Specific Adrenergic Responses of Smooth Muscles in the Vascular Wall of Guinea Pig Pulmonary Arteries during Ovalbumin Sensitization

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The adrenergic contractile responses of smooth muscles in the vascular wall of guinea pig pulmonary arteries were studied during ovalbumin sensitization. Sensitization was followed by inhibition of contractile responses to an α-adrenoceptor agonist mesatone, prevented endothelium-derived relaxation, and potentiated the contractile response to isoproterenol. Administration of a β-adrenoceptor agonist isoproterenol potentiated the increase in mechanical strain of smooth muscles in the pulmonary artery precontracted with high-potassium Krebs solution. Removal of the endothelium had no effect on the contractile response of smooth muscle segments from the pulmonary artery of intact and sensitized guinea pigs to β-adrenergic influences. The contractile responses of smooth muscles of the pulmonary artery are associated with activity of the cAMP-dependent signal system and play a role in the pathogenesis of ventilation-perfusion disturbances during atopic inflammation.

Key Words: *pulmonary arteries; smooth muscles; sensitization; adrenergic contractile responses*

Studying of the mechanisms for regulation of the vascular tone in the pulmonary circulation is still an urgent problem. The pulmonary artery (PA) and arterial branches maintain optimal ventilation-perfusion relationships, which contributes to specific regulation of the vascular tone in this region of the circulatory system. Impairment of the ventilation-perfusion relationships plays an important role in the pathogenesis of bronchial asthma and other diseases due to bronchomotor dystonia [2,4,8].

Here we studied adrenergic contractile responses of smooth muscles in guinea pig PA under conditions of ovalbumin sensitization.

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MATERIALS AND METHODS

Experiments were performed on isolated smooth muscle (SM) segments from the lobular and segmentary branches of guinea pig PA.

Thirty-eight animals of the treatment group were subcutaneously sensitized with 0.25% ovalbumin in physiological saline (0.1 ml per 100 g body weight) to cause bronchospasm. Ovalbumin was injected 3 times at 3-4-day intervals. The animals inhaled aerosol of the same solution on day 21. Inhalation was performed using a Musson-1M ultrasound nebulizer. Control animals (n=36) received physiological saline. The animals were euthanized. The heart and lungs were removed. PA and arterial branches were prepared. Annular arterial segments were isolated (length 2-3 mm). The

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endothelium was mechanically removed when required.

For evaluation of contractile activity, the segments were thermostated under constant perfusion with aerated Krebs solution of 120.4 mM NaCl, 5.9 mM KCl, 1.2 mM NaH_2PO4, 1.2 mM $MgCl_2$, 2.5 mM $CaCl_2$, and 11.5 mM glucose (pH 7.3-7.35) at 37°C. The precontracting (40 mM KCl) and test solutions were prepared on the basis of Krebs solution and contained the corresponding reagents. Krebs solution contained phentolamine, phenylephrine (Russia), isoproterenol, ovalbumin (ICN Biomedicals, Inc.), and forskolin (Sigma). Mechanical strain of segments was recorded under near-isometric conditions using FT10G force transducers.

The effect of the test solutions on mechanical strain of SM segments was estimated relative to the amplitude of precontraction in response to high-potassium Krebs solution (40 mM KCl). The results were analyzed by methods of variational statistics. Intergroup differences in the amplitude of contractile responses were evaluated by nonparametric Mann—Whitney test.

RESULTS

PA segments with intact endothelium from sensitized guinea pigs exhibited contractile response to phenylephrine (1 nM-100 μM). Table 1 shows that the maximum amplitude of contraction in treated animals (48.1±3.8%, n=6) is lower than in controls.

Removal of the endothelium had no effect on the adrenergic contractile response of SM segments from PA of sensitized animals (Table 1). However, this response in intact guinea pigs was endothelium-dependent. After removal of the endothelium, the maximum amplitude of contractions for segments from control animals (171.8±6.6%, n=6) was higher compared to segments with intact endothelium (71.5±7.8%, n=7, p<0.05).

Sensitization probably inhibits the α-adrenergic contractile response of PA smooth muscles and abolishes endothelium-derived relaxation.

After precontraction of SM segments from PA of control in high-potassium Krebs solution (40 mM KCl), isoproterenol in concentrations of 0.01-100 μM caused contraction of segments with intact

TABLE 1. Dependence of Mechanical Strain of SM Segments from Guinea Pig PA on the Concentrations of Phenylephrine, Isoproterenol, and Forskolin. Mechanical Strain is Expressed Relative to the Amplitude of the Response to High-Potassium Krebs Solution ($X \pm s_1$, %)

Parameter	Concentration, lg C					
	-9	-8	-7	-6	-5	-4
Phenylephrine						
intact rats						
segments with endothelium	11.2±4.1	11.2±3.4	12.9±5.1	48.5±1.8	68.1±6.1	71.5±7.8
segments without endothelium	0.9±1.1*	-2.5±5.6*	26.9±4.7*	151.5±3.5*	166.3±4.9*	171.8±6.6*
sensitized animals						
segments with endothelium	1.4±2.5	4.7±0.9+	6.1±1.6+	19.9±3.4+	39.9±3.1+	48.1±3.8+
segments without endothelium	8.6±3.1	9.5±4.2+	19.7±1.8+	41.1±3.3+	54.9±1.7+	57.3±3.4+
Isoproterenol after KCl treatment						
intact animals						
segments with endothelium	110.7±4.2	108.2±1.1	113.4±2.1	116.1±2.0	121.3±4.3	115.0±8.1
segments without endothelium	106.2±1.7	113.8±2.0	117.0±1.9	118.3±2.8	118.8±4.1	141.6±6.5
sensitized animals						
segments with endothelium	103.8±2.4	107.3±5.1	112.7±2.3	121.1±1.8	127.6±0.9	130.0±6.0+
segments without endothelium	102.7±2.0	111.0±1.4	121.7±1.9	127.1±1.4	131.7±4.2	144.6±8.9*
Forskolin after KCl treatment						
intact animals						
segments without endothelium	—	97.6±3.3	118.2±3.1	146.9±6.2	—	—
sensitized animals						
segments without endothelium	—	94.8±3.5	123.5±3.6	147.6±4.1	—	—

Note. KCl (40 mM), 100%. p<0.05: *compared to intact endothelium; +compared to intact animals.

endothelium (Table 1). Removal of the endothelium was followed by an increase in the maximum amplitude of contraction from 115.0±8.1 (n=6) to 141.6±6.5% (n=8, p<0.05). Pretreatment of segments with α-adrenoceptor antagonist phentolamine (5 μM) had no effect on isoproterenol-induced contraction.

Precontracted SM segments with intact endothelium from sensitized animals exhibited a contractile response to isoproterenol in concentrations of 0.01-100 μM (Table 1). The maximum amplitude of contraction (130.0±6.0%, n=7) was much higher compared to the control. The maximum amplitude of contraction in response to 100 μM isoproterenol increased to 144.6±8.9% after removal of the endothelium (n=5, p<0.05).

A specific feature of adrenergic regulation of PA smooth muscles (as compared to vessels of the systemic circulation) is the β-adrenergic contractile response. This response is inhibited by the endothelium, but increases after sensitization of animals with ovalbumin.

Published data show that the effects of β-adrenergic agonists on vascular smooth muscles are associated with activation of adenylate cyclase (AC) and increase in intracellular cyclic AMP (cAMP) concentration [3,4]. Experiments with AC-activating agent forskolin confirmed the cAMP-dependent type of contractile responses in SM segments from PA of intact and sensitized animals (Table 1). Our results indicate that the contractile response of PA segments to β-adrenoceptor agonists is related to activity of the cAMP-dependent signal system in PA smooth muscles.

Sensitization and provocative inhalation of ovalbumin serve as a model of atopic inflammation. Atopic sensitization is followed by infiltration of the mucosal and submucosal membrane in guinea pig bronchi with eosinophils and basophils. Eosinophil-derived cytokines (particularly interleukin-5) cause functional reconstruction of bronchial smooth muscles, which results in bronchial hyperactivity and bronchospasm [3,9].

Our results indicate that sensitization of guinea pigs decreased the α-adrenergic contractile response and potentiated the β-adrenergic contractile response of PA smooth muscles. Sensitization partially (isoproterenol) or completely (phenylephrine) abolishes endothelium-derived relaxation.

We conclude that sensitization modulates the adrenergic contractile response of PA smooth muscles. The decrease in the contractile response to α-adrenoceptor agonists is probably associated with reduced expression of adrenoceptors on the membrane of SM cells or desensitization of these cells [4,5,7]. The inhibition of endothelium-derived relaxation to adrenergic contractile responses can be related to endothelial damage or functional insufficiency during inflammation. The impairment of endothelium-derived relaxation was observed in experimental and clinical trials [1,3,6,9].

The contractile response to β-adrenergic influences is a specific feature of PA smooth muscles. It may be associated with the processes that are initiated by protein kinases of smooth muscles in response to an increase in intracellular cyclic nucleotide concentration. These changes result in an increase in intracellular calcium concentration or high sensitivity of the contractile apparatus in SM cells. Ovalbumin sensitization is followed by an increase in the contractile response of SM segments from PA to isoproterenol.

The adrenergic contractile response of smooth muscles from the vascular wall of guinea pig PA probably plays a compensatory role during ovalbumin sensitization. The sensitivity of bronchial smooth muscles to adrenergic influences decreases during atopic inflammation and bronchospasm and is accompanied by the reduction of adrenergic contractile responses in PA smooth muscles [9]. These changes probably maintain the optimal ventilation-perfusion relationships.

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