Histamine induced pulmonary vasodilatation in the rat: site of action and changes in chronic hypoxia

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ABSTRACT: Histamine constricts postcapillary lung vessels and also causes dilatation, site unknown. In chronically hypoxic rats, pulmonary arterioles are muscularized and histamine-containing mast cells increase. We wanted to determine a) whether vasoreactivity to histamine changes in chronic hypoxia; b) whether dilatation is due to H2 receptors; and c) which vessels dilate.

We perfused isolated lungs of normal (C) and chronically hypoxic (CH) rats. Histamine was tested during hypoxic vasoconstriction. To examine effects on arteries alone, we raised alveolar (inflation) pressure above outflow pressure; during inflation, pressure/flow (P/Q) lines were measured during normoxia, and hypoxia, and after histamine during continued hypoxia.

Dose-related dilatation was seen, which was abolished by cimetidine and enhanced in CH rats. A mast cell-discharging agent, but not exogenous histamine, caused constriction, which was abolished by chlorpheniramine. P/Q lines differed in C and CH rats in a manner which suggests that hypoxia constricts larger "extra-alveolar" vessels in C rats, but mainly muscularized arterioles exposed to alveolar pressure in CH rats. Histamine restored the P/Q line to nearly its normoxic position; it therefore dilated those vessels which constrict in hypoxia in each rat group.

It is concluded that histamine has a strong H2 dilator effect, enhanced in chronic hypoxia, which might be an important attenuating factor in hypoxic pulmonary hypertension.


Histamine is present in large quantities in the lung, mainly in mast cells, which are found adjacent to blood vessels and bronchi and in the pleura. It is presumed to play an important role in allergic conditions, but no physiological function has been forthcoming. Vasoconstriction was observed first and led to the suggestion that, because histamine was also a bronchoconstrictor, it would assist ventilation/perfusion matching [1]. In normal animals and man, there is little or no pulmonary vascular tone. Thus, the dilator action of histamine was only detected when studied in the lamb fetus, during vasoconstriction and in other conditions of high pulmonary artery pressure [2–4]. Dilatation proved to be an action on H2 receptors in cats and dogs [5].

Histamine was proposed as a mediator of hypoxic pulmonary vasoconstriction. Haas and Bergofsky [6] described histamine release and mast cell discharge during hypoxic vasoconstriction in the cat. Hauge [7] and Hauge and Melmon [8] provided good evidence that histamine was involved in hypoxic vasoconstriction in the rat. However, only huge doses caused vasoconstriction in this species [3, 7]. More recently, however, constriction has been demonstrated in a different strain of rat [9]. The hypothesis broke down when it was found that histamine caused dilatation during hypoxic vasoconstriction in the rat and the calf [10, 11]. This dilator action of histamine during hypoxia in the rat was not proven to be an H2 action, because early H2 antagonists (as well as H1 antagonists) abolished hypoxic vasoconstriction in this species. Thus, there are species and strain differences in the effects of histamine on pulmonary vessels. The functions of the two opposing actions of histamine are not known. In some species, notably the rat, there is a conspicuous increase in lung mast cells in chronic hypoxia, particularly beside the newly muscularized arterioles [12].

Our aim in this work was to see whether, in association with these new cells, there is a change in pulmonary vascular response to histamine in rats chronically exposed to hypoxia, and also to determine the cause of histamine dilatation in this species. Chronically hypoxic rats have pulmonary hypertension and muscularization of small arterioles. The site of histamine vasoconstriction appears to be postcapillary [13], but the site of histamine vasodilatation has not been described. Our second aim was, therefore, to determine the site of histamine vasodilatation by the use of the lung inflation method, which separates events upstream from the alveolar region in the pulmonary circulation from those on the venous side. Permutt and Riley [14] showed that, when alveolar (inflation) pressure is...
raised above left atrial pressure, left atrial pressure (or venous pressure) must rise above alveolar pressure before it affects pulmonary artery pressure; events on the arterial side of the alveoli are reflected in arterial pressure. With this technique, we demonstrated a probable peripheral shift in the main site of hypoxic vasoconstriction on the arterial side of the circulation from muscular “extra-alveolar” vessels to newly muscularized “alveolar” arterioles in chronically hypoxic rats [15], and showed that two dilator drugs act at the same site as hypoxia [16]. A preliminary communication on histamine has been published [17].

Methods

Male Wistar rats (A. Tuck and son) were obtained at ca. 28 days of age and allowed to adapt to the laboratory for a few days before they were placed in a normobaric environmental chamber at 10% O2 for 2–5 weeks (CH rats) as described previously [18]. On removal from the chamber, they were compared with litter-mate controls kept in the same room in air (C rats).

Isolated perfused lungs

Both C and CH rat lungs were perfused with blood in situ by a method previously described [18]; CH lungs were perfused within 4 h of removal from the chamber. After pentobarbitone anaesthesia (60 mg·kg−1 i.p.) the chest was opened and cannulae were inserted into the pulmonary artery and left atrium. Blood of normal haematocrit, taken from C rats themselves, or, for CH rats, from stock Wistar rats, was used in all experiments (the polycythemic blood of CH rats raises pulmonary artery pressure (Ppa) [19]). It was pumped from a reservoir kept at 38°C into the pulmonary artery with a Watson-Marlow roller pump, escaped freely from the left atrium to the reservoir and was recirculated to the pulmonary artery; left atrial pressure was zero. Ppa was measured close to the arterial cannula with an electromanometer (Electromed) and recorded on a pen recorder (Bryans). Flow rate was constant at 20 ml·min−1 in all rats, because we observed that lung vascular volume was similar in C and CH rats despite retarded growth in CH rats [18]. The lungs were ventilated through a tracheal cannula with air + 5% CO2 (normoxia), or 2% O2 + 5% CO2 + balance N2 (hypoxia). The pH of the circulating blood was adjusted to 7.35–7.45 during ventilation with air + 5% CO2.

Pressure/flow (P/Q) lines

Blood flow was measured with an electromagnetic flowmeter [20] inserted into the inflow line, and plotted against Ppa on an XY recorder (Bryans). P/Q lines were measured, as described previously [15], during arrest of ventilation and during inflation of the lungs by blowing the appropriate gas over a water trap. The trap consisted of a T-piece, whose vertical limb was immersed in water to the required depth; this inflation pressure (alveolar pressure (Palv)) was fixed first at 5 and then at 15 mmHg. P/Q lines were linear except at low flow rates (<5 ml·min−1). The slope of this linear portion, together with its projected intercept on the pressure axis, was recorded. The intercept is the effective downstream pressure for the linear part of the line and is attributed to collapsible vessels behaving like Starling resistors. These resistors are caused either by the alveolar pressure, or by small collapsible vessels in a state of tone which act in the same manner [14].

Protocol

Experiment 1. After adjustment of pH and a few minutes for stabilization of Ppa, two or three hypoxic tests were made to establish a reproducible response. Ppa rose to a plateau or slowly declining value (figs 1 and 2); at least 5 min was allowed between tests. Thereafter, in six C and six CH rats (mean±SEM weight 274±17 and 208±8 g, respectively), increasing doses of histamine were given at the height of hypoxic pressor responses, one dose per hypoxic response (1, 10, 100, 250, 500 µg, 1 and 10 mg); these were cumulative dose-response curves. After the last dose, 100 µg was repeated to test for tachyphylaxis. In some experiments, very large doses (20 mg) were given at the end of the experiment in an attempt to cause vasoconstriction.

Experiment 2. In seven rats, to test for an H2 action of histamine, 10 µg was given during two successive hypoxic tests. Cimetidine, 2.5 mg, was then given during the next normoxic period, followed by two further 10 µg doses of histamine during the next two hypoxic tests. To look again for vasoconstriction, 20 mg histamine was then given during normoxia. Several minutes later, the nonspecific mast cell-degranulating agent BW 48/80 was given (200 µg). During maximum constriction due to this drug, 1 mg of the H1 receptor antagonist, chlorpheniramine, was given.

In six further rats, the repeatability of response to 10 µg histamine was tested during three hypoxic tests, followed by cimetidine during normoxia and 10 µg histamine during a fourth hypoxic tests.

Experiment 3. In nine C and nine CH rats, ventilation was arrested and P/Q lines were measured during inflation, first to 5 and then 15 mmHg Palv: a) during normoxia; b) during hypoxia; and c) after 100 µg histamine during continued hypoxia.

Drugs

Histamine acid phosphate (Evans Medical), cimetidine (Smith, Klein and French), chlorpheniramine (Pirion; Allen and Hanbury’s) and Burrows Wellcome 48/80 were used, all dissolved in saline. The last was instilled via the blood reservoir, the rest into the inflow tubing close to the pulmonary artery as bolus doses (0.1–0.2 ml); 0.9% NaCl was administered in similar volumes as a control.
Statistics

Means and standard errors of the mean were calculated. A two-way analysis of variance with repeated measurements was carried out to examine the dose-response to histamine during hypoxic vasoconstriction.

For Experiment 2 a Student's t-test was used to compare the effect of histamine before and after cimetidine.

Results

Cumulative dose-response curves to histamine during acute hypoxia (Experiment 1)

Because of the near absence of vascular tone in the normal rat, histamine dilatation was studied in both C and CH rats during preconstriction by hypoxia. Over a wide dose range (1 µg to 10 mg), histamine caused transient or prolonged dilatation during hypoxic vasoconstriction in both groups of rats; it had little or no effect on either group during normoxia. Figure 1 compares the effect of 100 µg histamine, a dose which caused maximal dilatation, in one C and one CH rat. In each rat, there is prolonged dilatation, but the reduction in hypoxic vasoconstriction is much greater in the CH than the C rat. The figure also shows tachyphylaxis; later in the experiment, after tests with larger doses, a second dose of 100 µg has little effect in either rat.

Figure 2 shows the effect of a very large dose, 10 mg, again in one C and one CH rat. In the C rat, there is a brief dilatation, but in some C rats, after the dilatation, there was a brief rise above the preinjection level, indicative of weak constriction. In the CH rat, a sharp fall in pressure is succeeded by a transient rise above the preinjection value, a second fall, a second slight rise, followed by a slow decay; it is not possible to say whether this decay is due to histamine dilatation or to decay of the hypoxic response. The response to histamine in the CH rat suggests an unstable balance between two opposing actions.

Figure 3a shows a log dose-response curve to histamine during hypoxia for the six C and six CH rats. Maximum dilatation is recorded, although, as already stated, doses differed not only in their maximum effect but in their duration and, sometimes, in their biphasic characteristics. The absolute fall in Ppa was greater in CH and C rats at all doses, particularly in the dose range 1–100 µg. Optimum dilatation took place at 100 µg histamine in both groups of rats. This dose had a four times greater effect in CH than C rats. At higher doses (1–10 mg) dilatation was reduced but was still greater in CH and C rats. The greater dilatation caused by 10 mg than 1 mg in CH rats is somewhat misleading; the maximum dilatation was greater after 10 mg but was succeeded by oscillations, as shown in figure 2. There was a significant difference in the dose-response curves between the C and CH group (F=26.7; df 6.5; p<0.001). Testing the differences in response between the two groups at each dose separately, there was a significant difference at each dose (Mann-Whitney test, all p<0.01).

Figure 3b shows the results from figure 3a calculated as the percentage reduction in hypoxic vasoconstriction. This calculation is important because hypoxic constriction was greater in CH than C rats, as found previously.
It shows that the reduction in vasoconstriction is greater in the CH rats. Attenuation of constriction increased with doses up to 100 µg, and then declined. It must be emphasized that, for the larger doses, evaluation and interpretation of responses is difficult because of tachyphylaxis, cumulative dosage, and probably mixed dilator and constrictor effects. No overt pulmonary oedema was observed after any histamine dose in either rat group. The percentage inhibition of hypoxic response by histamine was also significantly different between the C and CH groups (F=7.13; df=6.5; p<0.024). However, only at the doses ≤100 µg was there evidence of a difference between the percentage inhibition in the two groups.

Both normoxic (between test) Ppa and the size of the hypoxic response were influenced by the repeated histamine dosage, which suggested persistence of histamine in the circulating blood. Mean normoxic Ppa was higher in CH than C rats (29.7±1.4 vs 16.9±0.4 mmHg), and climbed more during the course of experiments (29.7 to 45.2 vs 16.9 to 21.7 mmHg), as noted previously [18]. However, during the dose-response tests, the steady increase in normoxic Ppa was interrupted by a fall, between doses 250 µg and 1 mg, especially in CH rats. A reduction in normoxic Ppa after 100 µg histamine during hypoxia is shown in figure 1. CH rats showed a greater first and maximum pressor response to hypoxia than C rats, as seen previously [18]; (ΔPpa 30.0±2.8 vs 11.1±1.5, and 33.3±2.0 vs 14.0±1.4, respectively). These pressor responses remained stable during the first few tests with histamine, but after the 100 µg dose they were significantly reduced in both rat groups (CH p<0.01; C p<0.05).

Effect of the H2 inhibitor, cimetidine (control rats only) (Experiment 2)

Figure 4b shows one of seven rats in which the dilator action of histamine was abolished by an H2 receptor antagonist. Two doses of 10 µg histamine, given during hypoxia, caused dilatation, but a third dose after cimetidine, 2.5 mg, was ineffective. Occasionally, as here, cimetidine caused a small rise in normoxic Ppa. Figure 4a shows one of six rats in which three tests with 10 µg were given before cimetidine to exclude tachyphylaxis. Figure
We did not see unequivocal vasoconstriction with histamine from the normoxic baseline (even after 20 mg), during hypoxic vasoconstriction or after blockade of the H2 dilator action with cimetidine. However, after cimetidine, the mast cell-releasing agent BW 48/80 (200 µg) caused vasoconstriction during normoxia. During peak constriction by this agent, the H1 histamine antagonist, chlorpheniramine, 1 mg, reduced the rise in Ppa by 55.2±6.2%. This suggests that histamine released from mast cells could cause vasoconstriction in normal rat lungs.

Location of histamine dilatation; pressure/flow lines

When alveolar pressure exceeds left atrial pressure, changes in Ppa reflect changes in upstream pulmonary vessels, unless there is a rise in downstream pressure which exceeds alveolar pressure. We measured P/Q lines at 5 and 15 mmHg Palv, both of which values exceeded Pla. Figure 6 is a typical trace of lines in a C and

CH rat during normoxia and hypoxia; mean lines for nine C and nine CH rats are shown in figure 7 a and b. On the left are shown the mean lines during normoxia and hypoxia; on the right the hypoxic lines are repeated, whilst those measured after 100 µg histamine during continued hypoxia are also shown. In both C and CH rats, the lines measured during normoxia are wide apart and the distance between them is close to ΔPalv (10 mmHg). During hypoxia, the lines become steeper in both rat groups. In C rats, there is, invariably, only a small shift in the line when Palv is increased from 5 to 15 mmHg; whereas, in CH rats this Palv change, also

Fig. 6. – The effect of inflation on pressure/flow (P/Q) relationships during normoxia and hypoxia: a) in a control (C) rat; and b) in chronically hypoxic (CH) rat. P/Q lines were measured on an XY recorder during inflation at 5 (A) and 15 (B) mmHg Palv during normoxia, and similar inflations (C and D) during hypoxia. The dashed lines project the straight portion of the relationships to the pressure axis. P/Q lines A and B are separated by approximately the rise in inflation pressure in both rats during normoxia; during hypoxia, the C P/Q lines are close together whilst the CH lines are wide apart.

Action of BW 48/80; constrictor action of histamine

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Discussion

Dilator and constrictor actions of histamine

In early work, bronchoconstrictor and pulmonary vasconstrictor actions of histamine were demonstrated. Later a vasodilator effect was demonstrated when pulmonary vascular tone was high [2, 3]. The two actions were inhibited by two types of drug, H1 and H2 antagonists [4, 5], although the dilator effect of histamine was not proven to be an H2 action in the rat. We think that current work with cimetidine establishes this for the first time. Up till now, we have not been able to demonstrate histamine vasoconstriction in our strain of rat, although this has now been reported for another rat strain [9]. However, several observations suggest that, when many doses had been given, some constrictor effect was taking place. The reduced dilator action with large doses, the oscillations in pressure after some doses (fig. 2) and rises in baseline, normoxic, Ppa after several doses could all be explained if two opposing influences were at work. Also, the rise in pressure after BW 48/80 could be due to histamine release from mast cells, a possibility rendered more likely since this pressure rise was reduced by an H1 antagonist. We cannot explain why exogenous histamine was ineffective while a presumed release of histamine from mast cells caused vasoconstriction, unless histamine has a different effect on vascular muscle when applied from the outside compared with from the vascular lumen.

Changed effects of histamine in chronically hypoxic rats

The H2 dilator effect of histamine was greater in CH and C rats. One hundred micrograms nearly abolished hypoxic vasoconstriction in CH rats and reduced the subsequent normoxic pressure, thereby reducing the high basal tone in these rats (fig. 1). These rats have increased numbers of mast cells, many of which lie adjacent to small newly-muscularized arterioles; these could be an endogenous source of histamine [12].

Site of dilator action of histamine

The experiments with raised alveolar pressure suggest that histamine dilates vessels on the arterial side of the circulation. In a previous study, we showed that lung inflation, during hypoxic vasoconstriction, had very different consequences in C and CH rats [15]. We suggested that this was because CH rats have new muscle in previously nonmuscular vessels, which extends far peripherally into vessels within the acinus surrounded by alveoli. We suggested that the main site of hypoxic vasoconstriction in C rats was upstream from alveoli in "extra-alveolar vessels", whereas in CH rats the main site was in vessels affected by alveolar pressure. It was reasonable to suppose that these were the newly muscularized arterioles. Our tentative explanation for the findings for P/Q lines in the two groups of rats during hypoxic vasoconstriction is again summarized here: the lines measured during hypoxia in the current study (fig. 7) are very similar to those seen in the earlier work [15]. During hypoxia at Palv 5 mmHg, in C rats, the slope and intercept increase, while a rise in Palv from 5 to 15 causes only a small shift in the line. We suggested that hypoxic vasoconstriction caused a Starling resistor to develop in upstream vessels; the very high Palv, 15 mmHg, then opened up this resistor to become again the effective downstream pressure, as in figure 8; the P/Q line moved by the difference between the alveolar pressure and the effective pressure caused by the Starling resistor. An alternative explanation is that radial traction on extra-alveolar vessels during high lung inflation, attenuates hypoxic vasoconstriction. In CH rats, the slope and

Fig. 7. – Mean pressure/flow (P/Q) lines for: a) 9 control (C) rats; and b) 9 chronically hypoxic (CH) rats. In the left-hand panels, the solid lines (——) were measured at 5 and 15 mmHg alveolar pressure (Palv) during normoxia and the short-dashed lines (-----) during hypoxia. In the right hand panels, the hypoxic lines are repeated and the long-dashed lines (——) were measured after histamine, 100 µg, during continued hypoxia. Note the differences between C and CH rats during hypoxia illustrated in figure 6. After histamine, the P/Q line for both C and CH rats is restored close to the normoxic line, although hypoxia persisted.

invariably, causes a wide shift >ΔPalv. On the right, it can be seen that histamine causes the lines to revert to positions close to those during normoxia, although the hypoxic state persists. Histamine appears to dilate those vessels, in each rat group, which constrict in hypoxia.
intercept also increased during hypoxia at Palv 5 mmHg; however the increase in Palv to 15 mmHg caused a large upward movement in the line ≥ΔPalv. We suggested that in these rats a Starling resistor was caused by hypoxia in vessels in the alveolar region, and that the rise in Palv to 15 mmHg enhanced their collapse. The results with histamine suggest that it dilates "extra-alveolar vessels" in C rats and "alveolar vessels" in CH rats.

Significance of the two actions of histamine

It has been suggested that the vasoconstrictor and bronchoconstrictor actions of histamine may be one method whereby ventilation/perfusion are regulated [1]. The bronchoconstrictor action of histamine was shown, in cats, to affect peripheral airway muscle round alveolar ducts and alveoli, supplied by the pulmonary circulation [21]. This effect, combined with constriction of small vessels would effectively close down small lung units. However, the fact that the H1 action of histamine is thought to be mainly venous and, therefore, liable to cause microvascular leakage, would not favour such a regulatory role. HAAS and BERGOVSKY [6] observed histamine release and mast cell degranulation during hypoxia in cats; in this circumstance, peripheral airway closure and hypoxic vascular narrowing would also close down units. However, in rats, mast cell degranulation could not be detected in hypoxia [12]. The profound H1 dilator action of histamine cannot be fitted into any meaningful function at this time. It could be yet a further regulatory role. Hauge A. Role of histamine in hypoxic pulmonary hypertension in the rat. I. Blockade and potentiation of endogenous amines, kinins and ATP. Circ Res 1968; 22: 371–383.


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