Effects of Exogenous Nitric Oxide on Neurotransmitter Secretion and Ionic Currents in Motor Terminals

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Extracellular recording was used to study the effect of sodium nitroprusside, a donor of NO, on endplate transmitter release and ionic currents in frog cutaneous pectoris muscle. Exogenous NO inhibits induced transmitter secretion, and this effect is antagonized by extracellular Ca^{2+}. Exogenous NO increases potential-dependent outward potassium current and inhibits Ca^{2+}-activated potassium current in the motor nerve terminals.

Key Words: *synapse; nitric oxide; nerve terminal ionic currents; transmitter secretion*

Nitric oxide (NO) is a key regulator of various physiological processes. Of particular importance is the role of NO in nerve cells and synapses in the central and peripheral nervous systems. NO acts as a second messenger of intracellular signaling, transmitter in cell-cell signal transmission, activity modulator in nerve cell neurotransmitter systems, *etc.* [5,6,8,12,14]. One of the methods to reveal the effects of NO and decipher the mechanism of its action is to increase the level of exogenous NO by using NO donors, *i.e.* the substances releasing NO in aqueous solutions [10]. Experiments with sodium nitroprusside (SN) as the NO donor showed that exogenous NO decreases the amplitudes of endplate potentials (EPP) evoked by motor nerve stimulation and frequency of miniature endplate potentials (MEPP) in frog neuromuscular synapse, without affecting their amplitude and dependence on membrane potential. These data suggest that exogenous NO has a presynaptic effect that moderates quantal secretion of the transmitter from motor terminals without modifying the function of chemosensitive ionic channels in the postsynaptic membrane [4,10]. However, the mechanisms of the presynaptic effects of NO are poorly understood.

Our aim was to study the effects of exogenous NO on transmitter secretion and ionic currents in frog motor nerve terminals.

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MATERIALS AND METHODS

Experiments were carried out on isolated neuromuscular preparations of cutaneous pectoris muscle of *Rana ridibunda* at room temperature. In most experiments we used a low-calcium physiological solution (pH 7.2-7.4) containing (in mM): 115 NaCl, 2.5 KCl 2.4 $NaHCO_3$, 0.2-0.4 $CaCl_2$, and 2 $MgCl_2$. Some experiments were carried out with a standard solution (in mM): 115 NaCl, 2.5 KCl, 2.4 $NaHCO_3$, and 1.8 $CaCl_2$. Blockade of action potentials and muscle contraction, as well as attenuation of endplate ionic currents were performed by adding of tubocurarine (0.02 mM) into the perfusion solution. The evoked responses of nerve terminals, EPP, and MEPP were recorded with extracellular glass microelectrodes (tip diameter 2-5 μ) filled with 1 M NaCl [1-4]. Quantal composition of the endplate currents was calculated by falling (probability) analysis [9]: $m=\ln n/n_0$, where n is the number of stimuli and n_0 is the number of realizations.

The motor nerve was stimulated by suprathreshold stimuli at a rate of 1 pulse per second.

The synaptic signals were recorded with the help of an L-1230 digitizer incorporated in a PC. The data were analyzed using Origin and Excel software.

RESULTS

In nerve-muscle preparations placed in low-Ca^{2+}/high-Mg^{2+} solution, SN (100 μM) rapidly and significantly

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decreased the amplitudes of the averaged extracellularly recorded EPP without pronounced changes in MEPP amplitude. As soon as after 10 min, the amplitude of endplate currents decreased to 34.03±0.16%, and after 50 min it dropped to 17.11±0.03% of the initial value (n=12, p<0.05, Fig. 1). The rate of the inhibitory effect depended on SN concentration. The effect of SN was poorly reversible: 20-30-min perfusion of the preparation with initial solution did not restore EPP amplitude. Attenuation of EPP amplitude under the action of SN was associated with the decrease in the quantal content of EPP, which dropped to 45.6±5.3% and 11.26±1.1% of initial level during 10 and 50 min, respectively.

The inhibitory effect of SN on the amplitude and quantal composition of EPP was not observed in hemoglobin-containing solutions (10^{-5} μM, Fig. 1).

To study the effect of SN on evoked neurotransmitter secretion at normal concentration of Ca^{2+} (1.8 mM), the action potentials in muscle fibers were blocked by tubocurarine. Under these conditions, SN (100 μM) only little reduced the amplitude of EPP: to 89.4±10.6% and 86.8±15.03% of the initial value after 15- and 50-min application, respectively (n=7, p<0.05, Fig. 1).

SN inhibited both evoked and spontaneous neurotransmitter secretion and progressively decreased the frequency of MEPP (to 22.7±5.3% of the initial value after 40 min application, n=9, p<0.05).

In addition to the decrease in the amplitude and quantal content of EPP in low-Ca^{2+} solution (0.2-0.4 μM), SN considerably transformed the shape of evoked response of nerve terminal recorded extracellularly (Fig. 2). In the proximal segments of frog nerve terminals this response had a characteristic triphasic shape [2]. Numerous studies with specific ionic channel blockers showed that the negative high-amplitude phase II of the response reflects inward Na^+ currents, while the positive phase III is produced by outward K^+ currents through the membrane of nerve terminals [1-3]. SN gradually increased the amplitude of phase III to 201±0.92% and 275±1.13% after 15 and 60 min, respectively (n=12, p<0.05 compared to the initial value).

In a high-Ca^{2+} medium (1.8 mM), SN produced a less pronounced increase in the amplitude of phase III: to 124.85±11.8% and 111.42±12.5% after 15 and 50 min, respectively (Fig. 2). The temporal and amplitude parameters of phases I and II were not affected by SN (Fig. 2), which indicates that SN modifies only K^+ currents in nerve terminals.

The outward potassium currents in nerve terminals are predominantly mediated through potential-dependent and Ca^{2+}-activated K^+ channels [1,3]. To identify the type of potassium channels involved in SN effect, we used 4-aminopyridine (0.1 mM), a specific blocker of potential-dependent K^+ channels [13]. Perfusion of the nerve-muscle preparation with 4-aminopyridine (Ca^{2+} concentration in the medium was 0.3 mM) led to a minor increase in phase III of nerve terminal response, enhancement of neurotransmitter secretion, and to appearance of the repeated activity of nerve terminals (Fig. 2). All these effects are characteristic of the blockage of voltage-dependent K^+ channels resulting in prolongation of the repolarization

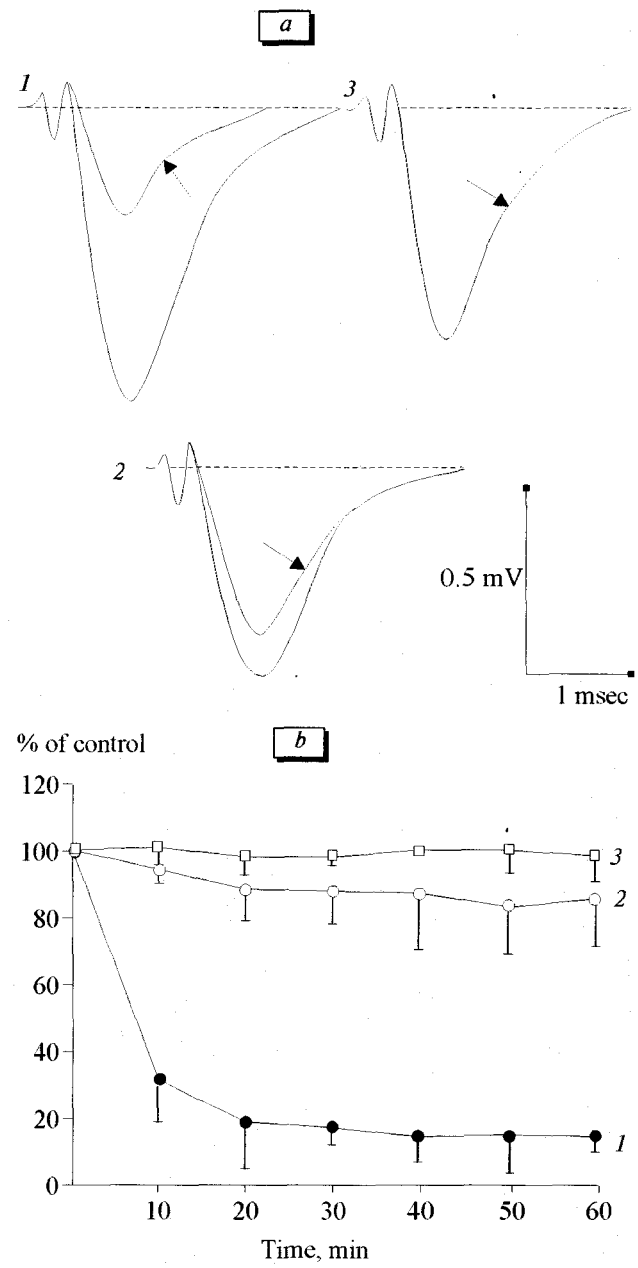

Fig. 1. Effect of sodium nitroprusside on evoked secretion of neurotransmitter in neuromuscular synapse. *a*) averaged endplate potentials (n=32) in the control and 30 min after application of 100 μM sodium nitroprusside (arrows); *b*) dynamics of evoked secretion of neurotransmitter (averaged data from several experiments) in the presence of 100 μM sodium nitroprusside. Concentration of external Ca^{2+}: 0.4 mM (*1,3*) and 1.8 mM (*2*); in 10^{-5} M hemoglobin is added (*3*).

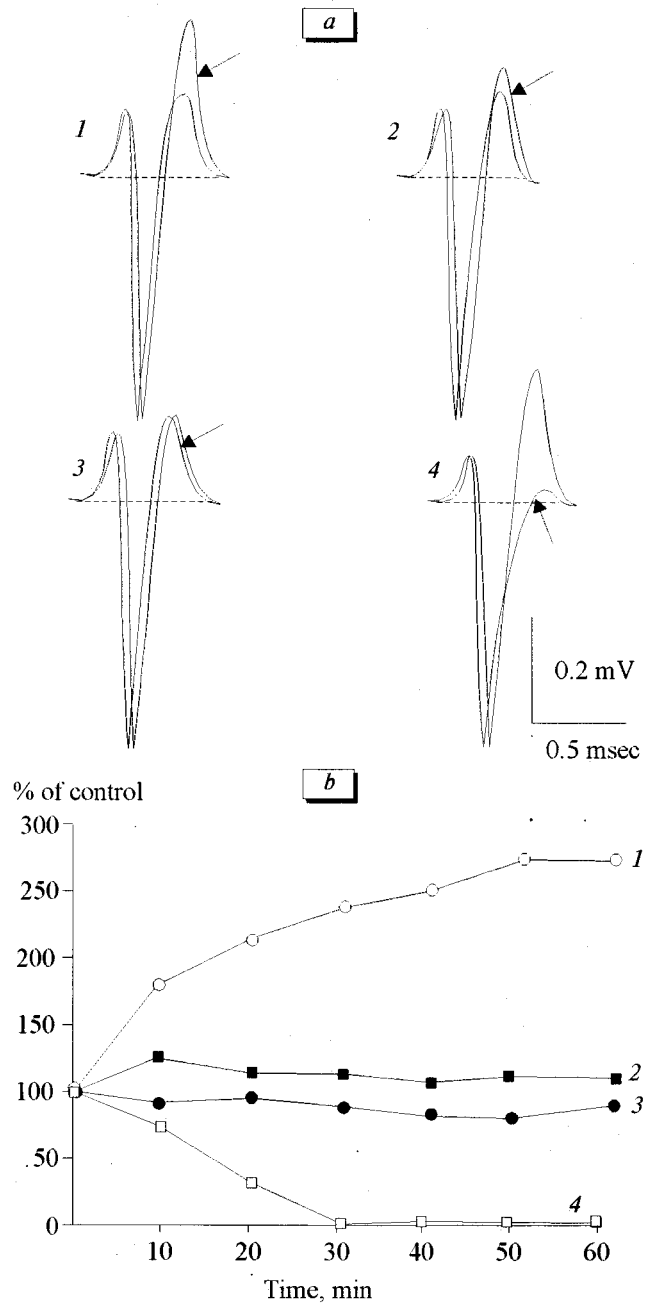

Fig. 2. Effect of sodium nitroprusside on ionic currents in nerve terminal. *a*) averaged potentials of nerve terminals (n=32) before and 30 min after application of 100 μM sodium nitroprusside (arrows); *b*) changes of phase III of nerve response. Concentration of external Ca^{2+}: 0.4 mM (*1,3*) and 1.8 mM (*2,4*). 0.1 mM 4-aminopyridine is added (*3,4*).

phase of presynaptic action potential and the increase in inward calcium current. When applied against the background of 4-aminopyridine, SN did not change the amplitude of phase III of the nerve terminal response (Fig. 2).

In high-calcium medium (1.8 mM), 4-aminopyridine produced a pronounced increase of phase III of nerve terminal response, while addition of SN to 4-aminopyridine-containing solution markedly attenuated this phase; in some experiments this phase disappeared and the signal became biphasic (Fig. 2).

Our data showed that SN inhibited evoked and spontaneous secretion of neurotransmitter in the synapse, which was demonstrated by a decrease in MEPP frequency and EPP quantal content (Fig. 1). This inhibition is undoubtedly caused by NO generated by SN in aqueous solution, since hemoglobin effectively binding NO completely abolished these effects [10].

The data indicate that the effect of exogenous NO on evoked transmitter release is inversely proportional to extracellular and, correspondingly, intracellular Ca^{2+} concentration (Fig. 1). Indeed, EPP amplitude was inhibited by 83% in low-Ca^{2+} medium (0.2-0.4 mM) and only by 13% in high-Ca^{2+} medium (1.8 mM).

The inhibitory effect of exogenous NO on transmitter secretion can be explained by its influence on outward potassium currents in nerve terminals. The increase in the phase III amplitude produced by SN in low-Ca^{2+} medium and the lack of this effect after the blockage of voltage-dependent K^+-channels with 4-aminopyridine attests to activating (positive) effect of NO on voltage-dependent K^+ channels in nerve terminals. It cannot be excluded that exogenous NO changes the kinetics of ionic channel [11]. The increase of voltage-dependent K^+ current shortens the repolarization phase of action potential, attenuates inward Ca^{2+} current, and, correspondingly, inhibits neurotransmitter secretion [13].

To reveal NO effect on Ca^{2+}-activated K^+ channels in nerve terminals, we increased concentration of external Ca^{2+} [3,7]. These channels are activated by high internal Ca^{2+} concentration [3]. In high-Ca^{2+} medium containing 4-aminipyridine, NO inhibited phase III of the nerve response, which attests to a decrease in Ca^{2+}-activated K^+ current. Since this current limits Ca^{2+} current [3], exogenous NO in the presence of K^+ channel blockers prolongs presynaptic action potential, increases inward Ca^{2+} current, and enhances secretion of neurotransmitter [7].

Thus, exogenous NO exerts two opposite effects on voltage-dependent and Ca^{2+}-activated outward potassium currents in motor nerve terminals. It modifies performance of potassium system in motor terminals and changes the duration of presynaptic action potential, amplitude of inward Ca^{2+} current, and evoked neurotransmitter secretion in dependence on internal Ca^{2+} concentration. This agrees with the fact that the increase in phase III of nerve response and inhibition of evoked transmitter secretion by NO in high-Ca^{2+} (1.8 mM) medium are less pronounced than in low-Ca^{2+} (0.4 mM) medium (Figs. 1 and 2). In low-Ca^{2+} medium, Ca^{2+}-activated K^+ current is negligible, and the positive effect is exerted only on voltage-dependent K^+ channels. At high calcium concentrations this

effect is accompanied by the negative influence of NO on Ca^{2+}-activated K^+ channels.

At the same time, the inhibition of spontaneous transmitter secretion under the effect of exogenous NO suggests its direct effect on the molecular structures responsible for exocytosis of synaptic vesicles, which include exocytosis protein complex and Ca^{2+} channels [13]. However, this mechanism requires further investigations.

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