Dilatation Reserve of Pulmonary Arteries at Stages of the Chronic Obstructive Pulmonary Disease Model

INTRODUCTION

Vascular abnormalities accounting for the development of severe complications (pulmonary hypertension, chronic cor pulmonale) of chronic obstructive pulmonary disease (COPD) play an important role in the COPD pathogenesis [1, 2]. A chain of changes, leading to pulmonary hypertension, starts with endothelium functional impairment at the early disease stages: this is manifested by an imbalance in vasoactive agents released by the endothelium, reduction in the production of endothelial relaxing factors (nitrogen oxide (NO), prostacyclin, endothelium-derived hyperpolarizing factor), and synthesis enhancement of substances possessing vasoconstrictive effects (such as endothelin-1, angiotensin II, serotonin, thromboxane A2, and superoxidated anions) [3-5]. Vasoconstriction contributes to the remodeling of pulmonary arteries, which is characterized by the proliferation of poorly differentiated smooth muscle cells and deposition of elastic and collagen fibers [5, 6].

Medications used in compliance with the current standards of COPD care, such as corticosteroids, bronchodilators, and vasodilators, do not enable a persistent disease control and prevention of the development and progression of life-threatening conditions. At present, it remains unclear to what degree the defective vascular responsiveness promotes COPD generation and progression and in what way the development of this pathologic state influences smooth muscle vascular wall dysfunction.

The objective of the study was to assess the state of pulmonary vascular mediator systems in the process of staged generation of the COPD experimental model.

OBJECTIVES: To assess the state of pulmonary vascular mediator systems during the stepwise formation of the chronic obstructive pulmonary disease (COPD) model.

MATERIALS AND METHODS: The COPD model was induced in rats by nitrogen dioxide (NO2) inhalation for 60 days. At different stages of COPD (15, 30, and 60 days), the effect of reagents-vasodilators (β-adrenoceptor agonist isoproterenol, nitric oxide donor nitrosorbide, acetylcholine, activator of C-fibers capsaicin, corticosteroid beclometasone) on the isolated pulmonary arteries (diameter <0.5 mm) was studied. Vascular reactivity was assessed by determining isometric contraction (tension in milligrams) of arterial rings by using an electromechanical transducer.

RESULTS: All vasodilators dose-dependently decreased the vascular tone of pulmonary arteries isolated from intact rats. After 15 days of NO2 exposure, dilatation effect of low doses of vasodilators did not differ from that of intact specimens. The functional state of the adrenergic system deteriorated faster than that of the nonadrenergic noncholinergic system as reflected by the weakening of the isoproterenol relaxation effect. On prolonged NO2 exposure, pulmonary arteries responded to the impact of all vasodilators with smaller relaxation. Dose dependence of the dilatation reaction disappeared for isoproterenol, capsaicin, beclometasone, and was less expressed for nitrosorbide and acetylcholine after 60 days of exposure.

CONCLUSION: In the course of COPD model generation, the functioning of almost all neurotransmitter systems of pulmonary artery wall was negatively affected. This led to a decrease in the influence of vasodilators on pulmonary artery vascular tone and could facilitate the development of pulmonary hypertension, which is typical of COPD.

KEYWORDS: Chronic obstructive pulmonary disease, nitrogen dioxide, pulmonary arteries, smooth muscle, vasodilatation

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MATERIALS AND METHODS

The ethics committee for experimental animal studies of Pavlov University approved the study (number: 19734826/211). Experiments were performed in 34 male Wistar rats weighing 150-170 g. All procedures and experiments were carried out in accordance with the internationally accepted guidelines for the care and use of laboratory animals [7]. Rats were housed in cages (250 cm²/rat) with free access to drinking water and standard lab rodent chow under conditions of 20-22°C air temperature and 55%-60% humidity.

The experimental model of COPD formation was based on nitrogen dioxide (NO₂) exposure [8]. Rats (n=27) were placed in a chamber mounted into the exhaust hood and connected with a NO₂-generating laboratory device. A mixture of nitrogen oxides was produced in a chemical reaction between sodium nitrite and sulfuric acid and pumped into the exposure chamber provided with an outlet tube. Colorless NO reacted with atmospheric oxygen and was converted to the more stable yellow-brown NO₂. NO₂ concentration in the chamber was 30-40 mg/m³ (15-19 ppm) as determined by the colorimetric method. Rats were exposed to NO₂ in an intermittent regime: three 30-min exposures/day with 30-min intervals for 15, 30, or 60 days. Euthanasia was carried out with cervical dislocation after 15 (n=9), 30 (n=9), and 60 (n=9) days of NO₂ exposure. Seven rats were not exposed to NO₂ representing the intact group.

Contractile activity of pulmonary artery smooth muscles was evaluated in vitro. Four samples in the form of rings (3-4 mm wide) were isolated from pulmonary arteries (0.5 mm in diameter) for each animal. These samples were placed into a thermostatic flow bath (volume of 2.5 mL) perfused with the Krebs-Henseleit solution with the following composition (mM): NaCl 118.0, KCl 4.8, CaCl₂ 2.5, MgSO₄ 1.2, NaHCO₃ 11.9, KH₂PO₄ 1.2, and glucose 11.0 (pH 7.4) by a peristaltic pump (Zampl, Poland) with a flow rate 0.6 mL/min. The solution was aerated with air by a microcompressor (MKM-7, Praktik-NC, Russia). To obtain pH 7.4, the sodium bicarbonate concentration was reduced to 11.9 mM [9]. The temperature was maintained at 37-37.5°C with the aid of an ultrathermostat (U10, Medingen, GmbH, Germany). One side of the vessel ring was fastened to the bath bottom with tungsten needles, the opposite side was connected with an electromechanical transducer (SPA “Introtest”, Russia). For arterial muscle tension of 500 mg, arterial rings were exposed for 60 min to establish a balance. To increase the original tone, the agonist of α-adrenergic receptors phenylephrine (5 µg/mL) was perfused via the bath containing arterial samples starting 5 min before the experiment and continuing through the experiment. The changes in isometric contraction of arterial samples (expressed as tension force in milligrams) induced by electrical stimulation were evaluated. Smooth muscle tension converted into an electrical signal was fed to an analog-to-digital converter (L-CARD 14-140, Russia) and recorded on a computer.

To assess vascular responsiveness, reagents-vasodilators (1.0 mL, at the rate of 1 mL/min) were added to the bath containing the perfusate. The substances were presented with increasing concentration. Each successive substance concentration was administered into the bath after vascular tone had been stabilized. Following the use of each substance, arterial rings were washed with a saline solution with phenylephrine for 15 min. The following substances were used: β-adrenoceptor agonist isoproterenol (0.1-10 µg); nitrosorbide (0.1-100 µg)-nitric oxide donor, the mediator of the nonadrenergic noncholinergic (NANC) inhibitory system; acetylcholine (1-1,000 µg), the mediator of the cholinergic system; capsaicin, the activator of C-fibers (1-100 µg); and glucocorticosteroid beclometasone (0.1-10 µg).

Statistical Analysis

Statistical evaluation was performed using Microsoft Excel 7.0 software including calculations of mean values for maximal contraction amplitudes of arterial smooth muscles and the SEM. Comparison of mean values was performed using Student’s t-test, and pairwise comparison methods. A value of p<0.05 was considered significant.

RESULTS

Stimulation of pulmonary artery preparations of intact rats with the agonist of α-adrenergic receptors phenylephrine resulted in vascular tone increasing by 83.9±4.1 mg. The same increment in muscle contraction caused by phenylephrine was demonstrated by the pulmonary arterial rings of rats that were exposed to NO₂ for 15 and 30 days. After 60 days of NO₂ exposure, the effect of phenylephrine was less pronounced (70.9±1.8 mg, p<0.05). In the presence of enhanced tone caused by phenylephrine, the changes in the pulmonary arterial dilator activity in response to exposure to vasodilator agents interacting with different pulmonary vascular mediator systems were assessed.

In the intact group, all the applied pharmacological agents decreased initial smooth muscle tone of isolated pulmonary artery samples in a dose-dependent manner (Figure 1). Arteries demonstrated the greatest susceptibility to β-adrenoceptor agonist isoproterenol: a dose of 0.1 µg caused smooth muscle relaxation of up to 43.1±2.6 mg (p<0.05). Capsaicin, beclometasone, and nitrosorbide showed a similar dilatory effect on pulmonary arterial smooth muscle with a dose 10 times greater than that of isoproterenol, while acetylcholine showed a similar dilatory effect with the dose 100 times more than the dose of isoproterenol. When dilatory effect of both isoproterenol and the mediator donor of the NANC inhibitory system, nitrosorbide, was compared, it became evident that isoproterenol in the...
Effect of vasodilators on the tone of pulmonary arteries

Following 15 days of NO₂ exposure, the dilatation effect of vasodilators used in low doses indeed did not differ from that of intact specimens (Figure 2). A 0.1-μg dose of beclometasone caused greater relaxation in pulmonary arterial smooth muscle: -44.8±2.3 mg (-30.3±1.8 mg in the intact group, p<0.01). The effect of larger doses of beclometasone did not significantly differ from the effect seen in pulmonary arterial specimens in the intact group. Dilatation effect of capsaicin and acetylcholine (in all doses used) showed a tendency to decrease, while simultaneously not demonstrating significant differences from the effect manifested in specimens from intact rats. The relaxation of pulmonary arterial smooth muscles caused by isoproterenol at doses of 1.0 and 10 μg (-50.2±2.9 and -50.8±2.9 mg, respectively) was significantly less than that in the intact group (-60.6±2.9 and -65.3±2.3 mg, respectively, p<0.05) (Figure 2). The same change in smooth muscle contractile force was seen in response to nitrosorbide exposure at a dose of 100 μg (-64.8±3.6 mg vs. -72.8±2.9 mg in the intact group, p<0.05). Following 15 days of NO₂ exposure, the dilator reaction of pulmonary arteries in response to isoproterenol addition to the perfusate deteriorated faster than that under the action of nitrosorbide. Thus, when the isoproterenol dose increased from 0.1 to 10 μg, the pulmonary arterial smooth muscle contractile force decreased by 6.9 mg (by 22.7 mg in the intact group, p<0.05). When nitrosorbide was added to the perfusate in a similarly increasing dose range, the pulmonary arterial contractile amplitude decreased by 24.1 mg (by 34.1 mg in the intact group, p<0.05). It can be assumed that the functional state of the mediator adrenergic system deteriorated more rapidly than that of the inhibitory NANC system, which was manifested in the weakening of the relaxation effect of isoproterenol. The dilatation effect of capsaicin and acetylcholine (at all doses used) showed a tendency to decrease but did not significantly differ from that in the intact group.

Prolongation of NO₂ exposure up to 30 and 60 days was accompanied by a further decrease in pulmonary arterial smooth muscle relaxation caused by vasodilator agents. Following 60 days of exposure, the greatest changes were observed in response to the administration of high doses of reagents-vasodilators into the perfusate (Figure 3). In the case of isoproterenol, capsaicin, and beclometasone, the dose dependence of pulmonary arterial relaxation was negligible. When adding nitrosorbide and acetylcholine to the perfusate, the dose-dependent character of the response remained unchanged but was less pronounced than that in the case of pulmonary arterial specimens of intact rats or after 15 days of NO₂ exposure (Figure 3). In the intact group, the amplitude of pulmonary arterial smooth muscle contraction caused by nitrosorbide (in the dose range of 1.0-100 μg) decreased by 27.9 mg; following 60 days of NO₂ exposure decreased, it only by 11 mg (p<0.05). The dilator response of pulmonary arteries to acetylcholine significantly differed from that of intact specimens only after 60 days of NO₂ exposure and with a dose of 1,000 μg (-42.4±4.4 mg versus -56.2±3.7 mg for intact specimens, p<0.05).

DISCUSSION

In the process of experimental COPD pattern generation, the functioning of virtually all pulmonary artery wall mediator
systems was impaired, though the intensity and rate of manifestation of these impairments varied. The number and/or sensitivity of pulmonary arterial α-adrenergic receptors significantly decreased only following 60 days of NO\textsubscript{2} exposure as evidenced by pulmonary arterial tone decrease under the action of phenylephrine. The decrease in the number of β-adrenoceptors was noted from 15 days of NO\textsubscript{2} exposure and lasted up to 60 days of NO\textsubscript{2} exposure. Their functioning mostly remained unchanged. In response to the administration of 0.1 µg isoproterenol into the perfusate, the amplitude of pulmonary arterial smooth muscle relaxation was 43.1±2.6 mg and 39.4±3.0 mg, respectively, after 15 and 60 days of NO\textsubscript{2} exposure (-42.6±2.5 mg for intact specimens, p>0.05). In addition, pulmonary arterial specimens of COPD rats (after 60 days of NO\textsubscript{2} exposure) did not demonstrate dose dependency in the isoproterenol dilatation effect. The amplitudes of pulmonary arterial wall relaxation for isoproterenol at doses of 0.1 and 10 µg were -39.4±3.0 and -41.2±3.2 mg, respectively, (p=0.05), while in the intact group, the difference in the dilatation effect for these doses was 22.7 mg.

A decrease in the phenylephrine vasoactive effect was observed only after 60 days of NO\textsubscript{2} exposure. Vasoconstriction in response to administration of the α-adrenoceptor agonist phenylephrine is associated with the influx of extracellular Ca\textsuperscript{2+} and its exit from intracellular stores [10]. The removal of calcium ions from the extracellular medium or the blockade of Ca channels restricts the influx of Ca\textsuperscript{2+} ions into cells [11]. It is supposed that the increase in pulmonary arterial tone under the influence of the α-adrenergic receptor agonist phenylephrine, seen in the intact group and following 15 and 30 days of NO\textsubscript{2} exposure, was mediated by the interaction of Ca channels with the smooth muscle cell sarcolemma or the release of Ca from intracellular depots.

The vasodilator effect mediated by afferent capsaicin-sensitive C-fibers is caused by the release of biologic active substances: P-substance and calcitonin gene-related peptide. These substances along with acetylcholine promote the discharge of NO from endotheliocytes [12]. Previous studies have demonstrated that during COPD generation under the influence of chronic hypoxia, C-fiber inactivation occurs [13] accompanied by a decrease in neurotransmitter emission, which triggers the mechanism of vascular smooth muscle relaxation. Considering that the dilatation effect of capsaicin and acetylcholine on COPD rat pulmonary arteries significantly differed from that of nitrosorbide, it can be assumed that under the influence of an oxidant pollutant (NO\textsubscript{2}), the synthesis/release of mediators may be compromised, but not the function of NO receptors. This has been confirmed in the literature: in the case of chronic hypoxia, NO-dependent vasodilatation decreased owing to reduced expression or increased deterioration of soluble guanylate cyclase, and this consequently decreased the production of cyclic guanosine monophosphate that serves as a signaling molecule for smooth muscle relaxation [14]. A decrease in NO discharge, associated with endothelium function deterioration, is observed at the earliest COPD stage, as one of the causes of vasoconstriction and triggering of pulmonary arterial wall remodeling, followed by the development of pulmonary hypertension and cor pulmonale [5].

The administration of low doses of beclometasone at early stages of COPD model generation enhanced pulmonary arterial smooth muscle relaxation. A similar effect was seen in pulmonary vessels of patients with mild asthma by using low doses (0.6 µg) of R-albuterol owing to the restoration of β\textsubscript{2}-adrenoceptor vasodilator function [15]. The increase in dilatation properties of prednisolone, revealed in the rat bronchial specimens following 15 days of NO\textsubscript{2} exposure, is accounted for by a decrease in the release of tachykinins from C-fiber terminals under the action of corticosteroids [16]. Some authors associate the decrease in the glucocorticosteroid dilatation effect with changes in noradrenergic transmission and increased influence on vascular α-adrenoceptors [17].

In conclusion, the present study demonstrated the dynamic pattern of receptor structure damage involved in pulmonary arterial vascular tone formation during the development of an experimental COPD model. The functional impairment of virtually all ergic systems of the pulmonary arterial wall was noted, which resulted in the decreased effect of vasodilators on vascular tone. As a pathologic state developed, the dilatation effect of agents varied irregularly, which could explain why certain medications efficiently influence vascular tone at early COPD stages (glucocorticosteroids, β-adrenoceptors agonists. NO donors), while others cause vasodilation both at early and late disease stages (NO donors); this could be taken into account in clinical practice.

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