BIOPHYSICS AND BIOCHEMISTRY

Effect of Inhibitors of Cyclic Nucleotide Phosphodiesterases on Electrical and Contractile Activity of Smooth Muscle Cells

I. V. Kovalev, A. G. Popov, M. B. Baskakov, I. L. Minochenko, A. A. Kilin, Yu. L. Borodin, Ya. D. Anfinogenova, L. V. Kapilevich, and M. A. Medvedev

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Double sucrose gap experiments were carried out to study the effect of phosphodiesterase inhibitors and penetrating analogs of cyclic nucleotides on action potential and contraction of guinea pig ureteral smooth muscle cells. 3-Isobutyl-1-methylxanthine (10 μM) and dibutyryl-cAMP (20 μM) shortened the plateau of action potential and inhibited contraction of smooth muscle cells by increasing potassium permeability of their membrane. Vinpocetine (1 μM) and dibutyryl-cAMP (100 μM) strengthened contraction of smooth muscle cells and shortened action potentials by decreasing sodium permeability of their membrane.

Key Words: *smooth muscle cells; cyclic nucleotide phosphodiesterases; membrane ionic permeability*

Myogenic reactions in smooth muscle cells (SMC) depend on the level of cytoplasmic Ca^{2+} [6,7,14], which can be modulated by many biologically active substances, in particular by cyclic nucleotides [6,10,12]. Intracellular concentration of cyclic nucleotides can be changed by activation of their synthesis or inhibition of their degradation (inhibition of phosphodiesterases) [5,13]. However, identification of the effects of cAMP and cGMP is difficult because of a large number of cross-reactions at the level of both the effector molecular mechanisms and intermediary biochemical stages [4-6,12].

We reported previously that in contrast to SMC of rat aorta [2], elevation of cAMP level in guinea pig ureter activates contraction [3], while adenylate cyclase activator forskolin and dibutyryl-cAMP inhibit action potential (AP) and contraction [7,11, 12]. This difference makes it possible to separate the effects of cAMP and cGMP at the level of physiological response of smooth muscles.

Our aim was to study the effect of inhibitors of cAMP- and cGMP-stimulated phosphodiesterases and penetrating analogs of cyclic nucleotides on excitation-contraction coupling in SMC of guinea pig ureter.

MATERIALS AND METHODS

Double sucrose gap technique was used to record simultaneously the electrical and contractile parameters of SMC [1]. Preparation of SMC and the procedure of recording of membrane potential and contraction in SMC were described previously [3].

Krebs saline for the experiments contained (in mM): 120.4 NaCl, 5.9 KCl, 2.5 $CaCl_2$, 1.2 NaH_2PO_4, 15.0 Tris-(hydroxymethyl)aminomethane, 1.2 $MgCl_2$, and 11.5 glucose (37°C; pH 7.35).

Test solutions contained the following agents dissolved in Krebs saline: sodium nitroprusside

Department of Biophysics, Department of Normal Physiology Siberian Medical University, Tomsk. ***Address for correspondence:*** kovalev@mail2000.ru. Kovalev I. V.

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(Sigma), 3-isobutyl-1-methylxanthine (IBMX, Sigma), tetraethylammonium chloride (TEA, Serva), dibutyryl-cAMP and dibutyryl-cGMP (Boehringer Mannheim GmbH), vinpocetine (cavinton, Gedeon Richter), and methylene blue (Reakhim).

The data were processed statistically using nonparametrical Wilcoxon test for paired samples. The values of AP and contraction amplitude in Krebs saline were taken as 100%.

RESULTS

Addition of IBMX (10 μM), a non-specific phosphodiesterase inhibitor [4], to Krebs saline reversibly inhibited contraction (to 56%, n=6, p<0.05) and shortened AP plateau (to 68%, n=6, p<0.05) in comparison with the control (Fig. 1, *a*). In the presence of potassium channel blocker TEA (5 mM) the inhibitory effect of IBMX on AP and contraction of ureteral SMC was diminished (n=6, p<0.05, Fig. 1, *b*). These data indicate that the effects of IBMX are mediated via elevation of potassium permeability in SMC membrane.

Another phosphodiesterase inhibitor vinpocetine (1 μM) produced an opposite effect: it stimulated SMC contraction by 33% and shortened AP by 22% (Fig. 1, *c*). When applied against the background of vinpocetine, IBMX (10 μM) shortened AP plateau and reduced contraction amplitude (59 and 78%, respectively; n=6, p<0.05).

These data attest to opposite effects of the examined phosphodiesterase inhibitors on SMC. For elucidation of the possible mechanism of vinpocetine action, the experiments were carried out with activator (sodium nitroprusside, 100 μM) and inhibitor (methylene blue, 10 μM) of soluble fraction of guanylate cyclase [2,3].

When applied against the background of sodium nitroprusside, vinpocetine produced a further increase in contraction amplitude by 23% (n=6, p<0.05, Fig. 2, *a*). However, the stimulating effect of sodium nitroprusside and vinpocetine on ureteral SMC contraction was eliminated in the presence of methylene blue (Fig. 2, *b*).

These data suggest that vinpocetine produced its effect on contraction of guinea pig ureteral SMC via elevation of intracellular cGMP concentration.

What mechanisms underlie the myogenic effects of this cyclic nucleotide? The duration of ureteral AP plateau is determined by synchronous changes in membrane permeability for Ca^{2+}, Na^{+} and K^{+} ions [6,8]. There is no unambiguous view on the effect of cGMP on calcium channels: the data on its activating [11] and inhibitory [15] effects are controversial.

To study the mechanisms of cGMP action, we carried out the experiments with histamine, which increases the duration of AP plateau and contraction amplitude of ureteral SMC by enhancing of so-

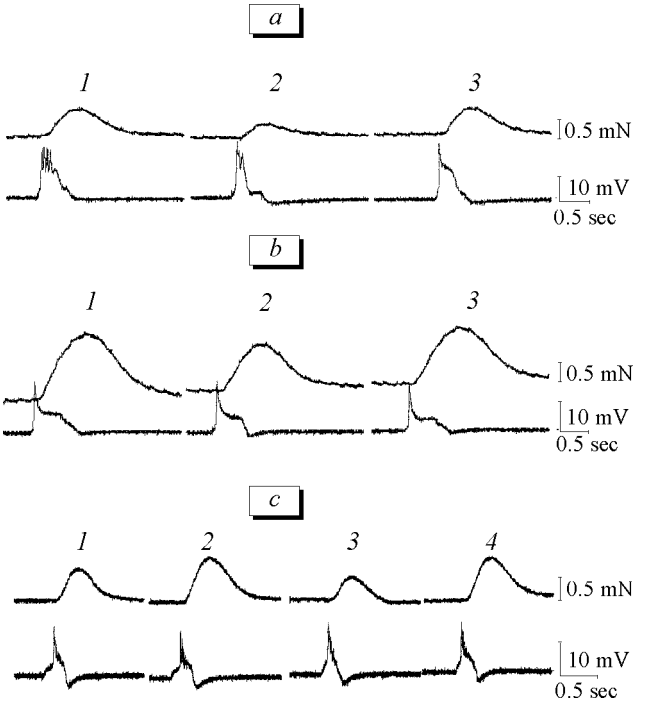

Fig. 1. Effect of 3-isobutyl-1-methylxanthine (IBMX, 10^{-5} M) on contractile (top curves) and electrical (bottom curves) activities of SMC in guinea pig ureter. *a: 1*) Krebs saline; *2*) IBMX; *3*) washout; *b*: *1*) TEA, 5×10^{-3} M; *2*) IBMX; *3*) washout; c: *1*) Krebs saline; *2*) vinpocetine, 10^{-6} M; *3*) IBMX; *4*) washout.

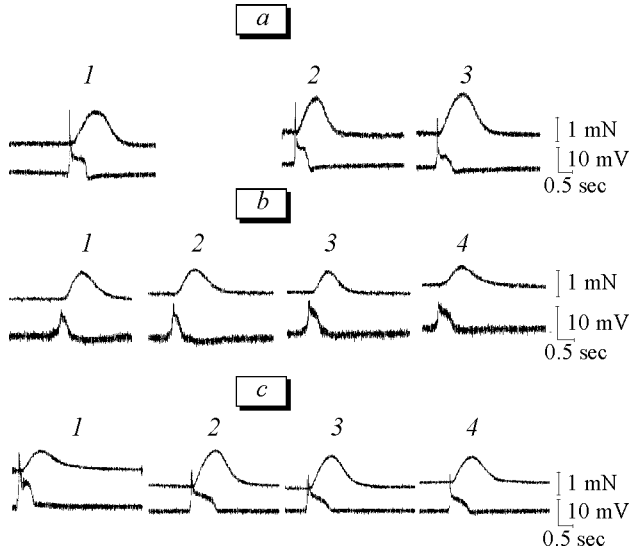

Fig. 2. Effect of vinpocetine (10^{-6} M) on contractile (top curves) and electrical (bottom curves) activities of guinea pig ureteral SMC. *a: 1*) Krebs saline; *2*) sodium nitroprusside (10^{-4} M); *3*) vinpocetine; *b*: *1*) Krebs saline; *2*) methylene blue (10^{-5} M); *3*) sodium nitroprusside; *4*) vinpocetine; c: *1*) Krebs saline; *2*) histamine; *3*) vinpocetine; *4*) washout.

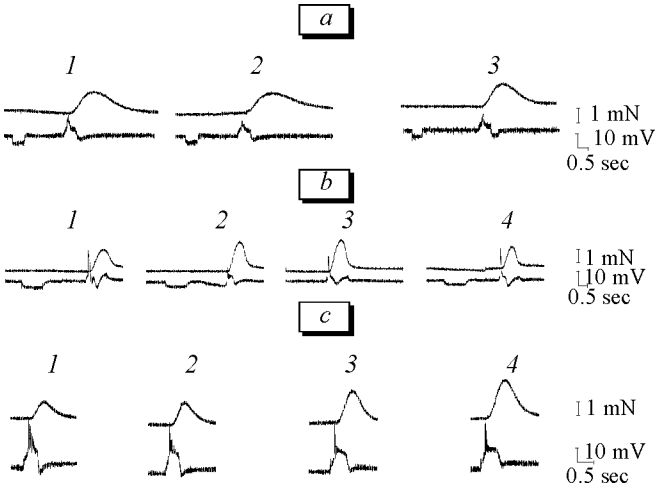

Fig. 3. Effect of penetrating analogs of cyclic nucleotides on contraction (top curves) and action potentials (bottom curves) of guinea pig ureteral SMC. *a: 1*) Krebs saline; *2*) dibutyryl-cAMP (2×10^{-5} M); *3*) washing; *b*: *1*) Krebs saline; *2*, *3*) dibutyryl-cAMP (2×10^{-4} M), spontaneous and evoked activity, correspondingly; *4*) washing; c: *1*) Krebs saline; *2*, *3*) dibutyryl-cAMP (10^{-4} M), exposure time 10 and 30 min, respectively; *4*) washing.

dium permeability of its membrane [6]. When applied against a background of histamine (10 μM), vinpocetine (1 μM) inverted its activating effect: it inhibited SMC contraction and decreased AP plateau (Fig. 2, *c*). These data attest to a possible inhibition of sodium permeability in ureteral SMC membrane by vinpocetine. Similar effect of vinpocetine and cGMP on neuronal membrane is described [9].

There are data on inhibitory effect of vinpocetine on type 1 phosphodiesterases, which use cGMP as the substrate [5,10]. It seems that the effects of IBMX on AP and contraction in SMC are mediated via elevation of intracellular cAMP concentration. According to numerous data, this cyclic nucleotide inhibits electrical and contractile activity of ureteral SMC by activation of potassium permeability of its membrane [6,9, 12,14].

Further analysis of the action of cyclic nucleotides on electrical and contractile properties of SMC was performed with penetrating analogs of cAMP and cGMP (Fig. 3). Similar to IBMX, dibutyryl-cAMP (20 μM) inhibited ureteral SMC contraction (Fig. 3, *a*). By contrast, the activating effect of dibutyryl-cGMP (100 μM) on ureteral SMC contraction was similar to the effects of vinpocetine and sodium nitroprusside (Fig. 3, *c*).

It is noteworthy that in contrast to dibutyryl-cGMP, the effects produced by dibutyryl-cAMP depended on its concentration. For example, increasing the concentration of dibutyryl-cAMP to 200 μMm reversed its effect on SMC contraction: an increase of contraction was accompanied by a decrease in the duration of AP plateau (Fig. 3, *b*). In this connection, it can not be excluded that the final effect of the change in the intracellular content of cyclic nucleotides is markedly modulated by the ratio of cAMP and cGMP concentrations. It is possible, that at high concentrations of cAMP, SMC also accumulates cGMP due to substrate inhibition of shared phosphodiesterases pool [4,5].

Therefore, the data obtained attest to a possibility of independent and opposite effects of cGMP and cAMP on excitation-contraction coupling in ureteral SMC within a certain concentration ratios. cAMP shortens AP plateau and inhibits contraction due to activation of potassium permeability of SMC membrane, while cGMP augments contraction of SMC and decreases sodium permeability of its membrane. Activation of ureteral SMC contraction can be related to increased contribution of reticular calcium in generation of SMC contraction.

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