Vasorelaxation by Pinacidil in Isolated Perfused Lungs is Enhanced in Rats with Hypoxic Pulmonary Hypertension but is Dependent on the Constrictor

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Summary
The potassium channel opening drug, pinacidil, has been examined in isolated perfused lungs taken from rats with hypoxic pulmonary hypertension (housed in 10 % oxygen for 7 days) and control rats. Inhibition by pinacidil (1 to 30 μM) of noradrenaline (NA)-induced vasoconstriction (NA infusions; β-adrenoceptors blocked) and of hypoxic pulmonary vasoconstriction (HPV; ventilation for 3.5–4.5 min with 0–1 % oxygen) were compared.

The vasoconstrictor responses in preparations from control and hypoxic rats, respectively, were (mm Hg) NA 6.6±0.68 (6); 8.2±1.45 (9); HPV 7.8±1.03 (12); 8.8±0.93 (13). These responses were reversibly inhibited by pinacidil. In lungs from control rats pinacidil was 10-fold less potent against NA than against HPV, but in lungs from hypoxic rats it was equipotent against NA and HPV. When tested against NA, but not HPV, pinacidil was significantly more potent in lungs from hypoxic rats than control rats. It is postulated that NA-induced vasoconstriction in lungs from hypoxic rats, and HPV in both groups of rats, involve calcium influx through voltage-operated calcium channels. Consequently, these responses are readily inhibited by drugs such as pinacidil which open potassium channels and hyperpolarise the cell membrane. In contrast in lungs from control rats, NA-induced constriction may involve mainly intracellular calcium release and thus be less readily inhibited by the hyperpolarising effect of pinacidil.

Key words
Pinacidil - Rat perfused lungs - Pulmonary hypertension - Hypoxic pulmonary vasoconstriction - Noradrenaline

Introduction
Treatment with vasodilator drugs is one of the few therapeutic options that is available for patients with pulmonary hypertension. The most successful drugs have been prostacyclin (Long and Rubin 1987) and the calcium entry blocking drugs (Rich et al. 1992), but the "ideal" pulmonary vasodilator for pulmonary hypertension has yet to be found. Potassium channel opening drugs may be a valuable alternative to the current drugs. To date this group of drugs has not been systematically evaluated in pulmonary hypertension although there are isolated reports of the successful use of diazoxide in some patients with this disease (Chan et al. 1987). In animal experiments various potassium channel opening drugs have been shown to be good vasorelaxants in isolated preparations of large (conduit) pulmonary arteries (Kay et al. 1990, Wanstall and O'Donnell 1992, Rodman 1992), but it is also important to determine the pharmacological properties of these drugs on the resistance vessels of the pulmonary circulation, especially in experimental models of pulmonary hypertension.

The aim of this study was to examine the pulmonary vasorelaxant effects of the potassium channel opening drug, pinacidil, in isolated perfused lungs (pulmonary resistance vessel preparation) from normal rats and rats with chronic hypoxic pulmonary hypertension. The effects of pinacidil have been determined against two different vasoconstrictor responses, i.e. hypoxic pulmonary vasoconstriction (HPV) and noradrenaline (NA)-induced vasoconstriction.
Methods

Treatment of rats

Male Wistar rats (6 weeks, 170–235 g) were housed for 1 week in normobaric hypoxic chambers (10% oxygen; hypoxic rats) or in room air (21% oxygen; control rats) as described previously (Wanstall et al. 1992). They were then anaesthetized with pentobarbitone (90 mg kg\(^{-1}\) i.p.), the thorax was opened and heparin (2500 IU kg\(^{-1}\)) was administered directly into the right ventricle. A blood sample was removed for determination of the haematocrit. The lungs were removed, together with the heart and trachea, and were set up for perfusion of the pulmonary circulation (see below).

At the end of the experiment, the heart was separated from the lungs, divided into right ventricle (RV) and left ventricle plus septum (LV+S), blotted and weighed. The ratios RV/(LV+S) and RV/body weight were significantly higher (P<0.001) in hypoxic rats (0.52±0.02 mg/mg and 1.15±0.03 mg/g, respectively, n=22) than in control rats (0.31±0.01 and 0.71±0.02, respectively, n=17). Thus the hypoxic rats had right ventricular hypertrophy. This provided evidence that the hypoxic rats had pulmonary hypertension since the presence of right ventricular hypertrophy is a reliable indicator of elevated pulmonary artery pressure (Ghodsi and Will 1981, Wanstall and O'Donnell 1992, Wanstall et al. 1992).

The hypoxic rats also had elevated haematocrit (65±1.4%) when compared with control rats (47±0.6%), i.e. the hypoxic rats had polycythemia.

Isolated perfused lung preparations

The lungs were set up in a humidified chamber maintained at 37 °C. They were ventilated via a cannula in the trachea with a normoxic gas mixture containing 21 % O\(_2\)/5 % CO\(_2\)/balance N\(_2\) (60 strokes x min\(^{-1}\); inspiratory pressure 9 cm H\(_2\)O; end expiratory pressure 2.5 cm H\(_2\)O; Ugo Basile rodent ventilator).

The pulmonary circulation was perfused at constant rate (0.035 ml x g body weight\(^{-1}\) x min\(^{-1}\)), via a cannula in the pulmonary artery, with physiological salt solution (PSS) containing 4% w/v bovine serum albumin (BSA), 10 \(\mu\)M \(\text{NO}\)-nitro-L-arginine methyl ester (L-NAME; nitric oxide synthase inhibitor), and 3 \(\mu\)M indomethacin (cyclooxygenase inhibitor). The effluent perfusate was not recirculated. Perfusion

Fig. 1
Method for perfusing rat lungs \textit{in vitro} and experimental protocol for determining the inhibitory effect of pinacidil on vasoconstrictor responses induced by alveolar hypoxia (HPV) or by noradrenalin infusion (NA).
pressure was measured via a side arm in the perfusion line (Trantec pressure transducer Model 60-800, Ugo Basile Gemini recorder). A diagrammatic representation of the method is shown in Fig. 1. The composition of the PSS was (mM): NaCl 119, KCl 4.7, MgSO4 1.17, CaCl2 3.2, KH2PO4 1.18, NaHCO3 22.6, glucose 5.5, sucrose 50 (McMurtry 1984).

**Experimental protocol and analysis of data**

In one series of experiments, hypoxic pulmonary vasoconstrictor (HPV) responses were induced by replacing the normoxic gas mixture ventilating the lungs with a hypoxic gas mixture containing 0–1 % O2/5 % CO2/balance N2 (Fig. 1). In these experiments angiotensin II (2.5 nM; a concentration just below the threshold for vasoconstriction) was included in the PSS. In a second series of experiments vasoconstrictor responses were induced by infusion of noradrenaline (NA) into the perfusate just proximal to the pulmonary artery cannula (Fig. 1). The rate of infusion was ≤ 2 % of the perfusion rate and the final concentration of NA in the perfusate was 1 μM in experiments on lungs from control rats and 0.3 μM in lungs from hypoxic rats. The different concentrations were selected in order to match the size of the constrictor responses to NA in lungs from the two groups of rats. In the NA experiments propranolol (1 μM) was included in the perfusate PSS to block any effect of NA on β-adrenoceptors. Each vasoconstrictor response (HPV or NA) took 3.5 to 4.5 min to reach equilibrium; 4 min was allowed to elapse between successive responses.

The experimental protocol for examining the vasorelaxant effect of pinacidil is shown in Fig. 1. Vasoconstrictor responses (HPV or NA) were repeated until two consecutive control responses were reproducible. Two further vasoconstrictor responses were then obtained in the presence of pinacidil which was infused into the perfusate PSS to block any effect of NA on β-adrenoceptors. Each vasoconstrictor response (HPV or NA) took 3.5 to 4.5 min to reach equilibrium; 4 min was allowed to elapse between successive responses.

**Table 1**

<table>
<thead>
<tr>
<th></th>
<th>Control rats</th>
<th>Hypoxic rats</th>
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<tr>
<td>Resting perfusion pressure (mm Hg)</td>
<td>8.2±0.51</td>
<td>12.6±0.39***</td>
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<tr>
<td></td>
<td>(18)</td>
<td>(22)</td>
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<td>Vasoconstrictor responses (Δ mm Hg):</td>
<td></td>
<td></td>
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<tr>
<td>HPVa</td>
<td>7.8±1.03</td>
<td>8.8±0.93</td>
</tr>
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<td></td>
<td>(12)</td>
<td>(13)</td>
</tr>
<tr>
<td>NA b</td>
<td>6.6±0.68</td>
<td>8.2±1.45</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(9)</td>
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</tbody>
</table>

Values are means ±S.E.M. Numbers of lung preparations are in parentheses. All lung preparations were perfused with PSS containing 4 % w/v BSA, 10 μM L-NAME and 3 μM indomethacin. a hypoxic pulmonary vasoconstriction induced by ventilation of lungs with hypoxic gas mixture (0–1 % oxygen). All (2.5 nM) present in the PSS. b vasoconstriction induced by infusion of noradrenaline into lung perfusate (noradrenaline concentration in perfusate: control rats 1 μM, hypoxic rats 0.3 μM). Propranolol (1 μM) present in the PSS. *** Value in hypoxic rats significantly higher than value in control rats P<0.001 (Student’s t test)
Results

The resting perfusion pressure was significantly higher in lungs from rats exposed to chronic hypoxia than in those from control rats (Table 1). The vasoconstrictor responses were the same whether induced by ventilation with a hypoxic gas mixture (HPV) or by NA infusion, and whether obtained in lungs from control rats or hypoxic rats (Table 1).

Pinacidil caused concentration-dependent, reversible inhibition of HPV or NA-induced vasoconstriction. Concentration-response (inhibition) curves for pinacidil are shown in Fig. 2. This figure allows data for pinacidil to be compared (a) between the two different types of vasoconstriction and (b) between lungs from control and hypoxic rats.

In lungs from control rats, pinacidil was significantly less effective in inhibiting NA than HPV; the difference in potency was about 10-fold (Fig. 2). In contrast, in lungs from hypoxic rats pinacidil was equally potent against NA and HPV (Fig. 2). When tested against NA, the effects of pinacidil were significantly greater in lungs from hypoxic rats than in those from control rats but no significant difference between the two groups of rats was seen when pinacidil was tested against HPV (Fig. 2).

Discussion

There were two main observations from this study on the vasorelaxant effects of pinacidil in perfused lungs from rats. Firstly, in lungs from control rats, the potency of pinacidil in inhibiting vasoconstrictor responses was dependent on the method of inducing the vasoconstriction, i.e. pinacidil was less potent when constriction was induced with NA than when it was induced with acute alveolar hypoxia (HPV). Secondly, any difference in the vasorelaxant effects of pinacidil between lungs from pulmonary hypertensive and control rats was also dependent on the vasoconstrictor, because the potency of this drug was enhanced in lungs from pulmonary hypertensive rats when it was tested against NA but not when tested against HPV.

These data in perfused lungs reflect previous data obtained in ring preparations of the main pulmonary artery. In arteries from control rats the
The potency of pinacidil was found to be dependent on the vasoconstrictor spasmogen used to contract the preparations, i.e. it was less potent against NA and endothelin than against PGF$_{2\alpha}$ (O'Donnell et al. 1991) or U46619 (Wanstall, unpublished data). Furthermore, in arteries from pulmonary hypertensive rats the potency of pinacidil was enhanced, when compared with control rats. This enhancement was also spasmogen-dependent, i.e. it was seen if arteries were contracted with NA or endothelin but not if contracted with PGF$_{2\alpha}$ or U46619 (Kay et al. 1990, Wanstall and Kay, unpublished data). Responses to another potassium channel opening drug, levromakalim, have also been shown to be enhanced in pulmonary arteries from pulmonary hypertensive rats (Rodman 1992); in that study only one vasoconstrictor spasmogen was used, i.e. phenylephrine ($\alpha$-adrenoceptor agonist).

The differences in the potency of pinacidil between NA and HPV, or between lungs from the two groups of rats, could not be attributed to differences in the magnitude of the various vasoconstrictor responses because care was taken to match these for size. For NA-induced vasoconstriction this necessitated the use of different concentrations of NA in lungs from the two groups of rats since we found in preliminary studies that responses to a fixed concentration of NA were larger in lungs from hypoxic rats than in those from control rats. For HPV the presence of L-NAME in the PSS ensured that the constrictions were of the same magnitude in lungs from the two groups of rats. We have previously shown that, in the absence of L-NAME, HPV responses were larger in lungs from hypoxic rats than in those from control rats. However L-NAME potentiated HPV responses in lungs from control, but not hypoxic, rats, thereby eliminating the difference in the size of the HPV response in the two groups of rats (Wanstall, unpublished data).

A hypothesis which could explain the findings of this study, and which takes into consideration the mechanisms of action of pinacidil, NA and HPV, is illustrated in Fig. 3. The primary mechanism whereby pinacidil relaxes smooth muscle cells is through the opening of potassium channels; this leads to potassium efflux and hyperpolarisation of the cell membrane (Southerton et al. 1988). We have previously shown that inhibition of HPV in isolated lungs by pinacidil is prevented by the potassium channel blocking drug, glibenclamide, confirming an action of pinacidil on potassium channels in these preparations (Wanstall, unpublished data). Drugs, such as pinacidil, that act by hyperpolarisation are most effective against vasoconstrictor responses that involve calcium influx through voltage-operated calcium channels (VOCs).
The pulmonary vasoconstriction that is induced by alveolar hypoxia (HPV) is known to involve calcium influx through VOCs (McMurtry 1985). Thus our observation that HPV was readily inhibited by pinacidil was predictable. Since pinacidil was equally effective against HPV in lungs from hypoxic rats as it was in control rats we suggest that the mechanism of HPV remains the same in rats with hypoxic pulmonary hypertension (Fig. 3). On the other hand, NA-induced vasoconstriction may involve mainly intracellular calcium release in lungs from control rats, but calcium influx through VOCs in lungs from hypoxic (pulmonary hypertensive) rats (Fig. 3). This hypothesis would explain why pinacidil was less effective against NA than against HPV in control rats, but equally effective against the two types of vasoconstriction in lungs from hypoxic rats.

In summary, pinacidil is an effective pulmonary vasodilator in rat perfused lungs. Since, in control rats, it was more effective against HPV than against NA pinacidil might preferentially dilate hypoxic regions of the lung in these rats, thereby exacerbating any ventilation-perfusion mismatch. This disadvantage may not exist in pulmonary hypertensive rats where pinacidil was found to be just as effective against NA as against HPV. If it should be equally effective against other endogenous pulmonary vasoconstrictors then, in pulmonary hypertension, pinacidil should cause vasodilation throughout the whole lung (i.e. in both hypoxic and normoxic regions), and ventilation-perfusion mismatch would be minimized. It remains to be established whether the findings of this study on rat lungs are reflected in lungs from humans.

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References

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