PHYSIOLOGY

Effect of Exogenous Nitric Oxide on Electrogenesis in Myelinated and Unmyelinated Nerve Fibers

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Extracellular recording was used to study the effect of sodium nitroprusside, a donor of NO, on parameters of action potential and ionic currents in single sciatic nerve fibers and unmyelinated nerve terminals in the sternal muscle in frogs. Sodium nitroprusside significantly decreased the duration of action potential in Ranvier node and the amplitude of afterdepolarization. In motor nerve terminals bathed in low Ca^{2+} saline, sodium nitroprusside increased phase III amplitude of the nerve terminal response corresponding to outward potassium currents. Blockade of voltage-dependent potassium channels with 4-aminopyridine abolished the effects of NO. These data indicate that exogenous NO reduced the duration of action potential and afterdepolarization through enhancement of voltage-dependent potassium currents.

Key Words: *sodium nitroprusside; nitric oxide; nerve fiber; action potential; ionic currents*

Recently, considerable attention was focused on the effects of nitric oxide (NO) on various biological systems. NO regulates function of neurons and synapses in the central and peripheral nervous system, acts as a second messenger in the intracellular signaling and as a retrograde messenger in the cell-cell signal transmission, modulates activity of neurotransmitter systems, *etc*. [4,6,8,10]. Experiments showed that the effects of NO are realized via modulation of transmembrane ionic currents. In neurons, NO modifies Ca^{2+} and Ca^{2+}-activated K^+ channels [2,6] and modulates Ca^{2+} currents via NMDA channels [10].

Our aim was to study the effect of exogenous NO on the amplitude and duration of action potential (AP) and transmembrane ionic currents in myelinated and unmyelinated nerve fibers in frogs.

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MATERIALS AND METHODS

Experiments were carried out on single myelinated nerve fibers of *Rana ridibunda* with closed Ranvier node using the Kato—Tasaki technique with some modifications [3]. The internodal segment of nerve fiber was isolated, while AP were recorded from intact Ranvier node in the nerve trunk. Monophasic AP, were recorded after blockade of the distal node (application of 0.2% procaine). AP was recorded using non-polarizable calomel electrodes placed at both sides of isolated segment of the nerve fiber. Ringer's solution (pH 7.2-7.4) containing (in mM): 111.0 NaCl, 2.5 KCl, 1.8 $CaCl_2$, and 1.2 $NaHCO_3$ was used. All experiments were carried out at 20°C. The nerve was stimulated with single rectangular pulses (0.1 msec) delivered via silver electrodes. AP of a single nerve fiber was fed to a DC amplifier and digitized. The amplitude of AP was measured from zero level to the peak, and the duration of AP was determined at $^1/_3$

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peak level (Fig. 1, *a*). The phases of AP repolarization and afterdepolarization (AD) were approximated by straight lines to their crossing point. The distance from this point to the zero line was considered as AD amplitude (Fig. 1, *a*). Each series consisted of 6-7 experiments.

Experiments with unmyelinated fibers were carried out on motor nerve terminals (NT) of neuromuscular synapses in the sternal muscle from *Rana ridibunda*. The preparation was placed into a bath with Ringer's solution. The nerve was sucked into a plastic pipette. The pipette and two silver stimulating electrodes were mounted on the bath wall. The nerve was stimulated with suprathreshold stimuli at a rate of 1 Hz. The preparation was perfused with Ringer's solution containing (in mM): 111.0 NaCl, 2.5 KCl, 0-0.4 $CaCl_2$, 4.0 $MgCl_2$, and 1.2 $NaHCO_3$. Postsynaptic signals were blocked with tubocurarine (3×10^{-5} M).

Action potentials in unmyelinated NT cannot be directly recorded because their extremely small diameter (1-2 μ). Therefore, electrogenesis in unmyelinated NT evoked by nerve stimulation was studied using extracellular glass electrodes (tip diameter 2-5 μ). To this end, electrodes filled with 1 M NaCl were pressed close to NT. The responses were recorded in the proximal part of NT at distance of 10-30 μ from myelin segment. This response represents ionic currents across the membrane during excitation [1,9]. Under normal conditions, a high-amplitude triphasic response recorded in the proximal part of NT consisted of positive phases I and III and negative phase II. Phase I is formed by passive outward current induced by arriving AP, while phases II and III correspond to inward sodium and outward potassium currents in NT, respectively [1]. The calculated parameters of NT response are shown on Figure 1, *b*. The data were analyzed statistically by Student's *t* and Wilcoxon's tests using Excel, Origin, and SigmaStat software.

RESULTS

Stimulation of isolated myelinated nerve fiber evoked AP with amplitude 51-88 mV (72.52±3.40 mV) and duration 1.64±0.14 msec. A 10-fold amplification (Fig. 2, *a*, *1*) showed gradual transition from AP to AD with an amplitude 1.40-3.04 mV (2.35±0.33 mV). Sodium nitroprusside (20 mM) slightly decreased the amplitude (to 95.66±2.96% of the initial value) and considerably decreased of the duration of AP due to shortening of the repolarization phase (to 84.7± 4.2% of the normal, $p<0.05$). The amplitude of AD (Fig. 2, *b*, *2*) dropped to 70.4±8.0% of the initial value ($p<0.05$).

It is established that the rate of repolarization and AD parameters depend on the ratio between residual sodium permeability (determined by sodium inactivation rate) and potassium permeability [12,13]. Hence, changes in AP induced by exogenous NO can be determined by acceleration of sodium inactivation or enhancement of potassium permeability. This point was clarified in experiments with blockade of voltage-dependent K^+ channels with 4-aminopyridine. This blocker (2 mM, 5-min application) prolonged the polarization phase of AP, increased AD amplitude, and in 50% cases triggered generation of repeated AP in Ranvier node (Fig. 2, *c*, *1*). In the absence of recurrent spikes, AP duration and AD amplitude increased to 5.85±2.23 msec and 5.42±1.84 mV, respectively (Fig. 2, *c*, *2*). Sodium nitroprusside (20 mM) added to Ringer's solution with 4-aminopyridine (2 mM) did

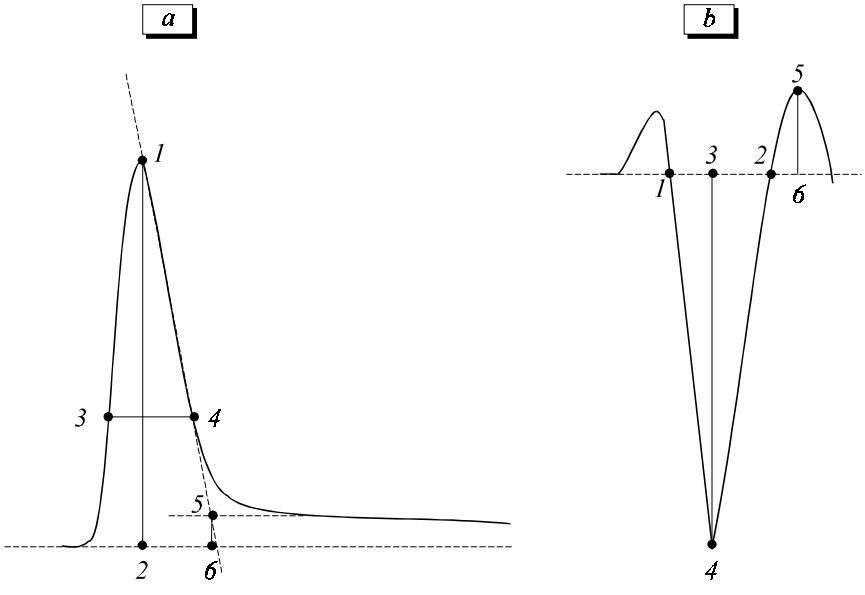

Fig. 1. Amplitude and time parameters of single nerve fiber action potential (*a*) and proximal nerve terminal response (*b*). *a*) amplitude of action potential (*1-2*), duration of action potential (*3-4*), and afterdepolarization amplitude (*5-6*); *b*) phase II duration of nerve terminal response. Dash line corresponds to zero voltage.

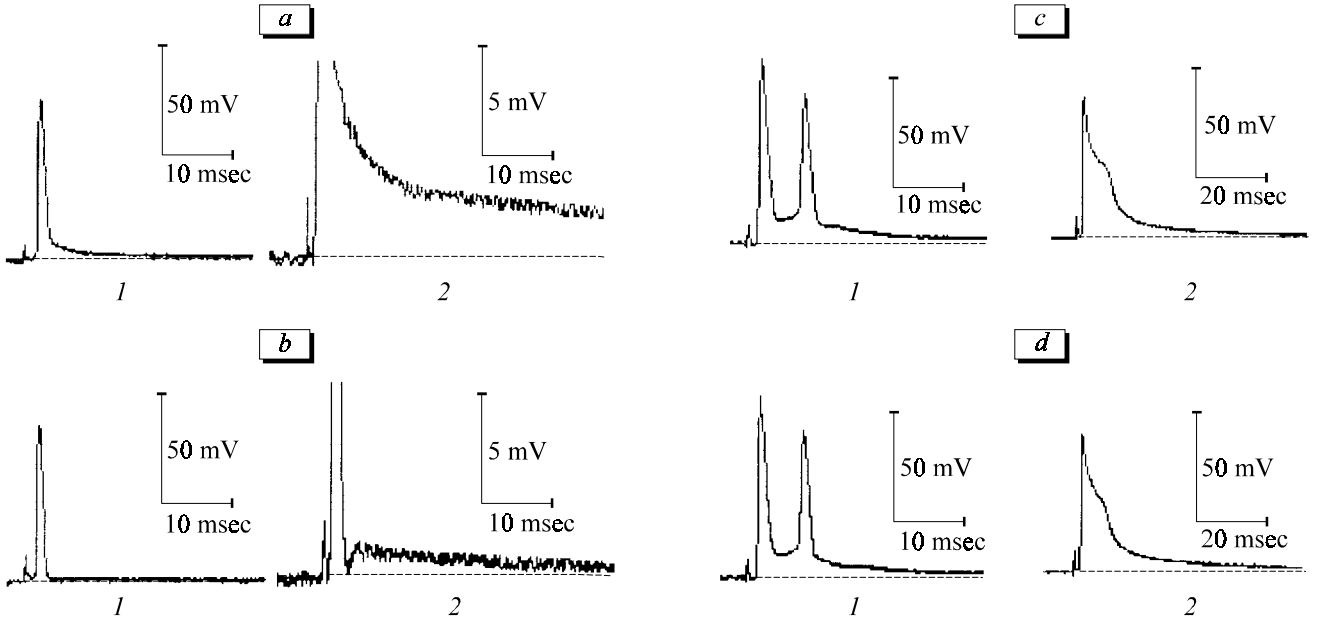

Fig. 2. Effect of sodium nitroprusside on action potential and afterdepolarization in isolated nerve fiber. Action potential in standard Ringer's solution before (*a*) and after addition of 20 mM sodium nitroprusside (*b*) recorded with low (*1*) and high (*2*) amplification. Action potentials evoked by a single pulse in nerve fiber bathed in Ringer's solution with 2 mM 4-aminopyridine before (*c*) and after addition of sodium nitroprusside (*d*), single (*2*) and multispike responses (*1*).

not eliminate the multispike response and did not change the parameters of AP and AD (Fig. 2, *d*). All these data indicate that exogenous NO increases potassium permeability of Ranvier node membrane without affecting inward sodium current.

Apart from voltage-dependent Na^+ and K^+ channels, NT possess also Ca^{2+}-activated K^+ channels and Ca^{2+} channels [7]. Therefore, to match the experimental conditions for myelinated and unmyelinated nerve fibers, we used Ca^{2+}-free or low Ca^{2+} solutions, which decreased or eliminated inward Ca^{2+} current and prevented the appearance of Ca^{2+}-activated K^+ current. In this case, AP is formed by the same types of ionic currents in myelinated and unmyelinated nerve fibers. Sodium nitroprusside (0.1-1.0 mM) added to low Ca^{2+} (0-0.4 mM) solution increased phase III (positive) amplitude of NT response reflecting the kinetics of outward K^+ currents (Fig. 3, *a*). Fifteen minutes after application this parameter increased to 201.00±17.92% compared to the initial value (n=12, p<0.05). Both the amplitude and duration of phase II of NT response remained unchanged, *i.e.* sodium nitroprusside had no effect on Na^+ currents in NT (Fig. 3, *a*).

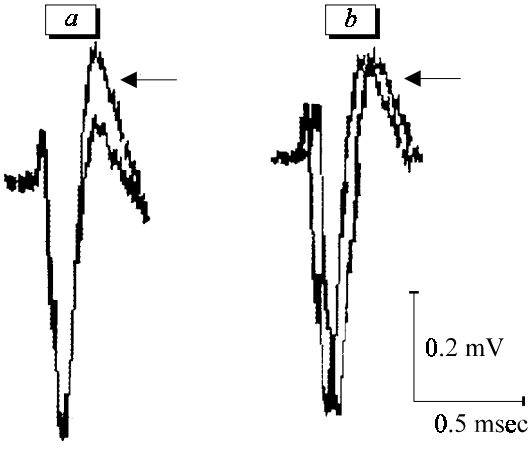

Fig. 3. Responses of a nerve terminal before and 30 min after (arrows) application of 100 μmol/liter sodium nitroprusside in standard Ringer's solution (*a*) and against the background of 0.1 mM 4-aminopyridine (*b*).

Application of 4-aminopyridine (0.1 mM) slightly decreased phase III amplitude of NT response and in some experiments triggered multispike activity. All these phenomena are characteristic of blockade of voltage-dependent K^+ channels [1]. Sodium nitroprusside applied with 4-aminopyridine did not change phase III amplitude of NT response (Fig. 3, *b*). These findings confirm the assumption on modulation of voltage-dependent K^+ channels in nerve fibers with exogenous NO.

These data suggest that exogenous NO produces significant and similar effects in myelinated and unmyelinated nerve fibers: shortening of AP and attenuation of AD due to enhancement of voltage-dependent K^+ currents.

The effective concentrations of sodium nitroprusside for myelinated nerve fibers several times surpassed those for unmyelinated fibers. This difference can be explained by the fact that unmyelinated NT is covered only by Schwann cell, while Ranvier node in myelinated fiber located in nerve trunk is surrounded by

connective tissue and membrane structures and other nerve fibers, which impedes the diffusion of agents.

The mechanisms underlying the enhancement of potassium transmembrane permeability by exogenous NO, as well as the sources of endogenous NO near the nerve fibers are still unknown. It can be hypothesized that NO either increases permeability of K^+ channels or modifies voltage dependence of their activation and/or inactivation via the intracellular messenger system [8]. Under natural conditions NO can be produced by Schwann cells [11]. Further studies are needed to answer these questions.

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