Effect of Nitro Derivatives on Electromechanical Coupling in Ureteral Smooth Muscle Cells

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 129, No. 5, pp. 539-541, May, 2000
Original article submitted October 6, 1999

Double sucrose gap experiments revealed differences in the effect of nitroglycerin and sodium nitroprusside on action potential and contraction of ureteral smooth muscle cells. Unlike sodium nitroprusside, nitroglycerin inhibited voltage-dependent Ca^{2+} membrane permeability. It was concluded that cGMP-independent mechanisms of the effects of nitro derivative reflect the peculiarities of excitation-contraction coupling in smooth muscles.

Key Words: *smooth muscle cells; nitro derivatives; ionic membrane permeability*

It was previously reported that sodium nitroprusside (SN) induces relaxation of precontracted smooth muscle strips and repolarization of smooth muscle cell (SMC) membrane accompanied [2,3]. The effects of SN are assumed to depend on activity of soluble guanylate cyclase, because the increase in cGMP level [8] modulates calcium and potassium permeability in SMC membrane [2,3,9,14]. However, cGMP-independent pathways of relaxation induced by NO-derivatives cannot be excluded [5,15]. Solution to this problem is impeded by a great variety of smooth muscle preparations and NO-derivatives used in the experiments [7-9,11], as well as by peculiarity of biological transformation of the test drugs [4,5].

The effects of NO-derivatives on electrical and contractile properties of SMC is not clearly understood. Our aim was to compare the effect of NO-derivatives on ionic mechanisms of excitation-contraction coupling in SMC of guinea pig ureter.

MATERIALS AND METHODS

Simultaneous recording of electrical and contractile parameters of SMC was performed using double sucrose gap technique [1]. The method and mode of recording of SMC membrane potential and contraction were described previously [2,3].

The ureters were isolated from outbred guinea pigs. After removing the connective tissue, 10-12-mm segments of the ureter were isolated.

Test solutions containing SN (Sigma), nitroglycerin (NG, Nitroject, SUN), tetraethylammonium chloride (TEA, Serva), dibutyryl-cGMP (Boehringer Mannheim), and methylene blue (Reakhim) were prepared on the basis of Krebs saline [2,3].

Sodium-free solution was prepared by replacing NaCl, NaH_2PO_4, $NaHCO_3$ in Krebs saline with Tris-(hydroxymethyl)aminomethane (Reakhim) in equimolar concentrations.

The data were analyzed statistically.

RESULTS

After conditioning in Krebs solution during 40-45 min, ureteral smooth muscle strips were stimulated with electrical pulses (0.5-1.5 μA). The resulting cation- and anion-electrotonic potentials reflected the typical current-voltage relationships [6]. Millimolar concentrations of NN and SN had practically no effect on these relationships, which suggests the absence of the effects of test NO-derivatives on poten-

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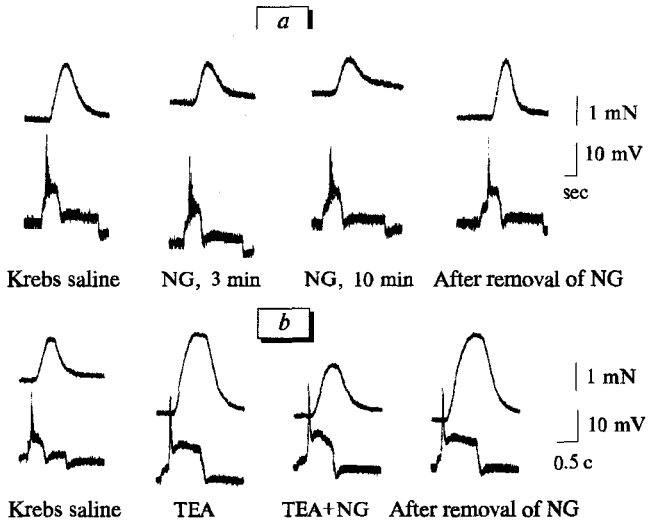

Fig. 1. Effect of nitroglycerin (NG) alone (*a*) and in combination with tetraethylammonium chloride (TEA) (*b*) on contraction of ureteral smooth muscle cells (top panels) and action potentials (bottom panels).

tial-dependent potassium permeability of SMC membrane [6].

When the membrane potential in response to depolarizing stimulus (0.8-1.5 μA) reached the threshold value, the action potentials typical for ureteral SMC [6] were recorded in parallel with contraction (Fig. 1, *a*). NG (10 μM) inhibited electrical and contractile activity of ureteral SMC 3-5 min after addition to Krebs saline: the amplitude of contractions and the duration of plateau decreased to 73.4±9.3 and 91.3± 6.0% of the initial values, respectively (n=8, p<0.05). This effect was reversible and removal of NG from the bathing solution restored AP and contraction parameters.

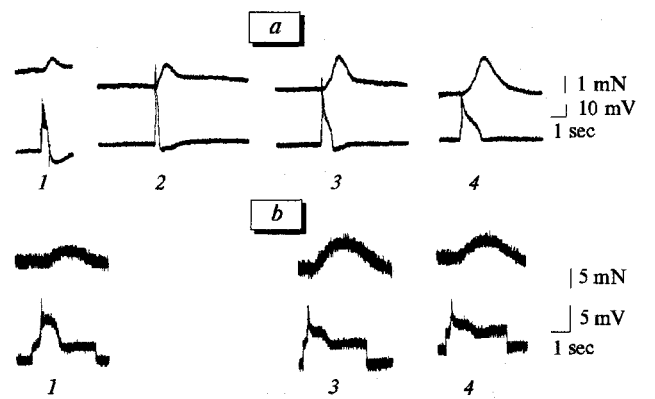

Fig. 2. Effect of sodium nitroprusside (*a*) and nitroglycerin (*b*) on contraction of ureteral smooth muscle cells (top panels) and action potentials (bottom panels) in sodium-free solutions. *1*) Krebs saline, *2*) sodium-free saline, *3*) sodium-free saline with tetraethylammonium chloride, *4*) sodium-free saline with tetraethylammonium chloride and NO-derivative.

The inhibitory effect of NG was dose-dependent: in the presence of 100 μM NG the amplitude of contraction and AP plateau duration decreased to 65.7±9.3 and 84.9±7.7% (n=8, p<0.05), while in the presence of 500 μM the corresponding parameters decreased to 39±9 and 79.7±11.0% (n=8, p<0.05), respectively.

Unlike NG, SN (0.1-5 mM) produced no changes in AP parameters, although it increased the amplitude of ureteral SMC contractions.

Excitation-contraction coupling in SMC is mediated by Ca^{2+} ions [6,11-13] and can be modulated by other ions [6]. For instance, the inward sodium-calcium and outward potassium currents participate in the formation of AP plateau in ureteral SMC [6]. To evaluate the role of outward potassium currents in the effects of NO-derivatives, we used tetraethylammonium chloride.

Tetraethylammonium chloride in a concentration of 10 mM increased the amplitude and duration of AP, as well as the contraction force of ureteral SMC (Fig. 1, *b*). Under these conditions NG (100 μM) shortened AP plateau to 71±11% and decreased contraction amplitude to 74±14% (n=8, p<0.05). NG applied in the same concentration in the absence of tetraethylammonium produced a stronger effect, which suggests the involvement of potassium permeability in the effect of NG, because NO-derivatives are known to enhance potassium permeability in vascular SMC [3,9,11].

The prolongation of AP plateau and the increase in contraction amplitude after blockade of potassium permeability with tetraethylammonium are explained by activity of slow voltage-dependent calcium channels [6]. These channels were blocked by NG, but not SN, and this blockade was manifested in our experiments by significant decrease of contraction amplitude and shortening of AP plateau in ureteral SMC.

In sodium-free Krebs solution containing an equimolar concentration of Tris(hydroxymethyl)aminomethane, the amplitude of SMC contractions increased, while the plateau of AP decreased (Fig. 2, *a*). Addition of 10 mM tetraethylammonium extended the plateau and potentiated contractions. Under these conditions NG inhibited the peak component of AP and suppressed contractions of ureteral SMC, while NN had no effect on these parameters (Fig. 2, *a, b*), *i.e.* NG, but not NN, suppressed potential-dependent calcium permeability of ureteral SMC membrane.

There is evidence on inhibition of L-type calcium channels of individual SMC with cGMP and cGMP-dependent protein kinases [10], although these data contradict with other reports [11,14]. It is known that NG and SN can participate in cGMP-dependent processes acting as exogenous NO donors, activators of soluble guanylate cyclase [2-5,7]. In our experiments, methylene blue (10 μM) added to bathing solution had no effect on the parameters of AP and ureteral SMC

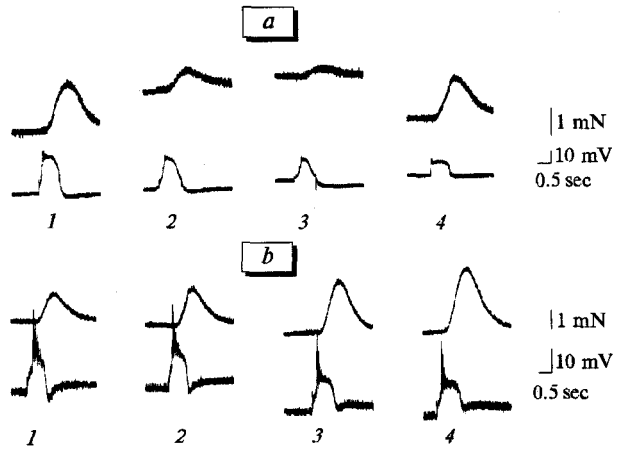

Fig. 3. Effect of nitroglycerin (NG) (*a*) and dibutyryl-cGMP (*b*) on contraction of ureteral smooth muscle cells (top panels) and action potentials (bottom panels). *1*) Methylene blue (*a*) and Krebs saline (*b*), *2*) and *3*) 3 and 5 min after addition of NG (*a*) and 10 and 30 min after addition of dibutyryl-cGMP (*b*), *4*) initial saline after removal of test agents.

contraction (Fig. 3, *a*) and did not abolish the inhibitory effect of 100 μM NG on AP and contraction of ureteral SMC.

Dibutyryl-cGMP (0.1-1 mM) did not affect AP, but potentiated contraction of ureteral SMC (Fig. 3, *b*). However, there is evidence on its capacity to reproduce (in this concentration range) the relaxing effects of NO-derivatives in precontacted vessels [7]. The effects of dibutyryl-cGMP and SN on AP and contraction of ureteral SMC were similar, which suggests the involvement of cGMP in stimulating effects of both these substances. It cannot be excluded that the potentiaton of ureteral SMC contraction results from additional accumulation of Ca^{2+} ions in the extra- and/or intracellular stores, which is characteristic of cGMP-dependent protein kinases [9, 11-13].

Thus, in contrast to SN, the effect of NG on AP and contraction of ureteral SMC is cGMP-independent and results from inhibition of calcium membrane permeability. In contrast to the vessels [2,3, 7,12] the increase in intracellular cGMP in these smooth muscles produces no relaxation. It seems that the effect of NO-derivatives is tissue-specific and depends on peculiarity of excitation-contraction coupling, which does not exclude the possibility of mediation of SMC myogenic effects via cGMP-independent pathway.

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