Interactions between hypoxic and almitrine-induced vasoconstriction in the rat lung

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1. To test whether almitrine might improve the arterial partial pressure of $O_2$ in patients with chronic obstructive airways disease by improvement of ventilation–perfusion matching, we looked at the interaction between hypoxic and almitrine-induced vasoconstriction in isolated rat lungs perfused with blood at constant flow. Increases in pressure represented increases in resistance.

2. Almitrine, given in increasing doses between challenges with 2% $O_2$, enhanced hypoxic vasoconstriction at low doses but attenuated it at high doses.

3. Stimulus–response curves to hypoxia of increasing severity gave a sigmoid curve.

4. Almitrine solvent caused small changes in pulmonary artery pressure and shifted the stimulus–response curve slightly in a parallel fashion.

5. Small doses of almitrine enhanced the action of mild to moderate hypoxia, medium doses attenuated moderately severe hypoxia, whereas high doses depressed vasoconstriction due to all degrees of hypoxia.

6. These effects of almitrine on hypoxic vasoconstriction were compared with the effect of solvent by analysis of variance; the results substantiated significant enhancement of hypoxia by small doses and attenuation by large doses.

7. In patients, if similar effects apply, small doses of almitrine would assist ventilation–perfusion matching, but large doses might worsen it.

8. Almitrine-induced vasoconstriction was attenuated by a fall in perfusate temperature in a similar manner to hypoxic vasoconstriction. It was also attenuated by three drugs, chlorpheniramine, propanolol and diethylcarbamazine, all of which also decrease hypoxic vasoconstriction. The similarity between hypoxic and almitrine-induced pulmonary vasoconstriction is further confirmed.

INTRODUCTION

Almitrine bimesylate simulates the actions of hypoxia in two places. At the carotid body it causes reflex stimulation of ventilation, whereas in the pulmonary circulation it causes locally mediated vasoconstriction [1, 2]. We found in isolated rat lungs that there was a strong correlation in different subjects between the vasoconstrictor response to hypoxia, which is notoriously variable, and the pulmonary vasoconstrictor response to almitrine. Both responses, unlike those to other pulmonary vasoconstrictor agents, were abolished by the $Ca^{2+}$-channel blocker verapamil [2]. However, there are circumstances in which large doses of almitrine, during prior hypoxic constriction, causes dilatation [2–4]. There are two possible, not mutually exclusive, explanations: that almitrine sometimes causes secondary release of a dilator substance which we have so far failed to identify, or that almitrine acts at a site where it interferes with the vasoconstrictor response to hypoxia. Furthermore, it is still disputed as to whether the rise in the arterial partial pressure of $O_2$ ($Pao_2$) which takes place during almitrine treatment in patients with chronic obstructive airways disease is due to improvement in ventilation–perfusion matching through enhancement of vasoconstriction in hypoxic areas of lung, or to better ventilation of these regions through a carotid body reflex [5]. Some workers have reported that almitrine enhances hypoxic pulmonary vasoconstriction in animal experiments, whereas others have not detected any such action [6, 7]. Our aim in this study was to investigate interactions between the effects of almitrine and hypoxia on the pulmonary circulation. Secondly, in order to further identify possible similarities between the actions of almitrine and hypoxia, we investigated whether stimuli which selectively modify hypoxic pulmonary vasoconstriction, such as temperature changes and certain relatively specific receptor inhibitors, would also modify the action of almitrine. Our results help to reconcile previously conflicting animal and clinical investigations. A preliminary report has been published [8].

METHODS

Animals and lung preparations

Tucks male Wistar SPF rats were used. Their isolated lungs were perfused in situ (after pentobarbitone.

Key words: almitrine, hypoxic pulmonary vasoconstriction.

Abbreviations: DEC, diethylcarbamazine; $Pao_2$, arterial partial pressure of $O_2$; $Ppa$, partial pressure of $O_2$; $Ppa$, pulmonary artery pressure; $\Delta Ppa$, change in pulmonary artery pressure.

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anaesthesia, 60 mg/kg intraperitoneally) by a method
described previously [9]. Briefly, blood of normal pH and
packed cell volume was perfused at constant flow (20 ml/
min) into the pulmonary artery and returned from the left
atrium to a reservoir held at 38°C. Lungs were ventilated
with air/5% CO₂ (normoxia) or 2% O₂/5% CO₂/balance
N₂ (hypoxia), which caused strong vasoconstriction. In
this constant flow preparation increases in pulmonary
artery pressure (Ppa) reflect increases in resistance. Ppa
was measured with an electromagnetic (Druck Ltd) and
was displayed on a pen recorder (Advance; Bryan's). The
rise in Ppa during hypoxia was measured at the maximum
pressure. In most preparations the Ppa then remained
stable, but in others there was a decline. The duration of
hypoxic tests was variable because the time to maximum
Ppa rise varied with the degree of hypoxia and was modi-
fied by temperature (and also possibly by almitrine); the
interval between tests was at least 5 min, but was contin-
ued until a stable baseline pressure was reached. Gas par-
tial pressures in samples of effluent blood were measured
immediately with a Corning blood gas analyzer. Values for
the partial pressure of O₂ (Po₂) and the partial pressure of
CO₂ did not differ before and after almitrine (see below).

Protocols of experiments

In all experiments several hypoxic tests were
performed at the outset until a reproducible response was
seen; the first one or two responses were usually less than
those seen later.

1. Dose–response curves to almitrine (0.1, 1.0, 2.0,
5.0, 50 and 200 μg) during normoxia (n = 11 rats). Since
the effects of large doses were persistent, doses were given
in a constant order, small doses first, and were considered
cumulative.

2. Effect of increasing doses of almitrine on repeated
hypoxic stimuli (2% O₂/5% CO₂) (n = 11 rats). The doses
given in protocol (1), in the same order, were given between
hypoxic challenges.

3. Effect of different doses of almitrine (1, 2, 5, 50
and 200 μg) on stimulus–response curves to a range of
low Po₂ values. Challenges with 7, 5, 2 and 0% O₂ (all
+ 5% CO₂) were given with a normoxic interval between
each test; they caused increasing pressor responses. The
different O₂ concentrations were given in random order.
After two complete stimulus–response curves had been
obtained (SR1 and SR2), a single dose of almitrine, its
solvent or saline in equivalent volume was administered,
followed by two further stimulus–response curves (SR3
and SR4) to hypoxia (four tests in all). Eight rats were
used for each dose of almitrine and solvent or saline
controls.

4. Effect on the vasoconstriction caused by almitrine
and hypoxia of cooling the perfusate from 38 to 15°C and
correlation between the two reactions at high and low
temperatures for two series of experiments. In experiment
1 (n = 9) hypoxia (2% O₂) was tested at the high and low
temperatures. A dose of 50 μg of almitrine was then given
at the low temperature. Since this dose did not give a
repeatable response, the perfusate temperature was raised
when the maximal vasoconstriction was achieved at the
low temperature (n = 6). In a second experiment we used
1 μg of almitrine, which gave a repeatable response,
although less than that caused by hypoxia. We were there-
fore able to repeat tests with hypoxia and almitrine at both
temperatures.

5. Comparison of the effects of 1.0 mg of chlorphenir-
amine (n = 8), 2 mg of propanolol (n = 8) and 20 mg of
diethylcarbamazine (DEC) (n = 13) on the vasoconstric-
tor actions of hypoxia (2% O₂), almitrine (1 μg) and
angiotensin II (1 μg). All three drugs attenuate hypoxic
pulmonary vasoconstriction in the rat.

Drugs

Almitrine (Servier Laboratories, dissolved in a 50%
polyethylene glycol and water mixture), chlorpheniramine
(Piriton; Allen and Hanburys), DEC (Sigma), angiotensin
II (Hypertensin; Ciba), propanolol (Inderal; ICI) were all
dissolved in 0.9% (w/v) NaCl.

Statistics

Means and SEMS were calculated. Comparisons of
means before and after pharmacological antagonists were
made by using paired Student's t-tests. Correlations
between variables were made by using least squares linear
regression analysis.

To test whether almitrine modified the effect of
hypoxia on Ppa in the experiments under protocol (3), we
used a method which took into account changes in the
response to hypoxia with time, the effect of the solvent,
the effect of the rise in baseline pressure with time which
we observed with repeated doses of almitrine (protocols 1
and 2), as well as any inter-animal variation. For each level
of hypoxia an estimate was obtained from the analysis of
variance model of the effect on Ppa of each almitrine
dose compared with the effect of solvent alone.

The estimate for a particular hypoxia level and
almitrine dose is approximately equal to:

\[ \text{average post-almitrine } \Delta Ppa - \text{average pre-almitrine } \Delta Ppa \]

where the average is that of two hypoxic tests in eight rats
before or after drug or solvent and ΔPpa is the rise in
Ppa. Since there were a few missing values the estimates
were not identical with the differences of the averages.

RESULTS

Dose–response to almitrine: protocol I

Fig. 1 shows the changes in Ppa when increasing doses
of almitrine were given into the pulmonary circulation.
The cross-hatched columns show the normoxic Ppa,
whereas the superimposed empty columns show the rise
in Ppa which followed each dose. After the first dose, the
baseline Ppa failed to return to its initial value, although
an interval was allowed between doses for it to stabilize.
Although in isolated rat lungs there was usually some rise
in basal Ppa over several hours [9], the rise here was greater and was attributable to the continued presence of the drug, known to accumulate in the lung [10]. The doses should thus be considered cumulative. Note that the solvent, unlike an injection of 0.9% (w/v) NaCl, appeared to cause a small rise in Ppa. Subsequent doses caused vasoconstriction, although with 200 μg of almitrine we observed a fall in all seven rats tested from the greatly increased baseline Ppa, shown by the criss-cross section of the last column. However, 200 μg of almitrine given as a single dose (protocol 3), when Ppa was low, caused a small rise in Ppa.

Effect of increasing doses of almitrine on a constant hypoxic stimulus: protocol 2

Fig. 2 shows the effect of increasing doses of almitrine on the response to a constant hypoxic stimulus, 2% O₂.

The cross-hatched columns show the normoxic Ppa, whereas the superimposed empty columns show the rise in Ppa during the hypoxic test. The dose of almitrine shown below each column was that given before that hypoxic test. Fig. 3(a) shows that the first hypoxic test given after a dose of almitrine was followed by a fall in baseline Ppa below that which had stabilized after the almitrine dose. The baseline pressure and pressor response to hypoxia displayed in Fig. 2 are therefore those of the second hypoxic test after the almitrine dose, as indicated in Fig. 3(a). The cause of this dilatation after hypoxia which succeeds a dose of almitrine is not yet explained but suggests the release of some relaxant during hypoxia. The baseline Ppa continued to rise, but the combined raised baseline Ppa and the Ppa reached during hypoxia gradually declined. A statistical analysis of the effect of almitrine on hypoxic vasoconstriction was performed on results from further experiments in protocol 3.

Fig. 3. Trace showing effect of almitrine on vasoconstriction caused by different levels of hypoxia. (a) Record of Ppa showing a rise after 1 μg of almitrine and two subsequent hypoxic tests. Note the fall in baseline Ppa after the first hypoxic test. The rise in Ppa in the second hypoxic test was used for Fig. 2. (b) Record of stimulus-response measurements to 7, 5, 2 and 0% O₂ before (top) and after (bottom) 1 μg of almitrine; the series of tests illustrated are those immediately before and after the dose of almitrine (see the text).

Effect of single doses of almitrine on the dose–response to increasing hypoxia: protocol 3

We showed interactions between almitrine and hypoxia in a second way (protocol 3), which proved more informative. Fig. 3(b) shows increased responses to four levels of hypoxia after 1 μg of almitrine. However, the results were
complex. In the first place, there was sometimes an increased response to repeat hypoxic stimulus–response curves. Secondly, as in Fig. 1, there was a gradual increase in baseline $P_{\text{pa}}$. Thirdly, there was an interaction between the effects of the dose of almitrine and the degree of hypoxia. The results are displayed in Fig. 4 and Table 1. Fig. 4 shows that the stimulus–response curve to progressively severe hypoxia is approximately sigmoidal. The rise in $P_{\text{pa}}$ is plotted against $P_{O_2}$ in effluent blood; $P_{O_2}$ ranges for different levels of hypoxia were as follows: $7\%$ $O_2$, $9-11$ kPa; $5\%$ $O_2$, $7-8.5$ kPa; $2\%$ $O_2$, $5-5$ kPa, $0\%$ $O_2$, $1-2.5$ kPa. Fig. 4(a) shows the four stimulus–response tests; the solvent was given between tests 2 and 3. Responses were increased between tests 1 and 2, still further increased after the solvent and were not changed between tests 3 and 4; the curves were all parallel. [In a similar series of eight rats $0.9\%$ (w/v) NaCl was given between tests 2 and 3 and no effect was recorded.] The other tests, in which doses of almitrine were given between the second and third hypoxic response curves, have been compared with the one in which solvent was given. Figs. 4(b), 4(c) and 4(d) show the effects of 1, 5 and 200 $\mu$g of almitrine, respectively; each of these doses modified the parallel displacement of the curve which followed solvent. After 1 $\mu$g and, to a lesser extent, 5 $\mu$g of almitrine, the curves became wider apart after moderate hypoxia but after 5 $\mu$g of almitrine the separation at severe levels of hypoxia was diminished, while after 200 $\mu$g of almitrine the response to all levels of hypoxia was depressed. Table 1 reports the rise in $P_{\text{pa}}$ caused by the least and most severe level of hypoxia (7 and 0% $O_2$) for all four hypoxic response curves before and after doses of almitrine.

The results of the analysis of variance described in the Methods section are given in Fig. 5 and Table 2. Fig. 5 shows a three-dimensional plot of the estimate of the difference between almitrine and solvent on the pressor response ($\Delta P_{\text{pa}}$) to each level of hypoxia. The estimated $\Delta P_{\text{pa}}$ related to the effect of solvent is plotted vertically, whereas the five almitrine doses and the four hypoxic levels are on the two horizontal axes. There is a change from a positive response (enhancement of hypoxic vasoconstriction) at low doses and moderate hypoxia to a negative response (attenuation) with higher doses and more severe hypoxia. The estimates are made up of three components: (1) the effects on $P_{\text{pa}}$ of the different almitrine doses relative to solvent, (2) the effects of the different degrees of hypoxia and (3) the interaction between the effects of hypoxia and almitrine. The broken line separates the positive from the negative effects of almitrine on hypoxic vasoconstriction. It is clear from the Figure that the difference between the effects of the different almitrine doses on $P_{\text{pa}}$ is the most important: the differences are highly significant ($F_{5,34}=61.3$, $P<0.001$), as are the differences between the different levels of hypoxia ($F_{5,522}=65.7$, $P<0.001$). The inter-

![Fig. 4. Stimulus–response curves to different levels of hypoxia before and after almitrine solvent and three increasing doses of almitrine. (a) 0.1 ml of almitrine solvent. (b) 1.0 $\mu$g of almitrine. (c) 5.0 $\mu$g of almitrine. (d) 200 $\mu$g of almitrine. $\Delta P_{\text{pa}}$ (ordinate) is plotted against $P_{O_2}$ (abscissa) in effluent blood. Two curves were measured before (——) and two after (---) almitrine or solvent. Note that the curve moves in a parallel fashion after solvent, is asymmetricaly moved after 1 and 5 $\mu$g of almitrine and that hypoxic vasoconstriction is almost totally suppressed after 200 $\mu$g of almitrine.](image-url)
Table 1. Effect of severe and moderate hypoxia on Ppa before and after varying doses of almitrine. Values are mean ± SEM. Abbreviation: SR, stimulus-response test.

<table>
<thead>
<tr>
<th>O₂ (%)</th>
<th>Before almitrine</th>
<th>Almitrine dose</th>
<th>After almitrine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SR1</td>
<td>SR2</td>
<td>Solvent</td>
</tr>
<tr>
<td>0</td>
<td>10.9 ± 0.6</td>
<td>12.7 ± 1.1</td>
<td>16.8 ± 1.7</td>
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<tr>
<td>7</td>
<td>5.4 ± 0.6</td>
<td>7.5 ± 0.9</td>
<td>11.3 ± 1.4</td>
</tr>
<tr>
<td>0</td>
<td>13.4 ± 1.1</td>
<td>16.3 ± 1.5</td>
<td>20.2 ± 1.5</td>
</tr>
<tr>
<td>7</td>
<td>3.4 ± 0.3</td>
<td>4.1 ± 0.8</td>
<td>13.9 ± 0.9</td>
</tr>
<tr>
<td>0</td>
<td>15.1 ± 2.0</td>
<td>18.2 ± 2.6</td>
<td>14.6 ± 2.6</td>
</tr>
<tr>
<td>7</td>
<td>1.9 ± 0.3</td>
<td>1.9 ± 0.3</td>
<td>8.5 ± 1.3</td>
</tr>
<tr>
<td>0</td>
<td>14.1 ± 1.3</td>
<td>18.5 ± 1.5</td>
<td>15.0 ± 1.2</td>
</tr>
<tr>
<td>7</td>
<td>4.8 ± 0.6</td>
<td>7.2 ± 1.1</td>
<td>12.6 ± 0.8</td>
</tr>
<tr>
<td>0</td>
<td>13.4 ± 1.6</td>
<td>18.5 ± 2.3</td>
<td>50 µg</td>
</tr>
<tr>
<td>7</td>
<td>4.8 ± 0.5</td>
<td>8.0 ± 1.2</td>
<td>6.8 ± 0.7</td>
</tr>
<tr>
<td>0</td>
<td>12.1 ± 1.2</td>
<td>13.2 ± 0.8</td>
<td>200 µg</td>
</tr>
<tr>
<td>7</td>
<td>5.7 ± 0.8</td>
<td>7.7 ± 1.1</td>
<td>2.5 ± 0.2</td>
</tr>
</tbody>
</table>

Fig. 5. Three-dimensional diagram showing the interaction between different doses of almitrine and different levels of hypoxia. The estimate of the difference in the effect of almitrine solvent and almitrine dose is plotted vertically (see the text for the derivation of this index). The almitrine dose is plotted on the right-hand abscissa and the O₂ concentration administered on the left-hand abscissa. Estimates above the broken line show enhancement of hypoxic vasoconstriction by almitrine and below this line diminution. Low doses of almitrine enhance constriction by mild hypoxia, high doses inhibit all levels of hypoxia and intermediate interactions are seen with medium doses and moderate hypoxia.

action effects of hypoxia and almitrine dose are much less dramatic but still significant ($F_{15,522} = 6.63, P < 0.001$). Fig. 5 and Table 1 show that 1 and 2 µg of almitrine enhanced mild hypoxic vasoconstriction but had no effect on more severe hypoxia. High doses of almitrine, 50 and 200 µg, shifted the estimate to negative values, especially with more severe hypoxia. Two micrograms caused inhibition and enhancement to severe and moderate hypoxia, respectively. Five micrograms was the dose after which the estimate of the difference between almitrine and solvent switched from positive to negative; there was little effect on moderate hypoxia but attenuation of severe hypoxia. A tenfold dose increase to 50 µg of almitrine caused no greater attenuation of severe hypoxia but now moderate hypoxia was attenuated. The highest dose of almitrine, 200 µg, caused near abolition of hypoxic vasoconstriction at all levels. Table 2 shows the results of the analysis of variance analysis.

**Effect of temperature on hypoxic and almitrine-induced vasoconstriction: protocol 4**

Table 3 shows the effect of changing the temperature of the perfusate from 38 to 15°C on vasoconstriction by hypoxia and by 50 µg of almitrine (first experiment). The pressor effect of 2% O₂ was reduced and developed more slowly. The almitrine produced a small rise in Ppa at the low temperature and a further rise when the temperature was raised. The vasoconstriction in response to angiotensin II (0.1–0.5 µg) was not reduced by cooling; in fact, larger responses were recorded at the low temperature, although they developed more slowly. Fig. 6 shows significant relationships between the constrictor effects of hypoxia and almitrine at the high and low temperatures. Results from the second experiment, in which both
almitrine and hypoxic tests were repeated at the high, low and then high temperatures, are shown in Fig. 7; responses to both stimuli were reversibly reduced at the low temperature.

Table 2. Analysis of variance in \( \Delta P_{pa} \) after almitrine in the presence of different levels of hypoxia. The \( P \) values are calculated assuming that the \( \Delta P_{pa} \) values are normally distributed around their expected values. ‘Occasions*’ means the occasions on which each test of the response to four levels of hypoxia was repeated. Occasions 1 and 2 were before and occasions 3 and 4 were after the almitrine dose. ‘Almitrine*’ means before and after the almitrine dose (i.e. occasions 1 and 2 or 3 and 4).

<table>
<thead>
<tr>
<th>Effect</th>
<th>Sum of squares</th>
<th>Degrees of freedom</th>
<th>Mean square</th>
<th>( F )-ratio</th>
<th>( P ) value</th>
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<tr>
<td>Between animals</td>
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<td>46</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dose</td>
<td>4094</td>
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<td></td>
<td></td>
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<tr>
<td>Residual</td>
<td>5658</td>
<td>41</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Total</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Within animals</td>
<td>17775</td>
<td>698</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between animals x occasions*</td>
<td>7420</td>
<td>140</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Almitrine*</td>
<td>429.8</td>
<td>1</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Almitrine x dose</td>
<td>4865</td>
<td>5</td>
<td>973.0</td>
<td>61.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Residual</td>
<td>2125</td>
<td>134</td>
<td>15.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within animals x occasions</td>
<td>10335</td>
<td>558</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoxia</td>
<td>4093</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Hypoxia x dose</td>
<td>624.4</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoxia x almitrine</td>
<td>835.6</td>
<td>3</td>
<td>278.5</td>
<td>65.7</td>
<td>&lt;0.001</td>
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<tr>
<td>Hypoxia x dose x almitrine</td>
<td>421.2</td>
<td>15</td>
<td>28.1</td>
<td>6.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Residual</td>
<td>2122</td>
<td>522</td>
<td>4.2</td>
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<tr>
<td>Total</td>
<td>27522</td>
<td>744</td>
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</table>

Effect of pharmacological blocking drugs on hypoxic and almitrine-induced vasoconstriction: protocol 5

In eight rats, 1.0 mg of chlorpheniramine reduced \( \Delta P_{pa} \) caused by hypoxia (2% \( O_2 \)) from 14.5 (SEM 1.5) to 2.8 (SEM 0.5) mmHg (\( P<0.001 \), paired  \( t \)-test) and \( \Delta P_{pa} \) caused by 1 \( \mu \)g of almitrine from 4.6 (SEM 0.8) to 2.3 (SEM 0.5) mmHg (\( P<0.005 \)). In 13 rats, 20 mg of DEC reduced the effect of hypoxia from 17.0 (SEM 2.0) to 1.7 (SEM 0.7) mmHg (\( P<0.001 \)) and that of 1 \( \mu \)g of almitrine from 13.0 (SEM 1.7) to 2.1 (SEM 0.5) mmHg (\( P<0.001 \)). In eight rats, 2 mg of propranolol reduced \( \Delta P_{pa} \) to hypoxia from 13.9 (SEM 1.8) to 1.0 (SEM 0.25) mmHg and the effect of the same dose of almitrine from 8.9 (SEM 1.7) to 2.8 (SEM 0.6) mmHg (\( P<0.001 \)). After all three drugs large vasoconstrictor responses to angiotensin II remained. In a second series of tests with DEC, angiotensin II was given both before and after the drug; as in the first series, the effects of hypoxia and almitrine were greatly reduced, but the action of angiotensin II was little affected. Fig. 8 shows the close correlation between the pressor response to almitrine and hypoxia in all these tests both before and after the three pharmacological inhibitors. Vasoconstrictor responses to angiotensin II did not correlate with responses to hypoxia and almitrine.
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DISCUSSION

The experiments designed to display any interaction between hypoxic and almitrine-induced vasoconstriction, which might possibly indicate that they act at a similar site, showed three things. There was enhancement of moderate hypoxic vasoconstriction by small doses of almitrine, continued enhancement of moderate hypoxia by a medium dose with simultaneous attenuation of responses to severe hypoxia (Figs. 4 and 5). It is not profitable to speculate on the meaning of these interactions at this time. A simple competitive interaction at a receptor site is not suggested, but the known dilator action of almitrine in large doses might mask such a relationship. The dose represented an approximate concentration in the perfusate of 100, 200 and 500 ng/ml and 5 and 20 μg/ml. It is not realistic to compare isolated perfused lungs with patients in detail, but our concentrations are in the same range as the blood levels reported during prolonged treatment with almitrine [11]. The Pao, values caused by ventilation with 7 and 5% O2 are closest to those found in hypoxic lung disease. Since submission of this work, a report has been published which also shows that almitrine enhances or depresses hypoxic pulmonary vasoconstriction, according to dose and degree of hypoxia [12].

Since almitrine is given with benefit to patients with hypoxic chronic obstructive airway disease, it may be of great importance that it sometimes enhances and sometimes attenuates hypoxic pulmonary vasoconstriction. Pao, is improved in these patients but the argument continues as to whether this is due to small changes in ventilatory pattern or to better ventilation/perfusion matching in the lung, which could also be due to improved ventilation of hypoxic areas [5]. Better matching could also be due to enhanced hypoxic vasoconstriction and diversion of blood away from such regions. Clearly, the current work, should similar conditions prevail in man, indicates that in a non-homogeneous lung either better or worse matching might result, being dependent on both the tissue levels of almitrine and the severity of hypoxia.

An important point for almitrine therapy is whether it has any effect, given clinically, on Ppa. A large European multi-centre trial [11] gave no evidence for increase in this pressure after 1 year of treatment. One small trial, however, did show rises [13]. Tests with almitrine given acutely led only to transient increases in Ppa [14]. In this connection, the dilator effect, observed by several groups as well as ourselves, must be born in mind [2–4]; this might counteract any tendency to a rise in Ppa caused by its vasoconstrictor action.

We have shown that pulmonary vasoconstriction induced by almitrine, like hypoxic pulmonary vasoconstriction, is temperature-sensitive, unlike constriction caused by angiotensin II. This fact, first demonstrated by Nilsen & Hauge [15], is one of the most important leads in our search for the still unresolved mechanism of hypoxic pulmonary vasoconstriction which contrasts with its vasodilator action on systemic vessels. Also, both the actions of hypoxia and almitrine in this circulation were selectively abolished by an H1-receptor blocker, a ß-adrenoceptor antagonist and a drug known to reduce, albeit non-selectively, the vasoconstriction caused by certain leukotrienes. The effect of each of these drugs was, in its time, thought to provide evidence that hypoxic vasoconstriction was caused by release of a specific transmitter. This hypothesis can no longer be held in a simple form, for no single substance explains the phenomenon in all species [16]. Thus in 1968 Hauge & Melmon [17] and Hauge & Melmon [18] found that hypoxic vasoconstriction in the isolated rat lung could be respectively abolished and enhanced by H1-receptor antagonists and histaminase.
inhibitors. Unfortunately, this elegant work could not be repeated in dogs and cats, and, indeed in the latter, an α-catecholamine transmitter seemed a more likely candidate [19, 20]; moreover, the main action of histamine in this species proved to be an H$_2$-receptor-mediated vasodilatation [20, 21]. More recently, other possible transmitters, in particular a leukotriene, have been considered ([22] and reviewed in [16]). Again, for no clear reason, propanolol also abolished hypoxic vasoconstriction in the rat [23]. Nevertheless, it is of interest that almitrine-induced pulmonary vasoconstriction is attenuated by three substances which have a relatively specific antagonistic effect on hypoxic vasoconstriction in the rat. Almitrine may prove a useful tool with which to study the mechanism of hypoxic pulmonary vasoconstriction. We have no information as to the cause of the dilator action of almitrine to add to our earlier suggestion that it might be due to release of a vasodilator substance [2]; a role for endothelial-derived relaxant factor has not been excluded.

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