Role of potassium channels and nitric oxide in the relaxant effects elicited by β-adrenoceptor agonists on hypoxic vasoconstriction in the isolated perfused lung of the rat

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1 The aims of this study were to compare, in the rat isolated perfused lung preparation, the antagonist effects of a nonselective β-adrenoceptor agonist (isoprenaline), a selective β_2-adrenoceptor agonist (salbutamol) and a selective β_3-adrenoceptor agonist (SR 59104A) on the hypoxic pulmonary pressure response, and to investigate the role of K^+ channels, endothelium derived relaxing factor and prostaglandins in these effects. K^+ channels were inhibited by glibenclamide, charybdotoxin or apamin, NO synthase and cyclo-oxygenase were inhibited by N^G-nitro-L-arginine methyl ester (L-NAME) and indomethacin, respectively.

2 Hypoxic ventilation produced a significant increase in perfusion pressure ($+65\%$, $P<0.001$) and L-NAME significantly increased this response further ($+123\%$, $P<0.01$). After apamin, L-NAME, indomethacin, post-hypoxic basal pressure did not return to baseline values ($P<0.001$).

3 Glibenclamide partially inhibited the relaxant effects of isoprenaline ($P<0.05$) and salbutamol ($P<0.001$) but not that of SR 59104A. In contrast, charybdotoxin and apamin partially inhibited the relaxant effects of SR 59104A ($P=0.053$ and <0.01, respectively) but did not modify the effects of isoprenaline and salbutamol. L-NAME partially inhibited the dilator response of salbutamol ($P<0.01$) and SR 59104A ($P<0.05$) but not that of isoprenaline.

4 We conclude that (a) EDRF exerts a significant inhibition of the hypoxic pulmonary response, (b) SK_{Ca} channel activation, EDRF and prostaglandins contribute to the reversal of the hypoxic pressure response, (c) the vasodilation induced by isoprenaline is mediated in part by activation of K_{ATP} channels, that of salbutamol by activation of K_{ATP} channels and EDRF. In contrast, SR 59104A partly operates through BK_{Ca}, SK_{Ca} channels and EDRF activation, differing in this from the β_1 and β_2-adrenoceptor agonists.

Keywords: Hypoxic pulmonary vasoconstriction; isoprenaline; salbutamol; SR 59104A; nitric oxide/endothelium derived relaxing factor; ATP-sensitive K^+ channels; Ca^{2+}-activated K^+ channels

Abbreviations: BK_{Ca}, large conductance Ca^{2+}-activated K^+; EDRF, endothelium derived relaxing factor; K_{ATP}, ATP-sensitive K^+; L-NAME, N^G-nitro-L-arginine methyl ester; NO, nitric oxide; SK_{Ca}, small conductance Ca^{2+}-activated K^+

Introduction

Pulmonary hypertension may be secondary to hypoxic vasoconstriction resulting in complex changes in the pulmonary vascular bed. Despite extensive research, the mechanism underlying hypoxic pulmonary vasoconstriction is yet unknown. Suppression of endogenous vasodilator substances such as endothelium derived relaxing factor (EDRF/NO) may be one mechanism mediating the hypoxic vasoconstriction (Liu *et al.*, 1991). The ability of potassium channels to modulate hypoxic vasoconstriction is suspected because of the excitatory effects of K^+ channel blockers such as tetraethylammonium or 4-aminopyridine (Hasunuma *et al.*, 1991a; Dumas *et al.*, 1994; Reeve *et al.*, 1997).

Currently, available therapies remain limited to correction of hypoxaemia and generalized non-specific pulmonary vasodilation (Leach & Treacher, 1995). The recent development of inhaled therapy (NO, prostacyclin and analogues) represents a significant advance in the management of hypoxic pulmonary vasoconstriction (Olschewski *et al.*, 1996; Mankte-

low *et al.*, 1997). However the therapeutic effects of this approach are limited by the short half-life of these compounds. A new pharmacological approach consists in prolonging the effects of prostacyclin by the addition of subtherapeutic doses of another aerosolized dilator agent limiting thereby the intrapulmonary shunt and systemic side effects (Ghofrani *et al.*, 1998). In this context, the development of selective compounds may be of interest. In the adrenergic system, 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 58611), but relatively insensitive to the conventional β-adrenoceptor antagonists are present in the vascular smooth muscle (Berlan *et al.*, 1994; Oriowo, 1994; 1995). In a recent study the relaxant effects of three β_3- adrenoceptor agonists (SR 58611A, SR 59104A and SR 59119A) have been investigated in the pulmonary circulation (Dumas *et al.*, 1998).

β-adrenoceptor agonists are usually regarded as agents mediating their effects through specific receptors coupled to adenylate cyclase, inducing smooth muscle relaxation by increasing the intracellular concentration of adenosine 3′, 5′-

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cyclic monophosphate (cyclic AMP) (Bylund *et al.*, 1994; Torphy, 1994). Recent evidence however suggests that *β*-adrenoceptor agonists can activate multiple signalling pathways. It has been shown that ATP-sensitive K^+ (K_{ATP}) channels (Randall & McCulloch, 1995; Nakashima & Vanhoutte, 1995; Chang, 1997) or Ca^{2+}-activated K^+ channels (Satake *et al.*, 1996; Huang & Kwok, 1997) or EDRF (Wang *et al.*, 1993; Hamdad *et al.*, 1996) are involved in the non selective- or the *β₂*-selective adrenoceptor agonists-induced relaxation of vascular smooth muscle. To our knowledge, these various mechanisms of action have not yet been investigated in the pulmonary circulation.

The purpose of this study was to investigate (1) the contribution of ATP sensitive K^+ channels, large conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels, small conductance Ca^{2+}-activated K^+ (SK_{Ca}) channels, EDRF, and prostaglandins to hypoxic vasoconstriction in rat lungs perfused with physiological solutions, and (2) the role of these mechanisms in the vasodilating actions exerted by the nonselective *β*-adrenoceptor agonist (isoprenaline), a selective *β₂*-adrenoceptor agonist (salbutamol) and a selective *β₃*-adrenoceptor agonist (SR 59104A).

Methods

Rat perfused isolated lung preparation

Twenty-eight groups ($n=4-9$ per group) of male Wistar rats (Dépré, St Doulchard, France), weighing $280-340$ g, were anaesthetized with sodium pentobarbitone (100 mg kg^{-1}) and tracheotomized. After thoracotomy, a polyethylene cannula was inserted into the main pulmonary artery and the lungs were removed quickly and allowed to equilibrate in the perfusion circuit maintained at 38°C by a surrounding water bath and consisting of a perfusion reservoir, a roller pump (Harvard 77, Ealing, Les Ullis, France), connecting tubing and bubble trap. Mean perfusion pressure which was measured from a side-arm of the arterial line (Harvard transducer, -50 to 300 mmHg), was recorded continuously (Ankersmit WR 3701 recorder, Graphtec Corp., Japan) and reflected pulmonary vascular resistances 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 (1 g 100 ml^{-1}, type 70, Sigma) was included as a colloïd (Hasunuma *et al*, 1991b). The lungs were ventilated with a Harvard rodent ventilator at a tidal volume of 10 ml kg^{-1} body weight and a frequency of 55 breaths min^{-1}. The end expiratory pressure was set at 2.5 cm H_2O. The pressure of airways was measured with a Validyne DP45 ($0-88$ cm H_2O) differential pressure transducer. A $20-30$ 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.

Experimental protocols

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. The lungs were then challenged with a hypoxic gas mixture (5% CO_2, 95% N_2) as described previously (Dumas *et al.*, 1996). Each hypoxic challenge (4 min) was followed by the addition of 0.25 μg angiotensin II in normoxic ventilation (4 min) and the pressure was allowed to return to baseline before the initiation of hypoxic ventila-
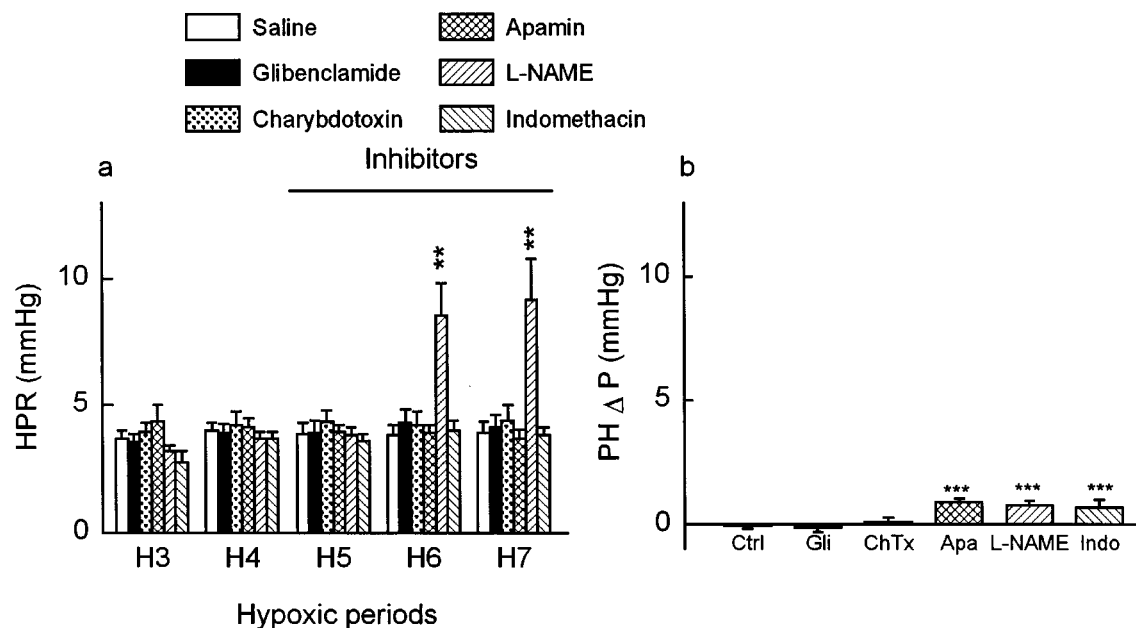

Figure 1 Influence of normal saline and inhibitors: glibenclamide (1 μM) (Gli), charybdotoxin (0.1 μM) (ChTx), apamin (0.05 μM) (Apa), L-NAME (100 μM) or indomethacin (10 μM) (Indo) on (a) hypoxic pressure response ($n=7$, 5, 5, 7 and 4, respectively), and (b) the post-hypoxic relaxation ($n=6$, 7, 5, 5, 7 and 4, respectively) in the isolated rat perfused lung. Values shown are increases of perfusion pressure above basal values (a) from the third to the seventh hypoxic period with infusion of the inhibitors from the fifth to the seventh hypoxic period (hypoxic pressure response: HPR), or (b) after the seventh hypoxic period (post hypoxic Δ pressure: PHΔP). Data represent mean$\pm$s.e.mean. Asterisks indicate responses that were significantly different from corresponding responses in control experiments (**$P<0.01$, ***$P<0.001$).

tion. The perfusate gas tensions were measured during hypoxic challenges, by collecting perfusate anaerobically from the arterial cannula and analysing it immediately (Corning 170 pH/blood gas analyzer). 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 (Dumas *et al.*, 1996). Drugs were tested after a stable response to hypoxia was reached.

Effects of isoprenaline, salbutamol and SR 59104A on pulmonary vasoreactivity: influence of K^+ channels, nitric oxide synthase and cyclo-oxygenase inhibitors Non-cumulative concentration-response curves to the three agonist compounds were obtained by perfusing lungs during six to seven hypoxic periods with a salt solution containing isoprenaline (0.001 – 1 μM), salbutamol (0.01 – 10 μM) or SR 59104A (0.3 – 100 μM). In another series of experiments, concentration-response curves were obtained in the presence of one of the following inhibitors: glibenclamide (1 μM), a K_{ATP} channel blocker. Charybdotoxin (0.1 μM), a BK_{Ca} channel blocker, apamin (0.05 μM), a SK_{Ca} channel blocker, N^G-nitro-L-arginine methyl ester (L-NAME 100 μM), a nitric oxide synthase inhibitor, or indomethacin (10 μM), a cyclo-oxygenase inhibitor were administered during the fifth, sixth and seventh hypoxic periods whereas the agonists were used during the sixth and seventh periods (Dumas *et al.*, 1997), at concentrations inducing about 75% inhibition of the pressure response to hypoxia (isoprenaline 0.03 μM, salbutamol 0.6 μM, SR 59104A 20 μM).

Chemicals/drugs

The drugs used were: SR 59104A (N- [6 hydroxy-1,2,3,4-tetrahydronaphtalen-(2**R**)-2yl) methyl]-(2**R**)-2hydroxy-2-(3-chlorophenyl) ethanamine hydrochloride (Sanofi-Midy, Research Centre, Milan, Italy), glibenclamide (Laboratoire

Hoechst, Paris la Défense, France), isoprenaline, salbutamol, angiotensin II, N^G-nitro-L-arginine methyl ester, indomethacin (Sigma Chimie, La Verpillère, France) and charybdotoxin and apamin (Latoxan, Rosans, France). Isoprenaline, salbutamol, SR 59104A, apamin, angiotensin II and N^G-nitro-L-arginine methyl ester were dissolved in distilled water, charybdotoxin in saline, glibenclamide in a mixture of dimethylsulphoxide-distilled water (1 : 1) and indomethacin in a carbonate salt solution (4 : 100). All the stock solutions were kept frozen until use and all the diluted solutions were prepared just before administration. The maximal concentrations of dimethylsulphoxide (2%) added to the bath did not by themselves exert any effect and did not modify the reactivity of the preparation.

Data analysis

Hypoxic pressure response was measured at the time of the peak increase and expressed as absolute changes from baseline values. For agonists, the one-half effective maximum concentration values expressed in the negative logarithm to base 10 form (pD_2) were determined from individual concentration-response curves. For the experiments performed with an inhibitor, the level of hypoxic pressure response being different in the presence of inhibitors, the effects of the latter were calculated as:

$$\left[1 - \frac{\dfrac{(R_I - R_{D+I})}{R_I}}{\dfrac{(R - R_D)}{R}} \right] \times 100$$

where (a) R_{D+I} and R_I are the hypoxic pressure responses observed with the inhibitor with and without the agonist, respectively, and (b) R_D and R are the corresponding hypoxic pressure responses observed in control experiments with and without the agonist, respectively.
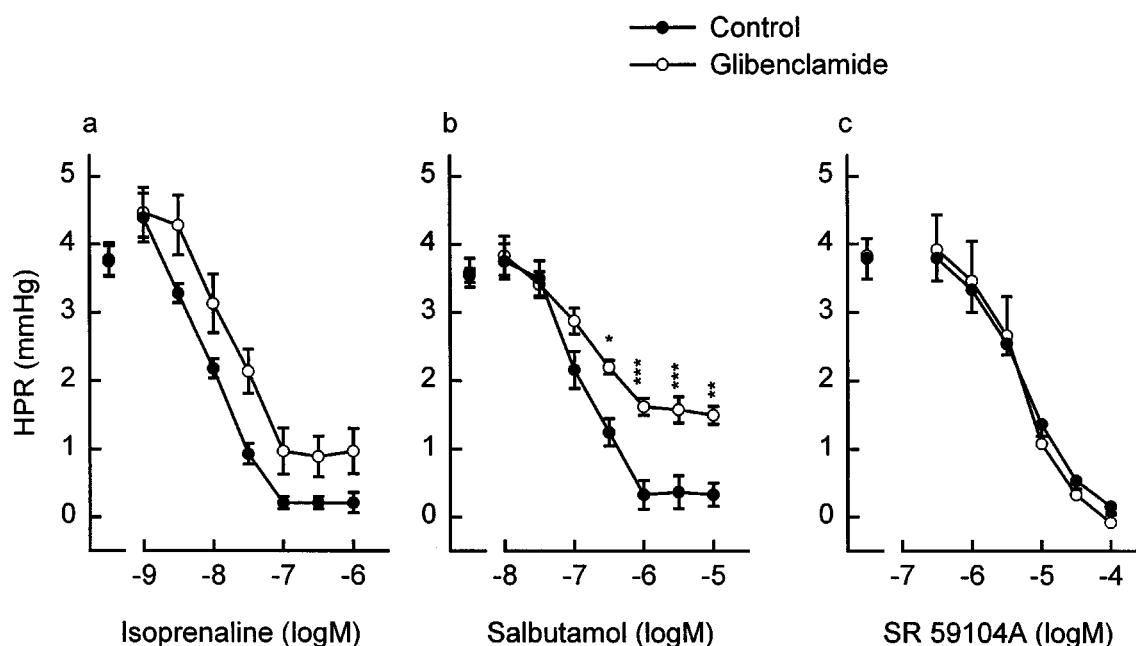

Figure 2 Effects of infusions of (a) isoprenaline (0.001 – 1 μM), (b) salbutamol (0.01 – 10 μM) or (c) SR 59104A (0.3 – 100 μM) on hypoxic pressor responses (HPR) in the absence (*n* = 7, 6 and 6, respectively) (●) and in the presence of glibenclamide (1 μM) (*n* = 9, 6 and 6, respectively) (○) in the isolated rat perfused lung. Values shown are increases of perfusion pressure above basal values. Data represent mean ± s.e.mean. Asterisks indicate responses with glibenclamide that were significantly different from corresponding responses obtained without glibenclamide (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

Data are shown as mean $\pm$ s.e.mean. Statistical significance was assessed with the Mann-Whitney U-test for simple comparisons and the ANOVA-Bonferroni multiple t-test for multiple comparisons; P values < 0.05 were considered significant.

Table 1 Potencies expressed as pD_2 values of isoprenaline, salbutamol and SR 59104A in pulmonary vessels contracted by hypoxia: Influence of glibenclamide

	pD_2	
Drugs	*Control*	*Glibenclamide* (1 μM)
Isoprenaline	7.84 ± 0.06	7.39 ± 0.19
Salbutamol	$6.73 \pm 0.10^{***}$	$5.83 \pm 0.18^{***\dagger\dagger}$
SR 59104A	$5.17 \pm 0.07^{***}$	$5.37 \pm 0.21^{***}$

Significant (***$P < 0.001$) against corresponding value obtained with isoprenaline. Significant (††$P < 0.01$) against corresponding value obtained in control experiments. Values are mean $\pm$ s.e.mean from 6–9 separate experiments per group.

Results

In lung preparations, the mean baseline inflation pressure was 10.32 ± 0.09 cm H_2O ($n = 192$) 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 (6.12 ± 0.09 mmHg, $n = 192$). Ventilation with a hypoxic mixture of gas produced a significant increase of the perfusion pressure ($+3.95 \pm 0.05$ mmHg, $n = 160$, $+65\%$ from baseline values, $P < 0.001$) which, starting from the fourth period of hypoxia, was reproducible for at least nine subsequent periods.

Effects of K^+ channel, EDRF and cyclo-oxygenase inhibitors on pulmonary reactivity

Glibenclamide, charybdotoxin, apamin, L-NAME and indomethacin did not affect significantly the baseline values of perfusion pressure ($+0.05 \pm 0.01$, $+0.08 \pm 0.17$, -0.15 ± 0.15, $+0.05 \pm 0.13$ and $+0.12 \pm 0.24$ mmHg respectively). As shown in Figure 1a, glibenclamide at 1 μM, charybdotoxin at 0.1 μM,

Table 2 Percentage of the maximal relaxation obtained with β-adrenoceptor agonists on hypoxic pulmonary vasoconstriction: Influence of charybdotoxin, apamin or L-NAME

β-adrenoceptor agonists	*Control*	*Charybdotoxin* (0.1 μM)	*Apamin* (0.05 μM)	*L-NAME* (100 μM)
Isoprenaline (0.03 μM)	73 ± 7	76 ± 5	68 ± 8	64 ± 3
Salbutamol (0.6 μM)	76 ± 6	75 ± 2	63 ± 5	$47 \pm 6^{**}$
SR 59104A (20 μM)	75 ± 5	$50 \pm 9\dagger$	$51 \pm 3^{**}$	$56 \pm 4^{*}$

Significant (†$P = 0.053$, *$P < 0.05$, **$P < 0.01$) against corresponding value obtained in control experiments. Values are mean $\pm$ s.e.mean from 5–9 separate experiments per group.
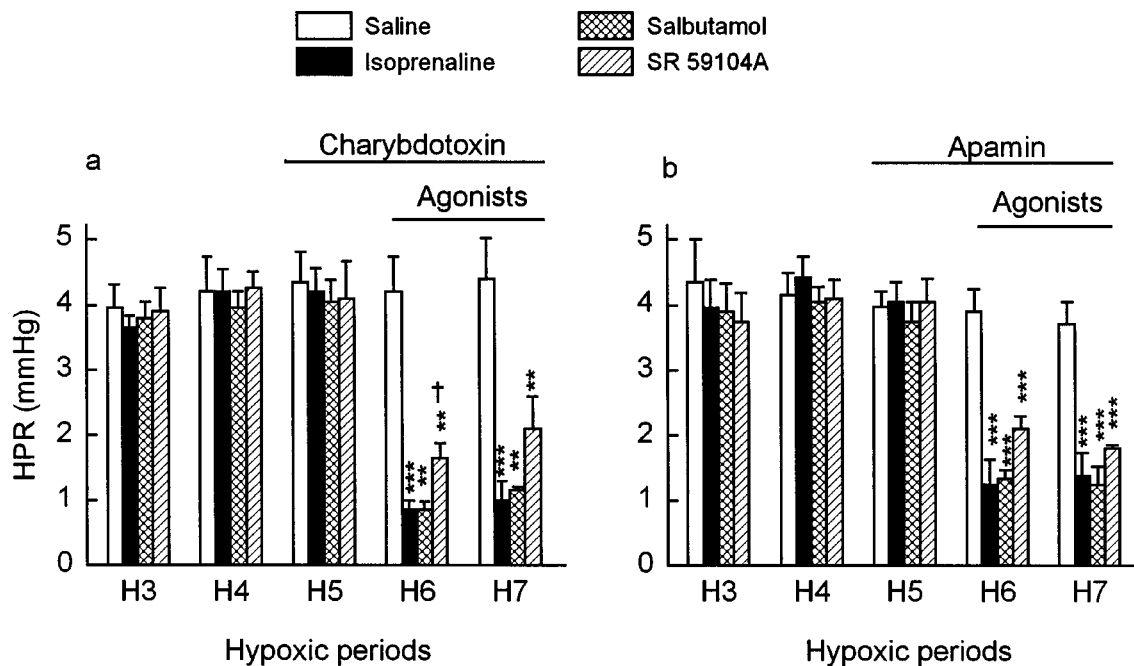

Figure 3 Influence of (a) charybdotoxin (0.1 μM) and (b) apamin (0.05 μM) on the effects of infusions of normal saline ($n = 5$ and 5, respectively), isoprenaline (0.03 μM) ($n = 5$ and 6, respectively), salbutamol (0.6 μM) ($n = 5$ and 6, respectively) or SR 59104A (20 μM) ($n = 5$ and 5, respectively) on hypoxic pressure responses (HPR) in the rat isolated perfused lung. Charybdotoxin and apamin were infused from the fifth hypoxic period and agonists were infused from the sixth hypoxic period. Values shown are increases of perfusion pressure above basal values. Data represent mean $\pm$ s.e.mean. Asterisks indicate responses to the agonists that were significantly different from corresponding responses obtained in the control period (**$P < 0.01$, ***$P < 0.001$). † indicates a response to SR 59104A that was significantly different ($P < 0.05$) from the corresponding response to salbutamol.

apamin at 0.05 μM and indomethacin at 10 μM did not by themselves affect the hypoxic pressure response. In contrast the hypoxic pressure response was significantly increased by L-NAME ($+9.17 \pm 1.60$ versus 3.92 ± 0.40 mmHg, $P < 0.01$). As shown in Figure 1b, post-hypoxic basal pressure did not return to baseline values after apamin ($+0.9 \pm 0.17$ mmHg, $P < 0.001$), L-NAME ($+0.79 \pm 0.18$ mmHg, $P < 0.001$) and indomethacin ($+0.69 \pm 0.34$ mmHg, $P < 0.001$).

Effects of isoprenaline, salbutamol and SR 59104A on pulmonary reactivity; influence of K^+ channel, EDRF and cyclo-oxygenase inhibitors

As shown in Figure 2, isoprenaline, salbutamol and SR 59104A concentration-dependently decreased the hypoxic pressure response ($P < 0.001$). Per cent inhibition of this response was 73% with isoprenaline 0.03 μM, 75% with salbutamol 0.6 μM and 75% with SR 59104A 20 μM. Table 1 shows that isoprenaline was significantly ($P < 0.001$) more potent than salbutamol and SR 59104A at decreasing the hypoxic pressure response, the decreasing order of potency being: isoprenaline > salbutamol > SR 59104A. During the hypoxic pressure response, the K_{ATP} channel inhibitor, glibenclamide produced a rightward shift of the concentration-response curves to isoprenaline and salbutamol ($P < 0.05$ and $P < 0.001$ respectively, Figure 2a and b), resulting in a significantly lower pD_2 value of salbutamol than in the control conditions ($P < 0.01$, Table 1). Glibenclamide inhibited the response to 0.03 μM isoprenaline and 1 μM salbutamol by

approximately 43 and 40% respectively. In contrast, the antagonist effects of SR 59104A against the hypoxic vasoconstriction remained unaffected by glibenclamide (Figure 2c and Table 1). The BK_{Ca} channel inhibitor, charybdotoxin, was devoid of any effect on the response to isoprenaline and salbutamol but reduced that to SR 59104A, there being in this respect a difference between the selective β_2- and β_3-adrenoceptor agonists ($P < 0.05$, Figure 3a). The antagonist effects of SR 59104A in presence of charybdotoxin were reduced by 33% as compared to those observed in control conditions ($P = 0.053$, Table 2). Similar results were observed with the SK_{Ca} channel inhibitor, apamin (Figure 3b) which showed no effect on the responses to isoprenaline and salbutamol but significantly decreased that to SR 59104A (-32%, $P < 0.001$, Table 2). As shown in Figure 4, L-NAME increased 2 fold the hypoxic pressure responses ($+8.53 \pm 1.30$ and $+9.17 \pm 1.60$ mmHg during the sixth and the seventh hypoxic periods as compared to $+3.71 \pm 0.26$ mmHg and $+3.92 \pm 0.40$ mmHg during the same hypoxic periods with saline only, $P < 0.01$). L-NAME (Table 2) did not significantly affect the relaxant effects of isoprenaline but significantly reduced those of salbutamol (-38%) and SR 59104A (-25%) ($P < 0.01$ and $P < 0.05$, respectively, Table 2).

Discussion

In this study, we investigated the effects of various β-adrenoceptor agonists on the pulmonary pressor response to hypoxia and the mechanisms involved, in a rat model of isolated perfused lung according to a method based on that described by McMurtry (1984). This model allows the investigation 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 vascular resistances (Madden *et al.*, 1985; Zhao *et al.*, 1993).

The baseline perfusion pressure under normoxic conditions was not influenced in our study by L-NAME, indomethacin and K^+ channel inhibitors suggesting that the low pulmonary vascular tone is not dependent upon EDRF or prostacyclin release, or upon K_{ATP}, BK_{Ca} channels (Leeman *et al.*, 1994; Dumas *et al.*, 1996; Nossaman *et al.*, 1997) and SK_{Ca} channels. It should however be stressed that other K^+ channels with a non-inactivating, voltaged gated potassium current identified as $I_{K(N)}$ in pulmonary artery rings (Osipenko *et al.*, 1998) or novel ATP dependent delayed-rectifier K^+ channels in pulmonary artery myocytes (Patel *et al.*, 1997) have been claimed to be involved in the maintenance of the pulmonary circulation resting tone. In our study, K^+ channels or cyclo-oxygenase inhibitors did not affect significantly the hypoxic pulmonary vasoconstriction, thus suggesting the absence of any modulating effect of K_{ATP}, BK_{Ca} channels (Hasunuma *et al.*, 1991a; Dumas *et al.*, 1996) and SK_{Ca} channels or of prostacyclin release. Although Nossaman *et al.* (1997) described a small increase in the pressor response to hypoxia after charybdotoxin, recent studies suggest that oxygen-sensitive K^+ channels involved in the modulation of vessel tone during hypoxia are not K_{Ca} channels but other K^+ channels identified as Kv1.2 and Kv1.5 channels (Wang *et al.*, 1997), novel Kv2.1/ Kv9.3 ATP dependent delayed-rectifier K^+ channels (Patel *et al.*, 1997) or K^+ channels with $I_{K(N)}$ currents (Osipenko *et al.*, 1998). In contrast to the lack of modulation of the hypoxic vasoconstriction observed in our study with glibenclamide, charybdotoxin, apamin or indomethacin, L-NAME enhanced the hypoxic vasoconstriction thus confirming that EDRF modulates the hypoxic pulmonary
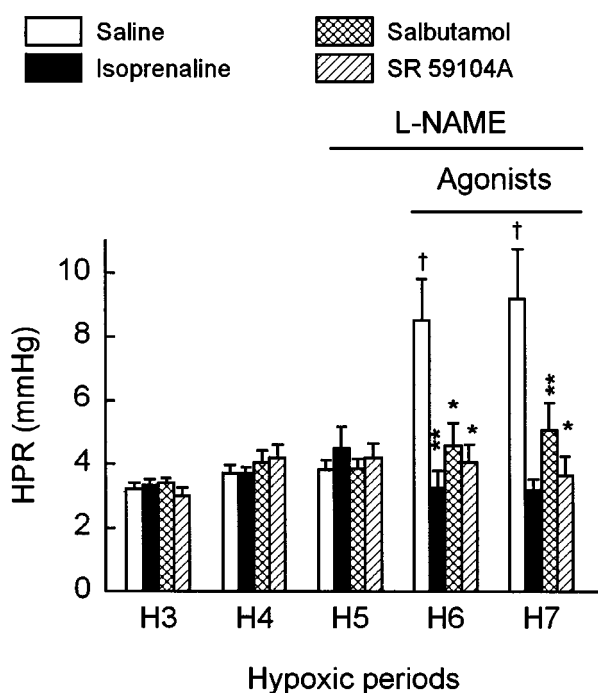

Figure 4 Influence of L-NAME (100 μM) on the effects of infusions of normal saline ($n = 7$), isoprenaline (0.03 μM) ($n = 5$), salbutamol (0.6 μM) ($n = 5$) and SR 59104A (20 μM) ($n = 5$) on hypoxic pressure responses (HPR) in the isolated rat perfused lung. Inhibitors were infused from the fifth hypoxic period and agonists were infused from the sixth hypoxic period. Values are increases of perfusion pressure above basal values. Data represent mean $\pm$ s.e.mean. Asterisks indicate responses to the agonists that were significantly different from corresponding responses obtained in the control period (*$P < 0.05$, **$P < 0.01$). † indicates responses to L-NAME that were significantly different ($P < 0.01$) from corresponding responses obtained during the fourth hypoxic period.

vasoconstriction as described previously (Liu *et al.*, 1991; Dumas *et al.*, 1994). A striking observation in our study was the partial inhibition of the post-hypoxic return to baseline pulmonary pressure induced by apamin, indomethacin and L-NAME which suggests that SK_{Ca} channels, prostacyclin and EDRF modulate post-hypoxic relaxation. These results support the view that the mechanisms involved in initiating the hypoxic smooth muscle contraction are different from those involved in initiating post hypoxic relaxation. The role of potassium channels and EDRF in the latter phenomenon deserve further investigations.

In this study, we investigated the effects of a non selective β-adrenoceptor agonist (isoprenaline), a selective $β_2$-adrenoceptor agonist (salbutamol) and a selective $β_3$-adrenoceptor agonist (SR 59104A, Croci *et al.*, 1995) on the pulmonary vascular response to hypoxia, a physiopathological condition that produces vasoconstriction, to study potential dilator agents for the treatment of diseases such as pulmonary hypertension (Dumas *et al.*, 1994). Isoprenaline, salbutamol and SR 59104A shared the ability to relax the pulmonary circulation during hypoxia (O'Donnell *et al.*, 1991; Dumas *et al.*, 1998) and in doing so, the relaxant potencies of isoprenaline and salbutamol were similar to or higher than those observed in pulmonary artery and in various other normoxic tissues (Jones *et al.*, 1990; Oriowo, 1994; Sooch & Marshall, 1996; Huang & Kwok, 1997). Our findings suggest that hypoxia does not influence the relaxant effects of β-adrenoceptor agonists, thus contrasting with other investigations which reported a negative influence of hypoxia on the relaxant effects of these drugs in pulmonary arteries precontrated with adrenaline or U46619 (McIntyre *et al.*, 1994; Wagner *et al.*, 1997). These discrepancies might be related to the different agents used to contract the preparations.

Our experiments with glibenclamide clearly show that K_{ATP} channels contribute to the vasodilation induced by isoprenaline and salbutamol but not to that produced by SR 59104A. As regards isoprenaline and salbutamol, our results are in agreement with previous studies that suggested that the relaxant effects of $β_1$- and $β_2$-adrenoceptor agonists in various smooth vascular tissues were mediated in part through activation of K_{ATP} channels (Nakashima & Vanhoutte, 1995; Randall & McCulloch, 1995; Ming *et al.*, 1997; Chang, 1997). As regards SR 59104A, glibenclamide failed to oppose its pulmonary relaxant effects. To our knowledge the role of K_{ATP} channels in the effects of $β_3$-adrenoceptor agonists have not yet been investigated and this study clearly shows these drugs exhibit a pharmacological profile different from that of $β_1/β_2$-adrenoceptor agonists in the pulmonary circulation. These data are consistent with our previous demonstration that in the same vascular bed propranolol antagonizes the effects of isoprenaline but not those of the $β_3$-adrenoceptor agonists SR 59104A, SR 59119A and SR 58611A (Dumas *et al.*, 1998). In another study investigating the influence of glibenclamide on the responses elicited by agents acting through direct activation of adenylate cyclase such as forskolin, or through specific receptors such as iloprost and prostaglandin E_1, we showed that only forskolin and iloprost, but not prostaglandin E_1, were partially inhibited by glibenclamide indicating that the role of cyclic AMP in these effects is not clear (Dumas *et al*, 1997). This may account for the discrepancies observed among drugs activating the cyclic AMP pathway through different β-adrenoceptors. Differential activation of protein kinases A and G may also be suggested as is the case in airway smooth muscle (Torphy,

1994). Other studies are consistent with the hypothesis of an interaction of $β_3$-adrenoceptor agonists with both G_s and G_i proteins (Chaudry *et al.*, 1993; Gauthier *et al.*, 1996; Roberts & Summers, 1998). Finally, it has been suggested that β-adrenergic activation could be linked *via* a cyclic AMP-independent and pertussis toxin-sensitive process to an activation of K^+ channels (Khac *et al.*, 1996), a hypothesis that might explain the effects observed with SR 59104A. Thus, at the high concentrations used, a mechanism of action other than the stimulation of β-adrenoceptors cannot be ruled out and further investigations are necessary to establish the pharmacological properties of $β_3$-adrenoceptor agonists according to tissues and/or species.

As regards K_{Ca} channels, charybdotoxin and apamin failed to block the relaxant effects of isoprenaline and salbutamol but inhibited partly those of SR 59104A, demonstrating that $β_3$-, but not $β_2$- and/or $β_1$-adrenoceptor agonists, act in part through BK_{Ca} and SK_{Ca} channels. Regarding isoprenaline, Nossaman *et al.* (1997) also failed to inhibit its relaxant effects with charybdotoxin in the rat isolated perfused lung. However other studies have suggested that activation of BK_{Ca} channels is one of the underlying mechanisms responsible for isoprenaline or $β_2$-adrenoceptor agonist-induced relaxation in various tissues and species as rat aorta (Satake *et al.*, 1996), mesenteric artery (Huang & Kwok, 1997) or guinea-pig mesenteric lymphatic vessels (Von der Weid *et al.*, 1996). However, these data were obtained in isolated vessels rings whereas our study was performed in the isolated whole lung preparation.

There are only few studies investigating the influence of the nitric oxide pathway on the pharmacological activity of β-adrenoceptor agonists in the pulmonary circulation (Priest *et al.*, 1997). In our study, L-NAME partly inhibited the relaxant effects of salbutamol and SR 59104A but not those of isoprenaline. These results are in agreement with previous reports where EDRF was involved in the $β_2$-adrenergic dilatation but not in that induced by $β_1$-adrenoceptor stimulation (Hamdad *et al.*, 1996) or isoprenaline (Bea *et al.*, 1994). These results suggest that the modulation of β-adrenoceptor mediated relaxation may be dependent upon the β-adrenoceptor subtypes. In contrast, in another report, it was shown that the relaxations induced by a non selective agonist, isoprenaline, and a $β_2$-adrenoceptor selective agonist, salbutamol, were both antagonized by NO-synthase inhibitors mainly in large pulmonary arteries and to a lesser extent in small pulmonary arteries suggesting differences according to the conduits rather than to the β-adrenoceptor subtypes (Priest *et al.*, 1997). Thus, the influence of EDRF in the modulation of the relaxant effects of β-adrenoceptor agonists in the pulmonary circulation deserves further investigations.

In conclusion, it appears from our data that in the pulmonary circulation: (a) the low resting tone is not modulated by K_{ATP}, BK_{Ca}, SK_{Ca} channels or the release of nitric oxide or prostaglandins, (b) EDRF exerts a significant inhibition of the hypoxic pulmonary vasoconstriction, (c) SK_{Ca} channels, EDRF and prostaglandins partially contribute to the reversal of the hypoxic pulmonary vasoconstriction, (d) isoprenaline, salbutamol and SR 59104A share the ability to oppose the hypoxic pulmonary vasoconstriction, (e) the vasodilation induced by isoprenaline is mediated in part by K_{ATP} channels, that of salbutamol by K_{ATP} channels and EDRF, and (f) SR 59104A also vasodilates the pulmonary vascular bed, partly through BK_{Ca} and SK_{Ca} channels activation and EDRF release, differing in this from $β_1$ and/or $β_2$-adrenoceptor agonists.

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