Balysum-2 Inhibits Evoked Activity of the Pyramidal Neurons in Hippocampus

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The effects of Balysum-2 on the responsiveness of field CA1 hippocampal pyramidal neurons was studied in experiments with cultured cerebral slices. Addition to the medium of 10^{-4}M Balysum-2 or its active ingredient (comenic acid) led to a reversible decrease in the peak amplitude of focal response (pop-spike) recorded in the pyramidal layer CA1 during stimulation of the radial layer. Perfusion of hippocampal slices with a solution containing the noncompetitive $GABA_A$-antagonist picrotoxin prevented the effects of Balysum-2 and comenic acid. Inhibition of hippocampal pyramids by Balysum-2 and comenic acid is probably caused by an increase in the inhibitory effect mediated by $GABA_A$-receptors.

Key Words: *Balysum, comenic acid; hippocampal slices; $GABA_A$-receptors; picrotoxin*

The new preparation Balysum-2 has been widely used in medicine [6]. This nontoxic drug is an affective therapeutic tool against drug-resistant staphylococci in the treatment of numerous diseases [5]. Balysum exhibits antistressor, antioxidant, and the growth stimulation activities [3].

The mechanisms underlying the effects of Balysum are practically unknown. It is not clear whether the central nerve system structures are sensitive to Balysum or whether its activity is limited to peripheral effects. To answer this question we studied the effect of Balysum-2 on evoked activity of hippocampal neuron. Hippocampus is the central structure that controls emotional behavior and visceral reactions [2]; it can be the target for drugs that modulate these processes.

MATERIALS AND METHODS

Experiments were carried out on hippocampal slices obtained from 3-6 week male Wistar rats [3]. The perfusion medium was a modified Ringer's solution for warm-blooded animals (in mM): NaCl (124), KCl (3), $CaCl_2$ (2.5), $MgSO_4$ (2.5), Na_2HPO_4 (1.25), $NaHCO_3$ (26), D-glucose (10), aeration with carbogen (95% O_2+5% CO_2), pH=7.25. Perfusion rate was 2.5-3 ml/min.

Electrical activity was recorded 1.5-2 h after preparation of slices. Focal potentials arising in response to stimulation of the radial layer were recorded in the pyramidal layer CA1 with the help of a glass microelectrode filled with 1.5 M NaCl. Stimulation was performed with single 0.1 msec rectangular pulses. The amplitude was chosen so that to adjust the amplitude of the peak component of the response (pop-spike, PS) to 30-50% of its maximum value. Drug effect was evaluated by a change in PS amplitude corresponding to responses of the pyramidal cell population to activation of the synaptic input.

Test substances were applied by switching the flow system for 15 minutes to the reservoir with 10^{-6}-10^{-4} M Balysum-2 or comenic acid. Picrotoxin (Sigma) was used to analyze the possible mechanisms of action of the preparations. The compound was dissolved in 0.1 N HCl and diluted with perfusion medium to the necessary concentration.

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RESULTS

At 10^{-6} M and 10^{-5} M, Balysum-2 produced no appreciable effect on the amplitude of evoked response or slightly inhibited PS in some preparations. In a concentration of 10^{-4} M Balysum-2 decreased PS amplitude in all hippocampal slices (n=30). This effect was observed 8-12 min after the start of infusion (Fig. 1) and in some cases (7 experiments) it was increased during rinsing of the preparation. The evoked responses were inhibited by 35±3% (n=30).

In most cases the effect of Balysum-2 was completely reversible: rinsing of the preparation resulted in PS amplitude restoration up the control level within 15-25 min. Only in 4 slices restoration was partial 30 min after rinsing, the PS amplitude being 90% of the control.

To reveal the active ingredient of Balysum-2, which is a mixture of organic ketonic acids [5], the same experimental model was used to study the effect of comenic acid, which is the most active component of the mixture. Comenic acid in a concentration of 10^{-4} M proved to produce a similar inhibition of the evoked response in hippocampal slices (25±3%).

PS amplitude corresponds to the number of neurons involved in the stimulus-evoked response

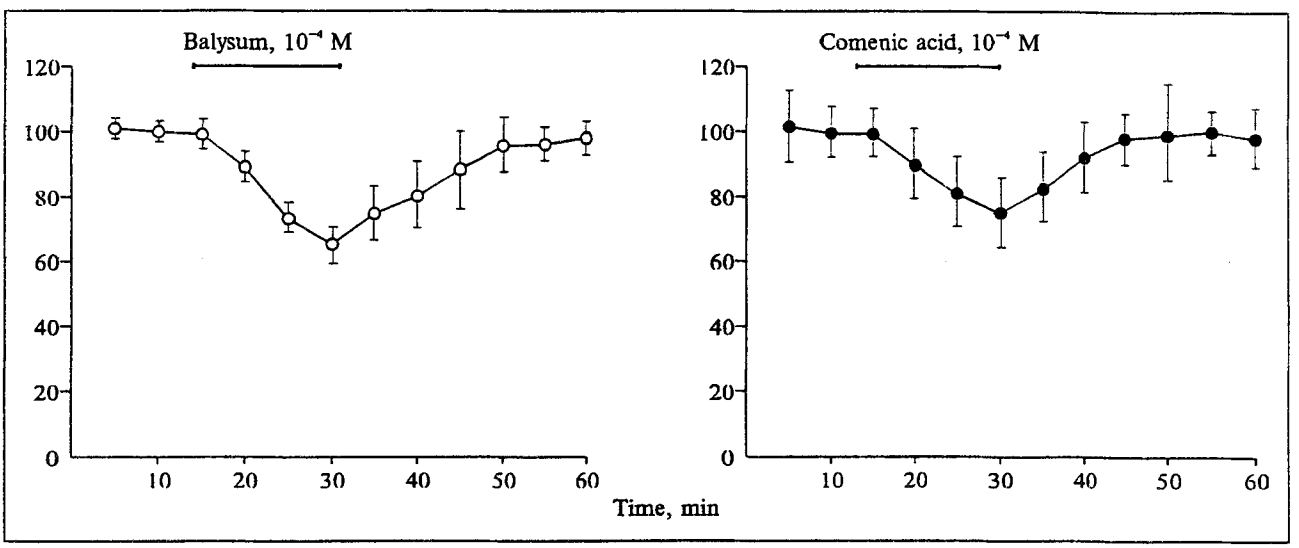

Fig. 1. Inhibition of evoked activity in hippocampal field CA1 neurons by Balysum or comenic acid. Here and in Fig. 2: ordinate, changes in the focal response (pop-spike) amplitude as percentage of initial mean amplitude of the response. Horizontal bars indicate drug infusion.

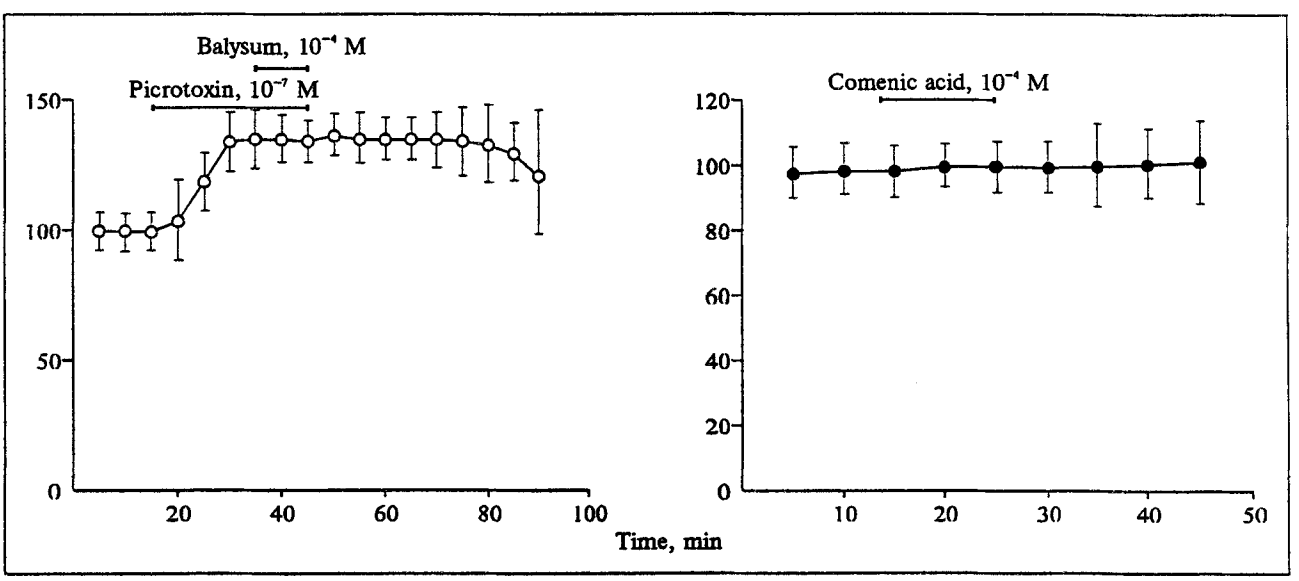

Fig. 2. $GABA_A$-antagonists picrotoxin prevents the effects of Balysum and comenic acid. In experiments with comenic acid, picrotoxin was added to the perfusion medium 30-40 min prior to the drug.

[6,7], and its decrease indicates attenuation of hippocampal pyramids responsiveness, which can be caused by augmentation of inhibitory influences. A similar inhibition of PS amplitude is produced by the substances that stimulate the interaction of gamma aminobutyric acid (GABA) with GAB_A-receptors, i.e., barbiturates and benzodiazepines [1].

To assess the involvement of $GABA_A$-receptors in the effects of Balysum-2 and comenic acid, both drugs were studied on sliced pretreated with picrotoxin, a non-competitive antagonist of $GABA_A$ receptors.

Picrotoxin in a concentration of 10^{-6} M increased PS amplitude by 33-41%. Against this background, Balysum-2 in a previously effective concentration of 10^{-4} M did not inhibit focal responses (Fig. 2). Comenic acid was also ineffective in picrotoxin-pretreated preparations (Fig. 2).

The lack of PS changes by infusion of Balysum-2 or its active ingredient under conditions of blocked the $GABA_A$-receptor-channel complex suggests that the effect of these substances on the responsiveness of hippocampal pyramids can be mediated by GABAergic system. It is noteworthy that comenic acid has a similar structure as the kojic acid, whose derivative is a $GABA_A$-agonist [8]. Comenic acid may directly interact with the $GABA_A$-receptor-channel complex. Analysis of the relationships between the examined substances and GABAergic processes requires further investigation.

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