PHARMACOLOGY AND TOXICOLOGY

Effect of Nitric Oxide and Cyclic Guanosine Monophosphate on the Desensitization of Choline Receptors in Snail Neurons

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Desensitization of choline receptors is studied after pharmacological effects on the intracellular level of nitric oxide and cyclic guanosine monophosphate in snail neurons. It is shown that inhibitors of NO synthase do not alter desensitization, whereas an activator of guanylate cyclase as well as intracellular injection of guanosine monophosphate boost it.

Key Words: *nitric oxide; cyclic 3',5'-guanosine monophosphate, desensitization of choline receptors; snail neurons*

The recently discovered second messenger nitric oxide (NO) [4,5] is an intermediate link between the transmitter receptor and soluble guanylate cyclase. Binding to the heme group of the enzyme, NO activates it and stimulates the synthesis of cyclic 3',5'-guanosine monophosphate (cGMP) [4,5].

As was previously shown by us, cGMP is involved in desensitization of the choline receptors of the identified RPa3 and LPa3 neurons of the snail when sodium nitroprusside, an activator of guanilate cyclase, is used [1]. Stimulation of soluble guanylate cyclase by endogenous NO points to its role in the activation of cGMP synthesis in neurons and its involvement in the regulation of choline receptor desensitization. In this connection this study was devoted to an investigation of the influence of NO synthase inhibitors, cGMP, and guanylate cyclase modulators on desensitization of choline receptors of snail neurons.

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MATERIALS AND METHODS

Experiments were carried out on the identified RPa3 and LPa3 neurons of the edible snail (*Helix lucorum*) in a preparation of isolated ganglia. Transmembrane ion currents were recorded using the technique of the double-electrode voltage-clamp on the membrane.

Desensitization of the choline receptors was assessed according to the suppression of the amplitude of the acetylcholine (AC)-induced inward current (AC current) during a series of 11-13 local rhythmic AC applications. To lower the amplitude of the AC current the first 10 stimuli were applied at a constant interval, the duration of which was 45-240 sec in series on different neurons. The next 1-3 stimuli were delivered at 10-min intervals for estimation of the extent and rate of restoration of the diminished reaction.

The following substances were used: inhibitors of NO synthase Nω-methyl-L-arginine acetate (L-NMMA, Fluka), the hydrochloride of the methyl ether of N-nitro-L-arginine (L-NAME, Sigma); the NO donor S-nitroso-N-acetylpenicillamine (SNAP, RBI); the inhibitor of soluble guanylate cyclase LY-

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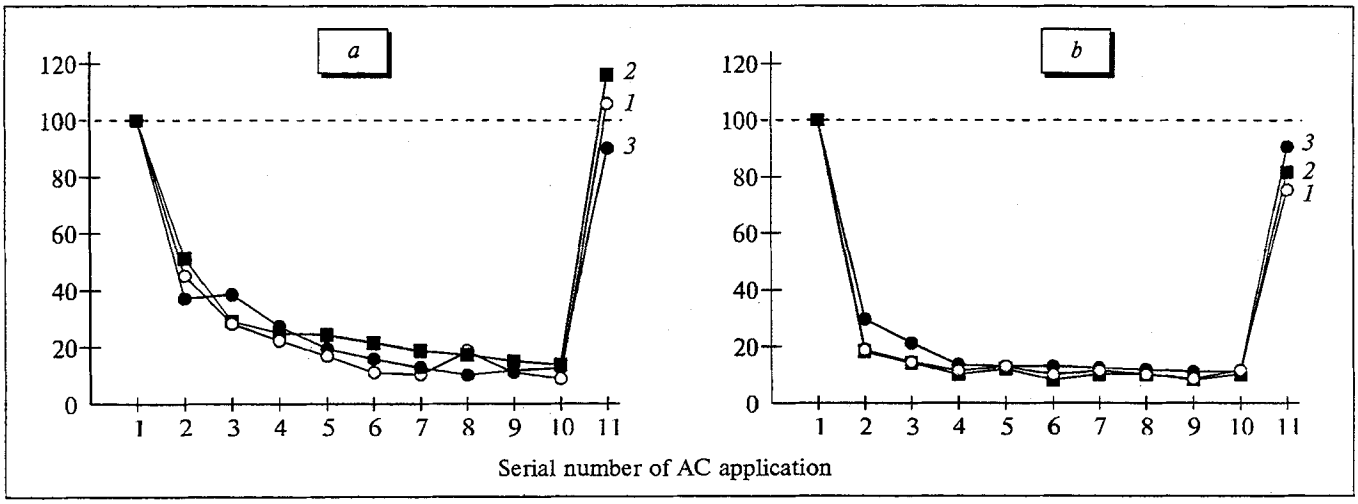

Fig. 1. Effect of L-NMMA (*a*) and L-NAME (*b*) on the frequency depression of the AC current in identified RPa3 and LPa3 neurons, respectively. *1*) frequency depression prior to pharmacological treatment; *2*) after extracellular L-NMMA application (500 μM, 20 min, *a*), L-NAME (200 μM, 30 min, *b*); *3*) after washing of preparation in Ringer solution for 20 min. Here and in Figs. 2 and 3: the ordinate is maximal amplitude of AC current expressed in percentage in relation to its level in response to the first stimulus in the series.

83,583 (RBI); the monosodium salt of guanosine-3',5'-cyclophosphate (cGMP, Fluka); and a cGMP analog which crosses the cell membrane, the sodium salt of 8-bromine-cGMP (8-Br-cGMP, RBI).

All substances apart from cGMP were applied directly to the perfusion chamber with a microsyringe in volumes of 1-5 μl. cGMP was ionophoretically injected into the neuron through a third intracellular microelectrode filled with an aqueous solution of cGMP (100 mM; resistance more than 200 MOhms) by passing a negative current (more than 300 nA in amplitude) during 10-50 min.

L-NMMA, L-NAME, cGMP, and 8-Br-cGMP were dissolved in water, LY-83,583 in ethanol or dimethyl sulfoxide (Sigma), and SNAP in dimethyl sulfoxide. The final concentration of ethanol and dimethyl sulfoxide did not exceed 0.5% in the perfusion chamber.

Reliability of the effects was estimated according to the nonparametric Wilcoxon tes and sign test. Statistical treatment of the results was performed with a PC using designated STADIA software.

Results were obtained on 68 neurons (31 RPa3 and 37 LPa3) in 68 preparations of ganglia. The membrane potential of the cells after microelectrode puncture ranged from -33 to -85 mV (-57.3±1.5 mV).

RESULTS

Inhibitors of NO synthase L-NMMA (100-500 μM) and L-NAME (200-300 μM) did not alter the ki-

TABLE 1. Influence of Modulators of Second Messengers NO and cGMP on the Depth of AC Current Depression in RPa3 and LPa3 Neurons Induced by Rhythmic Delivery of AC to Soma

Compound (concentration, μM)	Number of neurons studied			
	total	weakening of depression	strengthening of depression	lack of effect
L-NMMA (100-500)	13	3	4	6
L-NAME (200-300)	9	2	1	6
SNAP (50-500)	12	0	11** (-18.6±2.8)[1]	1
LY-83,583 (3-6)	6	2	3	1
LY-83,583 (20-40)	10	1	9* (-19.8±3.7)	0
cGMP[2]	11	0	11** (-18.1±3.3)	0
8-Br-cGMP (60-500)	8	1	2	5

Note. [1]The extent of AC current change (%) after pharmacological treatment. [2]Intracellular injection of the compound. *$p<0.01$; **$p<0.001$.

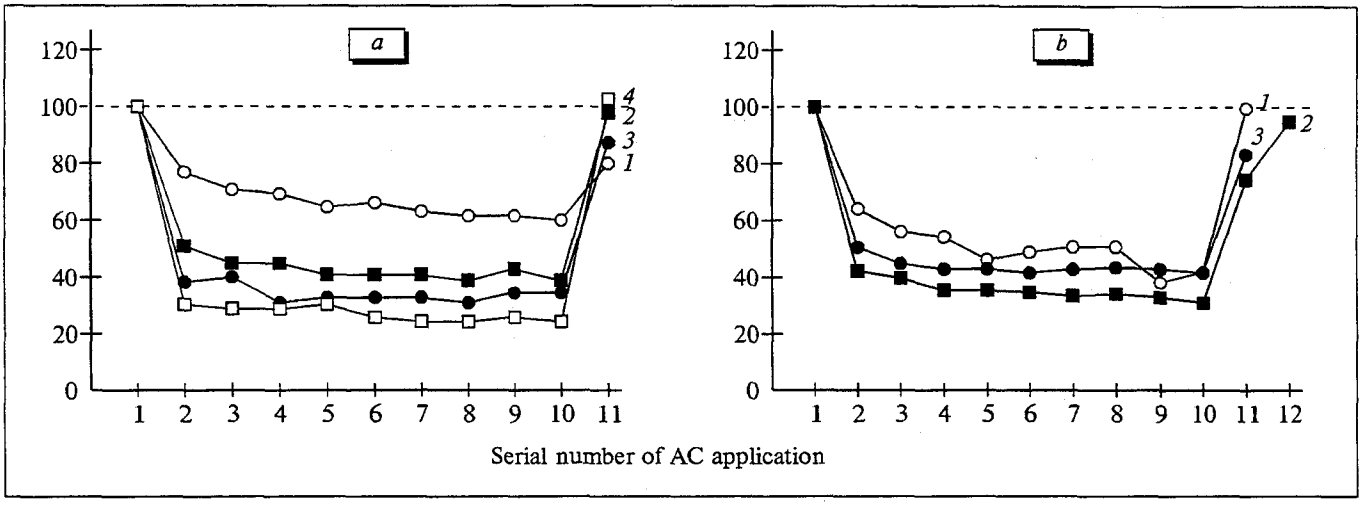

Fig. 2. Effect of SNAP (*a*) and LY-83,583 (*b*) on the frequency depression of the AC current in identified LPa3 neurons. *1*) frequency depression prior to pharmacological treatment; *2*) after extracellular SNAP application (150 μM, 20 min, *a*), LY-83,583 (20 μM, 30 min, *b*); *3*, *4*) after washing of preparation in Ringer solution for 20 min (*3*) and 40 min (*4*).

netics of the AC current depression (Table 1, Fig. 1). The NO donor SNAP (50-500 μM) increased this depression (Table 1, Fig. 2, *a*). The effect was irreversible. Sometimes the effect of the preparation was enhanced after washing (Fig. 2, *a*).

It was expected that the effect of LY-83,583 as an inhibitor of soluble guanylate cyclase would be opposite to that of SNAP, which activated of this enzyme through NO. However, in concentrations of 20-40 μM LY-83,583 acted on the AC current depression similarly as did SNAP — increasing it (Table 1, Fig. 2, *b*). Although the concentrations used were those recommended by the manufacturer, hypersensitivity of *Helix lucorum* neurons to this inhibitor could not be ruled out. Because of this, lower concentrations (3-6 μM) were examined. In this case LY-83,583 did not affect the depression of the AC current (Table 1).

An intracellular injection of cGMP (10-50 min) significantly increased the AC current depression (Table 1, Fig. 3, *a*). The cell-penetrating analog of cGMP, 8-Br-cGMP, did not have an influence on the depression (Table 1, Fig. 3, *b*) in a wide range of concentrations (60-500 μM).

The effects of these compounds on the degree of inhibition of the AC current are not associated with the influence of these substances on the AC current amplitude prior to the series of rhythmic AC applications, but are determined by modulation of the corresponding enzymatic systems in the neuron. Thus, the substances which did not affect the AC current depression either did not affect its amplitude:

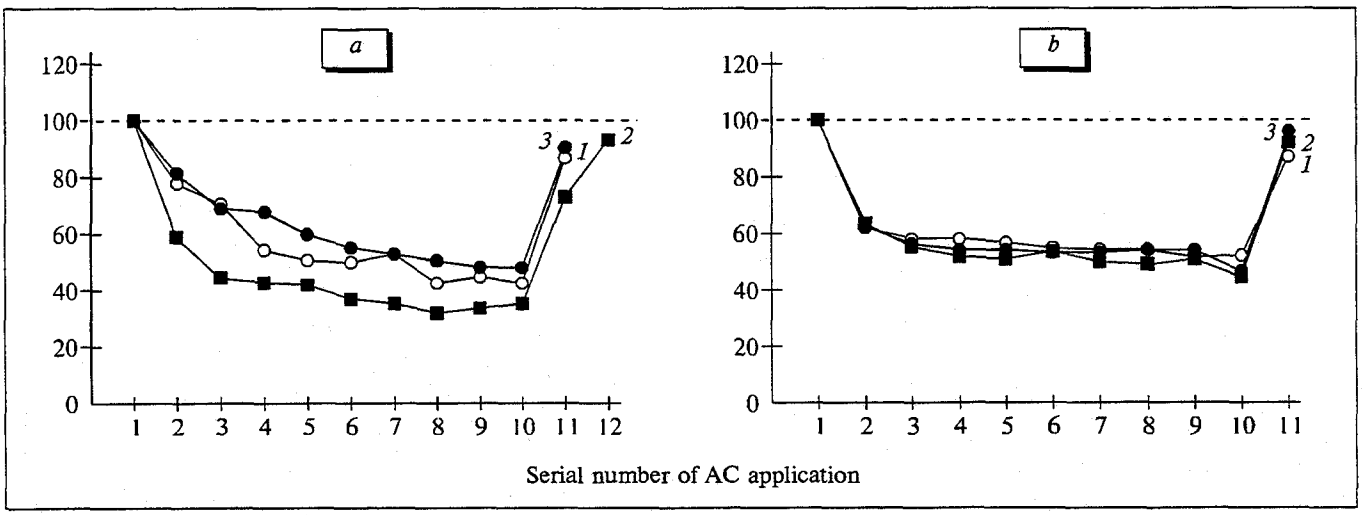

Fig. 3. Effect of cGMP (*a*) and 8-Br-cGMP (*b*) on the frequency depression of the AC current in identified LPa3 neurons. *1*) frequency depression prior to pharmacological treatment; *2*) 2 min after the end of the intracellular cGMP injection (45 min, *a*), after extracellular 8-Br-cGMP application (500 μM, 20 min, *b*); *3*) 50 min after the completion of intracellular cGMP injection (*a*); after washing of preparation with Ringer solution for 20 min (*b*).

L-NMMA (n=13) and L-NAME (n=9), or inhibited it: 8-Br-cGMP (by 21.8±2.7%; n=8, p<0.01). The compounds which increased the AC current depression either did not influence the AC current amplitude: cGMP (n=11) or suppressed it, as follows: SNAP (by 36.6±4.7%; n=9, p<0.01) and LY-83,583 (by 40.1±6.8%, n=10, p<0.01).

This study does not allow us to draw conclusions on the involvement of endogenous NO in the desensitization of choline receptors of RPa3 and LPa3 snail neurons. The results of a histochemical study [7] concerning the prevalence of NO-synthesizing activity in nerve fibers in comparison with neurons in snail ganglia agree well with our data.

The results regarding the involvement of cGMP in the plasticity of RPa3 and LPa3 cholinergic neurons are ambiguous. On the one hand, the augmentation of the desensitization of choline receptors by the NO donors sodium nitroprusside [1] and SNAP as well as after intracellular cGMP injection testifies that cGMP participates in choline receptor desensitization. On the other hand, the fact that desensitization is enhanced by the inhibitor of soluble guanylate cyclase LY-83,583 and the absence of an effect of 8-Br-cGMPt cast some doubt on such involvement of endogenous cGMP.

This contradiction can be explained. In a culture of endothelial cells the compound LY-83,583 boosts the formation of the oxygenated radicals superoxide/peroxide of hydrogen O_2^-/H_2O_2 [8], which may stimulate soluble guanylate cyclase and raise the cGMP level [5]. Consequently, the same compound (LY-83,583) can inhibit or activate soluble guanylate cyclase via different mechanisms, thereby diversely affecting the cGMP level in the cell. It may be assumed that LY-83,583 acts as an activator of guanylate cyclase, increasing the cGMP content in RPa3 and LPa3 snail neurons, which accounts for the greater choline receptor desensitization and additionally confirms the involvement of cGMP in the development of desensitization.

The results reported here attest to the lack of an effect of NO synthase inhibitors on the desensitization of choline receptors; moreover, the weak efficacy of the NO-synthesizing enzyme in snail neurons does not permit us to consider the NO—cGMP system as an effective component in the intracellular regulatory mechanism of choline receptor desensitization. It is known that arachidonic acid and its derivatives stimulate cGMP formation via the synthesis of soluble guanylate cyclase [6]. Endogenous lipoxygenase eicosanoids also make a significant contribution to the desensitization of choline receptors [2]. It may be assumed that stimulation of the choline receptors raises the cGMP level in neurons through the acyclic eicosanoids.

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