Comenic Acid Prevents Post-Stress Enhancement of Long-Term Potentiation in Rat Hippocampus

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In experiments on hippocampal slices from young rats subjected to immobilization-cold stress we observed a pronounced increase in the amplitude of long-term potentiation of focal responses in CA1 area. Daily injections of comenic acid during stress exposure normalized parameters of long-term potentiation in the hippocampus.

Key Words: *comenic acid; immobilization-cold stress; hippocampal slices; focal response; long-term potentiation*

Comenic (5-oxy-γ-pyrone-2-carboxylic) acid is the most efficient ingredient of Baliz-2 microbiological medicinal preparation, which is composed of a mixture of organic keto acids. Apart from antibacterial, antioxidant, and growth-stimulating action underlying the positive effects of Baliz-2 during treatment of wounds and ulcers, the spectrum of biological activity of this preparation also includes antistress action, which is characteristic of comenic acid [4].

Stress-factors modulate physiological and cognitive functions via activation of the hypothalamo-adrenocortical system and increase in the level of circulating corticosteroids. The chronic action of these factors induce the pathologic alterations in CNS and other physiological systems. One of the most stress-vulnerable structures in CNS is the hippocampus, which is characterized by high density of glucocorticoid receptors [9]. High sensitivity to the stress factors is also demonstrated by long-term potentiation (LTP) of hippocampal synaptic transmission [11,12], which is a manifestation of synaptic plasticity underlying adaptive processes and learning [8].

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For evaluation of the possible mechanisms of antistress action of comenic acid, we compared LTP values in hippocampal slices from rats exposed to immobilization and cold stress under control conditions and during administration of comenic acid.

MATERIALS AND METHODS

The study was carried out on 4 groups of male Wistar rats aging 6-8 weeks (n=41). The first (intact control) group (n=11) comprised rats not exposed to stress. The rats of the second (treatment control) group (n=8) were not subjected to stress, but received comenic acid dissolved in water (daily dose 1 mg) through a metal tube for 4 days. The concentration of 1 mg/ml is known to be optimal for producing the antistress effect [4]. The rats of the third and fourth groups were subjected to cold and immobilization stress for 4 days according to the routine regimen [4]: fasting with water ad libitum on days 1 and 3 and immobilization in a tight box at 4°C for 5 h. During stress exposure, the rats of the third (reference placebo) group (n=11) were daily injected with 1 ml distilled water through a tube, while the rats of the fourth (experimental) group (n=11) were given comenic acid (1 mg/ml).

On the next day, 17-19 h after the last stress exposure the rats were decapitated. Three or four transversal sections of the hippocampus were made and the

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specimens were placed into the experimental chamber to record the electrical activity. The slices were perfused with warm (28-29°C) physiological saline containing (in mM): 124 NaCl, 3 KCl, 2.5 $CaCl_2$, 2.5 $MgSO_4$, 1.25 Na_2HPO_4, 26.0 $NaHCO_3$, 10 D-glucose, aerated with 95% O_2+5% CO_2. Evoked potentials (focal responses) elicited in the pyramidal layer of CA1 hippocampal area were recorded using a glass pipette microelectrode filled with 1.5 M NaCl. Stimulation was performed with rectangular voltage pulses (0.1 msec, 1-50 V) delivered at the repetition rate of 0.1 Hz via bipolar glass electrodes filled with perfusion saline and placed into the radial layer of CA1 area. LTP of the peak component in the focal response (pop-spike) was induced by tetanization (100 Hz, 1 sec) via the same electrodes 2-4 h after preparing the slices. The amplitude of the pulses was equal to that inducing half-maximum pop-spike.

The dependence of pop-spike amplitude on stimulus strength, maximum pop-spike amplitude, and LTP value were determined in each slice. The latter parameter was measured 1 h after tetanization by the relative increment of the area under the stimulus-response curve (Fig. 1, *b*).

The data were recorded and processed with original software. They were analyzed statistically using Student's *t* test.

RESULTS

In hippocampal slices of control animals, tetanization of the synaptic input induced LTP of the pop-spike, whose value varied from 11.3 to 48.5%, the mean LTP being 29.6±4.5% (n=11). After tetanization, the amplitude increased, while the latent period decreased (Fig. 1, *a*). Moreover, after tetanization the same stimulus produced significantly greater averaged response. It is noteworthy that this difference was most pronounced at the stimulus strength of 20-30 V but decreased after increasing the stimulus strength to 40-50 V (Fig. 1, *b*).

In hippocampal slices from animals subjected to immobilization-cold stress (group 3), LTP more than 2-fold surpassed the control value (72.1±7.3%; n=11, p<0.001; Fig. 2, *3*). In animals treated with comenic acid LTP varied within the control range: 31.0±4.6% in intact rats (group 2, n=9; Fig. 2, *2*) and 21.4±2.3% in stressed rats (group 4, n=11, Fig. 2, *4*). Thus, stress exposure markedly increased LTP amplitude of focal response in the CA1 hippocampal area. Comenic acid had no effect on LTP under control conditions, but prevented stress-induced increase of LTP.

Comenic acid promoted the increase in responsiveness of hippocampal neurons. Analysis of the dependence of pop-spike amplitude (reflecting the number of the reacting neurons [7]) on stimulus strength showed that the maximum pop-spike amplitude reached 7.4±0.2 mV in hippocampal slices from non-stressed rats treated with comenic acid (Fig. 3, *2*), which significantly surpassed the control value (5.1±0.3 mV, p<0.001, Fig. 3, *1*). Similarly to other researchers [10, 13], we found no pronounced changes in pop-spike reactivity in animals exposed to stress. The maximum amplitude of pop-spike in hippocampal slices of rats subjected to stress was 4.9±0.2 mV (Fig. 3, *3*), which did not significantly differ from the control value of 5.1±0.3 mV. It is noteworthy that the maximum amplitude of hippocampal pop-spikes in animals recei-

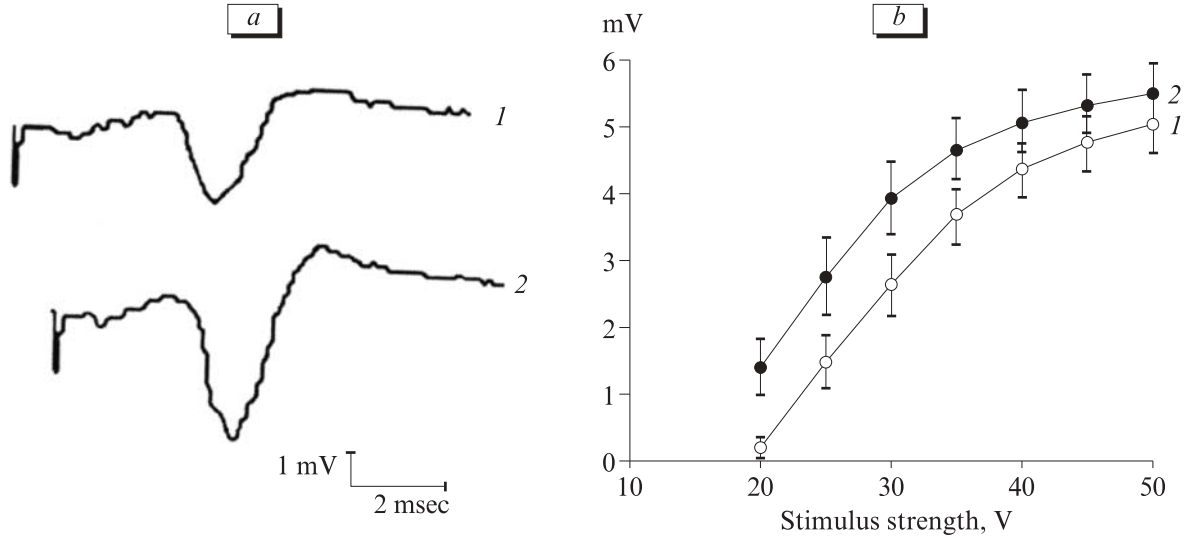

Fig. 1. Effect of high-frequency stimulation (tetanization) of synaptic inputs on long-term potentiation of the focal response in CA1 hippocampal area. *a*) focal responses before (*1*) and after (*2*) tetanization in a control experiment; *b*) averaged stimulus-response curves for pop-spike amplitude before (*1*) and after (*2*) tetanization in the control group.

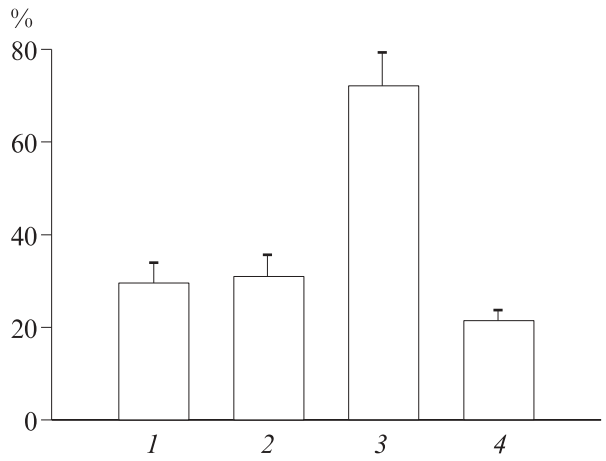

Fig. 2. Normalization of long-term potentiation by comenic acid in rats subjected to immobilization-cold stress. Ordinate: the mean value of long-term potentiation in percentage of control response amplitude. Here and in Fig. 3: *1*) control (saline); *2*) comenic acid; *3*) stress+saline; *4*) stress+comenic acid.

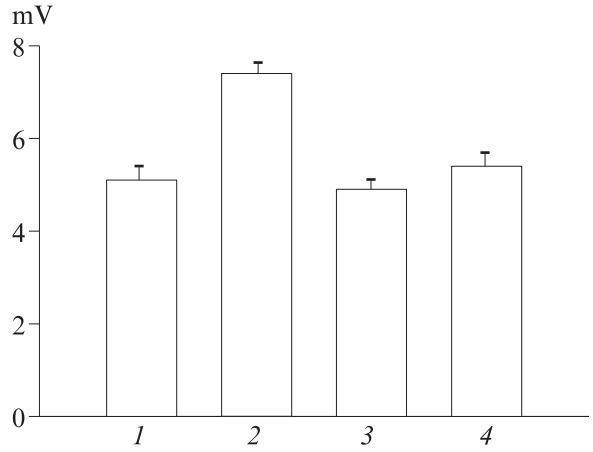

Fig. 3. Increase in responsiveness of hippocampal neurons induced by comenic acid in rats not subjected to immobilization-cold stress. Bars show maximum pop-spike amplitude.

ving comenic acid during stress (5.4±0.3 mV) also did not significantly differ from the control (Fig. 3, *4*).

Thus, administration of comenic acid augmented responsiveness of hippocampal neurons in rats not exposed to stress and normalized LTP in rats subjected to immobilization and cold stress. It is unlikely that stable increase in neuronal responsiveness in rats receiving comenic acid was determined by its direct action on hippocampal neurons, because application of this acid directly to hippocampal slices inhibited focal responses mediated by activation of $GABA_A$-receptors [3]. Considering various mechanisms of comenic action on reactivity of central neurons, it can be hypothesized that intraventricular administration of a chemical agent is a stress factor *per se*, which potentiates synaptic transmission in the hippocampus and produces a stable increase in responsiveness of hippocampal neurons in rats not exposed to more potent stress factors. It can be hypothesized that this weak stress stimulus triggers a mechanism attenuating the effects of more potent stress factors. As a result, comenic acid normalizes both responsiveness and LTP in the hippocampus of animals exposed to immobilization-cold stress. This explanation of the effects of comenic acid from the viewpoint of metaplasticity theory [6] widely used for explaining the effect of stress on functional parameters of hippocampal neurons [11,12] needs further experimental background.

The observed normalizing effect of comenic acid on plasticity of hippocampal neurons could be also induced by its antioxidant activity [5]. It is known that stress is accompanied by hyperproduction of free radicals, including reactive oxygen species. Since the primary LPO burst mobilizes the sympatho-adrenomedullar and hypothalamic-pituitary corticoadrenal systems, its suppression by an antioxidant agent should inhibit production of stress mediators (catecholamines and glucocorticoids [1,2]) thereby eliminating or moderating their effect on LTP.

Therefore, our study showed that comenic acid could modify responsiveness of hippocampal neurons and correct the stress-induced disturbances in hippocampal LTP. The mechanisms of comenic acid action need further studies.

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