Role of Cyclic Nucleotides in Regulation of Pulmonary Artery Tone in Rabbits

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The involvement of cAMP- and cGMP-dependent signal systems into the regulation of the contractile reaction of smooth muscles of the rabbit pulmonary arteries was studied using a mechanographic method. For modulation of intracellular level of cyclic nucleotides we used adenylate cyclase activators β-adrenoceptor agonist isadrin, guanylate cyclase inhibitor methylene blue, penetrating analog dibutyryl-cAMP, and phosphodiesterase inhibitors. The mechanisms of cAMP- and cGMP-dependent regulation of smooth muscle contractile activity were realized in close interrelationship, and the key component of this was cyclic nucleotide phosphodiesterases. The ratio of activities of phosphodiesterase subtypes in smooth-muscle cells can essentially modulate the adrenergic effects in the pulmonary artery wall and even invert them.

Key Words: *pulmonary arteries; smooth muscles; cAMP; cGMP; phosphodiesterases*

Smooth-muscle cells (SMC) of pulmonary artery (PA) branches are involved in the maintenance of adequate perfusion-ventilation relationships, and therefore investigation of the mechanisms regulating the tone of pulmonary vessels remains an important problem [4-7]. It was shown that contraction developing in response to β-adrenergic effects is an important characteristic of adrenergic regulation of SMC in pulmonary vessels [2]. The mechanism of the effects mediated by β-adrenergic receptors in SMC is traditionally explained by specific features of the signaling systems associated with metabolism of cyclic nucleotides (cAMP and cGMP) [1,3].

We studied the role of cyclic nucleotides in the regulation of contractile activity of smooth-muscle segments of rabbit pulmonary vessels.

MATERIALS AND METHODS

Isolated smooth-muscle segments of lobular and segmentary branches of rabbit pulmonary artery were used. After isolation of the heart and lungs, PA and its branches were isolated and vascular rings (2-3 mm long) were prepared. The endothelium was mechanically removed.

The rings were incubated at 37°C under conditions of constant perfusion with aerated Krebs solution containing (in mM): 120.4 NaCl, 5.9 KCl, 1.2 NaH_2PO_4, 1.2 $MgCl_2$, 2.5 $CaCl_2$, 15.5 $NaHCO_3$, 11.5 glucose, pH 7.30-7.35. Precontracting (KCl, 40 mM) and testing isadrin (1 nM-100 μM), cavinton (1 nM-100 μM), theophylline (1 nM-100 μM), 1-isobutyl 3-methylxanthine (IBMX, 1 nM-100 μM), dipyridamole (1 nM-100 μM), and dibutyryl-cAMP (1-50 μM) solutions were prepared on Krebs solution.

Mechanical tension (MT) of the vascular segments was recorded in a near-isometric mode using a 6MX1B mechanotrons. The effects of test preparations on mechanical tension of smooth-muscle segments were evaluated in percents of the precontraction amplitude, taken as 100%.

The data were processed by methods of variation statistics.

RESULTS

Isadrin in concentrations of 0.1-1.0 μM caused relaxation of intact segments, but in concentrations of 10

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to 100 μM it induced contractions of all segments (Fig. 1), MT increased to 150.2±4.8% (n=12, p<0.05). We found no reports about β-adrenergic contractile reactions of vascular SMC. Pretreatment of the segments with guanylate cyclase blocker methylene blue completely suppressed relaxation of segments, but the contractile component remained unchanged (Fig. 1). Stimulation of β-adrenoceptors in smooth muscles of pulmonary vessels activates adenylate cyclase and increases production of cAMP. cAMP activates cAMP-dependent protein kinase, which potentiates SMC relaxation via phosphorylation of myosin light chain kinase [1,2,7]. The operation and regulatory effects of the cAMP-dependent signaling system probably have some specific features in SMC of pulmonary vessels. This hypothesis is confirmed by the results of experiments with cAMP analog dibutyryl-cAMP penetrating through the membrane; in concentration of 10 μM this agent induced contractions of rabbit PA segments (127.3±3.9%, n=5, p<0.05).

For elucidation of the role of cyclic nucleotides in the regulation of PA tone, we carried out a series of experiments using blockers of various cyclic nucleotide phosphodiesterases (PDE).

In experiment I we studied the effect of IBMX, a low-selective blocker of all PDE types. IBMX in concentrations of 0.1-10.0 μM induced relaxation of PA segments to 58.6±5.8% (n=9, p<0.05; Fig. 2, *a*). Pretreatment of the segments with methylene blue (50 μM) inverted the effect of IBMX: the agent in concentrations of 0.1-1.0 μM induced contractions of PA segments to 152.6±3.5% (n=8, p<0.05, Fig. 2, *a*).

In series II we studied the effects of theophylline, type I PDE blocker, for which cAMP serves as the predominant substrate in the cell. All segments responded by dose-dependent relaxation (by 35.8± 2.7%, n=9, p<0.05) to application of theophylline in concentrations 0.01-10 μM (Fig. 2, *b*). After pretreatment of the rabbit PA segments with methylene blue (50 μM) theophylline in concentrations of 0.1-10 μM induced contraction of vascular segments (the amplitude of contractions reached 82.8±3.8% of the amplitude of K^+-induced contractions; n=7, p<0.05), while higher concentration induced their relaxation (Fig. 2, *b*).

In series III we studied the effects of type V PDE blockers characterized by high affinity to cGMP: dipyridamole and cavinton. Dipyridamole in concentrations 0.01-10 μM induced contractions of PA segments to 136.7±4.3% (n=8, p<0.05) and relaxation of the segments to the initial level at higher concentrations (Fig. 2, *c*). The contractile effect of dipyridamole did not disappear after pretreatment of PA rings with guanylate cyclase blocker methylene blue (50 μM),

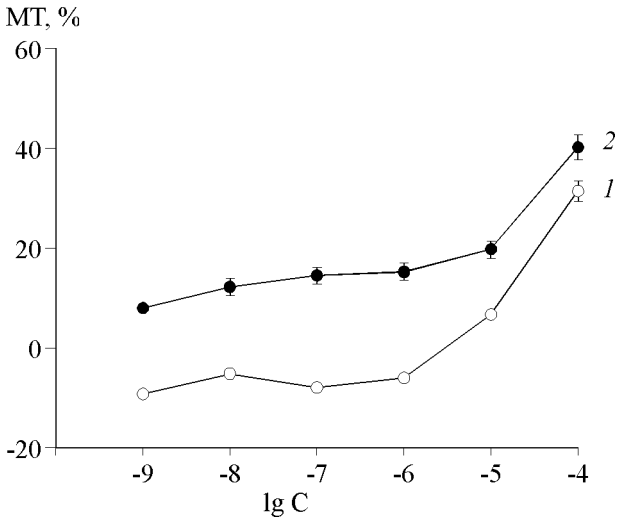

Fig. 1. Relationship between mechanical tension (MT) of the rabbit pulmonary artery smooth-muscle segments and isadrin concentration. Here and in Fig. 2: abscissa: decimal logarithms of the concentration; ordinate: mechanical tension of smooth-muscle segment in percent of amplitude of standard hyperpotassium contraction (40 mM KCl). *1*) intact segments; *2*) segments pretreated with methylene blue (10 μM).

but manifested only at concentrations of 1-20 μM (Fig. 2, *c*). Similar regularities were observed in experiments with cavinton: intact segments responded by 118.7±2.9% contractions (n=7, p<0.05) to cavinton in a concentration of 0.1 μM, while after pretreatment of the segments with methylene blue contractions (113.5±2.2%; n=5, p<0.05) were recorded only at cavinton concentration of 10 μM.

These results suggest that activation of the cAMP-dependent signaling system in smooth muscles of PA treated with isadrin and nonhydrolyzable cAMP analogs can have a contractile effect. Similarly to cardiomyocytes this can be due to cAMP-dependent phosphorylation of calciductin (calcium channel protein), which increases calcium permeability of the membrane [1,2]. This effect can predominate in smooth muscles of pulmonary vessels under certain conditions.

PDE inhibitors cavinton and dipyridamole characterized by high affinity to PDE and hydrolyzing mainly cGMP induced contractions of intact PA segments. Inhibition of guanylate cyclase with methylene blue increased the concentrations of these drugs required for induction of the maximum contractions.

Low-selective PDE inhibitor IBMX and mainly cAMP-hydrolyzing PDE inhibitor theophylline decreased mechanical tension of intact PA segments. Pretreatment with methylene blue inverted the effects of these drugs: contraction was recorded in all cases. This result confirms that in the SMC of pulmonary arteries the mechanisms of cAMP-dependent

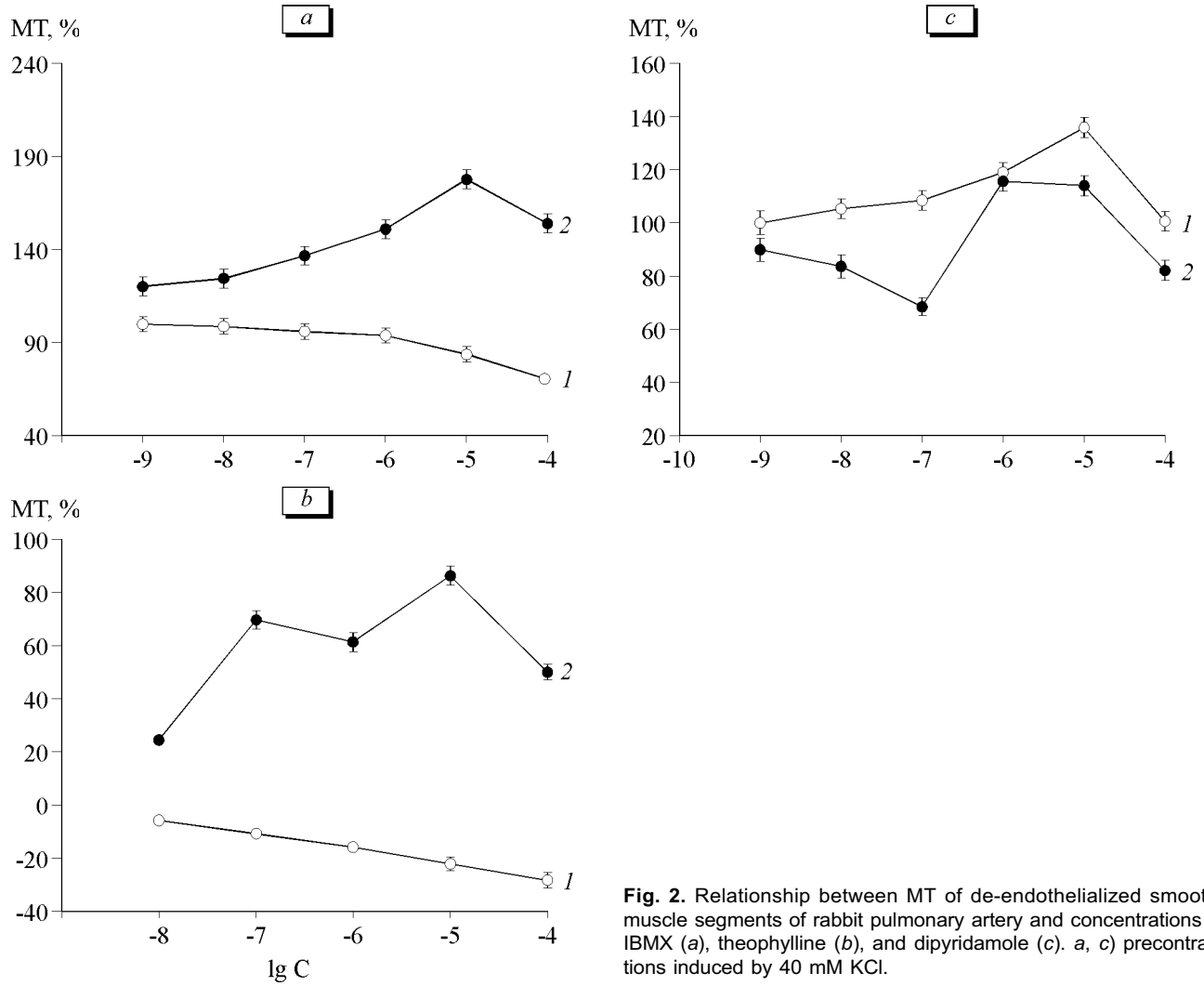

Fig. 2. Relationship between MT of de-endothelialized smooth-muscle segments of rabbit pulmonary artery and concentrations of IBMX (*a*), theophylline (*b*), and dipyridamole (*c*). *a*, *c*) precontractions induced by 40 mM KCl.

system operation mediating the effects of β-adrenoceptor agonists are closely related to activation of guanylate cyclase-cGMP system. Inhibition of guanylate cyclase modulates the realization of cAMP effects in cells.

It seems that the mechanisms of cAMP- and cGMP-dependent regulation of contractile activity of smooth-muscle segments of pulmonary vessels in rabbits are realized in close interactions between these two regulatory systems, the key component of which is cyclic nucleotide PDE. The balance of activities of various subtypes of this enzyme in SMC can modulate the adrenergic effects in PA wall and even cause their inversion.

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