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The Role of Intracellular Ca^{2+} Pool in Sodium Nitroprusside-Induced Relaxation of Rat Aorta Smooth Muscle Cells

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A relaxation effect of sodium nitroprusside on smooth muscle cells of rat aorta due to intracellular Ca^{2+}-store refilling is demonstrated using the double sucrose gap technique. It is suggested that sodium nitroprusside-induced repolarization of the smooth muscle cell membrane is associated with inhibition of Ca permeability and/or Ca-dependent K^+ permeability of the plasma membrane.

Key words: *sodium nitroprusside; Ca ions; smooth muscle cells*

Nitrate derivatives, in particular sodium nitroprusside (SN), activate cytosolic guanylate cyclase, which leads to a rise of intracellular cyclic GMP and triggers a cascade of cGMP-dependent events [4,9,10]. Activation of single potassium channels in smooth muscle cells (SMC) in the presence of cGMP and cGMP-dependent protein kinases is extensively discussed in the literature [9,15]. Some authors suggest that this mechanism underlies the nitrate-induced relaxation of vascular SMC [3,7,15], although this is in controversy [8-12].

It has been shown that cGMP inhibits both voltage-operated [8,14] and receptor-operated Ca^{2+} channels [7]. The possible effect of cGMP on Ca^{2+} utilization from the cytosol [2,6,13] and on the activity of contractile proteins in SMC [10,11] is intensively discussed. At the same time, simultaneous effects of nitrates on Ca^{2+} influx through membrane ion channels and Ca^{2+} release from SMC stores are disputable.

The aim of the present work was to study membrane and intracellular mechanisms by which cyclic GMP controls Ca^{2+}-dependent regulation of the vascular smooth muscle contraction.

MATERIALS AND METHODS

The membrane potential and muscle tension in SMC were simultaneously measured using the double sucrose gap method [1]. Electrical potentials were recorded via nonpolarizable electrodes using a C1-83 oscilloscope coupled to a KSP-4 recorder. Contractile activity was recorded on a 6MKh1B mechanotron in a nearly isometric regimen.

Male random-bred albino rats (200-250 g) were sacrificed and endothelium-denuded smooth muscle strips (0.6-0.7 mm wide, 10-12 mm long) were isolated from the middle portion of the thoracic aorta.

Isolated smooth muscle strips were kept in Krebs solution (36.8°C; pH 7.35) containing (in mM): 120.4 NaCl, 5.6 KCl, 15.5 $NaHCO_3$, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 2.5 $CaCl_2$, and 11.5 glucose for 40-45 min, and then treated with high-potassium (40 mM KCl) Krebs solution (Fig. 1). Test solutions were prepared on the basis of Krebs saline containing verapamil, SN (Serva), and caffeine; in the Ca-free solutions $CaCl_2$ was replaced with EGTA.

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The results were statistically analyzed using Student's *t* test.

RESULTS

The Ca^{2+} channel blocker verapamil (10 μM) induced membrane repolarization and relaxation of SMC (68±1 and 53±2%, respectively, n=6, p<0.05). Subsequent addition of 10 mM SN produced additional repolarization (53±2%, n=6, p<0.05) and complete relaxation of the smooth muscle strip. Verapamil added against the background of SN induced similar changes (Fig. 1).

It is accepted that concurrent membrane depolarization and contractile response in smooth muscle strip are associated with Ca^{2+} entry through voltage-dependent Ca^{2+} channels and Ca^{2+} mobilization from intracellular stores triggering muscular contraction [3,5,9]. Inhibition of Ca^{2+} channels by verapamil produced membrane repolarization and reduced smooth muscle tone; SN produces the same effect [8]. Similar effects of these two drugs on the membrane potential in SMC can be underlain by different mechanisms. For instance, catalytic subunits of cAMP-dependent and cGMP-dependent protein kinases significantly inhibit Ca^{2+} current through the L-type Ca^{2+} channels in rat vein SMC [14]. At the same time, cAMP-dependent and cGMP-dependent protein kinases increase K^+ permeability of the SMC membrane (specifically, Ca-dependent K^+ permeability [15]. In the presence of verapamil initially high K^+ permeability of vascular SMC membrane can lose a part of its Ca^{2+}-dependent component, and the effect of SN becomes negligible. Ineffectiveness of SN in the presence of tetraethylammonium, the potassium channel blocker, was previously reported [4].

It can be supposed that SN-induced relaxation and repolarization in rat aorta SMC results from inhibition of Ca^{2+} permeability and/or the rise of Ca^{2+}-dependent potassium permeability.

Relaxation of SMC depends on intracellular Ca^{2+} level and its utilization from the cytosole [5,12]. To study the effect of SN on these processes we used caffeine, an activator of Ca^{2+} release from cell stores [8], and Ca^{2+}-free solution (containing EGTA, Ca^{2+}-chelating agent) [2].

After recording the baseline membrane potential in the high-potassium saline, the strips were exposed to Ca^{2+}-free solution (1 mM EGTA). A decrease in the smooth muscle tone 50±3% and membrane depolarization by 31±3.5% were observed after 45 min (n=6, p<0.05). Caffeine (60 μM) added under these conditions induced membrane repolarization (by 11±5.5%) and restored the initial smooth muscle tension (51± 7.5%, n=6, p<0.05).

The addition of high-potassium solution after a 45-min incubation of SMC in Ca^{2+}-free medium induced a sustained membrane depolarization (25-30 min) by 45±1%, while muscle tension increased by 40±1.5% (n=6, p<0.05). The effect of high-potassium medium inversely depended on the duration of incubation with EGTA. However, membrane potential and smooth muscle tone became completely irresponsive to high-potassium Ca^{2+}-free solution only after a 80-100-min incubation with EGTA. Under these conditions caffeine (60 μM) induced membrane repolarization and increased smooth muscle tone.

SN (10 μM) added after a 45-60-min incubation with high-potassium Ca^{2+}-free solution completely abolished the rise of SMC contractility, the membrane potential being unaffected. Increasing of SN concentration to 0.1-1 mM had practically no effect on the studied parameters. Caffeine (60 μM) added after SN

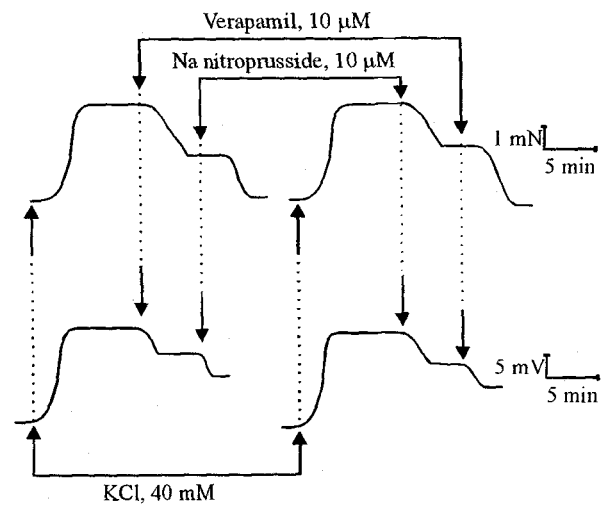

Fig. 1. Effect of sodium nitroprusside and verapamil on membrane potential (lower traces) and muscular tension (upper traces) of rat aorta SMC in high-potassium Krebs solution.

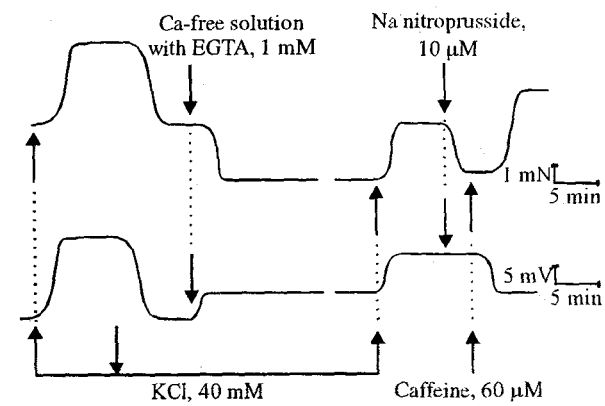

Fig. 2. Effect of sodium nitroprusside and verapamil on the membrane potential (lower traces) and muscular tension (upper traces) of rat aorta SMC in Ca-free Krebs solution with EGTA.

induced membrane repolarization and a rise of muscle tone (Fig. 2).

Thus, under depletion of extracellular Ca^{2+} pool, SN induces rat aorta SMC relaxation by activating of Ca^{2+} uptake in non-mitochondrial intracellular stores. The absence of changes in the membrane potential of SMC may be due to reduced Ca^{2+}-dependent potassium efflux and influx.

The relaxation and repolarization effects of SN on rat aorta SMC probably occur simultaneously due to decreased Ca^{2+} and increased K^+ membrane permeabilities, and enhanced Ca^{2+} utilization in intracellular stores.

REFERENCES

1. D. P. Artemenko, V. A. Buryi, I. A. Vladimirova, and M. F. Shuba, *Fiziol. Zh.*, **28**, No. 3, 377-380 (1982).
2. L. V. Baidan, S. M. Tishkin, and M. F. Shuba, *Fiziol. Zh. SSSR*, **73**, No. 11, 1569-1572 (1987).
3. I .V. Kovalev, A. A. Panov, M. B. Baskakov, *et al.*, *Ros. Fiziol. Zh.*, **83**, No. 7, 70-76 (1997).
4. V. P. Reutov and S. N. Orlov, *Fiziologiya Cheloveka*, **19**, No. 1, 124-137 (1993).
5. M. F. Shuba and V. A. Buryi, *Fiziol. Zh.*, **30**, No. 5, 545-559 (1984).
6. K. Baltensperger, E. Carafoli, and M. Chiesi, *Eur. J. Biochem.*, **172**, No. 1, 7-16 (1988).
7. P. Collins, A. H. Henderson, D. Lang, and M. J. Lewis, *J. Physiol. (Lond.)*, **400**, 395-404 (1988).
8. H. Karaki, H. Nakagawa, and N. Urakawa, *Br. J. Pharmacol.*, **81**, 393-400 (1984).
9. H. Karaki and B. G. Weiss, *Life Sci.*, **42**, No. 2, 111-122 (1988).
10. T. M. Lincoln, P. Komalavilas, and T. L. Cornwell, *Hypertension*, **23**, No. 6, Pt. 2, 1141-1147 (1994).
11. N. L. McDaniel, C. M. Rembold, and R. A. Murphy, *Can. J. Physiol. Pharmacol.*, **72**, No. 11, 1380-1385 (1994).
12. T. Nagao and P. M. Vanhout, *J. Physiol. (Lond.)*, **445**, 355-367 (1992).
13. L. M. Popercu, C. Panou, M. Heinetsu, and D. Nutu, *Eur. J. Pharmacol.*, **107**, No. 3, 393-394 (1985).
14. Z. Xiong, N. Sperelakis, and R. Fenoglio-Reiser, *J. Vasc. Res.*, **31**, No. 5, 271-279 (1994).
15. M. Yamakage, C. A. Hirshman, and T. L. Croxton, *Am. J. Physiol.*, **270**, No. 3, Pt. 1, 338-345 (1996).