Role of Histamine in Hypoxic Pulmonary Hypertension in the Rat

I. BLOCKADE OR POTENTIATION OF ENDOGENOUS AMINES, KININS, AND ATP

By Anton Hauge, M.D.

ABSTRACT

Pharmacological agents that block or potentiate the effects of naturally occurring vasoactive substances were used to try to determine which substance, if any, mediates the vasoconstrictor response to acute alveolar hypoxia in isolated rat lungs. Isolated and ventilated lungs of rats were perfused at 37°C with homologous blood at constant-volume, pulsatile inflow, andpressor responses to brief periods of ventilation hypoxia (2% O₂) were recorded (control, 21% O₂).

Antihistamines of four different chemical classes in concentrations of 70 to 140 μg/ml abolished all pressor responses to alveolar hypoxia without interfering with the effects of injected bradykinin, ATP, or serotonin. A histaminase-inhibiting compound, semicarbazide, potentiated the hypoxic pressor response. The hypoxic pressor response could not be abolished by α-receptor-, serotonin-, or ATP-blocking agents. The results suggest that endogenous histamine in the lung is involved in the vasoconstrictor response to acute alveolar hypoxia.

ADDITIONAL KEY WORDS rat lung serotonin catecholamines α-receptor-blocking agents bradykinin pulmonary vasoconstriction antihistamines

Experiments with isolated, perfused, and ventilated lungs from several animal species (1) have demonstrated that a mechanism within the lung itself elicits vasoconstriction during acute alveolar hypoxia. Since most systemic vascular beds and isolated vessels, even those taken from the lung (2), dilate and relax under hypoxic conditions, several investigators have suggested that the pulmonary vasoconstrictor response to alveolar hypoxia is mediated via the release or activation of a local hormone (3-5). However, there is no agreement about the chemical nature of such a substance. Although local accumulation of acid and release of catecholamines have been among the most frequently mentioned intrinsic mechanisms (4-7), several workers have failed to find that they are operative or necessary for the hypoxic effect (8, 9).

The present experiments were an attempt to single out the vasoactive substance, if any, responsible for the increased pulmonary vascular resistance during acute alveolar hypoxia. Agents that block or potentiate the effects of naturally occurring vasoactive substances were used. Because isolated, perfused rat lungs react reliably with pressor response to acute alveolar hypoxia (10), they were used in this investigation. The findings suggest that histamine may mediate the pulmonary vasoconstriction during alveolar hypoxia.
Methods

EXPERIMENTAL PREPARATION

Sprague Dawley rats (200 to 300 g) were anesthetized with pentobarbitone (3 to 4 mg/100 g, i.p.); a tracheostomy was performed and the chest opened during positive-pressure ventilation. The trachea and the lungs were freed from surrounding tissue, and heparin was injected (100 IU in 0.5 ml saline, iv) before the ligation of the caval veins. Stainless steel cannulas were placed in the pulmonary artery and the left atrium. The lungs, heart, and pulmonary vessels were transferred to a humidified constant-temperature chamber and perfused with 35 ml of heparinized (10 IU/ml) blood obtained by cardiac puncture from ether-anesthetized donor rats.

We began the perfusion within 12 minutes of the interruption of the animal's own circulation, using a Harvard Pulsatile Blood Pump (M-1405) giving constant-volume inflow. The inflow pressure was measured with a Statham P23AC pressure transducer connected to a Grass Model 5c Recorder. The baseline pressure usually changed less than 1 mm Hg/hr.

The perfusate was pumped from the temperature-controlled reservoir through the arterial cannula. All surfaces in contact with blood were siliconized. The left atrial pressure remained between 2 and 4 cm of water. Changes in pulmonary vascular resistance were thus directly reflected in changes of inflow pressure (ΔPia). Figure 1 is a diagram of the perfusion arrangement. The method was a modification of that described elsewhere (10).

VENTILATION

Positive-pressure ventilation was administered by a Starling "Ideal" pump1 using the ventilation overflow arrangement described by Konzett and Rossler (11). The peak inspiratory pressure was 10 cm of water and expiratory pressure was 1.5 to 3 cm of water. Ventilation overflow volume was recorded with a Minispirimeter model 118.2 This volume is the part of the ventilation pump stroke volume (9 to 10 ml) that is not accepted by the lung under a set peak inflation pressure. There was a tendency for the compliance of the lungs to decrease slowly throughout an experiment (Fig. 1). This was counteracted by gentle hyperinflations at intervals.

The standard gas mixture used for ventilation was 21% O2, 4% CO2, and 75% N2 (hereafter called 21% oxygen). Hypoxia was created by ventilating the lungs with 2% O2, 4% CO2, and 94% N2 (hereafter called 2% oxygen) except when stated otherwise; such periods are referred to as "alveolar hypoxia."

Po2 was recorded with one or two Beckman oxygen macroelectrodes. Effluent blood Po2 (PvO2) was followed continuously in all experiments; arterial Po2 (PaO2) was followed occasionally. The electrode was calibrated against air at 37°C. Zero setting was done using nitrogen. Control calibration was carried out after the end of each experiment. No calibration was done during the perfusion. The pH of the perfusate

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1C. F. Palmer, Ltd., London.
2Med-Science Electronics, Inc.

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FIGURE 1

Diagram of the perfusion arrangement.
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was measured with a Radiometer pH meter (model AME-1).

DRUG ADDITIONS

Vasoactive drugs were injected into the arterial tubing. All drugs were given in solute (0.9% NaCl) volumes of 0.2 to 0.5 ml. Equivalent volumes of saline were used as controls. Blocking agents were usually added to the blood reservoir into a 35-ml volume. The drugs used are listed in Table 1.

Results

A typical pressor response, arterial and venous oxygen tension, and ventilation over-flow volume during hypoxic ventilation are shown in Figure 2. The figure demonstrates that the pulmonary artery pressure started to increase 45 sec before the pulmonary artery oxygen tension started to decrease; when ventilation with 21% O₂ began again (second arrow), the pulmonary artery pressure had almost returned to the baseline before the pulmonary artery oxygen tension had again started to rise. Care was taken that the two oxygen electrodes and their recording apparatus had the same response times.

When 2- to 4-minute periods of alveolar hypoxia are repeated every 8 to 10 minutes, the pressor response follows a characteristic pattern (10): At the beginning of perfusion such "hypoxic tests" produce no pressor response. After two hypoxic periods the response gradually increases until there is a maximum effect for the given length of hypoxia. The number of equal maximum responses that can be obtained before responsiveness declines varies from lung to lung. The decline in responsiveness is gradual until alveolar hypoxia no longer produces a pressor effect. At this

<table>
<thead>
<tr>
<th>Compounds Used in Evaluation of Possible Vasoactive Mediators of the Pressor Response to Acute Alveolar Hypoxia</th>
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<tr>
<td><strong>Class</strong></td>
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<td>Possible Mediators</td>
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<td>Adenosine-5-triphosphate (ATP)</td>
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<td>Bradykinin (BRS-640)</td>
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<td>dl-Arterenol-HCl</td>
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<td>5-hydroxytryptamine (serotonin)</td>
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<td>Histamine dihydrochloride</td>
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<td>pH changes</td>
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<tr>
<td>Pharmacological Blocking Agents</td>
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<tr>
<td>Anti-ATP (2,4-xylenol)</td>
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<td>Antikinin (sodium salicylate)</td>
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<td>Anticatecholamines</td>
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<td>Phenolamine mesylate (Regitine)</td>
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<td>Phenoxybenzamine hydrochloride (Dibenzyline)</td>
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<td>Antiserotonin</td>
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<td>Methysergide bimaleate (UML 491)</td>
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<td>Antihistamines</td>
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<td>Peptide-B</td>
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<td>Of histamine</td>
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<td>Semicarbazide</td>
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<td>Thioglycollate</td>
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time the lungs will still respond to pharmacological vasoactive compounds for considerably longer periods.

In six preliminary experiments rat lungs were challenged with 3-minute hypoxic tests every 9 minutes to determine the range of variability of the hypoxic pressor responses and to find the most suitable periods in which to add pharmacological modifiers of the response. Figure 3 shows all the pressor responses to alveolar hypoxia that could be elicited in the best (A) and in the poorest (B) responding preparation from this control series. In succeeding experiments inhibitory drugs were tested while the hypoxic response was still increasing in magnitude or after the first two plateau responses were seen. Potentiating drugs were always tested when the hypoxic pressor responses had started to decline. In more than 50 perfusions, once the phase of decline had started, the hypoxic response never increased spontaneously. Thus, when drug effects were evaluated, each lung acted as its own control.

To detect nonspecific effects on the responsiveness of the vascular smooth muscle of the lungs, ATP and bradykinin were always injected before and after the drug in question. Both ATP and bradykinin are convenient to use in a recirculating system because they are rapidly inactivated by blood and have short-lasting vasoconstrictor effects.

**EFFECTS OF INCREASING PERFUSATE PH**

During the first half hour of the experiments, the pH of the perfusate varied between 7.42 and 7.48; thereafter it fell about 0.04 to 0.06 units per hour.

In two experiments a series of 3-minute hypoxic tests was carried out with alternating 4% CO₂ and 0% CO₂ in the hypoxic gas mixture (remainder of the gas was N₂). Effluent blood pH was measured toward the end of each period of hypoxia. In this way responses to alveolar hypoxia obtained with falling hydrogen ion concentration in blood, and presumably in the tissue fluids, could be compared with the responses obtained in periods in which hydrogen ion concentration was not decreasing. One experiment is shown in Figure 4. The pressor response to alveolar hypoxia was neither inhibited nor augmented by increasing pH within this range. If hydrogen ions had been the mediator of the hypoxic response...
pressor response, one might expect no response under conditions of rising pH.

**EFFECTS OF PHARMACOLOGICAL BLOCKERS OF SEROTONIN, CATECHOLAMINES, HISTAMINE, AND ATP**

**Methysergide Bimaleate (UML 491)**

The serotonin-blocking agent UML 491 (28.5-142.8 µg/ml) had no consistent effect on the hypoxic pressor response but always blocked pressor activity of serotonin (Table 2). None of the doses tested reduced the pressor responses caused by injection of ATP (300 µg) or bradykinin (15 µg). A typical example from this series of experiments is shown in Figure 5.

**Alpha-Receptor-Blocking Agents**

The effects of α-receptor-blocking agents, phentolamine mesylate (Regitine, 28.5 to 142.8 µg/ml), and phenoxybenzamine HCl (Dibenzyline, 57.1 to 62.8 µg/ml) were tested (Table 3). Constrictor effects of dl-arterenol were abolished by 1 mg of phentolamine or 2 mg of phenoxybenzamine.

The effects of phentolamine on the hypoxic

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**TABLE 2**

**Effects of Methysergide Bimaleate (UML 491) on the Pressor Responses to Alveolar Hypoxia**

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>Hypoxic periods (min)</th>
<th>Dose of drug (mg)</th>
<th>Flow (ml/min)</th>
<th>Responses to hypoxic tests (ΔPPA mm Hg)†</th>
<th>Change in response (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>5</td>
<td>16</td>
<td>12</td>
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<td>5</td>
<td>3</td>
<td>1</td>
<td>15</td>
<td>6</td>
<td>8</td>
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</table>

*Added to blood reservoir, 35-ml volume.

†The last two hypoxic pressor responses obtained before addition of drug and the two first obtained afterwards are given.

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pressor response were somewhat variable. A definite reduction of the response was seen in three experiments; no significant change was seen in seven experiments. In all but one (no. 6) of the experiments, no reduction of the pressor responses to bradykinin (15 μg) or ATP (100 to 300 μg) was observed after administration of phentolamine. In experiment 6 the pressor effects of both these drugs were reduced shortly after the addition of the blocking agent, suggesting that it produced nonspecific smooth muscle effects. The blocking agent itself caused slight reduction in pulmonary vascular resistance.

Phenoxybenzamine had no inhibitory effect on the pressor response to alveolar hypoxia at doses that blocked the effects of dl-arterenol but did not block the response to ATP and bradykinin.

**Antihistamines**

Five antihistamines were able to inhibit the pressor response to alveolar hypoxia. The effect was dose dependent (Table 4). The antihistamines did not alter the pressor responses to ATP, bradykinin, or serotonin. Although antihistamines are known to vary considerably in potency and specificity (12), the lack of effect on vasoconstriction stimuli other than acute alveolar hypoxia suggested that...
TABLE 3
Effects of \( \alpha \)-Receptor Blocking Agents on the Pressor Response to Alveolar Hypoxia

<table>
<thead>
<tr>
<th>Expt no.</th>
<th>Hypoxic periods (min)</th>
<th>Drug dose* (mg)</th>
<th>Blood flow (ml/min)</th>
<th>Responses to hypoxic drug (( \Delta P_{\text{hyp}} ) mm Hg)†</th>
<th>Change in response (%)</th>
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</table>

*Added to blood reservoir, 35-ml volume.
†The last two hypoxic pressor responses obtained before the drug addition and the two (or three) first obtained afterwards are given.

their action in this preparation was not due to a general depressant effect on smooth muscle contractility.

Several antihistamines are known to cause vasoconstriction (13); this was most clearly demonstrated in our experiments by triptelennamine HCl and chlorpheniramine maleate (Fig. 6). In general, the rat lung vascular bed was relatively insensitive to the constrictor effects of these agents, thus making it possible to use high doses. Chlorpheniramine caused edema when used in doses of 4 mg (114 \( \mu \)g/ml) or more. On a weight basis, this agent was the most potent inhibitor of the hypoxic pressor effect tested. Figure 6 demonstrates the effect of 2.5 mg of this agent given during a series of hypoxic tests.

The most logical way of evaluating the effects of antihistamines would have been to compare the vascular effects of injected histamine before and after addition of the inhibitor. Such an approach, however, was difficult in these experiments for the following reasons: (1) the pulmonary vascular bed of the rat was relatively insensitive to exogenous histamine; (2) histamine in doses large enough to cause increased pulmonary vascular resistance also produced a long-lasting reduction in compliance; (3) gradual edema formation usually followed addition of histamine. Therefore, histamine was usually given only after the addition of the antihistamine. The higher doses of antihistamine used protected the lung vessels and bronchial system from the pharmacologic effect of up to 8 mg (228 \( \mu \)g/ml) of histamine.

Figure 7 shows the effect of three antihistamines added to the perfusate during hypoxia-induced vasoconstriction. After delays of 20 to 35 seconds, the pressor rise was reversed in spite of continued ventilation with 2% oxygen. When given under these conditions, the vasoconstrictor effect of chlorpheniramine also is apparent.

Anti-ATP: 2,4-Xylenol

ATP constricts the pulmonary vascular bed in rats (10). Dimethylphenols including 2,4-xylenol specifically inhibit the ATP- and ADP-induced vasoconstriction in rabbit lungs (14). The possible role of ATP and ADP in the pressor response to alveolar hypoxia was evaluated in three experiments. Pressor responses to injected ATP were reduced by 10 to 70% in our experiments by 2,4-xylenol, but the hypoxic responses were not affected.
TABLE 4

Effects of Antihistamines on the Pressor Response to Alveolar Hypoxia

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>Hypoxic periods (min)</th>
<th>Drug dose (mg)</th>
<th>Blood flow (ml/min)</th>
<th>Responses to hypoxic tests (ΔPpa mm Hg)* Before drug</th>
<th>After drug</th>
<th>Change in response (%)</th>
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<td>Thioglycollate</td>
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</table>

*The last two hypoxic pressor responses obtained before the drug addition and the two (or three) first obtained afterwards are given.

EFFECTS OF PHARMACOLOGIC POTENTIATORS OF HISTAMINE

Semicarbazide

The major pathway for inactivation of histamine in the lungs is by its oxidative deamination to β-imidazoleacetaldehyde (15) by histaminase. One of the most selective in vitro and in vivo inhibitors of intracellular diamine oxidases is semicarbazide (15). In three of the four experiments with this agent, semicarbazide potentiated the hypoxic pressor response. In two experiments it more than doubled the subsequent responses. The results are given in Figure 8. Semicarbazide usually affected only the two or three hypoxic tests after its administration.

Thioglycollate

Thioglycollate in a concentration of 10 mM potentiates histamine release in the anaphylactic reactions of isolated rat mast cells (16). The effect has been attributed to a shift toward SH⁻ production, which then potentiates the anaphylactic mechanism through a freeing of tissue histamine.

Figure 9 shows the effect of thioglycollate at the stage of declining pressor responses to hypoxia. Thioglycollate (10 mM) markedly potentiated and prolonged the responses. When the concentration of thioglycollate was increased to 100 mM, a gradual rise in baseline pulmonary artery pressure took place. The
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40
30
20
10
0

PPA (mmHg)

120
60
0

PV (mmHg)

5
6
7
8
BRK

1 hr 7 min
1 hr 17 min
1 hr 27 min
1 hr 33 min
1 hr 45 min
2 hr 15 min

V.O.F. (ml)

FIGURE 6

Effect of chlorpheniramine maleate (CPM, 2.5 mg) on the pressor response to acute alveolar hypoxia. Test number and time after start of perfusion are given under each period of hypoxia. BRK = bradykinin, 15 μg. Preparation no. 12 in Table 4.

Figure 6 shows the pressor response to alveolar hypoxia with the addition of chlorpheniramine maleate (CPM, 2.5 mg). The pressor response caused by thioglycollate was readily reversed by ventilation with a gas mixture containing 96% oxygen (4% CO₂), but it could not be controlled with 21% oxygen. Figure 10 is a summary of the effects of additions of 10 mM thioglycollate.

AGENTS AFFECTING KININS

Bradykinin and kallidin elicit brief but marked vasoconstriction when injected into the pulmonary blood stream in most adult mammalian lungs (17), including rat lungs (10). These substances also have been shown to be produced in certain lung tissue (18). Therefore, kinins might play a role in the pressor response to alveolar hypoxia. The following two approaches were chosen in an effort to solve this question.

Sodium salicylate inhibits the constricting effect of bradykinin on smooth muscle in several test systems (19). If kinins were involved in the pressor response to alveolar hypoxia, one might expect that the hypoxic pressor response and the bradykinin response would be affected in the same manner by addition to the perfusate of sodium salicylate.

In four experiments, 1 to 18 mg of sodium salicylate was added to the perfusate without causing detectable changes in the hypoxic pressor response. However, even these high doses of salicylate did not abolish the pressor responses to injected bradykinin (10-15 μg).

The second approach to this problem was by the use of bovine peptide-B. In 1963 Gladner et al. (20) demonstrated that certain naturally occurring polypeptides released during the thrombin-catalyzed conversion of fibrinogen to fibrin could markedly potentiate the bradykinin-produced contraction of smooth muscle. The effects of the addition of peptide-B was therefore investigated in four experiments. Doses of 500 to 700 μg potentiated the pressor responses to injected bradykinin (15 μg), but no potentiating effect on the pressor response to hypoxia could be detected. One example from this series of experiments is shown in Figure 11.

Discussion

The most striking observation in this series of experiments was that various antihistamines inhibited the pressor response to alveolar hypoxia. Antihistamine compounds belonging to four chemically distinct classes were all able, when given in appropriate doses, to block the hypoxic pressor response without interfering with the pressor effects of injected bradykinin, ATP, or serotonin.
Antihistamines effectively antagonize the smooth-muscle-stimulating actions of histamine, including dilator and constrictor effects in various vascular beds (12) and the responses of the bronchial muscle. The antihistamines do not prevent histamine-liberating drugs from releasing endogenous histamine; indeed, some possess histamine-liberating properties themselves (12). However, antihistamines are capable of modifying the effects of histamine-releasing stimuli.

Their action as competitive pharmacological antagonists of released histamine probably depends on the intimate contact between the antagonist and the histamine receptors. Large doses of antihistamines may be necessary to abolish the pressor response to alveolar hypoxia in this preparation because endogenous histamine may have ready access to effector sites. Doses of antihistamine of the magnitude used in these experiments would be difficult to use in intact animals without inducing a rise in pulmonary vascular resistance and profound effects on the systemic blood pressure.

The results suggest that endogenous histamine in the lung may play a role in the pressor response to acute alveolar hypoxia. Such a role is emphasized by the finding that semicarbazide, one of the most specific diamine-oxidase inhibitors investigated, potentiates the hypoxic response. Semicarbazide also potentiates the actions of histamine on the guinea pig ileum (21). The potentiating effect of thioglycollate may be less specific

FIGURE 7

Effect of three antihistamines added to the perfusate during hypoxic ventilation (3 experiments). PMA = promethazine; CPM = chlorpheniramine maleate; PM = pyrilamine maleate.

FIGURE 8

Effects of semicarbazide on the pressor responses to acute alveolar hypoxia in 4 experiments. Solid circles = before, and open circles = after, addition of semicarbazide. Doses: expt. 1, 12 mg (342 μg/ml); expt. 2, 10 mg (285 μg/ml); expt. 3, 18 mg (514 μg/ml); expt. 4, 22 mg (625 μg/ml).
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and therefore more difficult to evaluate. Thioglycollate may act synergistically with histamine liberators, act as a weak diamine-oxidase inhibitor (15), or act as a reducing agent which interferes with oxidative metabolism. A more thorough discussion of the role for

![Graphs and diagrams related to histamine and alveolar hypoxia.](https://example.com/graphs)

*Figure 9*

Effect on the pressor response to acute alveolar hypoxia of addition of thioglycollate (10 mm) to the perfusate. Test number and time after start of perfusion are given under each hypoxic period. Blood flow was 20 ml/minute.

*Figure 10*

Effects of thioglycollate (10 mm) on the pressor response to acute alveolar hypoxia in 3 experiments. Solid circles = before, and open circles = after, addition of thioglycollate.

*Figure 11*

Effects of peptide-B on the pressor response to acute alveolar hypoxia and on the pressor responses to bradykinin (BRK). 700 μg peptide-B was injected into the arterial tubing (indicated by arrow). This injection had no apparent effect on the declining responses to 3 minutes of ventilation hypoxia, whereas the responses to bradykinin were increased fourfold during the following 40 minutes.
histamine in the hypoxic pressor response is given in the following article dealing with the effects of inhibitors of synthesis and releasers of biogenic amines (22).

When this work was started, one of its major objectives was to obtain information on the possible role of catecholamines in the pressor response to acute alveolar hypoxia. The results stress the difficulty of interpreting the effects of pharmacological blockade. Although the $\alpha$-receptor-blocking agent, phentolamine, was able to reduce the hypoxic pressor response in three of ten experiments, none of the doses used was sufficient to abolish the response. Doses used to produce the most marked effect produced a simultaneous reduction of responses to injected bradykinin and ATP. Such a generalized decrease in the responsiveness of the vascular smooth muscles suggests that phentolamine in high doses may have direct and nonspecific smooth muscle effect. That $\alpha$-receptor blockade does not seem responsible for inhibiting the hypoxic response is further suggested by the lack of effect of phenoxybenzamine. The delay of pressor response after the start of alveolar hypoxia was prolonged in several of the experiments involving the use of phentolamine. In part this may have been due to the vasodilation caused by this agent, in which case the standard hypoxic test time might have been too brief to allow development of the same absolute level in vascular resistance as seen before the drug was used.

Other suggested mediators of the hypoxic response include ATP, serotonin, and kinins, which are endogenous in the lung and are capable of increasing pulmonary vascular resistance. These studies in which inhibition of ATP (by 2,4-xyleneol) and serotonin (by UML 491) were produced did not reveal simultaneous reduction of the hypoxic response. Likewise, kinins do not seem to be probable endogenous mediators of the hypoxic response. Although inhibition of the constrictor effect of bradykinin was not produced by sodium salicylate, the potentiation of bradykinin by peptide-B did not change the response to alveolar hypoxia.

Hydrogen ions probably do not act as mediators of the hypoxic pressor response since there was no significant change in the hypoxic response in periods when decrease of hydrogen ion concentration in effluent blood (and presumably also in the extra- and intracellular fluids) were produced.

Histamine is suggested then as at least one mediator of the hypoxic pressor response in lungs. However, pharmacological blocking agents have multiple actions and may interfere with the action of more than a single pressor substance. The nonspecific depressant effects of blocking agents may lead to some erroneous conclusions regarding the role of their agonists. To further evaluate the role of histamine, and also of catecholamines and serotonin, in the pressor response to alveolar hypoxia, releasing and depleting agents were used in pretreatment of animals and in the in vitro test system. These results are reported in the following article.


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References
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