POTENTIAL INVOLVEMENT OF A PROPRANOLOL-INSENSITIVE ATYPICAL β-ADRENOCEPTOR IN THE VASODILATOR EFFECT OF CYANOPINDOLOL IN THE HUMAN PULMONARY ARTERY

The aim of our study was to examine whether non β₁/β₂-adrenoceptors participate in the relaxation of the human pulmonary artery. For this purpose the vasodilatory effect of the non-conventional partial β-adrenoceptor agonist cyanopindolol was examined. Cyanopindolol (1 - 300 µM), studied in the presence of the β₁/β₂-adrenoceptor antagonist propranolol, relaxed the human pulmonary artery preconstricted with serotonin 1 µM in a concentration-dependent manner (maximally by about 80%). This effect was diminished by bupranolol 10 µM (an antagonist of β₁/β₂-adrenoceptors and the low affinity state of the β₁-adrenoceptor) and CGP 20712 10 µM (known to antagonize the low-affinity state of the β₁-adrenoceptor at high concentrations). In further experiments, the effect of β-adrenoceptor ligands on the serotonin-induced vasoconstriction was examined. The concentration-response curve for serotonin was not affected by cyanopindolol 30 µM, bupranolol 10 µM and CGP 20712 10 µM but shifted to the right by cyanopindolol 100 and 300 µM; the serotonin 5-HT₂A receptor antagonist ketanserin 0.3 µM abolished the maximum contraction elicited by serotonin. In conclusion, the present study reveals that the vasodilatory effect of cyanopindolol in the human pulmonary artery consists of two components, i.e. activation of a propranolol-insensitive atypical β-adrenoceptor and antagonism against 5-HT₂A receptors.

Key words: cyanopindolol, atypical β-adrenoceptors, low-affinity state of the β₁-adrenoceptor, bupranolol, CGP 20712, human pulmonary artery.
INTRODUCTION

Vasorelaxation via $\beta$-adrenoceptors is related to the $\beta_2$ subtype although $\beta_1$-adrenoceptors also mediate vasorelaxation in some instances (for review, see 1). During the last decade, the occurrence of two additional $\beta$-adrenoceptor subtypes, insensitive to the $\beta_1-/\beta_2$-adrenoceptor antagonist propranolol or other antagonists of classical $\beta$-adrenoceptors, has been suggested in isolated vessels that is $\beta_3$-adrenoceptor and "atypical $\beta$-adrenoceptor" (for literature, see 2, 3). However, it is still not known, whether the vascular atypical $\beta$-adrenoceptor is closely related to, or identical with, the "low-affinity state of the $\beta_1$-adrenoceptor" (4), which was thoroughly studied in the heart (for review, see 5). So far, only few papers have appeared, in which the possible participation of non $\beta_1-/\beta_2$-adrenoceptors in the relaxation of human vessels was studied. Thus, mRNA for $\beta_3$-adrenoceptors has been identified in human coronary arteries (6), internal mammary artery (7) and corpus cavernosum (8). Moreover, in functional studies the $\beta_3$-adrenoceptor agonist BRL 37344 relaxed human coronary arteries preconstricted with endothelin-1; this effect was antagonized by bupranolol, an antagonist of $\beta_1-/\beta_2-/\beta_3$-adrenoceptors and the low-affinity state of the $\beta_1$-adrenoceptor, but not by the $\beta_1-/\beta_2$-adrenoceptor antagonist nadolol (6). Similarly, the vasodilatory effects of BRL 37344 in human corpus cavernosum (8) and of another $\beta_3$-adrenoceptor agonist, SR 58611A, in internal mammary artery (7) preconstricted with phenylephrine were diminished by the $\beta_3$-adrenoceptor antagonists SR 59230A and L-748,337, respectively; the SR 58611A-induced relaxation of the internal mammary artery was not affected by nadolol (7). The participation of $\beta_3$-adrenoceptors was also suggested in the vasodilatory effect of BRL 37344 in human umbilical arteries (9) preconstricted with serotonin but this conclusion was not confirmed by the use of $\beta$-adrenoceptor antagonists.

The tone in the human pulmonary vascular system is modulated in part by $\beta$-adrenergic relaxation. However, the $\beta$-adrenoceptor subtypes and their downstream vasorelaxing mechanism(s) in this vascular bed are poorly understood (for review, see 10). Thus, binding studies performed on human pulmonary vessels indicate the presence of $\beta_2$-adrenoceptors (11) or both $\beta_1$- and $\beta_2$-adrenoceptors with $\beta_1$-adrenoceptors as the predominating subtype (12). Moreover, a $\beta_3$-adrenoceptor-mediated relaxation in human pulmonary segmental arteries has been shown in functional experiments (13). So far, the possible participation of non $\beta_1-/\beta_2$-adrenoceptors in the modulation of the pulmonary tone has been examined in non-human vessels only. Thus, $\beta_2$-adrenoceptor agonists have been suggested to relax the canine pulmonary artery preconstricted with noradrenaline (14) or to have a relaxant effect with respect to the hypoxia-induced constriction of the rat isolated perfused lung (15). On the other hand, $\beta_3$-adrenoceptors have not been found in functional studies performed on the rat (16) and mouse (17) pulmonary arteries.
Relaxation of pulmonary arteries is of special interest for decreasing resistance in pathological situations such as pulmonary hypertension. Thus, the aim of our present study was to examine whether non \( \beta_1/\beta_2 \) adrenoceptors participate in the relaxation of the human pulmonary artery. For this purpose, the vasodilatory effect of the non-conventional partial \( \beta \)-adrenoceptor agonist cyanopindolol was examined; it activates the low-affinity state of the \( \beta_1 \)-adrenoceptor and \( \beta_3 \)-adrenoceptors at concentrations much higher than those at which it blocks \( \beta_1 \)- and \( \beta_2 \)-adrenoceptors. Cyanopindolol has been shown to relax the rat mesenteric artery preconstricted with serotonin via atypical \( \beta \)-adrenoceptors in our previous study (2). In interaction studies bupranolol and CGP 20712, both of which are antagonists of atypical \( \beta \)-adrenoceptors in high concentrations (18,19), were applied.

**MATERIALS AND METHODS**

All protocols were approved by the Human Ethics Committee of the Medical University of Białystok.

**Preparation of human pulmonary artery**

Human lung tissue was obtained from 21 patients (19 men and 2 women, mean age 62.5±1.4 years) undergoing lobectomy or pneumonectomy during resection of lung carcinoma. None of the patients had any clinical evidence of pulmonary hypertension or received \( \beta \)-adrenoceptor antagonists. Before the operation all patients received cephalosporins and low-molecular weight heparin as anti-infection and anti-thrombotic prophylaxis, respectively. The tissue was transported to the laboratory within half an hour in cold (4°C), pre-gassed Tyrode's bicarbonate solution (for composition, see below). Lobar and segmental pulmonary artery branches were cleaned from the lung parenchyma and cut into rings (from the middle portion of each artery; 3-5 mm length and 2-4 mm outer diameter).

**Organ Bath Technique**

The arterial rings were suspended on stainless-steel wires in 10 ml organ baths containing Tyrode's solution (concentrations in mM: NaCl, 139.2; KCl, 2.7; CaCl\(_2\), 1.8; MgCl\(_2\), 0.49; NaHCO\(_3\), 11.9; NaH\(_2\)PO\(_4\), 0.4; glucose, 5.5) and were gassed continuously with 95% \( O_2 \) and 5% \( CO_2 \) at 37°C and pH 7.4. Segments were allowed to equilibrate for 90 min, during which the bath fluid was exchanged every 10 min with fresh Tyrode's solution. The optimal resting tension was 2 - 2.5 g depending on the internal diameter. Muscle tension was recorded by a force displacement transducer (PIM 100RE, BIO-SYS-TECH, Białystok, Poland) and displayed on a computer.

**Concentration-response Curves**

After the equilibration period rings were constricted submaximally with serotonin (1 \( \mu \)M) and then functionality of endothelium was checked by the presence of at least 80% relaxation in response to acetylcholine 1 \( \mu \)M. After washout two different types of experiments were performed (in each individual preparation only one experimental curve was determined). In order to examine the vasodilatory effect of cyanopindolol, rings were constricted with serotonin 1 \( \mu \)M and cumulative concentration-response curves to cyanopindolol were created. In all these
experiments propranolol 0.3 µM was routinely present in the medium in order to exclude the participation of \( \beta_1/-\beta_2 \)-adrenoceptors in the effects of cyanopindolol. The vasodilatory effect of cyanopindolol was examined in the absence or presence of bupranolol 10 µM, CGP 20712 10 µM or the \( \alpha_1 \)-adrenoceptor antagonist prazosin 10 µM given 30 min before serotonin. In order to examine the antagonistic properties of \( \beta \)-adrenoceptor ligands against the serotonin-induced contraction rings were treated for 30 min with one concentration of \( \beta \)-adrenoceptor ligands or their vehicle (control tissues) and then a concentration-response curve for serotonin (0.01 µM - 100 µM) was created. The antagonist potencies of the serotonin 5-HT\(_{2A}\) antagonist ketanserin 0.3 µM and prazosin 10 µM against the serotonin-induced contraction were examined in the same way. In a separate series of experiments, a concentration-response curve for phenylephrine (0.1 - 300 µM) was constructed in rings treated (or not treated) with prazosin 10 µM 30 min beforehand.

Calculations and Statistical Analysis

The vasodilator activity of cyanopindolol was calculated as percentage change in the maximal tension of vessel rings after addition of serotonin. In order to quantify its potencies (under control conditions i.e. without any antagonist and in the presence of various antagonists) the concentrations causing a vasorelaxation by 25% (EC\(_{25}\) or its negative logarithm pEC\(_{25}\)) were determined graphically since full relaxation was not obtained. The contractions in the second type of experiments are reported as percentages of the maximal response to serotonin or phenylephrine. To determine the potency of serotonin (phenylephrine) in the absence and presence of adrenoceptor ligands EC\(_{50}\) values [concentration (M) of agonist that produces 50% of the respective maximum response] were calculated.

The antagonistic potency of the adrenoceptor ligands against the vasodilator effect of cyanopindolol (first type of experiments) or against the contractile response to serotonin (second type of experiments) was calculated from the equation: apparent pA\(_2\)=log(CR-1)-log[B], where [B] is the molar concentration of the antagonist and CR is the concentration ratio of the EC\(_{25}\) values of cyanopindolol or the EC\(_{50}\) values of serotonin in the presence and absence of the adrenoceptor ligands.

Results are expressed as mean ± SEM of n experiments. Mean concentration-response curves were analysed by using nonlinear regression (GraphPad Prism, GraphPad Software, San Diego, CA, USA). Statistical analyses were performed using the t-test for unpaired data. When two or more treatment groups were compared to the same control, one-way analysis of variance (ANOVA) followed by the Dunnett test was used. Differences were considered as significant when P<0.05.

Drugs Used

Acetylcholine chloride, (-)-phenylephrine hydrochloride, propranolol hydrochloride, ketanserin tartrate, CGP 20712 (±)-2-hydroxy-5-(2-((2-hydroxy-3-(4-(1-methyl-4-(trifluoromethyl)-1H-imidazole-2-yl)-phenoxy)propyl)amino)ethoxy)-benzamide monomethane sulphonate), serotonin (creatinine sulphate complex), prazosin (Sigma, München, Germany), cyanopindolol hemifumarate (Tocris-Cookson, Bristol, U.K.), bupranolol hydrochloride (Schwarz Pharma, Monheim, Germany). Drugs were dissolved in distilled water with the exception of cyanopindolol [mixture of water and dimethylsulphoxide (DMSO)] and serotonin (dissolved in 0.1 N HCl). Stock solutions of the latter drugs were further diluted with water in order to obtain the concentrations required for the experiments. The final concentration of the solvent in the organ bath was less than 0.1% v/v. The solvent solutions had no effect on the basal tone. Since the
highest concentration of cyanopindolol was dissolved in DMSO, in additional experiments we demonstrated that this organic solvent failed to affect the concentration-response curve for serotonin (Fig. 4A).

RESULTS

The human pulmonary artery was contracted by serotonin (0.01 - 30 µM) in a concentration-dependent manner, yielding a pEC$_{50}$ value of 6.18±0.11 and a maximal effect of 7.95±1.40 mN (n=18) (Fig. 1A). The vasoconstrictory action of serotonin was abolished by the 5-HT$_{2A}$ receptor antagonist ketanserin 0.3 µM but not affected by the α$_{1}$-adrenoceptor antagonist prazosin given at a concentration of 10 µM (Fig. 1A), which abolished the phenylephrine-elicited contraction of the pulmonary artery (Fig. 1B). The pEC$_{50}$ value for the concentration-response curve of phenylephrine was 5.41±0.11 and the contractile effect obtained at 300 µM (which was assumed to represent the maximum effect) amounted to 7.95±1.10 mN (n=11).

The vasodilatory effect of cyanopindolol was examined on the human pulmonary artery preconstricted with serotonin 1 µM, i.e. a concentration approximately equivalent to its EC$_{60}$. Cyanopindolol (1 - 300 µM) relaxed the serotonin-preconstricted human pulmonary artery in a concentration-dependent manner (Fig. 2; for typical traces see Fig. 3A). The relaxation obtained with the highest concentration was by about 80% and the pEC$_{25}$ value was 4.34±0.12 (n=11). The two β-adrenoceptor antagonists bupranolol and CGP 20712, given

![Fig. 1. Influence of prazosin and ketanserin on the contraction of the human pulmonary artery induced by serotonin (A) and phenylephrine (B). Results are expressed as percentages of the maximum response to serotonin or of the response to phenylephrine 300 µM. Means ± SEM of 4-18 tissues for each curve. For many points SEM is contained within the symbols.](image-url)
at a concentration of 10 µM (known to antagonize the low-affinity state of the β₁-adrenoceptor), shifted the concentration-response curve for cyanopindolol to the right, yielding apparent pA₂ values of 5.58±0.16 (n=6) and 5.35±0.09 (n=4),

![Figure 2. Relaxant effect of cyanopindolol and its interaction with prazosin, bupranolol and CGP 20712 in the human pulmonary artery preconstricted with serotonin 1 mM. In all experiments propranolol 0.3 µM was routinely present in the medium. Results are expressed as percentage relaxation of the tone induced by serotonin. Means ± SEM of 4-11 tissues for each curve. For many points SEM is contained within the symbols.](image)

**Table 1.** Potency of cyanopindolol for its vasodilatory effect and of serotonin for its vasoconstrictory effect in the absence and presence of various antagonists in the human pulmonary artery.

<table>
<thead>
<tr>
<th>Vasodilatory effect of cyanopindolol</th>
<th>Vasoconstrictory effect of serotonin</th>
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<td>no antagonist</td>
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<td>bupranolol (10 µM)</td>
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<td>CGP 20712 (10 µM)</td>
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<td>cyanopindolol (30 µM)</td>
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<td>cyanopindolol (300 µM)</td>
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Values are shown as means±SEM.

¹From Fig. 2.

²From Fig. 4.

³From Fig. 1A.

*P<0.05; **P<0.01; ***P<0.001, compared to the respective control.
respectively (Fig. 2; for typical traces see Fig. 3B and 3C). The vasodilatory effect of cyanopindolol was not affected by prazosin 10 µM (Fig. 2). The respective pEC\textsubscript{25} values, obtained in the presence of the antagonists under study, are given in Table 1. None of the antagonists affected the level of the serotonin-induced contraction by itself (results not shown).

In additional experiments, we examined the antagonistic effect of β-adrenoceptor ligands against serotonin. For this purpose a complete

Fig. 3. Traces from representative experiments showing the relaxant effect of cyanopindolol in the human pulmonary artery preconstricted with serotonin (1 µM). Experiments were performed in the absence (A) or presence of bupranolol (B) or CGP 20712 (C) given 30 min before the administration of serotonin. In all experiments propranolol 0.3 µM was routinely present in the medium.
The concentration-response curve for serotonin was constructed after exposure of the vessels to the β-adrenoceptor ligands or their vehicle (Fig. 4). As shown in Fig. 4A, cyanopindolol at a concentration of 30 µM, which relaxed the serotonin-preconstricted human pulmonary artery by about 20% (Fig. 2), failed to influence the vasoconstrictor response to serotonin. However, two higher concentrations of this drug, 100 and 300 µM, shifted the concentration-response curve for serotonin to the right without affecting its maximum contractile response. The apparent pA<sub>2</sub> values were 4.95±0.16 (n=4) and 4.73±0.17 (n=5), respectively (for pEC<sub>50</sub> values obtained in the absence and presence of antagonists, see Table 1). The β-adrenoceptor antagonists bupranolol and CGP 20712 did not affect (or only marginally affected) the serotonin-induced contraction of the human pulmonary artery at concentrations at which they diminished the cyanopindolol-elicited relaxation (Fig. 4B).

**DISCUSSION**

The aim of the present study was to examine whether non β<sub>1</sub>-/β<sub>2</sub>-adrenoceptors relax the human pulmonary artery. For this purpose we used cyanopindolol, one of the so-called non-conventional partial β-adrenoceptor agonists, i.e. drugs that activate the low-affinity state of the β<sub>1</sub>-adrenoceptor and β<sub>3</sub>-adrenoceptors at concentrations much higher than those at which they block β<sub>1</sub> and/or β<sub>2</sub>-adrenoceptors (5). For preconstriction of the vessel, we chose serotonin, which
plays a more important role as a vasoconstrictor in this vascular bed than catecholamines (10, 20).

We found that cyanopindolol produced a concentration-dependent relaxation of the serotonin-preconstricted isolated human pulmonary artery, which amounted to 80% at the highest concentration under study. What is the mechanism behind the vasodilatory effect of cyanopindolol? Since all experiments were performed in the presence of propranolol we can exclude the participation of \( \beta_1 \) - and \( \beta_2 \)-adrenoceptors. Another two \( \beta \)-adrenoceptor antagonists, i.e., bupranolol and CGP 20712, did antagonize the effect of cyanopindolol. Bupranolol is a non-selective antagonist of \( \beta_1 \)-, \( \beta_2 \)- and \( \beta_3 \)-adrenoceptors and still seems to be the best available tool to examine the interference of agonists with the low-affinity state of the \( \beta_1 \)-adrenoceptor (21, 22). CGP 20712 is an antagonist of \( \beta_1 \)-adrenoceptors and, at high concentration, also of the low-affinity state of the \( \beta_1 \)-adrenoceptor (18, 19, 23, 24) but at this high concentration still fails to counteract \( \beta_3 \)-adrenoceptor-mediated effects (18, 23). The fact that both drugs antagonized the effect of cyanopindolol suggests that its vasodilator effect is related to the activation of the low-affinity state of the \( \beta_1 \)-adrenoceptor but not due to the activation of \( \beta_3 \)-adrenoceptors. Whether the propranolol-insensitive atypical \( \beta \)-adrenoceptor involved in the effect of cyanopindolol is identical to the low-affinity state of the \( \beta_1 \)-adrenoceptor found in the heart, cannot be decided with certainty on the basis of the present data. The fact that the potencies of cyanopindolol, bupranolol and CGP 20712 in the present study were lower than those obtained at the low-affinity state of the \( \beta_1 \)-adrenoceptor in the isolated rat heart (18) suggests a difference.

Did the cyanopindolol-induced relaxation of the human pulmonary artery result only from the activation of an atypical \( \beta \)-adrenoceptor? The possibility that the drug relaxes the vessel due to direct blockade of the vasoconstrictor effect of serotonin has to be considered as a second mechanism. Such an explanation would be very plausible since various \( \beta \)-adrenoceptor antagonists including cyanopindolol exhibit an affinity for serotonin receptor subtypes; e.g., cyanopindolol has a high potency at 5-HT\(_{1B} \) receptors (25). In this context, it should also be considered that in previous studies on vascular preparations pre-constricted with the \( \alpha_1 \)-adrenoceptor agonist phenylephrine cyanopindolol and other drugs used for identification of the low-affinity state of the \( \beta_1 \)-adrenoceptor were active only in concentrations at which they already block \( \alpha_1 \)-adrenoceptors. Hence, one has to assume that their vasodilator effects, at least partially, are related to a simple blockade of the receptor activated by the vasoconstrictor agent (3, 16, 26). It is, however, unlikely that an \( \alpha_1 \)-adrenolytic effect of cyanopindolol contributed to its effect on the human pulmonary artery in the present study since a very high concentration of prazosin failed to influence the concentration-response curve of cyanopindolol.

In order to clarify whether the vasodilator effect of cyanopindolol is related to its antagonism at serotonin receptors, experiments were carried out in which the
influence of cyanopindolol, bupranolol and CGP 20712 on the concentration-response curve of serotonin for its contractile effect was determined. The latter two drugs did not significantly affect the concentration-response curve of serotonin at the concentrations under study. Cyanopindolol 30 µM also did not affect the serotonin-stimulated contraction, suggesting that the vasodilatation elicited by cyanopindolol up to 30 µM (which was by about 20%) was indeed due to the activation of an atypical β-adrenoceptor. However, higher concentrations of cyanopindolol, 100 and 300 µM, shifted to the right the concentration-response curve for serotonin, suggesting that a major part of the vasodilator effect of cyanopindolol is simply due to the fact that the drug interferes with serotonin receptors.

It was beyond the scope of the present paper to determine the serotonin subtype involved in the contractile effect of this monoamine. There are, however, two arguments suggesting that the contractile activity of serotonin in the present model is predominantly, if not exclusively, related to the activation of 5-HT\textsubscript{2A} receptors. First, the maximum effect of serotonin was totally abolished already by a submicromolar concentration of ketanserin (pK\textsubscript{i} value at the human 5-HT\textsubscript{2A} receptor, 8.1) (27). Second, the apparent pA\textsubscript{2} value of cyanopindolol against serotonin (4.84; mean of 4.95 and 4.73 determined for cyanopindolol 100 and 300 µM) was very similar to its affinity (pK\textsubscript{i} =4.5) at the 5-HT\textsubscript{2A} receptor (25).

In conclusion, the present study reveals that propranolol-insensitive atypical β-adrenoceptors (but not β\textsubscript{3}-adrenoceptors) may participate in the vasodilatory effect of cyanopindolol in the human pulmonary artery. Unfortunately we were not able to determine the real degree of relaxation due to the stimulation of this receptor because higher concentrations of cyanopindolol antagonized the serotonin-induced contraction. To our knowledge, this is the first demonstration of the involvement of an atypical (non β\textsubscript{1}-, β\textsubscript{2}-, β\textsubscript{3}-) β-adrenoceptor, pharmacologically similar to the low-affinity state of the β\textsubscript{1}-adrenoceptor, in the relaxation of a human vessel. So far it has been only shown that activation of the low-affinity state of the β\textsubscript{1}-adrenoceptor leads to a positive chronotropic, inotropic and lusitropic effects in the human heart (28). One should emphasize that the function of these receptors may be modulated by β-blockers like pindolol, alprenolol, bucindolol or carvedilol (24).

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