BIOPHYSICS AND BIOCHEMISTRY

Pyroglutamyl-Asparagine Amide Normalizes Long-Term Potentiation in Rat Hippocampal Slices

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Preapplication of peptide piracetam analogue pyroglutamyl-asparagine amide to rat hippocampal slices facilitates long-term potentiation of focal responses in the CA1 field after weak tetanization of the synaptic input (30 pulses, 100 Hz). This treatment normalized the development of long-term potentiation after standard tetanization (100 pulses, 100 Hz) impaired by ethanol.

Key Words: *pyroglutamyl asparagine amide; long-term potentiation; ethanol; hippocampus*

Pyroglutamyl-asparagine amide (PGA) is a synthetic peptide analogue of piracetam that acts as a potential ligand of nootropic receptors [2], improves learning and memory in rats in conditioned passive avoidance paradigm [3], and facilitates long-term potentiation (LTP) of synaptic responses in the hippocampus. These properties probably underlie mnesic activity of PGA [4].

Nootropic properties of compounds are usually studied on behavioral models with undertrained animals or impairment of memory consolidation by pharmacological compounds (scopolamine) and other factors (electrical shock and hypoxia) [1]. LTP forms the neurophysiological basis of learning and memory [6]. Here we studied the effect of PGA on hippocampal LTP during weak stimulation of synaptic input (model of undertraining) and inhibition with ethanol (model of impairment).

MATERIALS AND METHODS

Experiments were performed on hippocampal slices from young male Wistar rats weighing 90-180 g [4,5]. The focal response induced by stimulation of the radial layer with single rectangular pulses (0.1 msec, $^1/_{15}$ sec) was recorded in the pyramidal layer of hippocampal field CA1. LTP of the focal response was induced by high-frequency stimulation (HFS, 30 or 100 pulses, 100 Hz) of the synaptic input. The strength of stimulation was selected so that the peak response reflecting the total spike response of pyramidal neurons (pop-spike) had the half-maximal amplitude. The strength of stimulation remained unchanged during recording. Changes in reactivity of pyramidal neurons were estimated by deviations of the pop-spike amplitude from the mean value (20-30 min recording under baseline conditions). LTP was determined the average increase in the pop-spike amplitude over 45-60 min after HFS. The increase in the pop-spike amplitude no less than by 10% reflected the development of LTP.

PGA was synthesized from pentachlorophenyl ester of pyroglutamic acid and asparagine amide by the method of activated esters [3]. ^{1}H-nuclear magnetic resonance spectroscopy showed that the product had a diastereomeric purity of ≥98%.

Concentrated aqueous solution of PGA was frozen in microdoses. Its concentration was brought to 0.2 or 1 μM with perfusion medium immediately be-

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fore 20-min application. The flow system was switched to the corresponding reservoir 15 min before HFS. Reconnection was performed 5 min after HFS. The effect of PGA at a certain concentration was studied on one slice. Some series were performed with arginine-vasopressin AVP_{4-9} fragment (Sigma). It was stored and applied by the same method.

The data are expressed as means and standard errors. Intergroup differences were evaluated by Student's *t* test and exact Fischer test.

RESULTS

HFS of Schaffer collaterals was followed by long-lasting facilitation of the evoked potential in hippocampal field CA1. The degree and duration of changes depended on the intensity of HFS [10]. In our experiments standard HFS (100 Hz, 1 sec) increased the pop-spike amplitude in 11 of 12 slices (92%) by 143.3± 13.6%.

The induction of LTP was less pronounced after short HFS (30 pulses, 100 Hz). The pop-spike amplitude significantly increased over the first 5 min after short HFS (163±10%, n=8), but then progressively decreased. One hour after HFS the pop-spike amplitude was 130-140% (142.9±5.3%) in 4 of 7 slices (57%), but returned to the control level in other slices (102.9±1.4%, n=3).

Short HFS induced high-amplitude LTP (176.3± 12.7%) in 8 of 9 slices perfused with a medium containing 0.2 μM PGA (89%). Perfusion with a medium containing 1 μM PGA increased the incidence (3 of 4 slices, 75%) and amplitude of LTP (153.7±21.8%, n=3). However, changes induced by 1 μM PGA were less pronounced compared to those observed after treatment with 0.2 μM PGA. Preapplication of PGA in concentrations of 0.2 and 1 μM had no effect on the initial potentiation estimated 5 min after HFS: 163.1± 11.7 (n=10) and 177.3±24.1% (n=5), respectively, *vs.* 163±10% in the control (n=8).

These data show that PGA in submicromolar concentration facilitated the development of LTP after short HFS. This compound increased the incidence and amplitude of LTP. Significant differences were observed in temporal changes in the pop-spike amplitude after short HFS of control samples and slices perfused with 0.2 μM PGA (Fig. 1, *a*). The increase in the incidence and amplitude of LTP was statistically insignificant.

Our previous studies showed that the major metabolite of vasopressin AVP_{4-9} possesses similar but higher activity under conditions of LTP deficiency [5]. AVP_{4-9} and PGA have similar structure of the N-terminal part (pGlu-Asn). In the next series we compared the effects of PGA and AVP_{4-9} on another model of LTP deficiency (impairment with ethanol).

Perfusion with a medium containing 20-50 mM ethanol impaired the development of LTP in hippocampal slices after standard HFS [7,9]. Application of ethanol 15 min before HFS decreased the incidence of LTP to 50% (6 of 12 slices) and the mean increment of pop-spike amplitude after 1-h recording was 111.4±

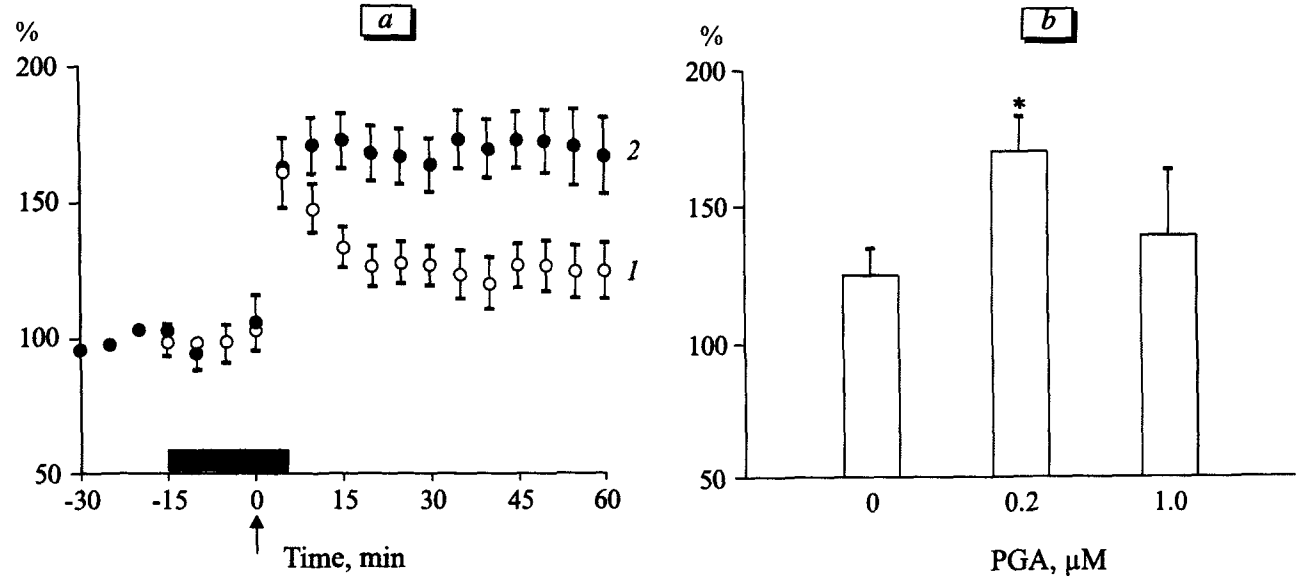

Fig. 1. Facilitation of long-term potentiation of the focal response in the CA1 field after short high-frequency stimulation of Schaffer collaterals in the presence of pyroglutamyl-asparagine amide (PGA). *a*) total temporal changes in the peak amplitude of the focal response in the control (*1*, n=7) and during perfusion with 0.2 μM PGA (*2*, n=9). *b*) Mean pop-spike amplitude over 45-60 min after stimulation of control samples (0) and slices perfused with 0.2 (n=9) and 1.0 μM PGA (n=5). Here and in Fig. 2: ordinate, changes in the pop-spike amplitude relative to the average level observed before PGA application; thick line, high-frequency stimulation. *p<0.05 compared to the control (Student's *t* test).

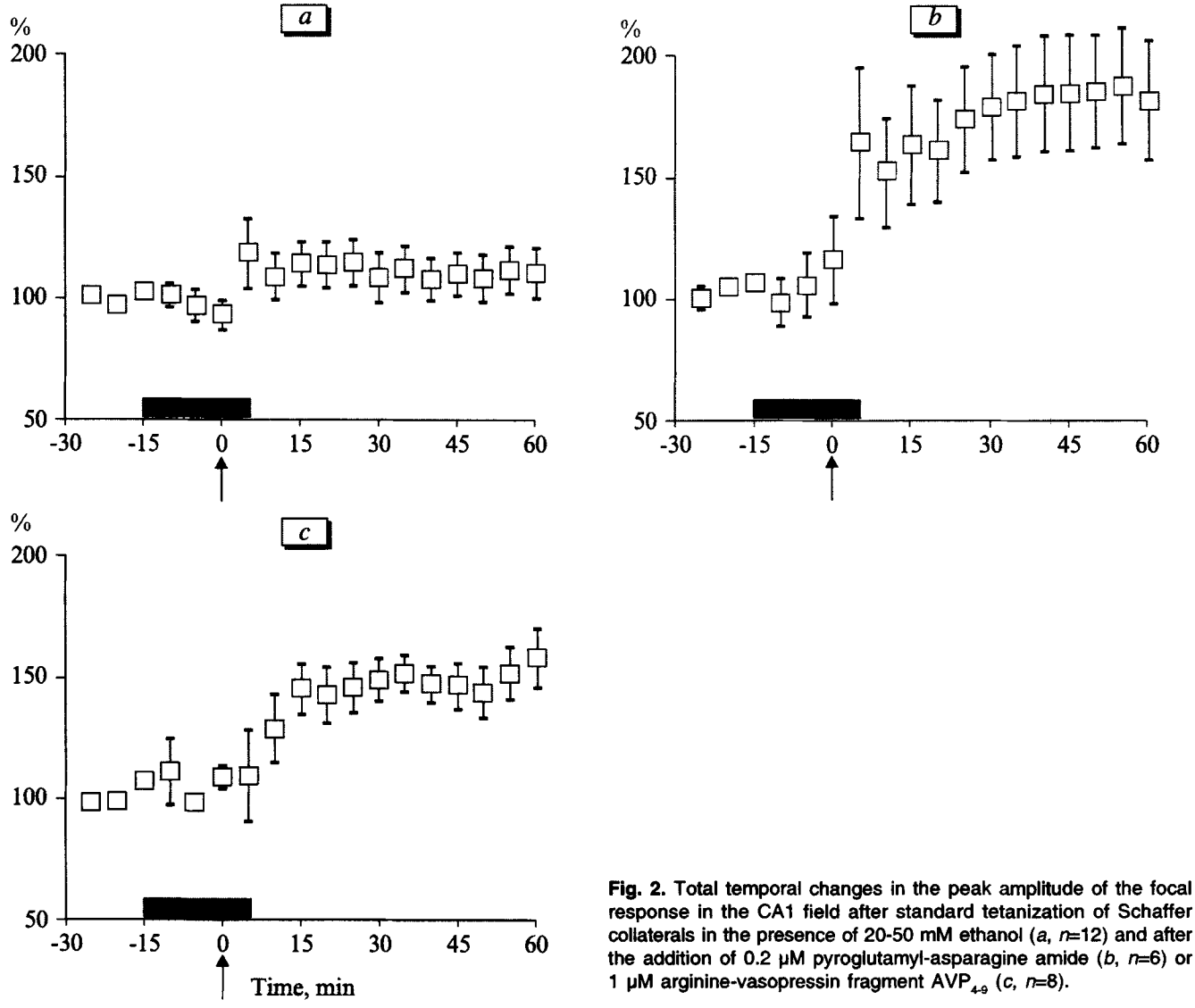

Fig. 2. Total temporal changes in the peak amplitude of the focal response in the CA1 field after standard tetanization of Schaffer collaterals in the presence of 20-50 mM ethanol (*a*, *n*=12) and after the addition of 0.2 μM pyroglutamyl-asparagine amide (*b*, *n*=6) or 1 μM arginine-vasopressin fragment AVP_{4-9} (*c*, *n*=8).

9.7% (*n*=12, Fig. 2, *a*). The amplitude of LTP in 3 slices did not differ from the control level (137.4± 10.2%, *n*=6).

The effect of short HFS was studied with PGA and AVP_{4-9} in concentrations of 0.2 and 1 μM, respectively. Published data show that peptides in these concentrations are most effective during LTP deficiency [5].

After standard HFS the mean amplitude of LTP in slices perfused with ethanol and PGA was 151.8±-10.3% ($p<0.05$, Fig. 2, *b*). Perfusion of slices with ethanol and 1 μM AVP_{4-9} increased the incidence (6 of 8 slices) and amplitude of LTP (211.0±19.7%, *n*=6, $p<0.01$, Fig. 2, *c*).

Our results show that both peptides normalized the development of LTP during perfusion with ethanol. PGA was potent in facilitating LTP induction. AVP_{4-9} significantly increased the amplitude of LTP.

The mechanisms underlying facilitation of LTP caused by PGA and vasopressin AVP_{4-9} fragment remain unknown. Induction of LTP in the synaptic system of Schaffer collaterals and CA1 pyramidal neurons depends on activation of NMDA glutamate receptors mediating Ca^{2+} entry into neurons and initiating intracellular events leading to considerable improvement of synaptic transmission [6]. The impairment of synaptic transmission with ethanol is primarily related to dysfunction of NMDA receptors [11,14]. The studied peptides facilitate or normalize the development of LTP by regulating functional activity of NMDA receptors or cascade reactions. This effect is realized via intracellular structures coupled with metabotropic peptide receptors. The vasopressin fragment AVP_{4-9} generates Ca^{2+} signals in hippocampal slices and activates the system of secondary messenger after the interaction with high-affinity specific receptors [12] and V_1 receptors for vasopressin [8,13]. PGA has stereoselective mnesic activity [3], which suggests that its effects are realized via receptor structures. Structural similarity of PGA and N-terminal part of AVP_{4-9}

suggests that PGA can act as a ligand of the specific receptor for AVP_{4-9}.

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