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Title: DILATATION RESERVE OF PULMONARY ARTERIES AT STAGES OF THE CHRONIC OBSTRUCTIVE PULMONARY DISEASE MODEL

Short Title: Pulmonary arteries and COPD

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ABSTRACT

OBJECTIVE: to assess the state of pulmonary vascular mediator systems during the stepwise formation of COPD model.

MATERIAL AND METHODS: Model of COPD was induced in rats by nitrogen dioxide inhalation for 60 days. At the stages of COPD (15, 30 and 60 days) the effect of reagents-vasodilators (β-adrenoreceptor agonist izoproterenol, nitric oxide donor nitrosorbid, 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 mg) of arterial rings using electromechanical transducer.

RESULTS: All vasodilators dose-dependently decreased vascular tone of pulmonary arteries isolated from intact rats. After a 15-day of nitrogen dioxide exposure dilatation effect of low doses of vasodilators do not differ from the intact specimens. Functional state of the adrenergic system has deteriorated faster than non-adrenergic non-cholinergic system as reflected in the weakening of the izoproterenol relaxation effect. As prolongation nitrogen dioxide exposure pulmonary arteries responded to the impact of all vasodilators with smaller relaxation. Dose-dependence of the dilatation reaction disappeared for izoproterenol, capsaicin, beclometasone and was less expressed for nitrosorbid and acetylcholine after 60 day of exposure.

CONCLUSION: In the course of forming the COPD model generation the functioning of almost all neurotransmitter systems of pulmonary artery wall was broken. 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, pulmonary arteries, smooth muscle, nitrogen dioxide, vasodilatation.

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 functioning impairment at the early disease stages, this is manifested by imbalance in vasoactive agents released by endothelium: reduction in endothelial relaxing factors production (nitrogen oxide, prostacyclin, endothelium-derived hyperpolarizing factor) and synthesis enhancement of substances possessing vasoconstrictive effect (endothelin-1, angiotensin II, serotonin, thromboxane A2, superoxidated anions etc.) [3, 4, 5]. Vasoconstriction contributes to the process of pulmonary arteries remodeling, which is characterized by proliferation of poorly differentiated smooth muscle cells and elastic and collagen fibers deposition [5, 6].

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

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

MATERIAL AND METHODS

The ethics committee of experimental animal studies of xxxx University approved the study. Experiments were performed in thirty-four 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 cm^2/rat) with free access to drinking water and standard lab rodent chow under the conditions of 20-22 °C and 55-60 % air temperature and humidity respectively.

An experimental model of COPD formation was based on nitrogen dioxide (NO_2) exposure [8]. Rats (n=27) were placed in a chamber mounted into the exhaust hood and connected with NO_2-generating laboratory device. A mixture of nitrogen oxides was produced in a chemical reaction between sodium nitrite and sulfuric acid, then being pumped into the exposure chamber provided with an outlet tube. Colorless nitrogen oxide (NO) reacted with air's oxygen and converted to more stable yellow-brown NO_2. Nitrogen dioxide concentration in chamber was equal to 30-40 mg/m^3 (15-19 ppm) as determined by colorimetric method. Rats were exposed to NO_2 in the intermittent regime: three 30-minute exposures/day with 30 min intervals for 15, 30 or 60 days. Euthanasia was carried out with cervical dislocation after 15 days (n=9), 30 (n=9) and 60 (n=9) days of NO_2 exposure. Seven rats were not exposed to NO_2, 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 thermostatic flow bath (volume of 2.5 ml) perfused with Krebs−Henseleit solution with the following composition (mM): NaCl 118.0, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 11.9, KH_2PO_4 1.2 and glucose 11.0 (pH 7.4) by peristaltic pump (Zampl, Poland) with flow rate 0.6 ml/min. The solution was aerated with air by microcompressor MKM-7 (Practik-NC, Russia). To obtain of pH 7.4, the sodium bicarbonate concentration was reduced to 11.9 mM (9). The temperature was kept at 37-37.5 °C with the aid of 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 the 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 mcg/ml) was perfused via 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 mg) induced by electrical stimulation were evaluated. Smooth muscle tension converted into electrical signal was fed to 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 the 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-adrenoreceptor agonist isoproterenol (0.1-10 mcg); nitrosorbide (0.1-100 mcg) – nitric oxide donor, the mediator of non-adrenergic non-cholinergic (NANC) inhibitory system; acetylcholine (1-1000 mcg) – the mediator of cholinergic system; capsaicin – the activator of C-fibers (1-100 mcg), and glucocorticosteroid beclometasone ((0.1-10 mcg).

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 S.E.M. Comparison of mean values was performed by 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 increase by 83.9±4.1 mg. The same increment in muscle contraction caused by phenylephrine was demonstrated by pulmonary arterial rings of rats that were exposed to NO_2 for 15 and 30 days. After 60 day NO_2 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 dose-dependently decreased initial smooth muscle tone of isolated pulmonary artery samples (Fig. 1). Arteries demonstrated the greatest susceptibility to β-adrenoreceptor agonist isoproterenol: in the dose of 0.1 mcg it caused smooth muscle relaxation up to 43.1±2.6 mg ($p<0.05$). Capsaicin, beclometasone and nitrosorbide showed similar dilatory effect on pulmonary arterial smooth muscle in the dose more than 10 times exceeding the dose of isoproterenol, while acetylcholine – in the dose more than 100 times exceeding the dose of isoproterenol. When dilatory effect of both isoproterenol and the mediator donor of NANC inhibitory system nitrosorbide was compared, it became evident that in the dose range 1.0-10 mcg isoproterenol increased relaxation only by 4.7 mg, whereas nitrosorbide – by 17.7 mg ($p<0.05$). That is effect of isoproterenol in the dose of

10 mcg approached its maximum value, while dilatation effect of nitrosorbide in the dose range 10-100 mcg kept growing up to 72.8±2.2 mg (Fig. 1).

Following 15 days of NO_2 exposure dilatation effect of vasodilators used in low doses indeed did not differ from that of intact specimens (Fig. 2). It was beclometasone in the dose of 0.1 mcg that caused greater relaxation in pulmonary arterial smooth muscle: -44.8±2.3 mg (in the intact group -30.3±1.8 mg, $p<0.01$). The effect of greater doses of beclometasone did not significantly differ from the effect seen in pulmonary arterial specimens in the group of intact animals. Dilatation effect of capsaicin and acetylcholine (in all doses used) showed the tendency to decrease, at the same time not demonstrating significant difference from the effect manifested on the specimens of intact rats. Relaxation of pulmonary arterial smooth muscles caused by isoproterenol in the doses of 1.0 and 10 mcg (-50.2±2.9 and -50.8±2.9 mg respectively) was significantly less than in the intact group (-60.6±2.9 and -65.3±2.3 mg respectively, $p<0.05$) (Fig. 2). The same change in smooth muscle contractile force was seen in response to nitrosorbide exposure in the dose of 100 mcg (-64.8±3.6 versus -72.8±2.9 mg in the group of intact rats, $p<0.05$). Following 15 days of NO_2 exposure, dilator reaction of pulmonary arteries in response to isoproterenol addition into the perfusate deteriorated faster than under the action of nitrosorbide. Thus, when the isoproterenol dose increased from 0.1 up to 10 mcg, pulmonary arterial smooth muscle contractile force decreased by 6.9 mg (in intact rat's specimens by 22.7 mg, $p<0.05$). When nitrosorbide in this increasing dose range was added to the perfusate the pulmonary arterial contractile amplitude decreased by 24.1 mg (in intact rat's specimens by 34.1 mg, $p<0.05$). It can be assumed that the functional state of the mediator adrenergic system deteriorated more rapidly than the inhibitory NANC system, which was manifested in the weakening of the relaxation effect of isoproterenol. Dilatation effect of capsaicin and acetylcholine (at all doses used) showed a tendency to decrease, but did not significantly differ from that of intact rats group.

Prolongation of NO_2 exposure up to 30 and 60 days was accompanied by further decrease in pulmonary arterial smooth muscle relaxation caused by vasodilator agents. Following 60 day exposure the greatest changes were observed in response to the administration of high doses of reagents-vasodilators into the perfusate (Fig. 3). In the case of isoproterenol, capsaicin, and beclometasone the dependence of pulmonary arterial relaxation on the dose was negligible. When adding nitrosorbide and acetylcholine to the perfusate dose-dependent character of the response remained unchanged, but was less pronounced than in the case of pulmonary arterial specimens of intact rats or after 15 days of NO_2 exposure (Fig. 3). In the intact group the amplitude of pulmonary arterial smooth muscle contraction caused by nitrosorbide (in the dose range between 1.0 and 100 mcg) decreased by 27.9 mg, and following 60 day NO_2 exposure – only by 11 mg ($p<0.05$). Dilator response of pulmonary arteries to acetylcholine significantly differed from that of intact specimens only after 60 day NO_2 exposure and in the dose of 1000 mcg (-42,4±4.4 mg versus -56.2 ±3.7 mg for intact specimens, $p<0.05$).

DISCUSSION

Thus, 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 sensibility of pulmonary arterial α-adrenergic receptors significantly decreased only following 60 day NO_2 exposure as evidenced by pulmonary arterial tone decrease under the action of phenylephrine. The decrease of β-adrenoreceptors number was noted from the 15 day of NO_2 exposure and lasted up to the 60 day. Their sensibility mostly remained unchanged. In response to the administration of 0.1 mcg isoproterenol into 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 day NO_2 exposure (-42,6±2.5 mg for intact specimens, $p>0.05$). In addition, pulmonary arterial specimens of COPD rats (after 60 day NO_2 exposure) did not demonstrate dose-dependent character of isoproterenol dilatation effect. The amplitudes of pulmonary arterial wall relaxation for isoproterenol doses 0.1 and 10 mcg were -39.4±3.0 and -41.2±3.2 mg respectively ($p>0.05$), while in the intact group the difference in dilatation effect for these doses was 22.7 mg.

Decrease of phenylephrine vasoactive effect was observed only after 60 days of NO_2 exposure. Vasoconstriction in response to administration of α-adrenoreceptor agonist phenylephrine is associated with the influx of extracellular Ca^{2+} and its exit from intracellular stores [10]. Removal of calcium ions from extracellular medium or blockade Ca channel blocks the influx of Ca ions into cells [11]. It is supposed that pulmonary arterial tone increase under the influence of α-adrenergic receptors agonist, phenylephrine, seen in the intact group and following 15- and 30 day NO_2 exposure, was mediated by the interaction with Ca channels of smooth muscle cell sarcolemma or Ca release from intracellular depot.

Vasodilator effect mediated by afferent capsaicin-sensitive C-fibers, is caused by biologic active substances release – P-substance and calcitonin gene-related peptide. These substances and acetylcholine as well, promote discharge of nitrogen oxide from endotheliocytes [12]. Previous studies demonstrated that in the process of COPD generation under the influence of prolonged NO_2 inhalation C-fibers inactivation occurs [13], that is accompanied by decrease in neurotransmitters emission, triggering the mechanism of vascular smooth muscle relaxation. Considering that capsaicin and acetylcholine dilatation effect on COPD rats' pulmonary arteries significantly differed from nitrosorbide effect, it can be assumed that under the influence of oxidant pollutant (NO_2) the synthesis/release of mediators mostly get compromised, but not function of NO-receptors. This finds confirmation in the literature: in the case of chronic hypoxia NO-dependent vasodilation decreased due to reduced expression or increased deterioration of soluble guanylate cyclase, and consequently, decreased production of cyclic guanosine monophosphate that serves as a signaling molecule for smooth muscle relaxation [14]. Decrease in nitrogen oxide discharge, associated with endothelium function deterioration, is observed at the earliest COPD stage being one of the causes of vasoconstriction and triggering of pulmonary arterial wall remodeling followed by development pulmonary hypertension and *cor pulmonale* [5].

The administration of low doses of beclometasone at early stages COPD model generation enhanced pulmonary arterial smooth muscle relaxation. Similar effect was seen on pulmonary vessels in patients with mild asthma using low doses (0.6 mcg) of R-albuterol due to restoration of β_2-adrenoreceptors vasodilator function [15]. Increase of prednisolone dilation properties, revealed in rats' bronchial specimens following 15 day NO_2 exposure, is accounted for decreased release of tachykinins from C-fiber terminals under the action of corticosteroid [16]. Some authors associate the decrease of glucocorticosteroid dilation effect with changes in noradrenergic transmission and increased influence on vascular α-adrenoreceptors [17].

Conclusion. The present study demonstrated the dynamic pattern of receptor structures damage involved in pulmonary arterial vascular tone formation during the development of experimental COPD pattern. Functional impairment of virtually all ergic systems of pulmonary arterial wall was found, which resulted in decreased effect of vasodilators on vascular tone. As pathologic state developed, dilatation effect of agents varied irregularly which to some extent explains why some medications efficiently influence on vascular tone at early COPD stages (glucocorticosteroids, β-adrenoreceptors agonists, nitrogen oxide donors), while others cause vasodilation both at early, and late disease stages (nitrogen oxide donors), that could be taken into account in clinical practice.

REFERENCES

1. Burger CD. Pulmonary hypertension in COPD: a review and consideration of the role of arterial vasodilators. COPD 2009;6:137-44.
2. Chhabra SK. Pulmonary hypertension associated with chronic obstructive pulmonary disease. Ind J Chest Dis Allied Sci 2010;52:29-40.
3. Nakazawa H, Hori M, Ozaki H, et al. Mechanisms underlying the impairment of endothelium-dependent relaxation in the pulmonary artery of monocrotaline-induced pulmonary hypertensive rats. Br J Pharmacol 1999;128:1098-104.
4. Yang Q, Underwood MJ, Hsin MK, et al. Dysfunction of pulmonary vascular endothelium in chronic obstructive pulmonary disease: basic considerations for future drug development. Curr Drug Metab 2008;9:661-67.
5. Zakynthinos E, Daniil Z, Papanikolaou G, et al. Pulmonary hypertension in COPD: pathophysiology and therapeutic targets. Curr Drug Targets 2011;12:501-13.
6. Malerba M, Romanelli G. Early cardiovascular involvement in chronic obstructive pulmonary disease. Monaldi Arch Chest Dis. 2009;71:59-65.
7. van Zutphen LFM, Baumans V, Beynen AC, editors. Principles of Laboratory Animal Science, rev. ed. Amsterdam: Elsevier; 2001. 428 p.

8. Lebedeva ES, Kuzubova NA, Danilov LN, et al. Experimental modelling of chronic obstructive pulmonary disease. Bull Exper Biol Med 2012;152:659-63. doi: 10.1007/s10517-012-1601-3.

9. Blattner R, Classen HG, Dehnert H, et al. Experiments on isolated smooth muscle preparations. Freiburg: Hugo Sachs Elektronik KG; 1978. 185 p. English edition prepared by Barnden IM, Colson R. 1980.

10. Timmermans PB, Chiu AT, Thoolen MJ. Calcium handling in vasoconstriction to stimulation of alpha 1- and alpha 2-adrenoreceptors. Can J Physiol Pharmacol 1987;65:1649-57.

11. Hamada H, Damron DS, Hong SJ, et al. Phenylephrine-induced Ca^{2+} oscillations in canine pulmonary artery smooth muscle cells. Circul Res 1997;81:812-23.

12. Taddei S, Virdis A, Ghiadoni L, et al. Role of endothelin in the control of peripheral vascular tone in human hypertension. Heart Fail Rev 2001;6:277-85.

13. [Fedin AN, Kuzubova NA, Danilov LN, et al. Dysfunction of airway nervous structures in rats inhaled with nitrogen dioxide. Russ J Physiol. 2007;93:1071-77].

14. Jernigan NL, Broughton BRS, Walker BR, et al. Impaired NO-dependent inhibition of store- and receptor-operated calcium entry in pulmonary vascular smooth muscle after chronic hypoxia. Am J Physiol Lung Cell Mol Physiol 2006;290:L517-25.

15. Mendes ES, Horvath G, Campos M, et al. Rapid corticosteroid effect on beta(2)-adrenergic airway and airway vascular reactivity in patients with mild asthma. J Allergy Clin Immunol 2008;121:700-4.

16. [Fedin AN, Kuzubova NA, Danilov LN, et al. Broncholytic effect of prednisolon in the rats inhaled with nitrogen dioxide. Russ J Physiol 2010;96:293-300].

17. Wanner A, Horvath G, Brieva JL, et al. Nongenomic actions of glucocorticosteroids on the airway vasculature in asthma. Proc Am Thoracic Soc 2004;1:235-38.

Figure captions

Fig. 1. Effect of vasodilators on the tone of intact rat's pulmonary arterial samples.

X-axis – dose of reagents-vasodilators, mcg; Y-axis – amplitude of pulmonary artery smooth muscle relaxation, mg.

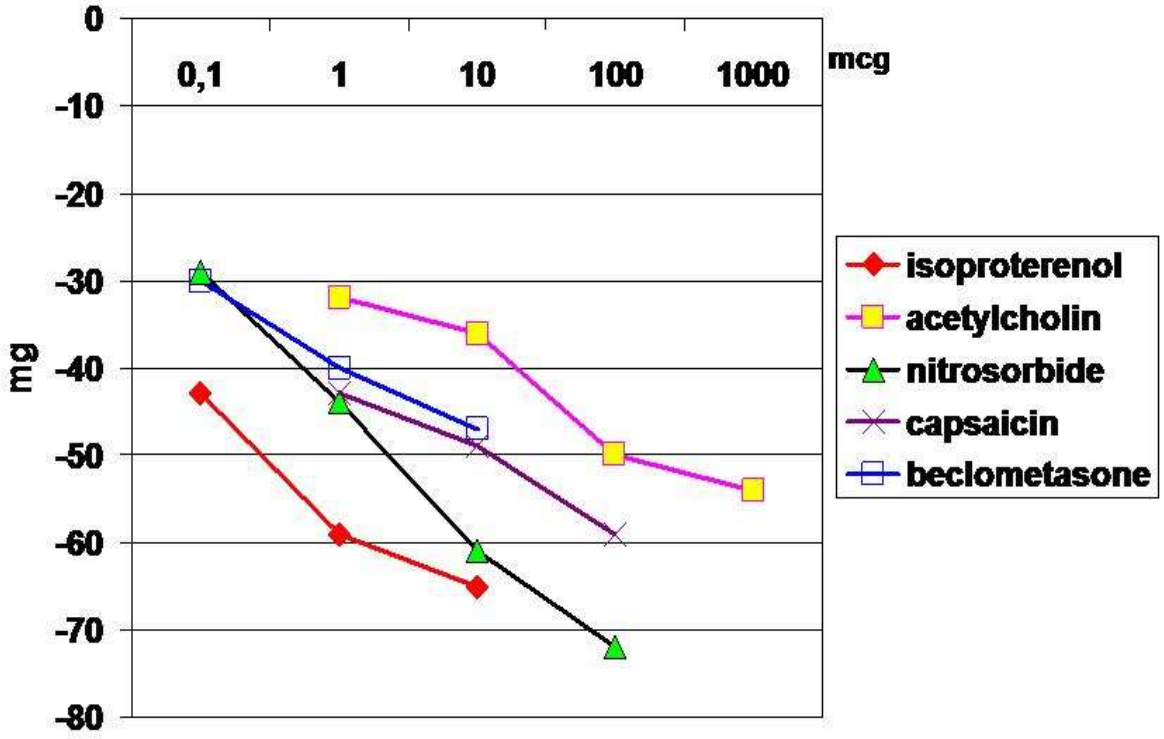

Fig. 2. Effect of vasodilators on the tone of pulmonary arteries isolated from rats exposed to nitrogen dioxide for 15 days.

X-axis – dose of reagents-vasodilators, mcg; Y-axis – amplitude of pulmonary artery smooth muscle relaxation, mg.

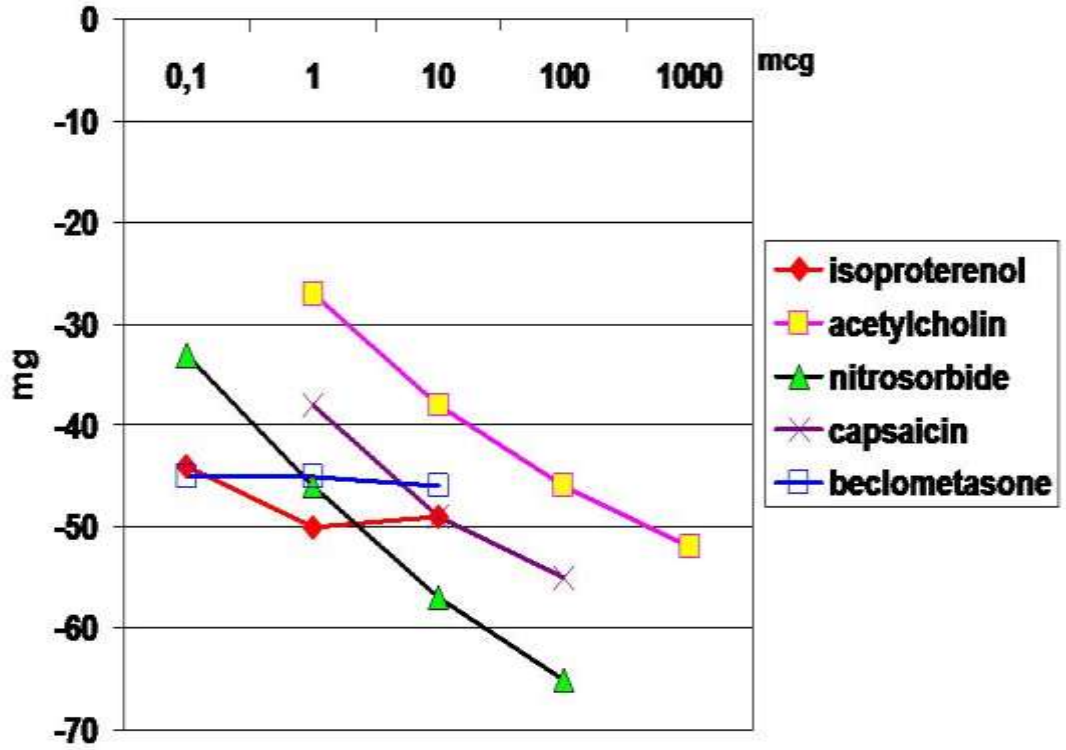

Fig. 3. Effect of vasodilators on the tone of pulmonary arteries isolated from rats with COPD model (exposed to nitrogen dioxide for 60 days).

X-axis – dose of reagents-vasodilators, mcg; Y-axis – amplitude of pulmonary artery smooth muscle relaxation, mg.