British Journal of Pharmacology (1996) 117, 633-638

Comparison of the effects of nicorandil, pinacidil and nitroglycerin on hypoxic and hypercapnic pulmonary vasoconstriction in the isolated perfused lung of rat

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1 The aims of this study were to compare in the rat isolated perfused lung preparation, the dilator actions of nicorandil, pinacidil and nitroglycerin on the hypoxic pulmonary pressure response with or without hypercapnic acidosis and to investigate the possible involvement of K channels and EDRF in these effects.

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 and gassed with $40\%CO_2+60\%N_2$ to produce hypercapnic acidosis. Glibenclamide (1 μM), charybdotoxin (0.1 μM), N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) and methylene blue (30 μM) were used to block K_{ATP} channels, K_{Ca} channels, EDRF synthesis and guanylate cyclase, respectively.

3 Hypoxic pressure response was significantly increased by hypercapnic acidosis (+115%, $P<0.001$), L-NAME (+111%, $P<0.001$), methylene blue (+100%, $P<0.05$) but not by glibenclamide or charybdotoxin. In contrast none of these inhibitors affected the hypoxic hypercapnic acidosis response.

4 Nicorandil, pinacidil and nitroglycerin caused relaxation during the hypoxic pressure response and hypoxic hypercapnic acidosis response. Nicorandil was more potent in the latter. Glibenclamide inhibited the relaxant effects of nicorandil and pinacidil but not those of nitroglycerin during hypoxia alone. In contrast, glibenclamide inhibited the relaxant effects of the three drugs during hypoxia + hypercapnia. Charybdotoxin inhibited the relaxant effect of pinacidil during normocapnia and hypoxia but not those of nicorandil or nitroglycerin. Methylene blue inhibited partially the dilator response to pinacidil but did not modify the effects of nitroglycerin or nicorandil.

5 It is concluded that in the rat isolated lung preparation, EDRF limits hypoxic pulmonary vasoconstriction but not hypoxic vasoconstriction potentiated by hypercapnic acidosis, whereas K_{ATP} or K_{Ca} channels are not involved in either case. Nicorandil and pinacidil dilate pulmonary vessels mainly through K_{ATP} channels but the effects of pinacidil may also involve an additional mechanism of action through K_{Ca} channels. Finally it is suggested that nitroglycerin may partly exert its relaxant effects through K_{ATP} channels.

Keywords: Hypoxia; acidosis; methylene blue; glibenclamide; charybdotoxin; hypoxic vasoconstriction

Introduction

Responses of arterial smooth muscle to chemical stimulation depend largely on the particular vascular bed studied. For example, the pulmonary vasculature constricts in response to hypoxic challenge whereas peripheral vascular beds dilate. The primary stimulus responsible for the local regulation of pulmonary blood flow is alveolar hypoxia reinforced by hypercapnia (Dawson, 1984). Despite extensive research, the biochemical mechanism underlying hypoxic pulmonary vasoconstriction is unknown. Suppression of endogenous vasodilator substances such as endothelium derived relaxing factor (EDRF) may be one mechanism mediating the hypoxic pulmonary vasoconstriction (Liu *et al.*, 1991). The ability of potassium channels to modulate hypoxic vasoconstriction is suspected because of the powerful inhibitory effects of potassium channel openers such as cromakalim, pinacidil or aprikalim (Eltze, 1989) and excitatory effects of K channel blockers such as tetraethylammonium (TEA) and 4-aminopyridine (Hasunuma *et al.*, 1991a; Post *et al.*, 1992).

With respect to the influence of hypercapnia, many studies have examined the effects of the blood acid-base status on hypoxic pulmonary vasoconstriction (Rudolph & Yuan, 1966; Malik & Kidd, 1973; Raffestin & McMurtry, 1987). These studies report discordant findings probably related to interspecies variability, experimental procedures and magnitudes of acid-base changes (Brimioulle *et al.*, 1990).

Among the vasodilators that may be investigated in these pathophysiological conditions, nicorandil is of special interest because this drug has at least two mechanisms of action. It can increase guanosine 3':5'-cyclic monophosphate (cyclic GMP) through a nitrate-like effect and enhance membrane potassium conductance by opening ATP-sensitive potassium (K_{ATP}) channels (Borg *et al.*, 1991). Its dilator action on the pulmonary circulation may be compared to that of nitroglycerin and pinacidil which are known to share the first and the second mechanism of action, respectively (Ignarro *et al.*, 1991; Quast, 1992). Interestingly, these two latter drugs also have been involved in K_{Ca} channel opening (Stockbridge *et al.*, 1991; Hamaguchi *et al.*, 1992). These various mechanisms of action have yet to be determined in the pulmonary circulation. The purpose of this study was to investigate (1) the effect of hy-

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percapnic acidosis on hypoxic vasoconstriction in rat lungs perfused with physiological solutions, (2) the involvement of K channels and EDRF in these effects (3) the vasodilator profile of nicorandil compared to those of other vasodilator drugs acting through different pharmacological mechanisms, i.e., pinacidil and nitroglycerin.

Methods

Rat perfused isolated lung preparation

Thirty two groups ($n=4$ 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 tracheotomized. After thoracotomy, a polyethylene cannula was inserted into the pulmonary artery for lung perfusion 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 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 Ulis, 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 (0.02 ml g^{-1} min^{-1}). 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 cmH_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 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 increased in excess of 10% (indicative of oedema) at the end of the experiment were discarded.

Experimental protocols

Vasoconstrictor responses to hypoxia with or without hypercapnic acidosis After the equilibration period, the pulmonary vasculature was precontracted twice, using a bolus injection of 0.25–0.5 μg of angiotensin II to prime the otherwise low vascular reactivity seen in salt solution-perfused lungs (McMurtry, 1984). Then the lungs were challenged with a hypoxic gas mixture (5% CO_2, 95% N_2). The perfusate gas tensions were measured at the beginning of, and during the experiments by collecting perfusate anaerobically from the arterial cannula and analyzing it immediately (Corning 170 pH/blood gas analyzer). P_{O_2} was maintained below 35 mmHg and the pH was between 7.3 and 7.4. 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 (Dumas *et al.*, 1994). After 3 or 4 hypoxic pulmonary vasoconstrictions the responses became reproducible and in a second series of experiments, the perfusate was then gassed during hypoxic period with a mixture of 40% CO_2 + 60% N_2 to produce hypercapnic acidosis. During these periods, P_{O_2} was less than 30 mmHg, P_{CO_2} was between 250 and 270 mmHg and pH was between 6.2 and 6.3 to obtain a significant and reproducible response from the preparation.

Effects of K channel and EDRF inhibitors on pulmonary reactivity The influence of a K_{ATP} channel blocker, glibenclamide (1 μM), a K_{Ca} channel blocker, charybdotoxin (0.1 μM), an inhibitor of EDRF synthesis, N^G-nitro-L-arginine methyl ester (L-NAME 100 μM) and an inhibitor of guanylate cyclase, methylene blue (30 μM), was tested on the baseline perfusion pressure in normoxic ventilation and on hypoxic pressure response with or without hypercapnic acidosis. Drugs were tested after a stable response to hypoxia was reached. Glibenclamide was infused during seven hypoxic periods. Charybdotoxin could not be administered for more than three hypoxic periods because of subsequent lung injury (oedema) development. In L-NAME experiments, the hypoxic pressure response was reproducible only during the 2nd and the 3rd periods of infusion of the inhibitor. In methylene blue experiments, the guanylate cyclase inhibitor could not be administered for more than two periods because of subsequent lung injury (oedema) development.

Effects of nicorandil, pinacidil and nitroglycerin on pulmonary vasoreactivity Non-cumulative concentration-response curves to the three drugs (0.1–100 μM) were obtained by perfusing lungs during hypoxic periods with salt solution containing nicorandil, pinacidil or nitroglycerin and the hypoxic pressure response was measured in experiments with or without hypercapnic acidosis. In other experiments concentration-response curves were obtained in the presence of glibenclamide to study the influence of the inhibitor of K_{ATP} channels on the relaxant effect of the three drugs. In experiments with other inhibitors, drugs were infused at concentrations inducing 80% inhibition of the pressure response to hypoxia (nicorandil, 10 μM, pinacidil, 1 μM, nitroglycerin, 1 μM). They were infused in the 6th and the 7th periods in charybdotoxin experiments and in the 6th period in methylene blue experiments.

Chemicals/Drugs

The drugs used were: nicorandil (Laboratoires Bellon, Neuilly sur Seine, France), pinacidil (Laboratoires Léo, Montigny-le-Bretonneux, France), nitroglycerin (Laboratoires Besins-Iscovesco, Paris, France), glibenclamide (Laboratoire Hoechst, Paris la Défense, France), angiotensin II, N^G-nitro-L-arginine methyl ester, methylene blue (Sigma Chimie, La Verpillère, France), charybdotoxin (Latoxan, Rosans, France). Nicorandil, angiotensin II, N^G-nitro-L-arginine methyl ester, methylene blue, were dissolved in distilled water, charybdotoxin in saline, pinacidil in a mixture of dimethylsulphoxide-distilled water (1:9) and glibenclamide in a mixture of dimethylsulphoxide-distilled water (1 : 1). Nitroglycerin was purchased as 4% in ethanol. The maximal concentrations of dimethylsulphoxide (2%) and ethanol (0.05%) 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. The results are expressed as absolute changes from baseline values. EC_{50}s are calculated from individual concentration-response curves. Data are shown as mean ± s.e.mean. Statistical significance was assessed with Student's t 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 10.77 ± 0.15 cmH_2O and was not significantly modified by hypoxic ventilation, hypercapnic acidosis or addition of the various drugs. The baseline perfusion pressure in normoxic ventilation was similar in all series of rats (5.60 ± 0.12 mmHg). The values from the different protocols were pooled as they were not significantly different from each other.

Vasoconstrictor responses to hypoxia: effects of hypercapnic acidosis

Ventilation with a hypoxic mixture of gas produced a significant increase of the perfusion pressure ($+3.04\pm0.31$ mmHg, $P<0.001$). The values of the pressure response obtained in hypoxic ventilation without hypercapnic acidosis were not significantly different from each other and were pooled. Hypoxia with hypercapnic acidosis also produced a significant increase of the perfusion pressure ($+6.54\pm0.74$ mmHg, $P<0.001$) and potentiated the hypoxic pressure response obtained without acidosis ($P<0.001$).

Effects of K channel and EDRF inhibitors on pulmonary reactivity

Glibenclamide, charybdotoxin, L-NAME and methylene blue did not affect significantly the baseline values of perfusion pressure (0.00 ± 0.01, $+0.20\pm0.12$, $+0.33\pm0.14$ and $+0.42\pm0.08$ mmHg, respectively). As shown in Figure 1a, glibenclamide did not affect normocapnic or hypercapnic hypoxic pressure response. In charybdotoxin experiments (Figure 1b), the pressure response potentiated by hypercapnia decreased significantly in the last 2 periods. In contrast in Figure 2, the hypoxic pressure response was significantly increased by L-NAME ($+7.00\pm0.85$ versus 3.31 ± 0.31 mmHg, $P<0.001$) and methylene blue ($+7.37\pm1.42$ versus 3.69 ± 0.34 mmHg, $P<0.05$), but the hypoxic pressure response obtained with hypercapnic acidosis was not further increased by these inhibitors.

Effects of nicorandil, pinacidil and nitroglycerin on pulmonary reactivity: influence of K channel and EDRF inhibitors

As shown in Figure 3, nicorandil, pinacidil and nitroglycerin concentration-dependently decreased the hypoxic pressure responses whether normocapnic or hypercapnic ($P<0.001$). The potencies of the three drugs expressed as EC_{50} values are shown in Table 1 and indicate that pinacidil and nitroglycerin were of similar potency against hypoxic pressure response, whether potentiated or not by hypercapnic acidosis. In contrast, nicorandil was far more active against hypercapnic hypoxia than against normocapnic hypoxic pressor response ($P<0.05$).

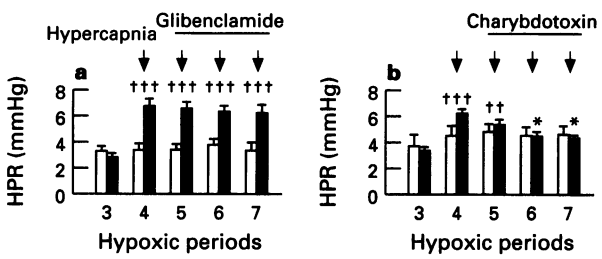

Figure 1 Effects of glibenclamide 1 μM (a) or charybdotoxin 0.1 μM (b) on hypoxic pressor response (HPR) without (open columns) or with hypercapnic acidosis (solid columns) in the isolated perfused lung of rat. Hypercapnia was induced from the 4th hypoxic period (arrows). Glibenclamide or charybdotoxin were infused from the 5th hypoxic period. Data represent mean $\pm$ s.e.mean from 5 to 9 separate experiments per group. †Indicates responses to hypercapnic acidosis that were significantly different from corresponding responses obtained in the 3rd period (††$P<0.01$, †††$P<0.001$), * indicates responses to charybdotoxin that were significantly different from corresponding responses obtained in the 4th hypoxic period ($P<0.05$).

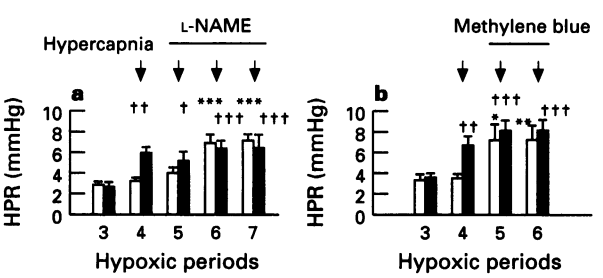

Figure 2 Effects of L-NAME 100 μM (a) or methylene blue 30 μM (b) on hypoxic pressor response (HPR) without (open columns) or with hypercapnic acidosis (solid columns) in the isolated perfused lung of rat. Hypercapnia was induced from the 4th hypoxic period (arrows). L-NAME or methylene blue were infused from the 5th hypoxic period. Data represent mean $\pm$ s.e.mean from 4 to 9 separate experiments per group. † Indicates responses to hypercapnic acidosis that were significantly different from corresponding responses obtained in the 3rd period (†$P<0.05$, ††$P<0.01$, †††$P<0.001$). *Indicates responses to L-NAME or methylene blue that were significantly different from corresponding responses obtained in the 4th hypoxic period (*$P<0.05$, **$P<0.01$, ***$P<0.001$).

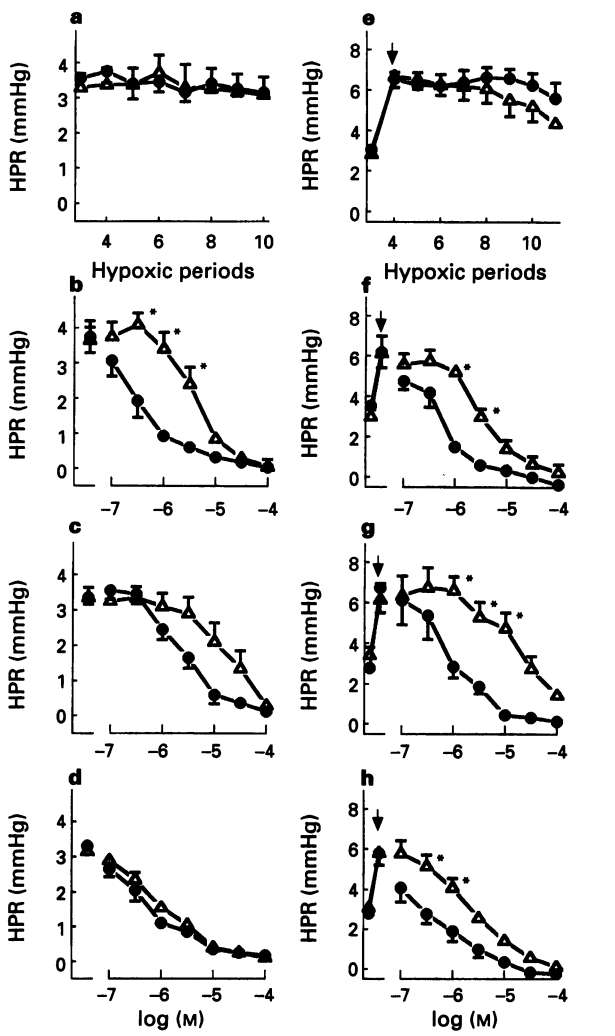

Figure 3 Effects of infusions of normal saline (a,e), 0.1–100 μM pinacidil (b,f), nicorandil (c,g) or nitroglycerin (d,h) on hypoxic pressor responses (HPR) without (a,b,c,d) or with hypercapnic acidosis (e,f,g,h) and without (●) or with glibenclamide (△) in the isolated perfused lung of rat. Hypercapnic acidosis was obtained from the 4th hypoxic period (arrow). Data represent mean $\pm$ s.e.mean from 5 to 9 separate experiments per group. * Indicate responses with glibenclamide that were significantly different from corresponding responses obtained without glibenclamide ($P<0.05$).

As shown in Figure 3, glibenclamide at 1 μM produced a rightward shift of the concentration-response curves to nicorandil and pinacidil ($P<0.001$) but not of that to nitroglycerin during hypoxic pressure response. In contrast glibenclamide inhibited the relaxant effects of the three drugs during hypoxia plus hypercapnia ($P<0.001$). Figure 4 shows the influence of charybdotoxin at 0.1 μM on the relaxant effects of the three drugs during hypoxia. This inhibitor reduced the response to pinacidil to approximately 80% of the control response but was without effect on the response to nicorandil or nitroglycerin. As shown in Figure 5, methylene blue at 30 μM did not affect significantly the relaxant effects of the three drugs. The response to pinacidil was inhibited by approximately 30%.

Discussion

In this study, we investigated the pulmonary pressor response to hypoxia, hypercapnic acidosis and various compounds 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 of all pulmonary vessels and particularly of small arteries and veins which are known to contribute to the greatest part of the pulmonary resistance (Madden *et al.*, 1985; Zhao *et al.*, 1993). The low perfusion rate used minimized the risk of oedema without altering the reactivity of the preparation.

In this study, the baseline perfusion pressure under normoxic conditions was not influenced by glibenclamide, charybdotoxin, L-NAME or methylene blue. These results suggest that this low pulmonary vascular tone was not dependent upon K channel activity or EDRF release as previously described (Mazmanian *et al.*, 1989; Hasunuma *et al.*, 1991a; Shaw *et al.*, 1992; Brayden & Nelson, 1992). Hypoxic pulmonary vasoconstriction was not significantly augmented by glibenclamide or charybdotoxin. In a previous report, TEA but not glibenclamide enhanced hypoxic pulmonary vasoconstriction suggesting the involvement of K channels other than the ATP-sensitive ones (Dumas *et al.*, 1994). The additional results of the present study indicate that these channels do not belong to the K_{ATP} or the K_{Ca} types. These findings contrast with those of Post *et al.* (1992) who showed that the pulmonary arterial pressure of the rat isolated perfused lung was increased to a level, similar to that induced by hypoxia, by the charybdotoxin-containing *Leiurus quinquestriatus* venom. These discrepancies may be due to the presence of an additional apamin-like K channel blocker in the venom (Post *et al.*, 1992). With regards to the effects of EDRF, and as previously described (Hasunuma *et al.*, 1991b; Liu *et al.*, 1991; Dumas *et al.*, 1994), the inhibition of nitric oxide synthesis and/or of guanylate cyclase enhanced hypoxic pulmonary vasoconstriction suggesting that the release of EDRF attenuated hypoxic vasoconstriction (Zhao *et al.*, 1991).

In this study we found a potentiation of hypoxic pulmonary vasoconstriction by hypercapnic acidosis. Both P_{CO_2} and pH affect the membrane potential and ionic permeability, but the respective contributions of these two factors to the pressor response is not clear. In dog, Malik & Kidd (1973) observed a potentiation of the hypoxic pulmonary vasoconstriction by CO_2, independent of the blood pH. In contrast, Brimioulle *et al.* (1990) suggested the involvement of protons in the pressor response but a predominant vasodilator effect of CO_2 in respiratory acidosis. With regards to the potentiation of the hypoxic pressor response in our model, we speculate that the specific H^+ increase is of greater importance than the dilator effect of molecular CO_2.

Glibenclamide did not affect significantly the hypoxic pressure response potentiated by hypercapnic acidosis. As the

Table 1 Concentrations of pinacidil, nicorandil and nitroglycerin producing 50% relaxant effect (EC_{50}) in pulmonary vessels contracted by normocapnic or hypercapnic hypoxia

	EC_{50} (μM)	
Drugs	*Normocapnic hypoxia*	*Hypercapnic hypoxia*
Pinacidil	0.38 ± 0.08	0.54 ± 0.02
Nicorandil	3.29 ± 1.42*	0.74 ± 0.09†
Nitroglycerin	0.64 ± 0.14	0.67 ± 0.20

Significant (*$P<0.05$) against corresponding value obtained with pinacidil. Significant (†$P<0.05$) against corresponding value obtained in normocapnic hypoxia. Values are mean ± s.e.mean from 5 to 9 separate experiments per group.

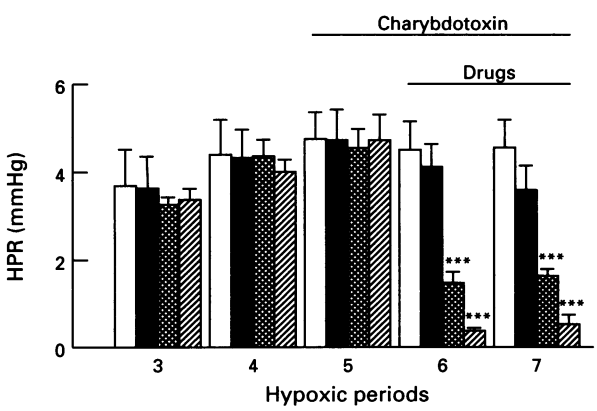

Figure 4 Influence of charybdotoxin 0.1 μM on the effects of the infusions of normal saline (open columns), pinacidil 1 μM (solid columns), nicorandil 10 μM (cross hatched columns) or nitroglycerin 1 μM (hatched columns) on hypoxic pressure responses (HPR) in the isolated perfused lung of rat. Charybdotoxin was infused from the 5th hypoxic period and drugs were infused from the 6th hypoxic period. Data represent mean ± s.e.mean from 5 separate experiments per group. * Indicates responses to the drugs that were significantly different from corresponding responses obtained in the 5th hypoxic period (***$P<0.001$).

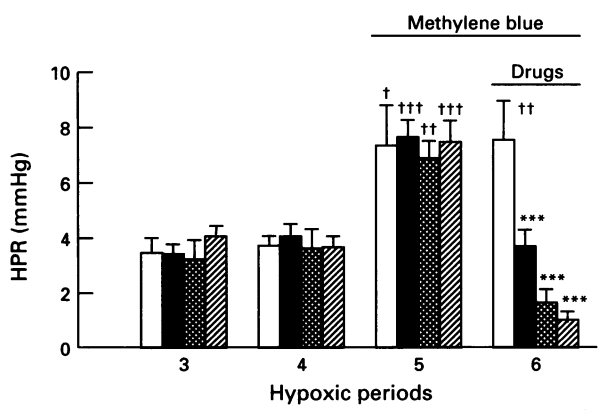

Figure 5 Influence of methylene blue 30 μM on the effects of the infusions of normal saline (open columns), pinacidil 1 μM (solid columns), nicorandil 10 μM (cross hatched columns) or nitroglycerin 1 μM (hatched columns) on hypoxic pressor responses (HPR) in the isolated perfused lung of rat. Methylene blue was infused in the 5th and 6th hypoxic periods and drugs were infused in the 6th hypoxic period. Data represent mean ± s.e.mean from 4 to 9 separate experiments per group. † Indicates responses to methylene blue that were significantly different from corresponding responses obtained in the 4th period (†$P<0.05$; ††$P<0.01$; †††$P<0.001$).* Indicates responses to the drugs that were significantly different from corresponding responses obtained in the 5th hypoxic period (***$P<0.001$).

effects of sulphonylureas are not decreased by acidosis (Meisheri *et al.*, 1993), it is extremely unlikely that K_{ATP} channels are involved in the modulation of the pressor response. These results are in agreement with findings in cardiomyocytes where intense extracellular acidosis (pH = 6.00) had no marked effect on K_{ATP} channel activity (Kwok & Kass, 1994). It appears from our data that neither L-NAME nor methylene blue influenced the pressor response to hypercapnic hypoxia. An inactivation of guanylate cyclase is unlikely (Ignarro *et al.*, 1981) and hence our data suggest that in our experimental conditions, nitric oxide was not involved in limiting the pressor response.

Our experiments clearly demonstrate the ability of nicorandil, pinacidil and nitroglycerin to relax the pulmonary vasculature in severe pathophysiological conditions. In hypoxia, the potency of pinacidil and nicorandil is in agreement with that previously observed in pulmonary artery rings contracted with KCl or noradrenaline (Eltze, 1989; Magnon *et al.*, 1994). In contrast the EC_{50} of nitroglycerin is 10 fold higher in our study than in the latter report. This discrepancy may be explained by a lower relaxant effect of nitroglycerin in pulmonary arterioles as compared to pulmonary artery as observed in coronary arterioles versus arteries (Winbury *et al.*, 1969).

Pinacidil behaved similarly in hypoxia with or without respiratory acidosis exhibiting a pH-independent effect on pulmonary vessels. This finding is in disagreement with the previously reported acidic pH-induced reduction in pinacidil efficacy (Meisheri *et al.*, 1993; Kwok & Kass, 1994). With regard to the effects of nitroglycerin, acidosis does not appear to have influenced the drug-induced dilatation in our study. Finally, nicorandil exhibited a greater relaxant effect in acidosis. These results are in agreement with those of Jahangir *et al.* (1994) who observed that whole cell acidification of isolated cardiomyocytes resulted in an increase of K_{ATP} channel currents in the presence of this compound. Because nicorandil is known to act through both nitrate-like and K_{ATP} channel opening properties, the relaxant effects of the three drugs have been investigated in the presence of various inhibitors.

Our experiments with glibenclamide clearly show that nicorandil and pinacidil act through K_{ATP} channels in hypoxia with or without respiratory acidosis (Meisheri *et al.*, 1993; Jahangir *et al.*, 1994). The shift of the concentration-response curves was similar for both drugs suggesting that nicorandil preferentially dilates pulmonary vessels through potassium channel opening rather than through its nitrate-like action. The prevalence of this mechanism of action seems to be mainly observed in resistance vessel preparations (Borg *et al.*, 1991). In the isolated perfused lung, dilatation of small arteries which contribute to the greatest part of vascular resistance is in favour of this hypothesis. Interestingly, the relaxant effect of nitroglycerin was reduced by glibenclamide during respiratory acidosis suggesting that under these experimental conditions the drug acts through K_{ATP} channels.

The inhibition by charybdotoxin of pinacidil-induced relaxation contrasts with its lack of effects against nicorandil and nitroglycerin and suggests a dilator action of pinacidil mediated through K_{Ca} channels. Previously, we had observed a partial inhibition of the relaxant effect of pinacidil by TEA supporting this hypothesis (Dumas *et al.*, 1994). Similar effects of pinacidil through K_{Ca} channels have been observed in various tissues (Stockbridge *et al.*, 1991; Quast, 1992) but in other studies, a lack of sensitivity of the pinacidil-induced relaxation to charybdotoxin (Nelson & Brayden, 1993) has also been reported, suggesting that differences exist dependent upon the tissues, the species or the experimental conditions. In contrast, the relaxant effects of nicorandil were insensitive to charybdotoxin as observed previously (Kitamura & Kamouchi, 1993). Thus despite sharing an action on K_{ATP} channels, nicorandil and pinacidil differ in their effects on pulmonary vessels. Charybdotoxin failed to block the relaxant effect of 1 μM nitroglycerin suggesting that this drug does not exert its repolarizing effect through K_{Ca} channels as has previously been postulated in the rabbit aortic rings (Hamaguchi *et al.*, 1992).

Methylene blue was also unable to oppose the dilator response to nitroglycerin as previously reported for the dog lung (Hofman *et al.*, 1992). The potentiation of the hypoxic pressure response by methylene blue observed in our study suggests that this dye probably inhibited guanylate cyclase. Hence, in addition to NO production, another additional mechanism is probably responsible for nitroglycerin relaxant effects. This hypothesis is in agreement with the fact that in a concentration range of 1 to 10 μM, the relaxant effects of nitroglycerin are partly insensitive to methylene blue (Hamaguchi *et al.*, 1992). Nitroglycerin might stimulate an isoform of guanylate cyclase or a compartment of the enzyme that is not accessible to inhibition or depletion by methylene blue as suggested by Miller & Vanhoutte (1989) for EDRF. Methylene blue also failed to inhibit the relaxant effect of nicorandil and pinacidil. The three drugs being insensitive to this blocker, the contribution of the cyclic GMP mechanism to nicorandil dilatation could not be assessed. Yet the relaxant response to nicorandil and nitroglycerin was inhibited by methylene blue to a lesser extent than that of pinacidil, suggesting a common additional effect for the two former compounds.

In conclusion, the present study suggests that if EDRF exerts a permanent limitation against hypoxic pulmonary response, neither K_{ATP} nor K_{Ca} channels appear to be involved in the modulation of normocapnic or hypercapnic hypoxic pulmonary vasoconstriction. It also demonstrates that nicorandil, pinacidil and nitroglycerin share the ability to oppose the hypoxic pulmonary vasoconstriction in extreme pathophysiological conditions such as intense hypercapnic acidosis. Finally, although pinacidil and nicorandil mainly operate through K_{ATP} channel activation, this study shows that other mechanisms of action are probably also involved in the relaxant effects of these two drugs as well as in those of nitroglycerin.

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(Received June 26, 1995
Revised October 2, 1995
Accepted October 19, 1995)