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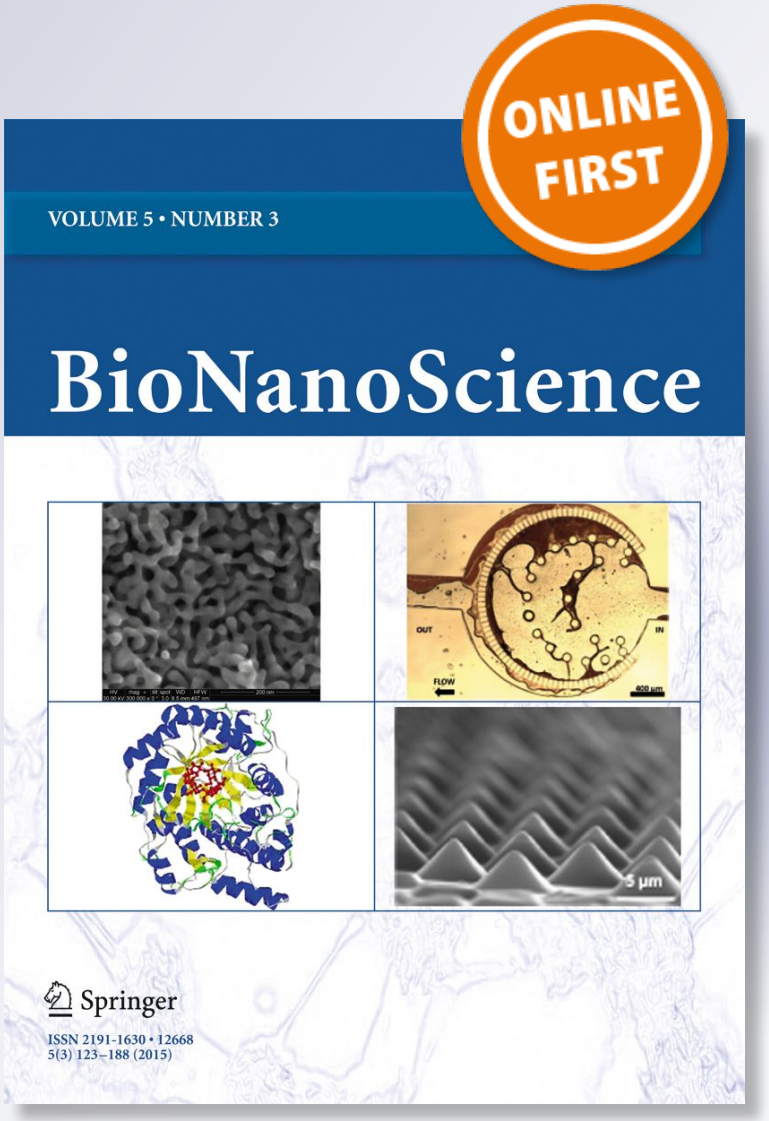

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Influence of Nonspecific Inhibitor of NO-Synthase L-NAME on Electric Characteristics of Premotor Interneurons of Terrestrial Snails

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Abstract
It has been found that the application of nonspecific inhibitor of NO-synthase L-NAME caused the depolarization shift of the membrane potential of premotor interneurons of defensive behavior of terrestrial snails. This effect is opposite to hyperpolarization shift of the membrane potential caused by the action of the nitric oxide donor—sodium nitroprusside.

Keywords Nitric oxide · L-NAME · Identified neurons · Membrane potentials · Snail

1 Introduction

The system of nitric oxide (NO) is one of the most studied systems of the body. However, despite today has accumulated a huge amount of data on the signaling targets of NO, a clear opinion on this matter is missing. NO is intra- and intercellular mediator that performs various signal functions; it is a molecule synthesized in response to physiological need in the cell from L-arginine with the participation of NO-synthase (NOS), activated by increased Ca^{2+} ions [1]. The effects of NO are associated with its action on ion channels, neurotransmitter secretion, calcium ion exchange, the cell's metabolism, and its genome. It is shown that NO plays a role as intercellular messenger and signaling molecule also in mollusks [2]. It is discovered that NO coordinates a number of behavioral programs in mollusks [3, 4]; it is found that NO is involved in the processes of learning and memory [5–8]. NO also controls the plastic properties of neurons: an inhibitor of NOS contributed to the development of habituation, and the NO donors caused the effect of sensitization [9]. It was shown the participation of NO in the plastic changes the synaptic transmission in various systems, including the nervous system of *Helix* [2, 10, 11]. In experiments on preparations of *Helix*, it was shown that NO donors increased the frequency of spikes and reduced the latency of spikes in the identified neurons [12] and that NO is released by two neurons, the cerebral giant cell (CGC) and the B2 buccal motor neuron in the isolated nervous system of the pond snail [13]. Thus, B2 has released more NO than the CGC neuron, but both cells were equally suppressed by the NOS inhibitor L-NAME. Earlier, we found that the NO donor caused the shift of membrane potential of premotor interneurons of defensive behavior of terrestrial snail [14]. Therefore, the aim of this work was to study effects of nonspecific NOS inhibitor L-NAME on the electrical characteristics of premotor interneurons of snail.

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2 Methods

The terrestrial snails *Helix lucorum*, the nervous system of which is well described, were used for the experiments. Before the experiments, the mollusks were in the active state for at least 2 weeks [15]. Analysis of electrical characteristics was carried out in the readily identifiable giant premotor interneurons LPa3 and RPa3 of the withdrawal reflex located in the rostral part of parietal ganglia (description and map in Balaban, 2002 [16]). The isolated nervous system was placed in a saline solution (SS) of the following composition: NaCl 80 mM, KCl 4 mM, $CaCl_2$ 10 mM, $MgCl_2$ 6 mM, $NaHCO_3$

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5 mM (or Tris 5 mM); pH 7.6–7.8. Measurements were carried out at room temperature (18–21 °C) using intracellular glass microelectrodes filled with 2.5 M KCl and having a resistance of 10–30 MΩ. Since the premotor giant interneurons LPa3 and RPa3 are normally silent, a one-electrode method of cell stimulation was used. To trigger the action potential (AP) in an isolated preparation through a recording microelectrode, a current pulse of rectangular shape lasting 1 s was applied to the cell using a bridge circuit for compensation of the constant current. A minimum current required to generate 3–5 APs was used. The following parameters of nerve cells were recorded: membrane resting potential—Vm (initial value before the beginning of each electrical stimulation) and a threshold of generation of AP—Vt (threshold potential). The threshold potential was scored as the difference between the membrane resting potential and the potential value during action potential generation at which its rate of increase (the first derivative of the potential with respect to time) reached a certain value of 1 V/s (proven by B.I. Khodorov, 1974 [17] and described in details in Andrianov et al., 2015 [18]). On the bases of the results of these measurements, the critical depolarization level (Ec) was calculated as the difference between Vm and Vt.

The effects of the application (during 30 min) of NOS inhibitor L-NAME (L-NG-nitro L-arginine methyl ester, Sigma, USA) (at a concentration of 10^{-4} mol/l [11, 19]), into the solution bathing the preparation of intact snails, on the membrane potential (Vm) of the premotor interneurons were studied.

The results are shown as mean ± SEM. The unpaired Student's *t* test and nonparametric Mann–Whitney test were used for comparison between two groups.

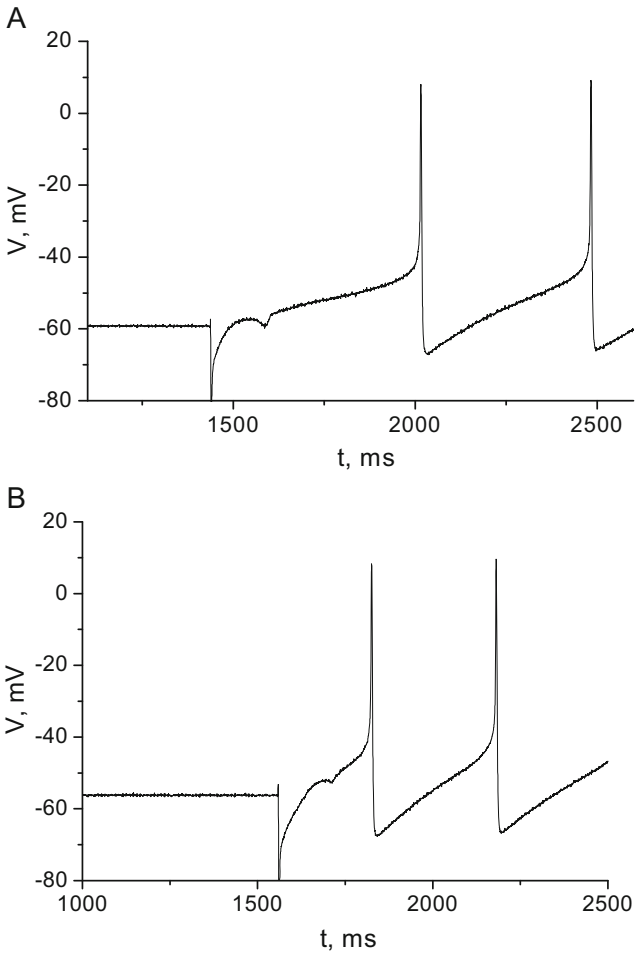

Fig. 1 The action potentials of premotor interneuron RPa3 before (**a**) and 30 min after application of L-NAME (**b**). Vertical axis shows value of potential, in mV; horizontal axis shows time, in ms

3 Results and Discussion

The measurements of electrical characteristics were carried out on premotor interneurons of defensive behavior of snail LPa3 and RPa3 [15, 16]. In the experiments, no significant differences in the values of Vm and Vt of premotor interneurons LPa3 and RPa3 were found. These results are similar to what were obtained by us earlier [20]. Figure 1 shows examples of the AP caused by the application of stimulating current in the neuron RPa3 before and 30 min after application of L-NAME. There is a depolarization shift of the resting membrane potential, which was later statistically processed. The changes of the threshold potential were measured by processing the records of AP. In the experiments, it was found that the application of NOS inhibitor L-NAME at a concentration of 10^{-4} mol/l into the solution bathing the preparation of the intact snails caused the reliable decrease of the membrane potential of the premotor interneurons from -60.2 ± 0.8 mV to -55.4 ± 1.7 mV, $n = 5$ (Fig. 2). The reliable difference from the control snails is $p < 0.05$. There are no observed changes in the threshold of the action potential. The changes of Vm and Vt are reflected in dynamics of Ec.

Thus, we have demonstrated that in certain neurons, the inhibition of synthesis of NO (i.e., reducing its quantities) may cause a depolarization of the membrane. This did not contradict the previously shown data that the increase in excitability of neurons in snails was dependent from NO in which it states that NO increased excitability by inhibiting calcium-activated K-current in neurons of the snail neurons [12]. In studying the effect of NO on Ca^{2+}-dependent potassium current in U-cells of the right parietal ganglion of *Helix pomatia* [21], it was found that NO reduced this current by activation of guanylate cyclase and subsequent activation of phosphodiesterase 2. The data available in the literature show that NO can both effectively increase and decrease neuronal excitability [10–12, 14, 22]; we associate the opposite effects of NO with different types of neurons or synapses. The depolarization shift of membrane potential in premotor interneurons under the action of inhibitor of NOS L-NAME allows to

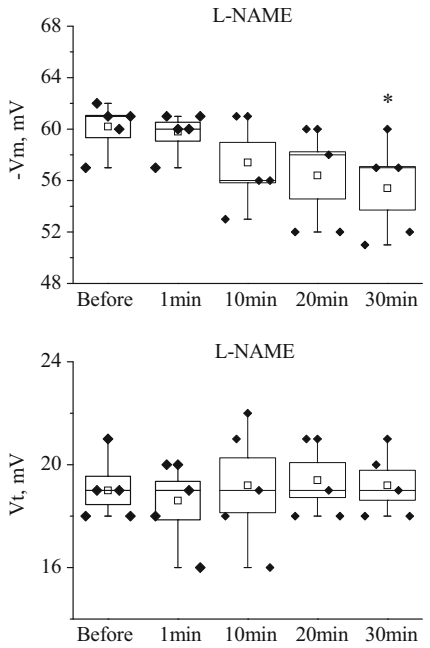

Fig. 2 Effect of bath application of NOS inhibitor L-NAME at a concentration of 10^{-4} mol/l on the membrane potential (Vm, $n = 5$), the threshold potential (Vt, $n = 5$), of the premotor interneurons of the snail's defensive behavior. On the chart, median and individual measurements are shown. Vertical axis shows value of potential, in mV. Asterisks (*) indicate significant difference ($p < 0.05$, independent t test)

assume that one of the possible mechanisms of decreasing of membrane potential of neurons, which we observed during the learning [18, 23], is the decrease of NO production. Previously [14], we analyzed the effect of the NO donor application on the electrical characteristics of the identified neurons and obtained a hyperpolarization effect, which was opposite to results obtained in the present work. This makes a suggestion about correlation between the level of NO in the neuron and its membrane potential.

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