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Influence of β-adrenoceptor agonists on the pulmonary circulation. Effects of a β_3-adrenoceptor antagonist, SR 59230A

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Abstract

The aims of this study were (a) to compare in the rat isolated perfused lung preparation, the effects of isoprenaline and of three β_3-adrenoceptors agonists, SR 59104A, (*N*-[(6hydroxy-1,2,3,4-tetrahydronaphtalen-(2*R*)-2yl)methyl]-(2*R*)-2-hydroxy-2-(3-chlorophenyl)ethanamine hydrochloride), SR 59119A (*N*[(7-methoxy-1,2,3,4-tetrahydronaphtalen-(2*R*)-2yl)methyl]-(2*R*)-2-hydroxy-2-(3-chlorophenyl)ethanamine hydrochloride) and SR 58611A (ethyl{(7*S*)-7-[(2*R*)-2-(3-chlorophenyl)-2-hydroxyethylamino]-5,6,7,8-tetrahydronaphtalen-2-yloxy}acetate hydrochloride) on hypoxia-induced pulmonary vasoconstriction, and (b) to investigate the potential existence of atypical β-adrenoceptors in these effects. Propranolol (0.1 μM) was used to antagonize β_1- and β_2-adrenoceptors whereas SR 59230A, 3-(2-ethylphenoxy)-1-[(1*S*)-1,2,3,4-tetrahydronapht-1-ylamino]-(2*S*)-2-propanol oxalate) (0.3 μM) was used to block β_3-adrenoceptors. Isoprenaline and the three β_3-adrenoceptors agonists caused concentration-dependent relaxations during the pulmonary pressure response. Propranolol and SR 59230A inhibited the relaxant effects of isoprenaline. SR 59230A but not propranolol inhibited those of SR 59104A. Finally, propranolol and SR 59230A failed to oppose SR 59119A- and SR 58611A-induced relaxant effects. In concentrations ≥ 1 μM, SR 59230A caused per se a relaxation of the hypoxic vasoconstricted lung. These results suggest the existence of atypical β-adrenoceptors in the rat pulmonary vessels.

Keywords: Hypoxic pulmonary vasoconstriction; Isoprenaline; β_3-Adrenoceptors agonist; Propranolol; SR 59230A

1. Introduction

In 1984, for the first time, studies using novel thermogenic β-adrenoceptor agonists suggested that catecholamines-induced stimulation of lipolysis in rodent brown and white adipose tissues was mediated by an atypical β-adrenoceptor, designed as the β_3-adrenoceptor (Arch et al., 1984). More recently, biological effects mediated by β_3-adrenoceptors have also been identified in the gastrointestinal tract, especially in the rat and human colon (Manara et al., 1996; De Ponti et al., 1996). There are indications that atypical β-adrenoceptors, which are sensitive to preferential pharmacological agonists that have little effects on β_1- and β_2-adrenoceptors (e.g., BRL 37344 and SR 58611A), but relatively insensitive to the conventional β-adrenoceptor antagonists, are also present in the myocardium (Kaumann and Molenaar, 1996), and in the vascular smooth muscle including the rat carotid artery (Oriowo, 1994), the rat thoracic aorta (Oriowo, 1995) and the canine cutaneous vessels (Berlan et al., 1994). Nevertheless, the similarity of these receptors with the β_3-adrenoceptor present in the rat adipose tissue remains to be demonstrated (Kaumann and Molenaar, 1996). The recent introduction of the first β_3-selective adrenoceptor antagonist, SR 59230A (Manara et al., 1995, 1996) prompted us to determine whether functionally relevant atypical β-adrenoceptors would also be present in the pulmonary vasculature. We therefore investigated the vasodilator profiles of three selective β_3-adrenoceptor agonists: SR 59104A, SR 59119A and SR 58611A on the hypoxic vasoconstriction in the rat isolated perfused lung preparation and compared them to that of the well known β-adrenoceptor agonist, isoprenaline.

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2. Materials and methods

2.1. Animal surgery and perfusion

Twenty two groups ($n = 5$ to 9 per group) of male Wistar rats (Dépré, St Doulchard, France) weighing 280–340 g, were anaesthetized with sodium pentobarbitone (100 mg kg^{-1}) and the lungs were removed for extracorporeal perfusion as previously described (Dumas et al., 1994). Mean perfusion pressure which was measured from a side-arm of the arterial line (Harvard transducer, −50 to 300 mmHg, South Natick, MA, USA), was recorded continuously (Ankersmit WR 3701 recorder, Graphtec, Tokyo, Japan) and reflected pulmonary vascular resistance because the flow rate was constant (0.025 ml g^{-1} min^{-1}). The lungs were perfused with a salt solution containing (mM): NaCl 116, KCl 5.4, NaH_2PO_4 1.04, $MgSO_4$ 0.83, $CaCl_2$ 1.8, $NaHCO_3$ 19 and D-glucose 5.5. Ficoll (0.01 g ml^{-1}, type 70, Sigma, La Verpillère, France) was included as a colloïd. The lungs were ventilated with a Harvard rodent ventilator (South Natick, MA, USA) at a tidal volume of 10 ml kg^{-1} body weight and at a frequency of 55 breaths min^{-1}. The end expiratory pressure was set at 2.5 cmH_2O. The pressure of airways was measured with a Validyne DP45 (0 to 88 cmH_2O) differential pressure transducer (Northridge, CA, USA). A 20- to 30-min equilibration period was allowed to establish a stable baseline for pulmonary airway and vascular pressures before experiments were started. During this period, the lungs were ventilated with a humid mixture of 21% O_2, 5% CO_2, 74% N_2 (normoxia). Lungs of which the weight had increased in excess of 10% (indicative of oedema) at the end of the experiments were discarded.

2.2. Experimental protocols

2.2.1. Vasoconstrictor responses to hypoxia

After the equilibration period, the pulmonary vasculature was precontracted twice, using a bolus of 0.25–0.5 μg angiotensin II to prime the otherwise low vascular reactivity seen in salt solution-perfused lungs. Then the lungs were challenged with a hypoxic gas mixture (5% CO_2, 95% N_2) as described previously (Dumas et al., 1994). Each hypoxic challenge (4 min) was followed by the addition of 0.25 μg angiotensin II under normoxic ventilation (4 min) and the pressure was allowed to return to baseline before the initiation of the next hypoxic ventilation episode. The perfusate gas tensions were measured at the beginning of, and throughout the experiments by collecting the perfusate anaerobically from the arterial cannula and analyzing it immediately (Corning 170 pH/blood gas analyzer, Ciba Corning Diagnostics, Medfield, MA, USA). During hypoxic periods, P_{O_2} was maintained below 35 mmHg and the pH was between 7.3 and 7.4. After three or four hypoxic pulmonary vasoconstrictions, the responses became reproducible for at least nine subsequent periods (Dumas et al., 1997). Drugs were tested after a stable response to hypoxia was reached.

2.2.2. Effects of isoprenaline, SR 59104A, SR 59119A and SR 58611A on hypoxic pulmonary vasoconstriction: influence of propranolol and SR 59230A

Non-cumulative concentration–response curves to the four compounds were obtained by perfusing lungs during nine to eleven successive hypoxic periods with a salt solution containing isoprenaline (0.001–1 μM), SR 59104A, SR 59119A or SR 58611A (0.3–100 μM). In another series of experiments, concentration–response curves were obtained in the presence of either the non-selective β-adrenoceptor antagonist, propranolol (0.1 μM), or of the selective β_3-adrenoceptor antagonist, SR 59230A (0.3 μM), or of both. Higher concentrations of SR 59230A could not be tested because of the development of intrinsic relaxant effects in this model. In these experiments, the concentrations of isoprenaline used were: 0.1–100 μM vs. propranolol, 0.01–10 μM vs. SR 59230A, and 0.1–100 μM vs. propranolol + SR 59230A.

2.2.3. Effects of SR 59230A and propranolol on hypoxic pulmonary vasoconstriction

Non-cumulative concentration–response curves to SR 59230A (0.03–30 μM) in the absence or in the presence of propranolol (0.1 μM), and to propranolol (0.03–100 μM) were obtained by perfusing lungs during eleven to twelve successive hypoxic episodes.

2.3. Drugs and solutions

The drugs used were: SR 59104A (*N*-[(6-hydroxy-1,2,3,4-tetrahydronaphtalen-(2*R*)-2yl)methyl]-(2*R*)-2-hydroxy-2-(3-chlorophenyl)ethanamine hydrochloride), SR 59119A (*N*[(7-methoxy-1,2,3,4-tetrahydronaphtalen-(2*R*)-2yl)methyl]-(2*R*)-2-hydroxy-2-(3-chlorophenyl)ethanamine hydrochloride), SR 586-11A (ethyl{(7*S*)-7-[(2*R*)-2-(3-chlorophenyl)-2-hydroxyethylamino]-5,6,7,8-tetrahydronaphtalen-2-yloxy}acetate hydrochloride), SR 59230A 3-(2-ethylphenoxy)-1-[(1*S*)-1,2,3,4-tetrahydronapht-1-ylamino]-(2*S*)-2-propanol oxalate) (Sanofi-Midy, Research Centre, Milan, Italy), propranolol (Laboratoires Zeneca Pharma, Cergy, France), isoprenaline chlorhydrate, angiotensin II (Sigma, La Verpillère, France). Angiotensin II and SR 59104A were dissolved in distilled water, SR 59119A in a mixture of dimethylsulfoxide–distilled water (1:4), SR 58611A in a mixture of ethanol–distilled water (3:7) and SR 59230A in a mixture of ethylene glycol–distilled water (1:1). Propranolol was supplied as a 1 mg ml^{-1} in citric acid–distilled water solution. All drugs were diluted in the perfusate. The

maximal concentrations of dimethylsulfoxide (0.25%) ethanol (0.5%), ethylene glycol (1%) and citric acid (0.15%) in the bath did not by themselves exert any effect and did not modify the reactivity of the preparation.

2.4. Data analysis

Hypoxic pressure responses were measured at the time of the peak increase and were expressed as absolute changes from baseline values. One-half effective maximum concentration values expressed in the negative logarithm to base 10 form (pD_2) were determined from individual concentration–response curves.

Data are shown as mean ± S.E.M. Statistical significance was assessed using the Student's *t*-test for simple comparisons and the analysis of variance (ANOVA)-Bonferroni multiple *t*-test for multiple comparisons. *P*-values < 0.05 were considered significant.

3. Results

In lung preparations, the mean baseline inflation pressure was 10.40 ± 0.11 cm H_2O ($n = 136$) and was not significantly modified by hypoxic ventilation or addition of the various drugs. After equilibration of the preparation, the baseline perfusion pressure in normoxic ventilation was similar in all series of rats (4.93 ± 0.08 mmHg, $n = 136$).

In a 1st control series, ventilation with the hypoxic gas mixture produced a significant increase of the perfusion pressure ($+3.81 \pm 0.05$ mmHg, $n = 136$, $+77\%$ from baseline values, $P < 0.001$) which, starting from the 4th period of hypoxia, was reproducible for at least nine subsequent periods (Fig. 1). As shown in Fig. 2, the non-selective β-adrenoceptor agonist isoprenaline, and the selective β_3-adrenoceptor agonists SR 59104A, SR 59119A and SR 58611A concentration-dependently decreased the hypoxic pressure response ($P < 0.001$). Percent inhibition of this response was 71% with isoprenaline 0.1 μM and 75%, 79% and 73% with SR 59104A, SR 59119A and SR 58611A 30 μM, respectively. Table 1 indicates the pD_2 values of the four drugs which show that the relaxant potency of isoprenaline was 125-, 110- and 270-fold greater than those of SR 59104A, SR 59119A and SR 58611A, respectively ($P < 0.01$). In contrast, there was no significant difference between the relative relaxant potencies of the three latter drugs.

Propranolol, 0.1 μM, and SR 59230A, 0.3 μM, did not affect by themselves the hypoxic pressure response (Fig. 1). During the hypoxic pressure response, SR 59230A, propranolol, and the propranolol + SR 59230A combination produced significant ($P < 0.001$) rightward shifts of the concentration–response curves to isoprenaline (Fig. 2), resulting in smaller pD_2 values than in control conditions

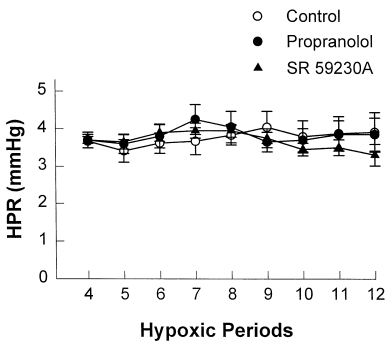

Fig. 1. Effects of infusions of normal saline on hypoxic pressor responses (HPR) recorded during nine successive periods of hypoxia in the absence ($n = 6$) (○) or the presence of 0.1 μM propranolol ($n = 5$) (●) or 0.3 μM SR 59230A ($n = 5$) (▲) in the isolated rat perfused lung. Values shown are increases of perfusion pressure above basal values. Data represent mean ± S.E.M.

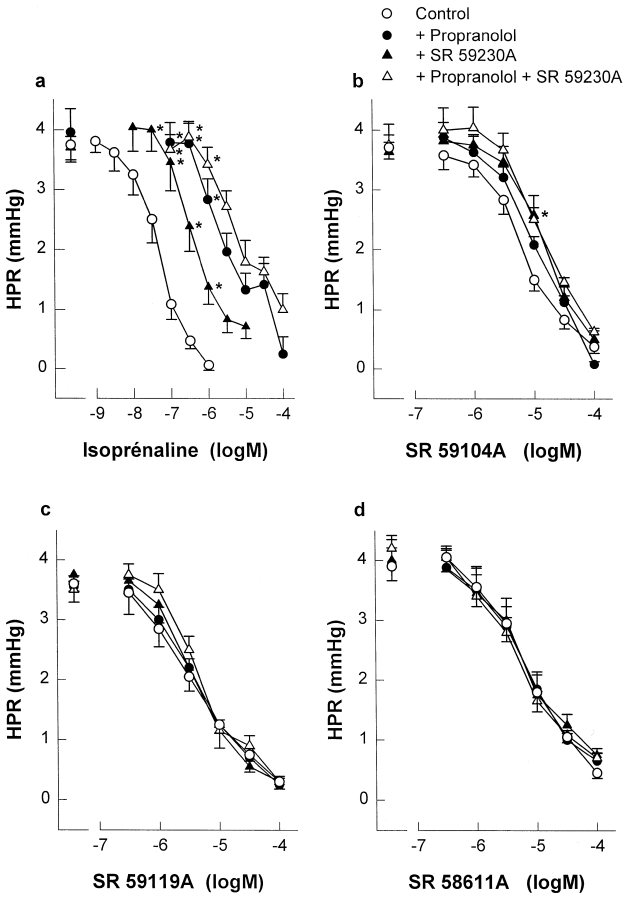

Fig. 2. Effects of infusions of isoprenaline 0.001–1 μM, 0.01–10 μM or 0.1–100 μM (a), SR 59104A 0.3–100 μM (b), SR 59119A 0.3–100 μM (c) or SR 58611A 0.3–100 μM (d) on hypoxic pressor responses (HPR) in the absence ($n = 8$, 7, 5 and 5, respectively) (○) or in the presence of either 0.1 μM propranolol ($n = 6$, 6, 5 and 5, respectively) (●), or 0.3 μM SR 59230A ($n = 6$, 7, 5 and 5, respectively) (▲), or 0.1 μM propranolol + 0.3 μM SR 59230A ($n = 6$, 6, 5 and 5, respectively) (△) in the isolated rat perfused lung. Values shown are increases of perfusion pressure above basal values. Data represent mean ± S.E.M. * Indicate responses with propranolol, SR 59230A or both that were significantly different from corresponding responses obtained in control experiments.

Table 1
Potencies expressed as pD_2 values of isoprenaline, SR 59104A, SR 59119A and SR 58611A in pulmonary vessels contracted by hypoxia. Influence of SR 59230A (0.3 μM), propranolol (0.1 μM) and both

Drugs	pD_2			
	Control	SR 59230A	Propranolol	SR 59230A + propranolol
Isoprenaline	7.36 ± 0.12	6.30 ± 0.13^{a}	5.36 ± 0.26^{b}	5.01 ± 0.24^{b}
SR 59104A	$5.26 \pm 0.10^{\ddagger}$	$4.71 \pm 0.08^{a\ddagger}$	4.84 ± 0.06^{a}	4.75 ± 0.09^{b}
SR 59119A	$5.32 \pm 0.13^{\ddagger}$	$5.41 \pm 0.11^{\dagger}$	5.27 ± 0.02	5.24 ± 0.04
SR 58611A	$4.93 \pm 0.11^{\ddagger}$	$5.11 \pm 0.25^{\dagger}$	5.01 ± 0.13	5.20 ± 0.06

Significant ($^{a}P < 0.05$, $^{b}P < 0.01$) against corresponding value obtained in control experiments.
Significant ($^{\dagger}P < 0.05$, $^{\ddagger}P < 0.01$)) against corresponding value obtained with isoprenaline.
Values are mean $\pm$ S.E.M. from 5 to 8 separate experiments per group.

(Table 1). However, as shown in Fig. 2 and Table 1, the propranolol + SR 59230A combination was not significantly more potent than propranolol alone at antagonizing isoprenaline relaxant effects.

SR 59230A and the SR 59230A + propranolol combination produced significant and similar rightward shifts of the concentration response curve to SR 59104A ($P < 0.01$) but these were smaller than those observed with isoprenaline. Propranolol also produced a small but nonsignificant rightward shift of the concentration–response curve to SR 59104A (Fig. 2). In contrast, SR 59230A, propranolol and the SR 59230A + propranolol combination were almost ineffective at antagonizing the relaxant effects of both SR 59119A and SR 58611A (Fig. 2, Table 1). In the presence of propranolol, the relaxant potency of isoprenaline became similar to those of the three selective β_3-adrenoceptor agonists (Table 1).

As shown in Fig. 3, SR 59230A and propranolol produced a concentration-dependent decrease of the hypoxic pressure response at concentrations ≥ 1 μM and ≥ 10 μM, respectively. Addition of 0.1 μM of propranolol to SR 59230A did not modify the concentration–response curve obtained with SR 59230A alone.

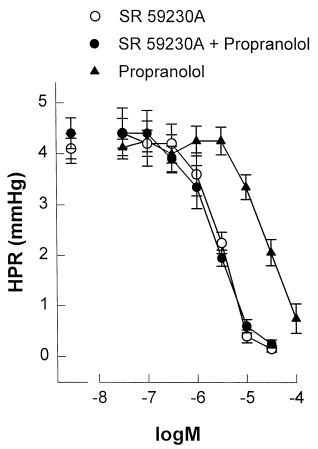

Fig. 3. Effects of infusions of SR 59230A, 0.03–30 μM, in the absence ($n = 5$) (○) and the presence of 0.1 μM propranolol ($n = 5$) (●), and of propranolol 0.3–100 μM ($n = 5$) (▲) on hypoxic pressor responses (HPR) in the isolated rat perfused lung. Values shown are increases of perfusion pressure above basal values. Data represent mean $\pm$ S.E.M.

4. Discussion

The rat isolated perfused lung preparation allows the exploration of the vasomotor tone in all pulmonary vessels and particularly in the small arteries and veins which are known to account for the greatest part of pulmonary vasculature resistances (Madden et al., 1985; Zhao et al., 1993). We investigated the effects of three phenylethanolaminotetralines reported to exert atypical β-adrenoceptor agonist properties (Bianchetti and Manara, 1990; Croci et al., 1995), SR 59104A, SR 59119A and SR 58611A, on the pulmonary vascular response to hypoxia. Their effects were compared to those of the well-known β-adrenoceptor agonist isoprenaline, and challenged with (a) propranolol, a non-selective $\beta_1 + \beta_2$-adrenoceptor antagonist, and (b) SR 59230A, an aroxylpropanolaminotetralin developed as a putative β_3-adrenoceptor antagonist in the gut (Manara et al., 1995).

To our knowledge this is the first report demonstrating the ability of three β_3-adrenoceptor agonists, SR 59104A, SR 59119A and SR 58611A to relax the pulmonary vasculature during hypoxia, a physiopathological condition that produces an interesting contracting stimulus to investigate potential dilator agents for the treatment of diseases such as pulmonary hypertension (Dumas et al., 1994). Relaxant effects in other vascular beds have previously been described with other β_3-adrenoceptor agonists such as BRL 37344 and CGP 12177 in the rat isolated carotid artery (Oriowo, 1994) and thoracic aorta (Oriowo, 1995), BRL 37344 and ZD 7114 in the rat thoracic aorta and pulmonary artery (Sooch and Marshall, 1996) and BRL 37344 and CL 316243 in the canine cutaneous vascular smooth muscle (Berlan et al., 1994). In our study, however, the three β_3-adrenoceptor agonists used were 100 (SR 59104A and SR 59119A) to 300-fold (SR 58611A) less potent than isoprenaline. Similar observations have been made with other β_3-adrenoceptor agonists such as ZD 7114 (Sooch

and Marshall, 1996) and with BRL 37344 and CGP 12177 (Oriowo, 1994). Finally, in our study SR 58611A was found to be 100- to 1000-fold less potent in the pulmonary vascular bed than reported in the rat distal colon (Manara et al., 1995) and the human colon (De Ponti et al., 1996). All these data support the concept of tissue-specific properties that could be explained by variations in the β-adrenoceptor subtypes density modifying the receptor mediated response (Oriowo, 1995), and (or) by the presence of spare receptors as suggested by Blin et al. (1994).

Besides the fact that our data confirm the ability of the non-selective $\beta_1 - \beta_2$-adrenoceptor antagonist, propranolol to oppose the relaxant effects of isoprenaline (Oriowo, 1994; Mc Kean and Mc Donald, 1995), they in addition demonstrate that the selective β_3-adrenoceptor antagonist, SR 59230A also induces a significant shift of the concentration–response curve for isoprenaline. This finding is in accordance with previous data which suggest that all β-adrenoceptor subtypes contribute to the isoprenaline-induced relaxation in smooth muscle from other tissues (Oriowo, 1994; De Ponti et al., 1996; Manara et al., 1996). However, in our study, the SR 59230A + propranolol combination was only slightly more potent than propranolol alone at shifting to the right the isoprenaline concentration–response curve. This observation suggests that propranolol, despite the low concentration used, might exert a weak blocking activity at β_3-adrenoceptors in this tissue (Mc Kean and Mc Donald, 1995), or that isoprenaline could elicit a pleiotropic agonist response with multiple G-proteins activation according to its concentration or the antagonist used (Bianchetti and Manara, 1990; Kenakin, 1995). Indeed further investigations are necessary to state the evidence of β_3-adrenoceptors or other β-adrenoceptor subtypes in the pulmonary circulation.

In the presence of the β_1- and β_2-adrenoceptor antagonist, propranolol, the concentration of SR 59230A necessary to obtain a shift by 3 of the concentration response curve of isoprenaline in our study, was 30-fold greater than that required to obtain a similar shift in smooth muscle from human colon (De Ponti et al., 1996) suggesting that a partial β_2-adrenoceptor antagonism of SR 59230A might be involved at this concentration. Nevertheless, this hypothesis is not in agreement with the effects observed with graded concentrations of SR 59230A since at concentrations ≥ 1 μM, this compound elicited a concentration-dependent relaxant effect against hypoxic pulmonary vasoconstriction which was resistant to propranolol. Different mechanisms could explain the relaxation induced by SR 59230A, e.g., a partial β_3-adrenoceptor agonist as described in transfected Chinese hamster ovary cells (Strosberg and Pietri-Rouxel, 1997), or a 'membrane-stabilizing effect' as observed with propranolol at high concentrations (Black and Prichard, 1973).

Regarding SR 59104A, its ability to relax hypoxic pulmonary vasculature, and the ability of SR 59230A, but not propranolol to antagonize these relaxant effects is in favour of the presence of atypical β-adrenoceptors in the pulmonary circulation. Nevertheless, the rightward shift of SR 59104A concentration–response curves induced by SR 59230A was smaller than that produced by the same drug vs. isoprenaline, but this is fairly consistent with the numerous differences in agonists' effects that have been reported in previous studies (Blin et al., 1994; Manara et al., 1996).

Whereas SR 59230A shifted to the right the concentration–effect curves to isoprenaline and SR 59104A, it failed to oppose the relaxant effects of SR 59119A and SR 58611A. This discrepancy could be explained by a change in the selectivity of these drugs in relation with different experimental conditions as suggested previously (De Ponti et al., 1996). And, indeed, SR 58611A has been described as a β_1-adrenoceptor agonist in the central nervous system. (Nisoli et al., 1996). However, this hypothesis can obviously be ruled out in our study as β_1-adrenoceptors are few in the vascular smooth muscle (Lands et al., 1967) and as propranolol failed to antagonize the pharmacological effects of SR 58611A. Likewise, the latter results are not in favour of β_2-adrenoceptor agonist properties for these compounds.

In conclusion, it appears from our data that in the rat pulmonary circulation: (a) the non-selective β-adrenoceptor agonist isoprenaline and the selective β_3-adrenoceptor agonists SR 59104A, SR 59119A and SR 58611A share the ability to oppose the hypoxic pulmonary vasoconstriction, (b) the antagonist effects exerted by the selective β_3-adrenoceptor antagonist SR 59230A vs. isoprenaline and SR 59104A, and the lack of antagonist effects of propranolol vs. the three selective β_3-adrenoceptor agonists suggest the existence of atypical adrenoceptors the evidence of which for a role in the pulmonary circulation, requires however further investigations, (c) the inability of SR 59230A to antagonize SR 59119A and SR 58611A effects underlines the versatility of these atypical-adrenoceptors, and (d) the intrinsic relaxant effects of SR 59230A at high concentrations suggest additional properties which deserve further investigation.

References

Arch, J.R.S., Ainsworth, A.T., Cawthorne, M.A., Piercy, V., Senitt, M.V., Thody, V.E., Wilson, C., Wilson, S., 1984. Atypical β-adrenoceptor on brown adipocytes as target for anti-obesity drugs. Nature 309, 163–165.

Berlan, M., Galitzky, J., Bousquet-Melou, A., Lafontan, M., Montastruc, J.L., 1994. β_3-adrenoceptor-mediated increase in cutaneous blood flow in the dog. J. Pharmacol. Exp. Ther. 268, 1444–1451.

Bianchetti, A., Manara, L., 1990. In vitro inhibition of intestinal motility by phenylethanolaminotetralines: evidence of atypical β-adrenoceptors in rat colon. Br. J. Pharmacol. 100, 831–839.

Black, J.W., Prichard, B.N.C., 1973. Activation and blockade of β-adrenoceptors in common cardiac disorders. Br. Med. J. 29, 163–167.

Blin, N., Mahmias, C., Drumare, M.F., Strosberg, A.D., 1994. Mediation of most atypical effects by species homologues of the β_3-adrenoceptor. Br. J. Pharmacol. 112, 911–919.

Croci, T., Cecchi, R., Guzzi, M., Landi, T., Menini, T., Parotti, L., Le Fur, G., Manara, L., 1995. The novel β_3-adrenoceptor agonists SR 59119A and SR 59104A are stereospecifically antagonized by SR 59230A. Br. J. Pharmacol. 116, 204P.

De Ponti, F., Gibelli, G., Croci, T., Arcidiaco, M., Crema, F., Manara, L., 1996. Functional evidence of atypical β_3-adrenoceptors in the human colon using the β_3-selective adrenoceptor antagonist SR 59230A. Br. J. Pharmacol. 117, 1374–1376.

Dumas, M., Dumas, J.P., Sgro, C., Advenier, C., Giudicelli, J.F., 1994. Effects of two K^+ channel openers, aprikalim and pinacidil, on hypoxic pulmonary vasoconstriction. Eur. J. Pharmacol. 263, 17–23.

Dumas, M., Dumas, J.P., Rochette, L., Advenier, C., Giudicelli, J.F., 1997. Role of potassium channels and nitric oxide in the effects of iloprost and prostaglandin E_1 on hypoxic vasoconstriction in the isolated perfused lung of the rat. Br. J. Pharmacol. 120, 405–410.

Kaumann, A.J., Molenaar, P., 1996. Differences between the third cardiac β-adrenoceptor and the colonic β_3-adrenoceptor in the rat. Br. J. Pharmacol. 118, 2085–2098.

Kenakin, T., 1995. Agonist-receptor efficacy: II. Agonist trafficking of receptor signals. Trends Pharmacol. Sci. 16, 232–238.

Lands, A.M., Luduena, F.P., Buzzo, H.J., 1967. Differentiation of receptors responsive to isoproterenol. Life Sci. 6, 2241–2249.

Madden, J.A., Dawson, C.A., Harder, D.A., 1985. Hypoxia-induced activation in small isolated pulmonary arteries from the cat. J. Appl. Physiol. 59, 113–118.

Manara, L., Badone, D., Baroni, M., Boccardi, G., Cecchi, R., Crochi, T., Giudice, A., Guzzi, U., Le Fur, G., 1995. Aryloxypropanolaminotetralins are the first selective antagonists for atypical (β_3) β-adrenoceptors. Pharmacol. Commun. 6, 240–245.

Manara, L., Badone, D., Baroni, M., Boccardi, G., Cecchi, R., Croci, T., Giudice, A., Guzzi, U., Landi, M., Le Fur, G., 1996. Functional identification of rat atypical β-adrenoceptors by the first β_3-selective antagonists, aryloxypropanolaminotetralins. Br. J. Pharmacol. 117, 435–442.

Mc Kean, J., Mac Donald, A., 1995. Contributions of β-adrenoceptor subtypes to responses to isoprenaline in rat distal colon. J. Pharm. Pharmacol. 47, 388–391.

Nisoli, E., Tonello, C., Landi, M., Carrura, M.O., 1996. Functional studies of the first selective β_3-adrenergic receptor antagonist SR 59230A in rat brown adipocytes. J. Pharmacol. Exp. Ther. 49, 7–14.

Oriowo, M.A., 1994. Atypical β-adrenoceptors in the rat isolated common carotid artery. Br. J. Pharmacol. 113, 699–702.

Oriowo, M.A., 1995. Different atypical β_3-adrenoceptors mediate isoprenaline-induced relaxation in vascular and non vascular smooth muscles. Pharmacol. Lett. 56, 269–275.

Sooch, S., Marshall, I., 1996. Evidence for atypical β-adrenoceptors in the rat vasculature. Br. J. Pharmacol. 117, 261P.

Strosberg, A.D., Pietri-Rouxel, F., 1997. The β_3-adrenoceptor constitutes indeed a versatile receptor. Trends Pharmacol. Sci. 18, 52–53.

Zhao, L., Packer, S., Rhoades, R.A., 1993. Pulmonary veins contract in response to hypoxia. Am. J. Physiol. 265, L87–L92.