Effect of Pyroglutamylasparagine Amide on Plastic Characteristics of Synaptic Transmission in the Hippocampus

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Preincubation of rat hippocampal slices with 0.05-0.5 μM pyroglutamylasparagine amide improved characteristics of long-term potentiation of focal responses in the synaptic system of Schaffer collaterals—CA1 field pyramids facilitating LTP development and increasing its amplitude and duration. Presumably, the positive modulation of plastic characteristics of synaptic transmission in the hippocampusis is responsible for facilitation of learning and memory induced by pyroglutamylasparagine.

Key Words: *pyroglutamylasparagine amide; hippocampus; focal response; long potentiation*

Pyroglutamylasparagine amide (PGA) was synthesized by T. A. Gudasheva as a peptide analog of piracetam within the framework of the concept on the existence of specific nootropic receptors and endogenous peptide nootrops [2,4]. This compound exhibited high and stereoselective mnestic activity in conditioned passive avoidance response (CPAR) [3], a reaction mediated by hippocampal structures [12]. In light of this it was interesting to study the effect of PGA on reactivity and plastic characteristics of the hippocampal neurons. Here we studied the effects of PGA on evoked responses in the synaptic system of Schaffer collaterals—hippocampal CA1 field pyramids and on the development of long-term potentiation (LTP) of synaptic responses after high-frequency stimulation, a plastic phenomenon considered as the cellular basis of learning and memory [8].

MATERIALS AND METHODS

The study was carried out on hippocampal slices from adult male Wistar rats (100-180 g) as described previously [6,7]. Focal response evoked in the CA1 field pyramidal layer by stimulation of the radial layer by single rectangular pulses at a frequency of $^1/_{15}$ sec was recorded. The intensity of stimulation was so selected that the amplitude of the peak component of the response reflecting the summary spike response of the pyramidal neuron population (pop-spike, PS) were approximately half of its maximum value. LTP of the focal response was induced by high-frequency stimulation (HFS; 100 Hz during 1 sec) of the same entry at the same stimulus intensity.

Changes in the reactivity of pyramidal neurons were evaluated by changes in PS amplitude in comparison with its mean value determined by a 20-30-min period of baseline recording.

L-Pyroglutamyl-L-asparagine amide (PGA) was synthesized using the method of activated esters from pentachlorophenyl pyroglutamic acid ester and asparagine amide as described previously [3]. According to ^{1}H-NMR spectroscopy, the purity of the resultant product was at least 98%.

Concentrated aqueous solutions of PGA were stored as frozen microdoses (50-100 μl). Solutions of the needed concentration were prepared directly before the use by dissolving in perfusion medium and applied by switching from the perfusion system to the appropriate reservoir. The effect of only one dipeptide concentration was tested on each slice.

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In the first series of experiments LTP characteristics were studied after HFS during application of 0.2-1 μM PGA and in the second series 60-90 min after the end of application. The possibility of LTP, its amplitude (relative increment of PS amplitude) and duration (60-min observation after HFS) were evaluated.

The data were computer-recorded and processed. The results were presented as means and mean errors ($M \pm m$). The data were statistically processed using nonparametric Mann—Whitney's and precise Fisher's tests.

RESULTS

Analysis of amplitudes of the focal responses revealed no appreciable changes during perfusion of slices with PGA in low concentrations (0.05-1 μM), while higher concentrations (10 μM) caused a reversible 20-30% suppression of PS. The relative changes in the amplitudes of responses 60 min after the end of application (in percent of the mean level during the entire preapplication period) were 106.0±29.8% (n=5), 103.6±17.4% (n=5), 109.0±11.8% (n=5), and 89.6±12.7% (n=6) after perfusion of slices with PGA in concentrations of 0.05, 0.2, 1, and 10 μM, respectively, which did not differ significantly from changes in the responses in control tests (101.8±6.6%, n=6). Hence, PGA in submicromolar concentrations corresponding to behaviorally active doses did not modulate the reactivity of hippocampal neurons.

In series I PGA facilitated LTP (100% *vs.* 86% in the control) and prevented its shortening: in the control LTP decreased from 146.6±26.9% to 115.4±6.9% over 60 min (n=6), while after application of 0.2 μM PGA it remained at the same level (143.9±23.1% and 148.1±23.0%, n=6, at the beginning and end of a 60-min observation, respectively; Fig. 1, *b*).

In series II PGA in doses of 0.05 and 0.2 μM not only facilitated LTP (100% *vs.* 67% in the control) and increased its duration, but also increased its amplitude (167.4±24.5, n=5, and 164.4±14.8%, n=5, respectively, *vs.* 138.1±19.0%, n=4, in the control; Fig. 2). High concentrations of PGA (10 μM) inhibited LTP by 50%, but if LTP developed, its value did not differ from the control (137.3±9.7%, n=3). Hence, PGA in submicromolar concentrations promoted the development of LTP of the focal response in the hippocampal CA1 field due to its facilitation and the increase in its amplitude and duration, while PGA in higher concentrations suppressed LTP induction.

The effects of nootropic drugs are better expressed under conditions of impaired or insufficient cognitive functions [1,4]. Therefore we evaluated the effect of PGA on a model of LTP deficiency caused by long-term survival of slices (Fig. 3). Gradual degeneration of the tissue limits the time of full-value *in vitro* functioning of the preparations by several hours. In our experiments HFS applied 5-6 h after the start of recordings induced LTP in only 3 of 14 cases (21%), although the main electrophysiological parameters (maximum amplitudes of responses, relationship between PS amplitude and stimulus intensity, characteristics of frequency and posttetanic potentiation) remained within the normal. Perfusion of slices with 0.05-0.5 μM PGA 60-90 min before HFS facilitates

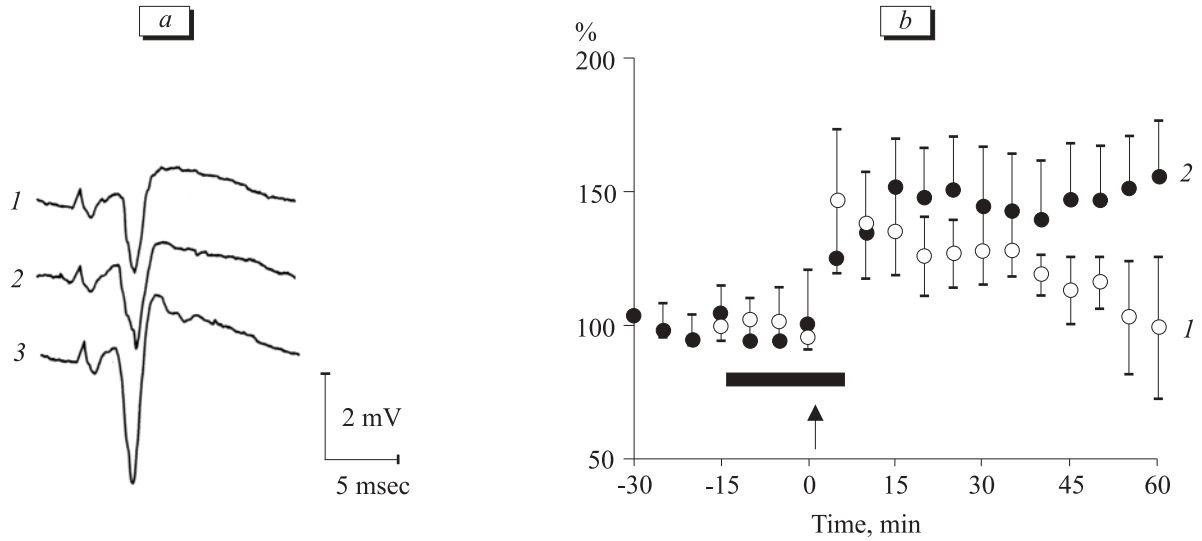

Fig. 1. Prolongation of long potentiation of focal response in CA1 field of the rat hippocampal slices by application of pyroglutamylasparagine (PGA) amide. *a*: focal response before (*1*), after 15-min perfusion with 0.2 μM PGA (*2*), and 30 min after tetanization (*3*); *b*: summary curves of time course of pop-spike amplitude in the control (*1*, n=6) and after application of 0.2 μM PGA (*2*, n=4). Here and in Fig. 2, *a*, and 3: ordinate: changes in pop-spike amplitude in comparison with the mean level before PGA application: the mark shows application period and the arrow shows the moment of tetanization.

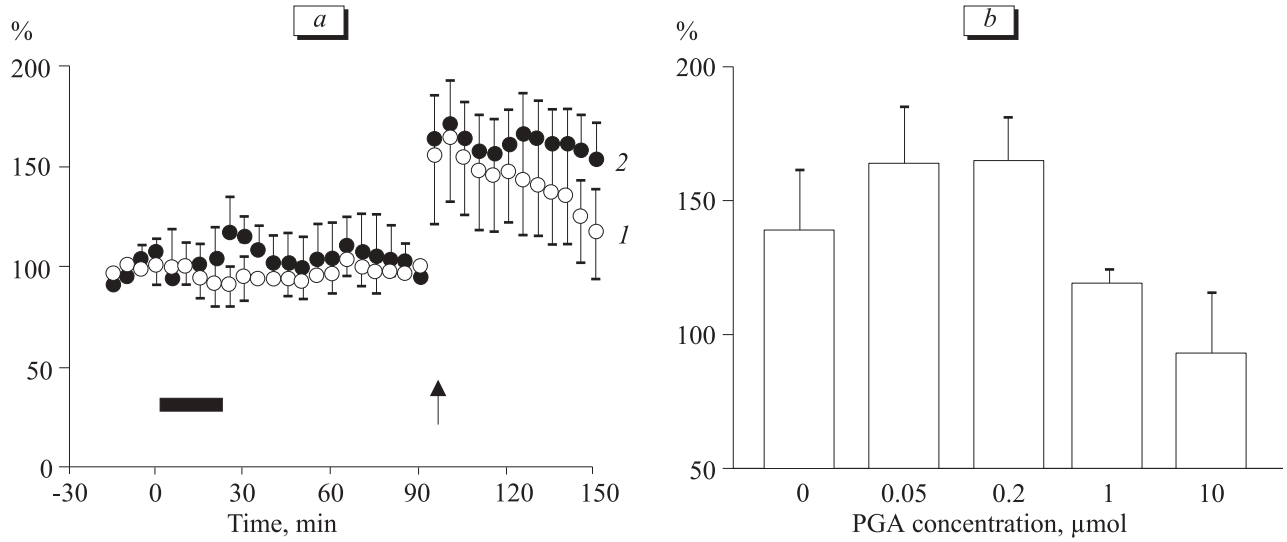

Fig. 2. Prolongation and intensification of long-term potentiation of focal response in the hippocampal CA1 field slices after preincubation with submicromolar concentrations of PGA. *a*: dynamics of focal response amplitude in the control (*n*=6) and after application of 0.2 μM PGA (*n*=5); *b*: mean value of changes in the reactivity 30 min after tetanization in slices preincubated with 0.05-10 μM PGA 60-90 min before tetanization.

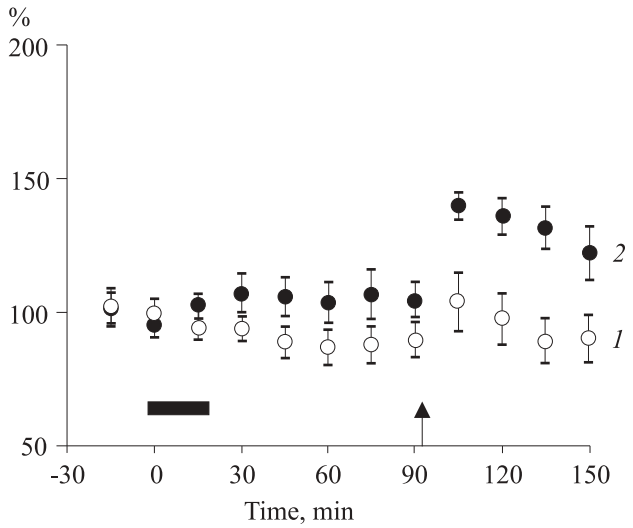

Fig. 3. Retained capacity to development of long-term potentiation of focal response in long-surviving hippocampal slices after preincubation with PGA. *1*) changes in pop-spike amplitude in the control (*n*=14)and *2*) after perfusion of slices with 0.05-0.5 μM PGA (*n*=8).

LTP (7 of 8 slices, 88%, $p<0.01$) without changing its amplitude (134.00±7.07%, *n*=7, *vs.* 132.7±4.8%, *n*=3, in the control). Summary curves were plotted in experiments on a model of LTP deficiency (Fig. 3). Hence, improvement of plastic characteristics of synaptic transmission manifesting as a trend on two first experimental models with relatively early HFS became significant under conditions of impaired LTP induction.

The characteristics of LTP in the Schaffer collaterals—CA1 field positively correlate with learning characteristics in some behavioral tests [8-11], and agents improving learning and memory have a positive impact on LTP development and characteristics [5-7]. Our data suggest that improvement of learning under the effect of PGA can be due to positive modulation of plastic properties of synaptic transmission in the hippocampus.

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