# PIRACETAM PEPTIDE ANALOG L-PGLU-D-ALA-$NH_2$ MAINTAINS THE PLASTIC PROPERTIES OF SYNAPTIC TRANSMISSION IN LONG-LASTING HIPPOCAMPAL SLICES

**A. N. Chepkova, N. V. Doreuli, R. U. Ostrovskaya, T. A. Gudasheva, and V. G. Skrebitskii**

UDC 612.825.014.46:[615.214.3:547.745.08

KEY WORDS: long-term potentiation, hippocampus, peptides, piracetam

Short-term high-frequency stimulation of afferent hippocampal inputs leads to increased reactivity of postsynaptic neurons, which may last many hours. This plastic phenomenon, known as long-term potentiation (LTP), is nowadays regarded as the neurophysiological basis of learning and memory [6, 9].

The mechanism of development and preservation of LTP have been studied most intensively on surviving hippocampal slices in the system of Schaffer's collaterals, connecting the two output systems of the hippocampus, namely the pyramids of areas CA3 and CA1 [6]. Several investigations have been conducted on this same synaptic system in order to study the action of substances affecting learning and memory on LTP [9].

The first attempts to evaluate the action of nootropic drugs — agents selectively improving memory and cognitive functions — on LTP gave negative results: the characteristics of LTP in the system of Schaffer's collaterals — area CA1 pyramids in hippocampal slices were unchanged after application of various does of the standard preparation (piracetam) and of a substance of different chemical nature (bifemelane) [7, 8]. However, nootropic drugs are known to be effective in the case of memory disturbances due to various causes, but have virtually no effect on normal memory. In a search for a more adequate model of damaged LTP, we directed our attention to the fact that LTP develops easily in hippocampal slices and persists for a long time during high-frequency stimulation (tetanization) of the input in about the first 4 h after preparation of the specimen and it rarely appears after tetanization in the later stages, although all other electrophysiological characteristics, including short-term plastic phenomena (frequency and post-tetanic potentiation) remain intact. The aim of the present investigation was to assess the effect of nootropic drugs on the development of LTP in long-surviving hippocampal slices, subjected to tetanization in the late periods. Another aim of the investigation was to compare the action of piracetam and its peptide derivative — pyroglutamyl-D-alanine (PGAA), which possesses higher activity and its effective at all stages of learning, as shown by behavioral tests [4].

## EXPERIMENTAL METHOD

Transverse hippocampal slices were prepared from the brain of male Wistar rats aged 4-8 weeks, and transferred in the course of preparation into a continuous-flow chamber filled with oxygenated medium, heated to 29-31°C, and fixed on a nylon grid, mounted 1-1.5 mm below the level of the liquid. The medium consisted of a salt solution of the following composition (in mM): NaCl — 124; KCl — 3; $CaCl_2$ — 2.5; $MgSO_4$ — 2.4; $KH_2PO_4$ — 1.25; $NaHCO_3$ — 26; D-glucose — 10. The solution was saturated with a gas mixture of 95% $O_2$ + 5% $CO_2$, with pH 7.2-7.4. The rate of flow was 1.5-2 ml/min, sufficient to ensure complete change of medium in the chamber during 2-3 min.

The data were recorded and processed by "Labtam" minicomputer. Recording activity began 2 h after preparation of the specimen. The focal response, reflecting the synchronized discharge of the pyramidal cells [5] was recorded in the pyramidal layer

Brain Research Institute, All-Union Mental Health Research Center, Academy of Medical Sciences of the USSR. Research Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 12, pp. 602-604, December, 1990. Original article submitted November 29, 1989.

0007-4888/90/0012-1647$12.50 

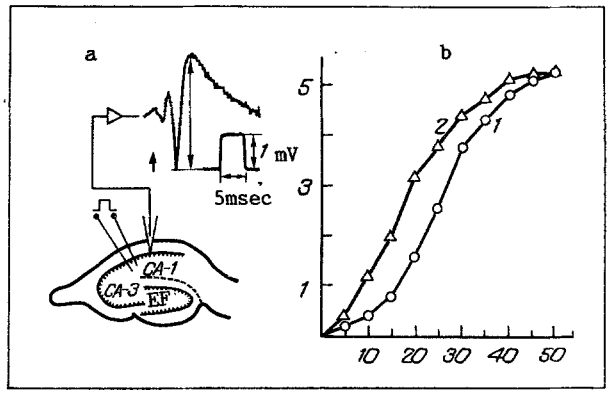


Fig. 1. Measurement of reactivity of pyramidal neuron population in hippocampal slices: a) focal response recorded in pyramidal layer of area CA1 in response to stimulation of radial layer (diagram represents displacement of electrodes on slice as seen from below). Amplitude of pop-spike measured as indicated by arrow; b) curve showing dependence of amplitude of pop-spike (in mV) on stimulus strength (in V) before (1) and after (2) tetanization of synaptic input.

of area CA1 by means of glass microelectrodes filled with 1.5 M NaCl, during bipolar stimulation of the radial layer in the region of the bundle of Schaffer's collaterals (single square pulses 0.1 msec in duration, 0-50 V in amplitude). The magnitude of the spike component of the response (pop-spike) was the measure of reactivity of the pyramidal neuron population (Fig. 1a). Changes in their reactivity were judged from the vertical shift of the curve of amplitude of the pop-spike as a function of stimulus strength (Fig. 1b), by calculating the relative changes in area beneath the curve in its linear region. Reactivity was tested with intervals of 15-30 min. Tetanization was carried out 6 h after preparation of the slice, using a stimulus strength sufficient to induce a response of half the maximal amplitude. Series of pulses (100 stimuli with a frequency of 100 Hz) were applied to the input twice with an interval of 1 min. The substances were added to the chamber by switching the flow system to the appropriate reservoir for 20 min, 80 min before tetanization.

The piracetam and sodium hydroxybutyrate used in the work were of USSR origin. PGAA was synthesized by T. A. Gudasheva by the method described previously [2].

## EXPERIMENTAL RESULTS

When tetanization was carried out 6 h after preparation of the hippocampal slices potentiation of the focal response was observed in only two of the eight preparations, and only in one of them did it last 1 h. Perfusion of the preparations with solution containing 100 $\mu$M piracetam did not lead to any significant improvement of ability to develop LTP, although in half of the preparations tested, it led to a significant increase in reactivity. The curves in Fig. 2a summarize changes in reactivity in the control experiments and experiments with piracetam.

Thus the results obtained with late tetanization of a model of damaged LTP do not differ from those of investigation of the action of piracetam on the normally developing LTP [7, 8].

Meanwhile, in all slices perfused after survival for 4-4.5 h in vitro in a solution containing 0.5 $\mu$M PGAA, late tetalization induced potentiation of the responses, which lasted in most preparations (in 6 of 7) into the 1-hour period of observation (Fig. 2b). With PGAA in a concentration an order of magnitude lower (0.05 $\mu$M) late tetanization did not lead to the development of LTP.

During perfusion (20 min) and subsequent rinsing out of the preparation, no regular changes in reactivity were observed when these two PGAA concentrations were used: in some slices responses were maintained at a relatively stable level or were slightly increased, whereas in others, reactivity gradually declined. In control experiments in which application was simulated by switching the flow to another reservoir containing normal solution, changes in reactivity in the period preceding tetanization were similar in character.

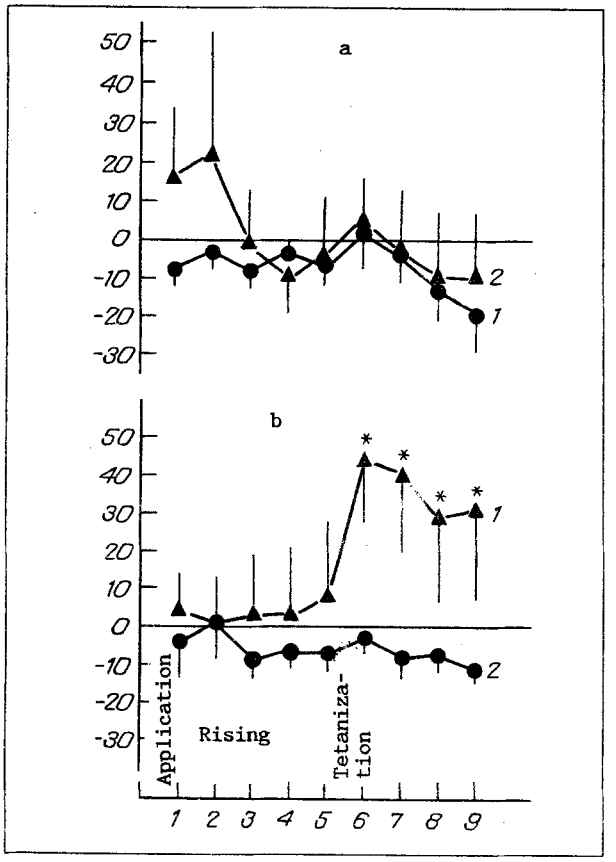


Fig. 2. Dynamics of changes in reactivity of pyramidal neuron population in experiments with late tetanization: a) curves summarizing changes of reactivity in control experiments (1; $n = 8$) and after incubation of preparations with 100 $\mu$M piracetam (2; $n = 4$); b) the same curves in experiments with addition of 0.5 $\mu$M PGAA (1; $n = 7$) and 100 $\mu$M sodium hydroxybutyrate (2; $n = 4$). Mean values of changes of reactivity relative to control level and errors of means (in %) 15 min after beginning of perfusion with solutions containing the substance (testing series 1 along lower scale), during rinsing of preparations for 1 h (series 2-4), and 15, 30, 45, and 60 min after tetanization (series 6-9) are shown. Control level of reactivity (zero line) in each experiment was determined from averaged values obtained during 3-4 testing series (these series were carried out at intervals of 15-30 min for 1.5-2 h).

It must be pointed out that if PGAA is added in high concentrations it can induce substantial facilitation of reactivity. We carried out a series of preliminary experiments to determine the concentration region causing changes in focal responses, and we found that an appreciable increase in the pop-spike is observed after addition of 50-100 $\mu$M PGAA, which is two orders of magnitude higher than the PGAA concentration affecting the development of LTP.

Thus incubation of slices with 0.5 $\mu$M PGAA, while not causing any significant changes of reactivity in the synaptic system of Schaffer's collaterals — pyramids of area CA1, helped to maintain the plastic properties of synaptic transmission during relatively long survival of the preparations in vitro. The results obtained on a model of damaged LTP are on the whole in good agreement with the results of behavioral studies, according to which the nootropic activity of PGAA is exhibited when the substance is used in doses hundreds of times smaller than the effective doses of piracetam [4]. This suggests that LTP in the late stages of survival of the hippocampal slices may prove to be a useful new model for comparing the efficacy of new compounds and for studying mechanisms of action of substances with nootropic activity.

Turning to the analysis of the mechanisms of action of PGAA, we first attempted to assess the possibility that antihypoxic effects are involved in preservation of ability to undergo LTP, for many nootropic drugs are known to have antihypoxic properties, which are linked with stimulation of the energy metabolism of cells [1, 3]. In pursuit of this aim we compared the action of PGAA with that of one of the most effective antihypoxic agents, namely sodium hydroxybutyrate.

The results given in Fig. 2b show that sodium hydroxybutyrate did not facilitate the appearance of LTP when tetanization was carried out in the late stages, although in some preparations it induced increased reactivity during injection, and stabilized reactivity to some extent later. The ineffectiveness of sodium hydroxybutyrate in relation to LTP suggests more specific mechanisms for maintenance of ability to develop LTP under the influence of PGAA and, in particular, the possibility of direct interaction of PGAA with mechanisms of LTP induction.

The study of interaction of PGAA with the critical stages of induction of LTP, as which, in the glutamatergic system of Schaffer's collaterals — pyramidal cells of area CA1, activation of glutamate receptors of NMDA-type and an increase in the intracellular $Ca^{2+}$ concentration have been identified [6], will be the subject for our future research.

## LITERATURE CITED


1. T. A. Voronina, Pharmacology of Nootropics [in Russian], Moscow (1989), pp. 8-19.
2. T. A. Gudasheva, R. U. Ostrovskaya, S. S. Trofimov, et al., Khim.-Farm. Zh., No. 11, 1322 (1985).
3. V. V. Davydov and V. S. Yakushev, Farmakol. Toksikol., **50**, No. 2, 99 (1987).
4. R. U. Ostrovskaya, T. A. Gudasheva, S. S. Trofimov, et al., Byull. Éksp. Biol. Med., **104**, No. 11, 576 (1987).
5. P. Andersen, T. V. P. Bliss, and K. K. Skrede, Exp. Brain Res., **13**, No. 2, 208 (1971).
6. B. Gustafsson and H. Wigström, Trends Neurosci., **11**, No. 4, 156 (1988).
7. H. R. Olpe and G. Lynch, Eur. J. Pharmacol., **80**, 415 (1982).
8. M. Satoh, K. Ishihara, and H. Katsuki, Neurosci. Lett., **93**, 231 (1988).
9. T. J. Teyler and P. DiScenna, Ann. Rev. Neurosci., **10**, 131 (1987).