PHARMACOLOGY AND TOXICOLOGY

NMDA Component in the Effects of Piracetam and New Nootropic Peptide GVS-111 on the Neocortical and Hippocampal EEG in Conscious Rats

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The effects of new nootropic dipeptide GVS-111 (N-phenylacetyl-L-prolylglycine ethyl ester) on EEG spectral characteristics were compared with those of piracetam. The EEG was recorded in the cortex and hippocampus of nonanesthetized nonrestrained rats with chronically implanted electrodes. GVS-111 and piracetam induced similar changes in EEG spectral profile in both structures increasing the α-band power and decreasing the power of the β- and δ-bands. These effects were prevented by intracerebral injection of 10^{-10} mol NMDA receptor antagonist (±)-3-(2-carboxypiperazine-4-il)-propyl-1-phosphonic acid. The data correlate with behavioral and neurochemical findings and suggest that NMDA receptors can be specifically involved in the mechanisms of nootropic effects of piracetam and GVS-111.

Key Words: *NMDA receptors; EEG; power spectra; piracetam; peptides*

Enhancement of α-activity and attenuation of δ- and θ-rhythms are typical EEG effects of different nootropic drugs including piracetam, pyritinol, centrophenoxine, cleregyl [2], fesam, orocetam, pantogam, and picamylon [5]. Proceeding from the original concept on the peptidergic nature of the nootropic effects of piracetam [4], GVS-111 dipeptide (N-phenylacetyl-L-prolylglycine ethyl ester) was synthesized and pharmacologically tested in the Institute of Pharmacology, Russian Academy of Medical Sciences [7,9]. The mnemotropic activity of GWS-111 2-4 thousand times surpassed that of piracetam. Neurochemical studies revealed a glutamatergic component in the mechanisms of action [8,10] of piracetam and GVS-111. The corresponding EEG data showed the involvement of NMDA glutamate receptors in modification of EEG frequency spectrum in rats [1].

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In this study we compared the effects of GVS-111 and piracetam on spectral characteristics of EEG in the cortex and hippocampus of free-moving rats and assessed the involvement of NMDA receptors in these effects. The choice of these two structures was determined by the data on cortical and subcortical effects of nootropic drugs [2].

MATERIALS AND METHODS

Experiments were carried out on 8 conscious free-moving male Wistar rats weighing 350-420 g. Nichrome electrodes (0.4 mm in diameter) were implanted in the symmetrical areas of the somatosensory cortex and dorsal hippocampus (CA1 area) of the right hemisphere under Nembutal anesthesia (50 mg/kg, subcutaneously). A cannula was implanted in the right lateral ventricle using a modified Meshcherski stereotaxic apparatus according to the rat brain atlas coordinates [11] AP=-0.4, L=3.2, H=3.7, α=20, where α is the angle between the sagittal plane and the cannula.

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The reference electrode was positioned in the nasal bone. The animals were adapted to experimental conditions during 4-5 days postoperation.

Piracetam (Sigma) and GVS-111 (Institute of Pharmacology, Russian Academy of Medical Sciences) were dissolved in isotonic saline and administered subcutaneously in doses of 400 and 0.2 mg/kg, respectively. Control group received saline. The NMDA receptor antagonist CPP ((±)-3-(2-carboxypiperazine-4-il)-propyl-1-phosphonic acid) in a dose of 10^{-10} mol or saline (5 μl) were injected intracerebroventricularly 10 min prior to the nootropic drugs. Before the experiment the rats were adapted to experimental conditions for 20-30 min (physiological sleep was induced). The EEG was recorded with an UBP 4-03 amplifier and processed on a computer 10 min before (baseline) and 30 sec postinjection. Consecutive 12-sec EEG fragments were sampled at 330 Hz and processed on-line using a modified method of periodogram analysis [3]. Integral amplitudes of rhythms in each of 20 bands within the 0.5-30 Hz range and their relations to the integral amplitude of all analyzed frequencies were computed. To compare the control and experimental data the individual spectra were averaged over 10-min periods with laboratory-developed software. The data were analyzed statistically using the Wilcoxon test.

RESULTS

In the control (subcutaneous administration of saline), significant changes in the EEG power spectra were observed only during the first 10 min postinjection: 20-26-Hz rhythms were enhanced, while 8-12 Hz rhythms were attenuated. Thereafter the EEG power

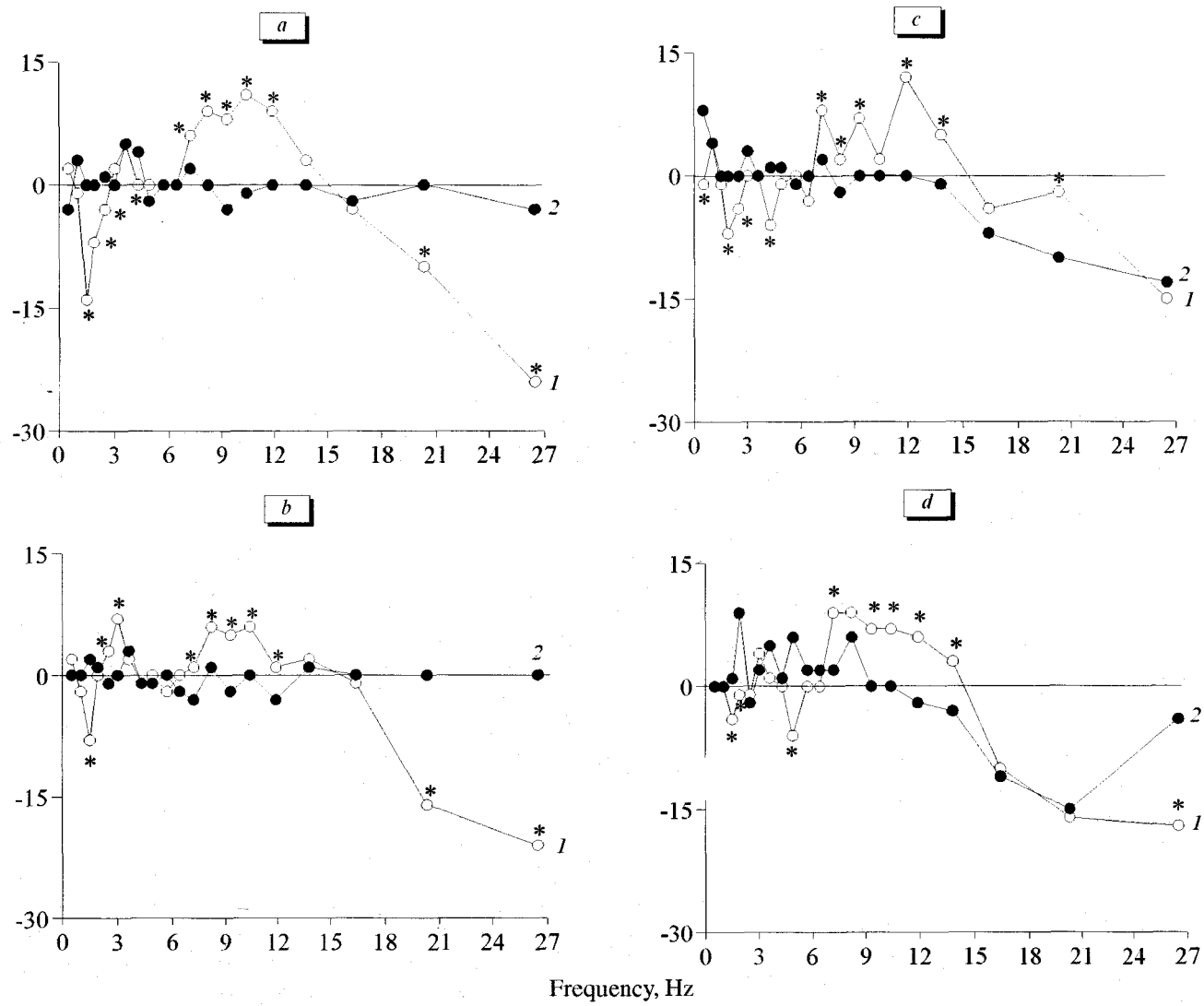

Fig. 1. Effects of piracetam and GVS-111 on spectral characteristics of cortical (*a, c*) and hippocampal (*b, d*) EEG in rats (n=8) 30-40 min (*a, b*) and 50-60 min (*c, d*) postinjection. *p<0.05 in comparison with saline (zero line). Here and in Fig. 2: ordinates: changes in EEG power spectra, %; *1)* saline+drug; *2)* CPP+drug.

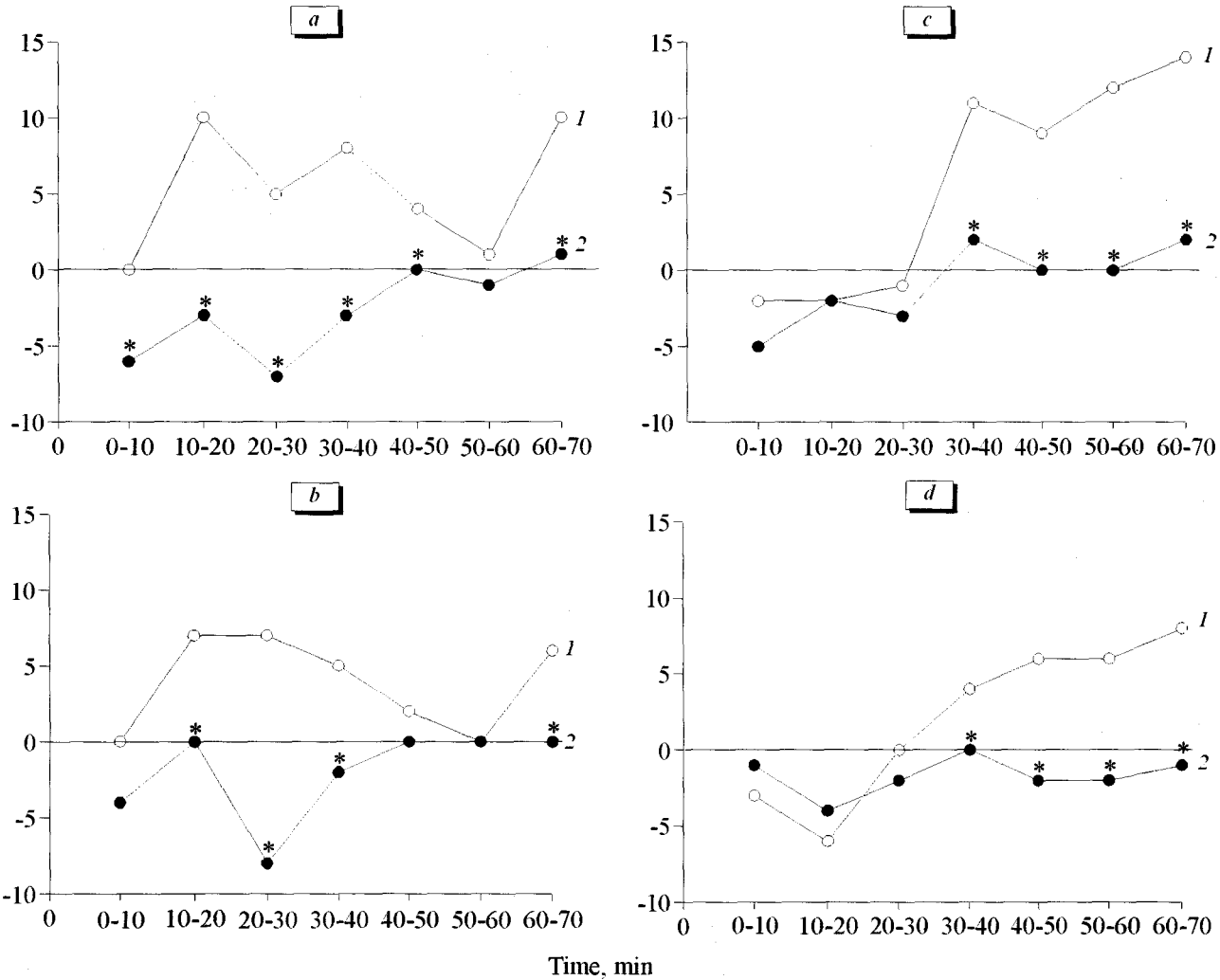

Fig. 2. Changes in 9-Hz (*a, c*) and 12-Hz power (*b, d*) bands under the influence of piracetam (*a, b*) and GVS-111 (*c, d*). *$p<0.05$ in comparison with saline.

spectra returned to the initial pattern characteristic of physiological sleep. Piracetam induced immediate shifts in the cortical EEG: the spectral power increased in a broad frequency range (7.2-11.9 Hz) and decreased within narrow bands of 1.5-2.5 Hz and 20-26 Hz (Fig. 1, *a, 1*). In the 9 Hz band, the power peaked 10-20 min postinjection, gradually decreased after 50-60 min and then increased again (Fig. 2, *a, 1*). Similar changes were observed in EEG power spectra of the hippocampus (Fig. 1, *b*; 2, *b*).

Piracetam-induced spectral changes in both cortical and hippocampal EEG were completely prevented by intraventricular injection of CPP (10^{-10} mol). The spectral profile formed by an equipotential dose of GVS-111 (0.2 mg/kg, subcutaneously) was similar to that caused by piracetam (Fig. 1, *c, d, 1*), but differed in some details: first, significant changes in the principal bands appeared with a considerable delay (30-40 min) and persisted to the end of recording (Fig. 2, *c, d, 1*); second, GVS-111 reduced the power in the 4-5 Hz band. Both frequency and time shifts caused by GVS-111 were abolished by CPP (Fig. 1, *c, d;* Fig. 2, *c, d*).

Our findings showed that piracetam and GVS-111 administered subcutaneously in equipotential doses of 400 and 0.2 mg/kg, respectively, produced similar changes in EEG power spectra in the cortex and hippocampus, in particular enhancement of α-rhythm and attenuation of β- and, to a lesser extent, δ-rhythms. All these changes were prevented by intracerebroventricular administration of CPP. The most pronounced difference between the effects of the two drugs was a shorter latency of piracetam-induced changes. Since GVS-111 is a precursor of cycloprolylglycine, an endogenous cyclopeptide with nootropic activity [6], the longer latency of its effect can be attributed to the time needed for its transformation to the active form.

Enhancement of α-rhythms and attenuation of δ-rhythms in EEG induced by nootropics were also observed in mental patients, elderly persons, patients with borderline mental disorders with predominant

asthenic syndrome [5], and children with learning disabilities [12]. The blockade of spectral changes by the NMDA antagonist revealed in our study suggests that this type of glutamate receptors can be involved in the mechanisms of the EEG effects of both piracetam and GVS-111. This suggestion is supported by the data on noncompetitive effects of piracetam on ^{3}H-piracetam binding to rat brain membranes *in vitro* [8], GVS-111-induced alleviation of amnesia caused by MK-801, a noncompetitive antagonist of NMDA receptors [9], CPP-induced reversal of the effect of GVS-111 on the release of dopamine from rat striatum *in vitro* [10].

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