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Role of β_3-Adrenoceptor Activation in Changes of Pulmonary Microhemodynamics after Experimental Pulmonary Thromboembolism

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Changes in pulmonary microhemodynamics during modelling of pulmonary thromboembolism against the background of nebivolol and mirabegron pretreatment were studied in isolated perfused rabbit lungs. In both cases, the pulmonary artery pressure and precapillary and pulmonary vascular resistance increased to a greater extent than in control animals, but the increase in capillary hydrostatic pressure was less pronounced. The postcapillary resistance did not change in pulmonary embolism against the background of nebivolol administration and increased in case of mirabegron pretreatment; capillary filtration coefficient after nebivolol pretreatment increased less markedly than after mirabegron administration. The increase in capillary filtration coefficient after activation of β_3-adrenoceptors with the specified drugs depended on the ratio of constriction of pulmonary veins, capillary hydrostatic pressure, and endothelial permeability.

Key Words: *pulmonary thromboembolism; capillary filtration coefficient; β_3-adrenoceptors; nebivolol; mirabegron*

In modern physiological and clinical literature, great attention is focused on endothelial β_3-adrenoceptors, because their stimulation promotes dilatation of pulmonary vessels as the result of enhanced NO synthesis [5,8,9,11,12]. High affinity β_1-adrenoceptor blocker nebivolol acts as an agonist of β_3-adrenoceptors [5,11,12]. In experimental monocrotaline-induced chronic pulmonary hypertension, nebivolol decreased blood pressure in the pulmonary artery (PA) [11]. The positive effect of β_3-adrenoceptor agonists BRL37344 and mirabegron on remodeling of the pulmonary arterial vessels was demonstrated on the venous model of chronic pulmonary hypertension [8]. However, there are no published data on changes in pulmonary microcirculation in response to pulmonary embolism (PE) after β_3-adrenoceptors activation.

Here we performed comparative analysis of changes in the pulmonary microhemodynamics during experimental acute PE against the background of treatment with $\beta_{1,2}$ and β_1-adrenoceptor blockers propranolol and nebivolol and of $\beta_{1,2}$ and β_3-adrenoceptor agonists isoproterenol and mirabegron.

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MATERIALS AND METHODS

The experiments were carried out on 35 rabbits weighing 3-4 kg under intraperitoneal anesthesia (500 mg/kg urethane and 50 mg/kg chloralose; Sigma) with sternotomy and jet ventilation performed with a Faza-9 apparatus. The parameters of ventilation (respiratory rate 30-40 breaths per min, tidal volume 15-20 cm^3/kg) were corrected to prevent hypoxic and acid-base balance disorders in animals. Blood gases were controlled using an ABL-50 blood-gas analyzer (Radiometer) [1]. The study was carried out according to Principles of Good Laboratory Practice (Order No. 199n of the Ministry of Health of the Russian Federation, April 1, 2016) and approved of the Local Ethic Committee of the Institute of the Experimental Medicine (protocol 3/19, April 25, 2019).

Five series of the experiments were carried out on *in situ* perfused isolated lungs using modified methods of dual-channels perfusion with a peristaltic pump of constant flow rate (136 ml/min; Institute of Experimental Medicine) [1]. The PA pressure and left atrial pressure were measured by MLT00699 (AD Instruments) pressure transducers. The mean capillary hydrostatic pressure, capillary filtration coefficient, and pre- and postcapillary vascular resistance of the perfused lungs were estimated by methods of the extracorporeal circulating blood volumometry [1]; the pre/postcapillary resistance ratio (Ra/Rv) were calculated. At the end of the experiments, the isolated lungs were weighed (30.2±2.7 g).

In series I (7 rabbits, control), acute PE was modelled by injection of 10-15 autologous cylindrical emboli (diameter 0.8 mm, length 1-1.5 mm) into PA directly through a T-shaped triplex catheter connected to a peristaltic pump [1]. In the next series, acute PE was modelled as described above in 5-10 min after injection of blockers or agonists of β adrenoceptors dissolved in 5 ml physiological saline.

In series II and III (8 and 7 rabbits, respectively) we used blockers of $\beta_{1,2}$ and β_1-adrenoceptors propranolol (2-2.5 mg/kg, ISIS Pharma) [3] and nebivolol (0.1-0.2 mg/kg, Berlin-Chemie AG) [5], respectively. In series IV and V (6 and 7 rabbits) we used agonists of $\beta_{1,2}$ and β_3-adrenoceptors of prolonged action isoproterenol (Isadrin) [3] and mirabegron (Betmiga, Avara Pharmaceutical Technologies Inc.), respectively. The mirabegron dose (5-10 μg/kg) was calculated according to published reports [10], taking into consideration the body weight of rabbits and volume of circulating blood (300 ml) in the perfusion system. In that case the concentration of this drug in blood plasma could attain ~100 ng/ml [10]. The isoproterenol dose (2.5-5 μg/kg) was chosen in such a way to induce a 10-15% decrease in PA pressure similar to the effect of mirabegron. The tablet forms of nebivolol, isoproterenol, and mirabegron were preliminarily dissolved in 10 ml physiological saline and then diluted to necessary doses.

The parameters of pulmonary microcirculation were compared over 5 min after emboli injection, when changes in PA pressure were maximum. The measured parameters of pulmonary microcirculation were digitized and sampled using L-Card L-783 converter. Then, the calculated parameters were processed using AST software.

The statistical analysis was performed using Student's t tests using standard (Axum 5.0, Math Soft, Inc.) and original programs designed in our laboratory. The initial values of the studied parameters and their changes (in %) were presented as the mean (*M*) and error of the mean (*m*).

RESULTS

The mean baseline values of the studied pulmonary microcirculation parameters in five experimental series are summarized in Table 1. After propranolol and nebivolol treatment, most hemodynamics parameters increased approximately to the same extent, which attested to enhanced activation of α-adrenoceptors in pulmonary vessels caused by the neurogenic sympathetic adrenergic influences [1].

After injection of the test drugs, PE was characterized by more pronounced (by 153-178%) elevation of PA pressure and precapillary and pulmonary vascular resistance in comparison with the control (Table 2). Hence, constrictor neurogenic and humoral influences on the pulmonary arterial vessels prevailed under these conditions. After nebivolol treatment, the capillary

TABLE 1. Parameters of Pulmonary Microcirculation in Perfused Rabbit Lungs after Administration of Blockers and Agonists of β Adrenoceptors (*M*±*m*)

Parameter	Initial	Propranolol	Nebivolol	Isoproterenol	Mirabegron
PA pressure, mm Hg	23±2	30±6**	27±4**	-11±3*	-14±5**
Left atrial pressure, mm Hg	4.7±0.2	6±3	-4±3	-2±2	-23±6**
PA flow, ml/min	136	0	0	0	0
Capillary hydrostatic pressure (Pc), mm Hg	7.9 ±0.2	16±4**	10±3*	-3±2	-13±4*
Pulmonary vascular resistance, dyn×sec×cm^{-5}	179±16	34±7**	36±8**	-14±5*	-12±3**
Precapillary resistance (Ra), dyn×sec×cm^{-5}	148±15	34±6**	37±8**	-18±6*	-16±3**
Postcapillary resistance (Rv), dyn×sec×cm^{-5}	31±4	30±5**	31±6**	0±2	0±2
Pre-/postcapillary resistance ration Ra/Rv	4.8±0.4	4±3	4±2	-16±5**	-14±6*
Capillary filtration coefficient (CFC), ml/min/100 g/mm Hg	0.04±0.01	25±7**	25±6**	0±2	-25±4**

Note. Here and in Table 2: minus shows a decrease in the parameter. *p<0.05, **p<0.01.

TABLE 2. Parameters of Pulmonary Microcirculation in Perfused Rabbit Lungs in PE against the Background Administration of Blockers and Agonists of β Adrenoceptors (*M*±*m*)

Parameter	Control	Propranolol	Nebivolol	Isoproter-enol	Mirabegron
PA pressure, mm Hg	90±10**	153±12**	178±16**	270±18**	272±28**
Left atrial pressure, mm Hg	-4±2	4±3	4±3	0±3	0±2
PA flow, ml/min	0	0	0	0	0
Capillary hydrostatic pressure (Pc), mm Hg	17±4**	22±5**	7±2**	13±5*	12±3**
Pulmonary vascular resistance, dyn×sec×cm^{-5}	111±18**	186±15**	209±15**	369±35**	333±27**
Precapillary resistance (Ra), dyn×sec×cm^{-5}	120±22**	209±61**	255±27**	479±28**	433±38**
Postcapillary resistance (Rv), dyn×sec×cm^{-5}	53±6**	51±16*	2±2	33±8**	26±5**
Pre-/postcapillary resistance ration Ra/Rv	43±7**	103±14**	247±28**	329±34**	322±38**
Capillary filtration coefficient (CFC), ml/min/100 g/mm Hg	25±4**	60±14**	20±6*	0±2	67±12**

Note. Parameters are shown in % of initial values (for control animals) or baseline values (without PE) after administration of blockers and agonists of β adrenoceptors.

hydrostatic pressure and capillary filtration coefficient in PE increased by 7±2% (p<0.01) and 20±6% (p<0.05), respectively, *i.e.*, to a lesser extent than after propranolol treatment: by 22±5% (p<0.01) and 60±14% (p<0.01), respectively, and the postcapillary resistance practically did not change (Table 2). It could be caused by nebivolol activation of endothelial β_3 adrenoceptors of the pulmonary venous vessels and enhanced NO synthesis [5,7,11,12]. It was shown [4] that after leukotriene C4 and noradrenaline applications, the constrictor reactions in pulmonary veins were less expressed than in pulmonary arteries due to more intensive NO production. However, in case of PE against the background of nebivolol treatment, the increase in capillary filtration coefficient (20±6%) did not statistically differ from that in control animals (25±4%), although in the control animals the postcapillary resistance increased by 53±6% (p<0.01).

We hypothesized that β_3 adrenoceptors activation can increase the endothelial permeability. It is known [6] that the intensification of NO production by endothelium promotes increasing of its permeability. To test this hypothesis, PE was modelled after administration of agonists $\beta_{1,2}$ and β_3 adrenoceptors isoproterenol and mirabegron.

After the injection of these drugs, PA pressure and precapillary and pulmonary vascular resistance decreased approximately to the same extent. However, in experiments in rabbits with mirabegron treatment, in contrast to isoproterenol, the capillary hydrostatic pressure, left atrial pressure and capillary filtration coefficient decreased (Table 1). It can be hypothesized that mirabegron caused more pronounced dilatatory effects on the pulmonary venous vessels than isoproterenol. The calculated parameter of postcapillary resistance did not change, which was a result of a decrease in the left atrial pressure.

In PE modeled after isoproterenol and mirabegron treatment, the increase in most analyzed hemodynamics parameters was approximately equal and more pronounced than in the control (Table 2). This could be caused by more potent constrictor reactions of pulmonary arterial and venous vessels after preliminary dilatation, because they depend on the initial vascular tone [2]. In case of PE after isoproterenol treatment, the capillary filtration coefficient did not change, but after mirabegron administration it increased by 67±12% (p<0.01). As the capillary hydrostatic pressure and postcapillary resistance increased approximately to the same extent in these cases, the elevation of the capillary filtration coefficient in PE modeled after mirabegron treatment could be a result of increased endothelial permeability. Hence, activation of endothelial β_3 adrenoceptors in pulmonary vessels promotes the decrease in the capillary filtration coefficient as a result of dilatation of pulmonary veins and the decrease of capillary hydrostatic pressure on the one hand, and the increase in this parameter due to enhanced endothelial permeability on the other hand.

Thus, we demonstrated distinctions between changes of the microhemodynamics in the pulmonary arterial and venous vascular beds during PE after activation of β_3 adrenoceptors with nebivolol and mirabegron. In both cases, PA pressure and precapillary and pulmonary vascular resistance increased to a greater extent than in control animals. After nebivolol treatment, the postcapillary resistance did not change, capillary hydrostatic pressure increased less markedly, but capillary filtration coefficient increased to the same extent, as in control. In case of PE after mirabegron

treatment, the increase in postcapillary resistance and capillary hydrostatic pressure was less pronounced, but the increase in capillary filtration coefficient was more pronounced than in control animals. This parameter depends from the ratio of pulmonary veins constriction, shifts in capillary hydrostatic pressure and endothelial permeability.

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