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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

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1 The aims of this study were to compare in the rat isolated perfused lung preparation, the antagonist effects of iloprost, a stable analogue of prostacyclin, and prostaglandin E_1 (PGE_1) on the hypoxic pulmonary pressure response, and to investigate the possible involvement of K_{ATP} and K_{Ca} channels and of EDRF (NO) in these effects. In addition, iloprost and PGE_1 effects were compared to those of adenosine and forskolin.

2 Isolated lungs from male Wistar rats (260–320 g) were ventilated with 21% O_2 + 5% CO_2 + 74% N_2 (normoxia) or 5% CO_2 + 95% N_2 (hypoxia) and perfused with a salt solution supplemented with ficoll. Glibenclamide (1 μM), charybdotoxin (0.1 μM), N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) were used to block K_{ATP}, K_{Ca} channels and NO synthesis, respectively.

3 Iloprost, PGE_1, adenosine and forskolin caused relaxation during the hypoxic pressure response. The order of potency was: iloprost > PGE_1 = forskolin > adenosine. EC_{50} values were $1.91 \pm 0.52\ 10^{-9}$ M, $3.31 \pm 0.58\ 10^{-7}$ M, $3.24 \pm 0.78\ 10^{-7}$ M and $7.70 \pm 1.68\ 10^{-5}$ M, respectively. Glibenclamide, charybdotoxin and L-NAME inhibited partially the relaxant effects of iloprost and forskolin but not those of PGE_1.

4 It is concluded that in the rat isolated lung preparation, iloprost and forskolin but not PGE_1 dilate pulmonary vessels partly through K_{ATP} channels, K_{Ca} channels and nitric oxide release. Furthermore our results suggest that the role of cyclic AMP in these effects is not unequivocal.

Keywords: Hypoxic pulmonary vasoconstriction; rat isolated perfused lung; iloprost; prostaglandin E_1; nitric oxide; K_{ATP} channels; K_{Ca} channels

Introduction

Pulmonary hypertension may be secondary to hypoxic vasoconstriction enhancing complex changes in the pulmonary vascular bed. Since endogenous prostaglandins play a role in the regulation of pulmonary blood flow, the use of exogenous prostaglandins that are known to dilate pulmonary vessels such as prostaglandin E_1 (PGE_1) and stable analogues of prostacyclin (PGI_2) have been advocated for the treatment of pulmonary hypertension (Dagher *et al*., 1993; Welte *et al*., 1993). PGI_2 and PGE_1 are usually regarded as agents mediating their effects through prostacyclin (IP)- and (or) EP-receptors coupled to adenylate cyclase, inducing smooth muscle relaxation by increasing the intracellular concentration of adenosine 3′,5′-cyclic monophosphate (cyclic AMP) (Coleman *et al*., 1994). However, recent evidence suggests that IP receptor agonists can activate multiple signalling pathways via the same IP receptor (Wise & Jones, 1996). It has been shown that K_{ATP} (or) K_{Ca} channels are involved in the PGI_2 stable analogue iloprost- and in the PGE_1-induced (a) relaxation of the dog carotid artery (Siegel *et al*., 1992), of the rat and rabbit coronary artery (Jackson *et al*., 1993; Bouchard *et al*., 1994) and of the rat aorta (Serebryakov *et al*., 1994), and (b) cardioprotection in the rabbit (Hide *et al*., 1995). These various mechanisms of action have yet to be investigated in the pulmonary circulation. This latter tissue is known to possess specific physiological properties evidenced by its ability to constrict in response to hypoxic challenge and to prostaglandin E_2 whereas the systemic vascular bed dilates (Kadowitz *et al*., 1975). The purpose of this study was to investigate the relaxant effects of a stable analogue of PGI_2, iloprost, and of PGE_1 on hypoxic vasoconstriction in rat lungs perfused with physiological solutions and the involvement of K channels and nitric oxide in these effects. We also studied the vasodilator profile of two other compounds acting through cyclic AMP either directly (forskolin) or through specific receptors (adenosine) to elucidate the role of this nucleotide.

Methods

Rat perfused isolated lung preparation

Nineteen groups ($n = 6$ to 9 per group) of male Wistar rats (Dépré, St Doulchard, France) weighing 260–320 g, were anaesthetized with sodium pentobarbitone (100 mg kg^{-1}) and the lungs were removed for extracorporeal perfusion as previously described (Dumas *et al*., 1996). 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 mg 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. The lungs were ventilated with a Harvard rodent ventilator at a tidal volume of 10 mg 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 to 88 cmH_2O) differential pressure transducer. A 20- to 30 min equilibration period was allowed to establish a stable baseline for pulmonary airway

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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, by 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.*, 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 ventilation. The perfusate gas tensions were measured at the beginning of, and during the experiments by collecting perfusate anaerobically from the arterial cannula and analysing it immediately (Corning 170 pH/blood gas analyzer). PO_2 was maintained below 35 mmHg and the pH was between 7.3 and 7.4. After 3 or 4 hypoxic pulmonary vasoconstrictions the responses became reproducible (Dumas *et al.*, 1996). Drugs were tested after a stable response to hypoxia had been reached.

Effects of iloprost, prostaglandin E_1, adenosine and forskolin on pulmonary vasoreactivity: influence of K channels and nitric oxide synthase inhibitors Non-cumulative concentration-response curves to the four compounds were obtained by perfusing lungs during six to eight hypoxic periods with a salt solution containing iloprost (0.0001–0.03 μM), PGE_1 (0.01–3 μM), adenosine (1–3000 μM) or forskolin (0.01–3 μM). In another series of experiments, concentration-response curves were obtained in the presence of K_{ATP} channel blocker, glibenclamide (1 μM). The K_{Ca} channel blocker, charybdotoxin (0.1 μM) could not be administered for more than three hypoxic periods because of subsequent lung injury development (oedema). The hypoxic pressure response obtained with the inhibitor of nitric oxide synthase, N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) was reproducible and significantly increased only during the 2nd and the 3rd periods of infusion of this compound. Charybdotoxin and L-NAME were then tested in the 5th, 6th and the 7th hypoxic periods. In these experiments, relaxant drugs were infused in the 6th and the 7th periods at concentrations inducing 75–95% inhibition of the pressure response to hypoxia (iloprost, 0.003 μM, PGE_1, 1 μM, forskolin, 3 μM, adenosine, 300 μM).

Chemicals/drugs

The drugs used were: iloprost (Laboratoires Schering, Berlin, Germany), prostaglandin E_1 (alprostadil, Laboratoires Upjohn, Paris La Défense, France), glibenclamide (Laboratoire Hoeschst, Paris la Défense, France), adenosine, forskolin, angiotensin II, N^G-nitro-L-arginine methyl ester (Sigma Chimie, La Verpillère, France) and charybdotoxin (Latoxan, Rosans, France). Angiotensin II and N^G-nitro-L-arginine methyl ester were dissolved in distilled water, adenosine and charybdotoxin in saline, forskolin in dimethylsulphoxide (DMSO) and glibenclamide in a mixture of DMSO-distilled water (1:1). Iloprost and alprostadil were supplied as 8 mg ml^{-1} in tometamol-ethanol and 0.5 mg ml^{-1} in ethanol solutions, respectively. All drugs were diluted in perfusate. The maximal concentrations of DMSO (2%) and ethanol (0.2%) 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. One-half effective maximum concentration values (EC_{50}s) were determined from individual concentration-response curves. The level of hypoxic pressure response being different in the presence of glibenclamide, charybdotoxin or L-NAME, the inhibitory effects of these compounds were calculated as:

$$\left[1-\frac{\dfrac{(R_1-R_{D+1})}{R_1}}{\dfrac{(R-R_D)}{R}}\right]\times 100$$

where (a) R_{D+1} and R_1 are the hypoxic pressure responses observed with the inhibitor with and without the drug respectively, (b) R_D and R are the corresponding hypoxic pressure responses observed in control experiments with and without the drug respectively.

Data are shown as mean ± 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.

Results

In lung preparations, the mean baseline inflation pressure was 11.19 ± 0.14 cm H_2O ($n=132$) and was not significantly modified by hypoxic ventilation or addition of the various drugs. After equilibrium of the preparation, the baseline perfusion pressure in normoxic ventilation was similar in all series of rats (5.42 ± 0.10 mmHg, $n=132$). In the first control series, ventilation with a hypoxic mixture of gas produced a significant increase of the perfusion pressure (+3.68 ± 0.06 mmHg, $n=69$, +68% from baseline values, $P<0.001$) which, starting from the 4th period of hypoxia, was reproducible for at least 9 subsequent periods (Figure 1). As shown in Figure 2, iloprost and PGE_1 concentration-dependently decreased the hypoxic pressure response ($P<0.001$). % inhibition of this response was 76% with iloprost 0.003 μM, and 75% with PGE_1 1 μM, thus demonstrating a higher relaxant potency of iloprost ($P<0.001$) as shown in Table 1. Figure 3 shows that forskolin and adenosine also dose-dependently and significantly ($P<0.001$) decreased the hypoxic pressure response, % inhibition of this response being 86% with forskolin 3 μM and 93% with adenosine 300 μM. EC_{50} values for the four other drugs are summarized in Table 1 which shows that their relaxant potency versus the hypoxic pressure response was in decreasing order; iloprost > PGE_1 = forskolin > adenosine.

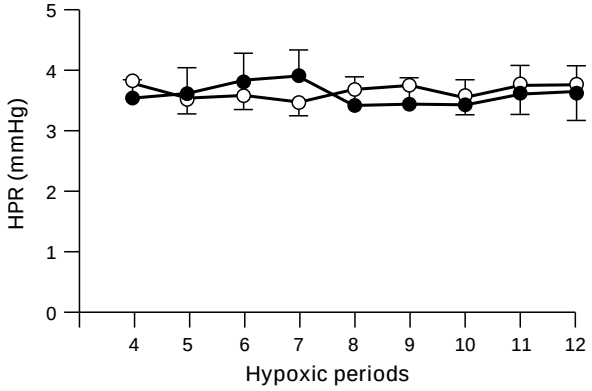

Figure 1 Effects of infusions of normal saline on hypoxic pressor responses (HPR) recorded during nine periods of hypoxia in the absence ($n=9$) (○) or the presence of 1 μM glibenclamide ($n=9$) (●) in the rat isolated perfused lung. Values shown are increases of perfusion pressure above basal values. Data repesent mean and vertical lines show s.e.mean.

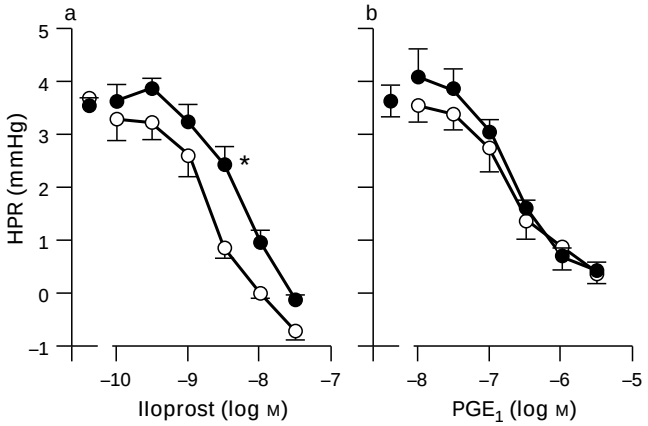

Figure 2 Effects of infusions of iloprost, 0.0001 – 0.03 μM (a), and of prostaglandin E_1, 0.01 – 3 μM (b), on hypoxic pressor responses (HPR) in the absence ($n=6$ and 8, respectively) (○) and the presence of 1 μM glibenclamide ($n=6$ and 8, respectively) (●) in the rat isolated perfused lung. Values shown are increases of perfusion pressure above basal values. Data represent mean and vertical lines show s.e.mean. *Indicate responses with glibenclamide that were significantly different from corresponding responses obtained without glibenclamide.

Table 1 Concentrations of iloprost, prostaglandin E_1, adenosine and forskolin producing a 50% relaxant effect (EC_{50}) in pulmonary vessels contracted by hypoxia: influence of glibenclamide

	EC_{50} (M)	
Drugs	*Control*	*Glibenclamide*
Iloprost	$1.91 \pm 0.52 \times 10^{-9}$	$5.76 \pm 1.66 \times 10^{-9}$†
Prostaglandin E_1	$3.31 \pm 0.58 \times 10^{-7}$***	$3.00 \pm 0.55 \times 10^{-7}$
Adenosine	$7.70 \pm 1.68 \times 10^{-5}$***	$32.41 \pm 9.38 \times 10^{-5}$†
Forskolin	$3.24 \pm 0.78 \times 10^{-7}$***	$14.58 \pm 4.34 \times 10^{-7}$

Values are mean ± s.e.mean from 6 to 9 separate experimentations per group. Significant (***$P<0.001$) against corresponding value obtained with iloprost. Significant (†$P<0.05$) against corresponding value obtained in control experiments.

Glibenclamide 1 μM did not by itself affect the hypoxic pressure response (+3.60 ± 0.15 versus +3.68 ± 0.10 mmHg) (Figure 1). During the hypoxic pressure response, this inhibitor produced a rightward shift of the concentration-response curves to iloprost as shown in Figure 2 ($P<0.001$), resulting in a significantly higher EC_{50} value than in the control conditions ($P<0.05$, Table 1). Glibenclamide inhibited the response to 0.003 μM iloprost by approximately 60%. In contrast, in the presence of this inhibitor of K_{ATP} channels, the antagonist effects of PGE_1 against the hypoxic vasoconstriction were similar to those observed in control experiments (Figure 2, Table 1). Figure 3 shows the influence of glibenclamide on the effects of forskolin and adenosine against hypoxic pressure response. The concentration-response curves were shifted to the right

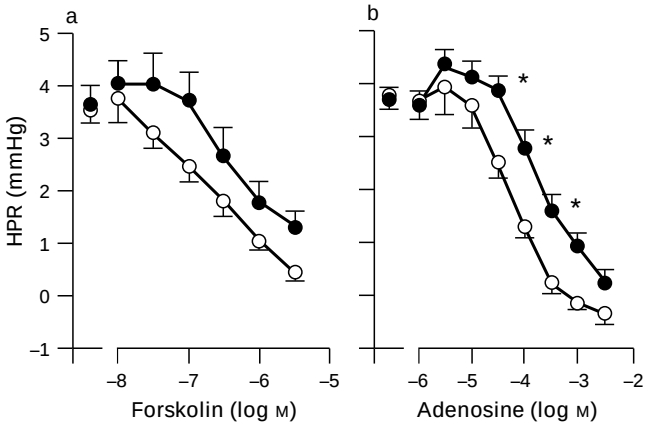

Figure 3 Effects of infusions of forskolin, 0.001 – 3 μM (a), and of adenosine, 1 – 3000 μM (b), on hypoxic pressor responses (HPR) in the absence ($n=6$ and 7, respectively) (○) and the presence of 1 μM glibenclamide ($n=9$ and 7, respectively) (●) in the rat isolated perfused lung. Values shown are increases of perfusion pressure above basal values. Data represent mean and vertical lines shown s.e.mean. *Indicate responses with glibenclamide that were significantly different from corresponding responses obtained without glibenclamide.

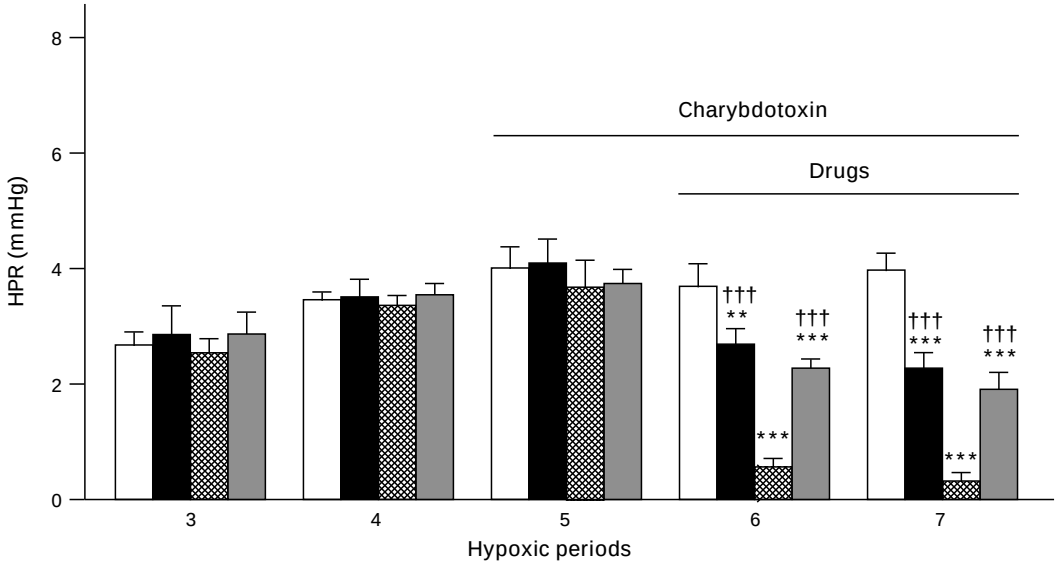

Figure 4 Influence of charybdotoxin 0.1 μM on the effects of infusions of normal saline ($n=6$) (open columns), iloprost 0.003 μM ($n=6$) (solid columns), prostaglandin E_1 1 μM ($n=6$) (cross-hatched columns) or forskolin 3 μM ($n=6$) (squared columns) on hypoxic pressure responses (HPR) in the rat isolated perfused lung. Charybdotoxin was infused from the 5th hypoxic period and drugs were infused from the 6th hypoxic period. Values shown are increases of perfusion pressure above basal values. Data represent mean ± s.e.mean. *Indicate responses to the drugs that were significantly different from corresponding responses obtained in the control period (**$P<0.01$, ***$P<0.001$). †Indicate responses to the drugs that were significantly different from corresponding responses obtained with prostaglandin E_1 (†††$P<0.001$).

($P<0.01$ and 0.001 respectively) exhibiting an inhibition of the effects of both drugs which reaches 30% at 3 μM forskolin and 40% at 300 μM adenosine.

As shown in Figure 4, charybdotoxin, 0.1 μM, did not affect by itself the hypoxic pressure response ($+4.00\pm0.34$ versus $+3.46\pm0.15$ mmHg). This inhibitor was without effect on the response to PGE_1 but reduced the response to both iloprost and forskolin, there being in this respect a difference between prostaglandin E_1 and the two other drugs ($P<0.001$). The antagonist effects of iloprost and forskolin in the presence of charybdotoxin were reduced by 40% as compared to those observed in control conditions.

As shown in Figure 5, L-NAME increased two fold the hypoxic pressure responses starting from the second hypoxic period of infusion ($+7.42\pm0.74$ versus $+3.72\pm0.24$ mmHg, $P<0.001$). L-NAME did not affect significantly the relaxant effects of PGE_1 but significantly reduced those of iloprost (-45%), forskolin (-55%) and adenosine (-60%), there being a significant difference ($P<0.001$) between the effects of L-NAME versus PGE_1 on the one hand, and versus iloprost, forskolin and adenosine on the other.

Discussion

In this study we investigated the effects of iloprost and PGE_1 on the pulmonary vascular response to hypoxia in the rat isolated perfused lung preparation described previously (Dumas *et al*., 1996), and we compared them to those of two other compounds known to enhance cyclic AMP formation either by direct activation of adenylyl cyclase (forskolin) or through stimulation of specific receptors (adenosine).

The decrease of the hypoxic vasoconstriction by iloprost and PGE_1 observed in our study confirms the pharmacological properties of these prostanoids in the pulmonary circulation (Kadowitz *et al*., 1975; Lock *et al*., 1980; Dagher *et al*., 1993). In our experimental conditions, the relaxant potency of iloprost was 10 fold greater than that described in the human isolated pulmonary artery (Haye-Legrand *et al*., 1987) and the relaxant potency of PGE_1 was 100 fold greater than that obtained in the rat isolated pulmonary arterial ring precontracted with phenylephrine (Fullerton *et al*., 1994). These discrepancies could be explained either by a different distribution of the IP- and (or) EP-receptors in the pulmonary vessels of the isolated perfused lung preparation, or by a species difference, or by an enhancement by hypoxia of the relaxant effects of prostaglandins as previously observed (Lock *et al*., 1980; Fukuda *et al*., 1994). Our study also demonstrates the ability of adenosine (Haynes *et al*., 1995) as well as that of forskolin to relax the pulmonary vasculature during hypoxia and at doing so, their potencies are in agreement with those previously observed in the rabbit coronary artery (Nakhostine & Lamontagne, 1993), the rabbit foetal ductus arteriosus (Smith & McGrath, 1994) and the guinea-pig tracheal smooth muscle (Hiramatsu *et al*., 1994). Our experiments with glibenclamide clearly show that K_{ATP} channels contribute to the vasodilatation induced by iloprost, adenosine and forskolin, but not to that produced by PGE_1. Forskolin, a direct activator of adenylyl cyclase was used to evaluate the contribution of cyclic AMP in this pathway and the results obtained in our study suggest a link between this nucleotide and the activation of K_{ATP} channels as previously shown in the rabbit coronary artery (Nakhostine & Lamontagne, 1994). As regards adenosine, our results confirm previous studies that suggested relaxant effects in rabbit (Nakhostine & Lamontagne, 1994) and porcine (Kuo & Chancellor, 1995) coronary vessels mediated through K_{ATP} channels. In the latter tissue it has been proposed that receptors that are coupled through a G protein to K_{ATP} channels are of the A_1 type (Nakhostine & Lamontagne, 1994). In contrast, in the pulmonary circulation, previous studies have suggested a vasodilator effect of adenosine mediated through A_2 receptors which activate adenylyl cyclase and do not involve the activation of K_{ATP} channels (Haynes *et al*., 1995; Cheng *et al*., 1996). These discrepancies indicate that the role of the adenosine receptor subtypes in vasodilating pulmonary vascular beds remains unclear and that the identity of the

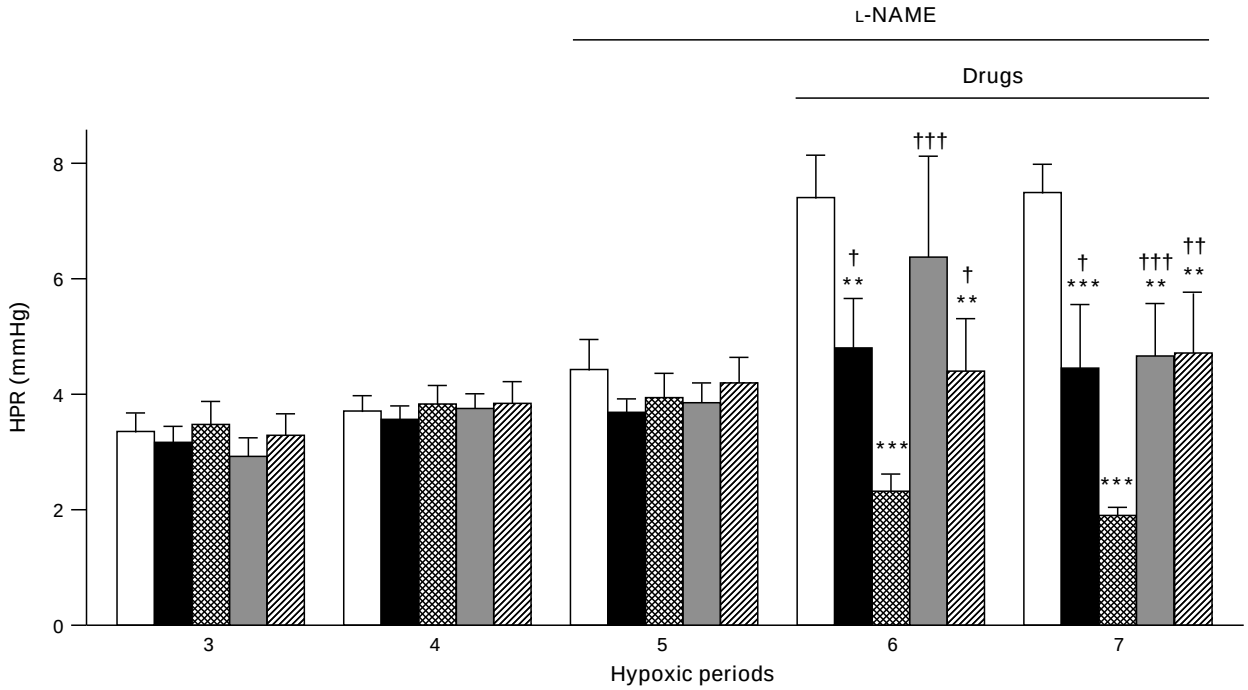

Figure 5 Influence of L-NAME 100 μM on the effects of infusions of normal saline ($n=9$) (open columns), iloprost 0.003 μM ($n=6$) (solid columns), prostaglandin E_1 1 μM ($n=6$) (cross-hatched columns), forskolin 3 μM ($n=6$) (squared columns) or adenosine 300 μM ($n=6$) (hatched columns) on hypoxic pressure responses (HPR) in the rat isolated perfused lung. L-NAME was infused from the 5th hypoxic period and drugs were infused from the 6th hypoxic period. Values are increases of perfusion pressure above basal values. Data represent mean$\pm$s.e.mean. *Indicate responses to the drugs that were significantly different from corresponding responses obtained in the control period. (**$P<0.01$, ***$P<0.001$). †Indicate responses to the drugs that were significantly different from corresponding responses obtained with prostaglandin E_1 (†$P<0.05$, ††$P<0.01$, †††$P<0.001$).

adenosine receptor coupled to the K_{ATP} channel and the involvement of cyclic AMP are uncertain (Cushing *et al*., 1991; Niya *et al*., 1994). As regards iloprost, our results are in agreement with previous studies suggesting that the relaxant effects of both PGI_2 and iloprost are mediated through K_{ATP} channels, e.g., in rabbit (Jackson *et al*., 1993) and rat (Bouchard *et al*., 1994) coronary arteries. Whether cyclic AMP is involved in these effects is still a matter of controversy. Jackson *et al*. (1993) indicate an activation of K_{ATP} channels by iloprost through a process independent of cyclic AMP production. In contrast, partial involvement of cyclic AMP is suggested by Nakhostine & Lamontagne (1994). In our study, the inhibition by glibenclamide of the relaxation induced by iloprost is greater than that observed with forskolin which is not in contradiction with the latter hypothesis. As regards PGE_1, a few studies only, have investigated the role of K_{ATP} channels in its effects. Ney & Feelish (1995) suggest that the vasodilator effects of PGE_1 in the coronary and systemic circulation of the rat are mediated by these channels. Our results are not in agreement with this hypothesis since we did not observe any shift of the concentration-response curve by glibenclamide. Thus, other pathways, such as hyperpolarization through the electrogenic Na^+ pump (Fukuda *et al*., 1992) could be responsible for PGE_1 effects in our study. This latter mechanism of action may be cyclic AMP-independent, or dependent upon one of the cyclic AMP compartments that are coupled to different functional responses (Murray *et al*., 1989).

As in glibenclamide experiments, we observed a partial inhibition by charybdotoxin of iloprost- and forskolin-induced relaxation. This demonstrates that the dilator action of iloprost or forskolin is mediated in part through K_{Ca} channels (Siegel *et al*., 1992; Hiramatsu *et al*., 1994). In forskolin-induced relaxation, the role of cyclic AMP in the stimulation of K_{Ca} channels has been suggested by Hiramatsu *et al*. (1994), but an additional hyperpolarization combined to the rise of cyclic AMP induced by iloprost has been proposed by Siegel *et al*. (1992). In contrast, we failed to block the relaxant effects of PGE_1 with charybdotoxin. Similar results have been obtained in the isolated working heart (Ney & Feelisch, 1995) excluding any role of K_{Ca} channels in PGE_1 effects. Once again, these discrepancies between the three compounds indicate that the responisibility of cyclic AMP is not clear.

There are only a few studies investigating the influence of the nitric oxide pathway on the pharmacological activity of prostaglandins (Armstead, 1995; Kaley & Koller, 1995; Qian & Jones, 1995). Our study clearly shows that L-NAME partly inhibits the relaxant effects of iloprost, adenosine and forskolin. Similar results have been obtained with L-NAME and cicaprost, another stable analogue of PGI_2 in rat isolated colon (Qian & Jones, 1995), and with N^{ω}-nitro-L-arginine (L-NOARG) and PGI_2 in porcine pial artery (Armstead, 1995), suggesting that IP-receptor agonists, in part, exert their effects by stimulating the release of nitric oxide possibly through inhibition of endothelin-1 (ET-1) secretion from endothelial cells (Razandi *et al*., 1996). It would be interesting to investigate ET_A- and ET_B- receptor antagonists to confirm these effects in the pulmonary circulation. An attenuation of the relaxant effects of forskolin by L-NOARG has also been observed previously, suggesting that cyclic AMP and cyclic GMP interact to produce a vasodilator response, the former inhibiting the breakdown of the latter (Rebich *et al*., 1995). However, the precise biochemical pathway responsible for nitric oxide release remains to be determined. Previous studies with nitric oxide synthase inhibitors do not support nitric oxide as a mediator of adenosine-induced vasodilatation in the pulmonary circulation (Cheng *et al*., 1996) or in other tissues (Santiago *et al*., 1994; Haynes *et al*., 1995). In contrast, in porcine coronary arterioles, data have been obtained, in agreement with our results, suggesting that adenosine receptor agonists release nitric oxide (Abebe *et al*., 1995; Kuo & Chancellor, 1995) through the opening of K_{ATP} channels (Kuo & Chancellor, 1995). The partial blockade by glibenclamide of the relaxant effect of adenosine observed in our study is not in contradiction with this hypothesis. Interestingly, once again, PGE_1 did not share the same mechanism of action since we failed to block its effects with L-NAME, as also previously observed in the cat mesenteric vascular bed (Santiago *et al*., 1994). This result confirms that PGE_1 acts through different pathways that are nitric oxide independent.

In conclusion, it appears from our data that in the pulmonary circulation: (a) iloprost, PGE_1, adenosine and forskolin share the ability to oppose the hypoxic pulmonary vasoconstriction, (b) the vasodilatation induced by iloprost and forskolin is mediated in part by K_{ATP} channels, K_{Ca} channels and nitric oxide, (c) PGE_1 does not share these mechanisms of action, and (d) the involvement of cyclic AMP in the differential effects of the four investigated compounds remains unclear and needs to be investigated further.

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