPSCs experiments with tetrodotoxin (TTX) application were performed. When TTX (1 μM) was added to the bathing solution, the larger IPSCs disappeared and almost all of the remaining currents had the amplitude of 47.07 ± 2.70 pA ($n = 6$) (Fig. 2b). The effect of TTX suggested that the larger IPSCs were caused by spontaneous action potentials in presynaptic neurons (sIPSCs) whereas IPSCs remaining in the presence of TTX were due to the release of transmitter independently from presynaptic TTX sensitive sodium action potentials and will be referred to as miniature IPSCs (mIPSCs). Mean frequency of mIPSCs ranged from 0.39 to 0.89 Hz; average 0.67 ± 0.07 Hz ($n = 6$). Amplitude histogram (Fig. 1e, control) clearly demonstrates that mIPSCs (less than 50 pA) make the main contribution into the number of IPSCs whereas number of larger TTX sensitive sIPSCs is significantly smaller.

In contrast to the relatively large effect of NP on sIPSCs, NP did not alter, mIPSC frequency (Fig. 1g, curve (2)) or amplitude (Fig. 2a and b) in any of the neurons tested. These results suggested that NP did not produce the changes in sIPSPs through action at inhibitory terminals or by altering GABA receptor sensitivity. Therefore we hypothesized that NP modulated firing rate of inhibitory interneurons.