PHYSIOLOGY

Peculiarities of Cholinergic Regulation of Smooth Muscles in Rabbit Pulmonary Arteries

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Cholinergic contractile reactions of smooth muscles in rabbit pulmonary arteries were studied by mechanography. The muscarinic receptor agonist pilocarpine induced biphasic relaxation of mesatone- or potassium-precontracted segments from lobar pulmonary arteries with intact endothelium. The low-threshold endothelium-dependent component of pilocarpine-induced relaxation was suppressed by denudation or nitric oxide synthase blocker L-NAME.

Key Words: *pulmonary arteries; endothelium; nitric oxide*

The mechanisms of regulation of pulmonary vessel tone attract much recent attention [5,7]. Smooth muscle cells (SMC) of pulmonary artery branches are involved in the maintenance of perfusion-ventilation interrelations impaired in bronchial asthma and other respiratory diseases [7]. Previous studies showed that pulmonary and systemic vessels display various physiological reactions [7].

Interrelations between the endothelium and smooth muscles play an important role in the mechanisms of local regulation of SMC tone. Vascular endothelium produces a variety of constrictor and relaxing factors and modulates contractile reactions of SMC to biologically active substances [4,8]. Nitric oxide (NO) plays the major role in the mechanisms of endothelium-dependent regulation. The physiological role of NO in the regulation of vascular tone was extensively studied [3,9].

Here we analyzed the peculiarities of cholinergic regulation of contractile activity of rabbit pulmonary artery SMC and the role of NO in this process.

MATERIALS AND METHODS

Experiments were performed on preparations from lobar branches of rabbit pulmonary arteries. The animals were euthanized by cervical dislocation, the heart and lungs were separated, and 2-3-mm arterial ring were prepared. The endothelium was removed when needed.

The segments were thermostated at 37°C under constant perfusion with aerated Krebs solution containing (in mM): 120.4 NaCl, 5.9 KCl, 1.2 NaH_2PO_4, 1.2 $MgCl_2$, 11.5 glucose, 2.8 $CaCl_2$, and 15.5 $NaHCO_3$ (pH 7.35). Precontracting solutions (40 mM KCl or 1 μM mesatone) and test solutions (0.1 nM-100 μM pilocarpine, 10 μM atropine, and 100 μM N(G)-nitro-L-arginine-methyl ester) were prepared in Krebs solution.

Mechanical strain (MS) of pulmonary artery segments was recorded under near-isometric conditions using 6MKh1B mechanotrons. Vascular relaxation was expressed in percents of the precontraction amplitude. Intact segments served as the control. The results were analyzed by variational statistics.

RESULTS

Pilocarpine induced relaxation of all segments with intact endothelium (Fig. 1). After maximum relaxation, MS were 50.9±2.5 and 51.9±3.3% of the precontraction amplitude in the presence of high-potassium solution and mesatone, respectively.

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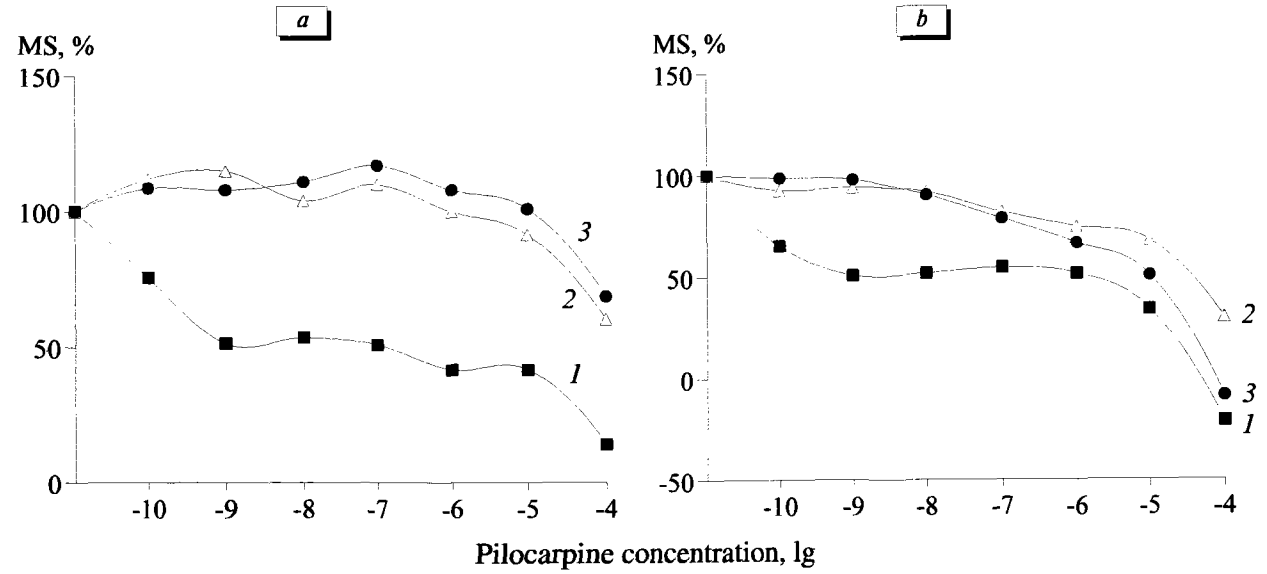

Fig. 1. Mechanical strain (MS) of segments from lobar pulmonary arteries precontracted with KCl (*a*) and mesatone (*b*) as a function of pilocarpine concentration: intact segments (*1*), denuded segments (*2*), and segments with the intact endothelium pretreated with 10 mM L-NAME (*3*).

Pilocarpine caused biphasic (consisting of low- and high-threshold components) relaxation independently on the type of precontraction stimuli. After precontraction caused by high-potassium solution and mesatone, half-maximal concentrations of pilocarpine (EC_{50}) producing low-threshold relaxation were 3.2±0.3 and 4.0±0.2 nM, respectively; high-threshold relaxation was induced by 2.5±0.3 and 6.3±0.4 μM pilocarpine, respectively.

Independently on the type of precontraction-induced stimuli, denudation completely blocked the low-threshold component of the pilocarpine-induced relaxation. However, relaxation caused by high doses of pilocarpine (10-100 μM) was retained.

Pretreatment of intact segments with the NO synthase blocker L-NAME produced similar changes. The segments were pretreated 15 min before precontraction induced by high-potassium Krebs solution or mesatone, and the effects of pilocarpine on MS were studied. L-NAME blocked the low-threshold endothelium-dependent component of pilocarpine-induced relaxation ($p<0.05$), but had no effect on the high-threshold component irrespectively of the type of precontraction stimuli (Fig. 1).

A comparison of these data with the results obtained on denuded segments showed that pretreatment of segments with intact endothelium with the NO synthase blocker simulated the effect of deendothelialization. Pilocarpine (0.1-10 nM) produced similar effects on MS of deendothelialized and intact segments treated with L-NAME.

This pilocarpine-induced biphasic relaxation of intact segments from pulmonary arteries differed from changes in systemic vessels caused by the activation of cholinoceptors. It was shown that pilocarpine produced single-phase relaxation of rat aortic segments (EC_{50}=1.1±0.2 μM), which was completely abolished by mechanical denudation [1,4].

To find out whether the biphasic relaxation in pulmonary arteries was related to the activation of muscarinic receptors, we studied the effects of pilocarpine after pretreatment of segments with the muscarinic receptor blocker atropine.

Pretreatment with 10 μM atropine completely blocked low-threshold, but had no effect on high-threshold components of pilocarpine-induced relaxation of arterial segments. The data indicate that pilocarpine in high doses produces nonspecific effects not related to the activation of muscarinic receptors, but probably associated with cross-reactions of the agonist with other receptors [5].

Hence, cholinergic relaxation of smooth muscles in rabbit pulmonary artery is biphasic and dose-dependent. The low-threshold component of pilocarpine-induced relaxation is endothelium-dependent. Activation of muscarinic-M_1 receptors induces Ca^{2+}-dependent production of endothelium-derived relaxing factor [6]. The effect of NO synthase inhibitors on this process indicates that the synthesis of endothelium-derived relaxing factor is associated with NO metabolism. The high-threshold component of pilocarpine-induced relaxation is myogenic and related to activation of smooth muscle cell receptors insensitive to atropine.

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