Lung mast cells in rats exposed to acute hypoxia, and chronic hypoxia with recovery

A. WILLIAMS, D. HEATH, J. M. KAY, AND P. SMITH

From the Department of Pathology, University of Liverpool

Williams, A., Heath, D., Kay, J. M., and Smith, P. (1977). Thorax, 32, 287–295. Lung mast cells in rats exposed to acute hypoxia, and chronic hypoxia with recovery. Exposure to acute hypoxia (barometric pressure 263 mmHg) for 8 hours did not lead to increased numbers of mast cells in the lungs of rats. In contrast, in adult rats kept for 35 days at a barometric pressure of 380 mmHg there was a proliferation of mast cells around the pulmonary blood vessels and in the alveolar septa. This hyperplasia of lung mast cells in response to chronic hypoxia was reversible on removal of the hypoxic stimulus. There was a correlation between the logarithm of the perivascular lung mast cell density (defined in the paper) and the logarithm of the right ventricular weight. There was no increase in the mast cells in the carotid bodies of the hypoxic rats. Young male, old male, young female, and old female rats which had been subjected for 39 days to a barometric pressure of 380 mmHg showed a proliferation of mast cells around the pulmonary blood vessels and in the alveolar walls. This response was greatest in the adult animals and independent of their sex. In the age and sex experiment there was a correlation between the perivascular lung mast cell density and the medial thickness of the muscular pulmonary arteries. Since mast cell hyperplasia has been reported as preceding right ventricular hypertrophy, it is conceivable that mast cell proliferation in the lung may be a defence mechanism to limit the severity of hypoxic pulmonary hypertension rather than to mediate it.

During the past few years we have carried out a series of experiments on the effects of chronic alveolar hypoxia on the lungs and heart. Among other things we have investigated the reversibility of the morbid anatomical changes induced in the heart and small pulmonary arteries (Abraham et al., 1971), and pulmonary trunk and carotid bodies (Heath et al., 1973), and the effects of age and sex on the response of the right ventricle and pulmonary vasculature to hypoxia (Smith et al., 1974). Recently the rôle of the mast cells in the hypoxic lung has aroused interest, and it has been suggested that in rats and guinea-pigs the perivascular pulmonary mast cell may be an important structure in the mediation of the pulmonary vasoconstrictor response to acute alveolar hypoxia (Haas and Bergofsky, 1972). Exposure of rats to chronic hypoxia for three weeks has been reported as leading to a proliferation of mast cells around small pulmonary blood vessels and within the alveolar walls (Kay et al., 1974; Mungall and Barer, 1975; Mungall, 1976). The magnitude of the mast cell hyperplasia correlates with the degree of right ventricular hypertrophy, suggesting that in the rat pulmonary mast cells may be connected in some way with chronic hypoxic pulmonary hypertension.

To investigate this problem further, we have exposed rats to severe acute hypoxia to see if hyperplasia of mast cells occurs in their lungs. We have also carried out a retrospective study on lung tissue derived from two previous chronic experiments on rats to determine whether the hyperplasia of mast cells was reversible once the hypoxic stimulus had been removed (Heath et al., 1973), and whether the hyperplasia was affected by the age and sex of the experimental animals (Smith et al., 1974). We were able to use paraffin-embedded material from previous experiments since in the rat mast cell granules are insoluble in aqueous fixatives. We have investigated the relation between the number of mast cells on the one hand and the right ventricular weight and medial thickness of muscular pulmonary arteries on the other. Finally we
counted the number of mast cells in the carotid body to see if they also proliferated under the influence of chronic hypoxia.

**Material and methods**

**RATS EXPOSED TO ACUTE HYPOXIA**

Ten adult female Wistar albino rats weighing from 210 g to 230 g were divided into five test animals and five controls. The test rats were subjected to a constant barometric pressure of 263 mmHg in a decompression chamber simulating an altitude of 7944 m above sea level for a period of 8 hours. The control animals were kept at sea level atmospheric pressure. On removal from the hypobaric chamber the test rats were anaesthetised with sodium pentobarbitone given intraperitoneally at 50 mg/kg body weight. The trachea was cannulated and 2% glutaraldehyde solution was infused into the lungs at a constant pressure of 20 cm H2O. The thoracic cage was gently massaged to allow escape of gases from the lungs. This procedure ensured that the lungs of different animals were fixed with a similar degree of expansion. Following fixation for 1½ hours at this constant pressure in situ the trachea was ligated and the thoracic viscera were removed in one block. The heart and lungs were dissected free and the lungs were placed in 2% glutaraldehyde for fixation. The lungs of the control rats were treated identically. After fixation one block of tissue from each of the five lung lobes of each rat was embedded in paraffin wax. One section from each block was cut, 5 μm thick, and stained with 0.2% metachromatic blue in 5% aluminium sulphate. The number and distribution of the pulmonary mast cells in the control and hypoxic rats were obtained according to the method described below.

**RATS EXPOSED TO CHRONIC HYPOXIA**

*Tissues from the recovery study of Heath et al.* (1973)

Three groups of 10 adult male Wistar albino rats weighing from 315 g to 425 g were used. A ‘hypoxic’ group was kept for 35 days in a hypobaric chamber and exposed to a barometric pressure of 380 mmHg, simulating an elevation of 5500 m, and then killed. A ‘recovery’ group was exposed to the same barometric pressure for 35 days and then allowed to recover in room air at sea level for a further five weeks. A control group was kept at sea level barometric pressure throughout the study. At necropsy the thoracic viscera were removed and the lungs were distended via tracheal cannulation with 4% formaldehyde until the pleural surfaces were smooth. The whole of the thoracic viscera were placed in the same solution for fixation. A block of tissue was taken from each lung and embedded in paraffin wax. The carotid bodies from each rat were fixed and embedded similarly. For the present study a section 5 μm thick was cut from the right and left lung blocks and stained with 0.2% metachromatic blue in 5% aluminium sulphate. A section, 5 μm in thickness, was also taken from near the centre of each block of carotid body tissue and stained identically. Counts of the pulmonary mast cells and carotid body mast cells of these sections were made as described below.

**Tissues from the age and sex study of Smith et al.* (1974)**

Four groups of four Wistar albino rats (young male, young female, adult male, and adult female) whose mean body weights were respectively 102 g, 100 g, 276 g, and 238 g, were kept in a decompression chamber for 39 days at a barometric pressure of 380 mmHg, equivalent to an elevation of 5500 m. Four similar control groups were kept at sea level barometric pressure. At necropsy the lungs were distented with 4% formaldehyde as described previously. A block of tissue from each of the five lung lobes of each rat was taken from each animal and embedded in paraffin wax. For the present study a section 5 μm thick was cut from each of the five blocks of lung tissue from each animal and stained with 0.2% metachromatic blue in 5% aluminium sulphate. Mast cell counts were performed on each of these sections from each animal as described below.

**PULMONARY MAST CELL COUNTS**

In the lung, mast cells are found in the pleura, the peribronchial connective tissue, the perivascular connective tissue (Fig. 1), and the alveolar septa. Pleural mast cells were regarded for the purposes of this study as extrapolmonary and were not counted. The mast cell counts were performed using a microscope fitted with a square graticule and at a magnification of ×120. The area of the section bounded by the graticule at this magnification was 0.392 mm². By moving the microscope stage vertically and horizontally in a systematic manner, mast cells within most of the lung substance bounded by the pleura were counted. Perivascular, peribronchial, and alveolar mast cells were counted separately in each field delineated by the graticule. Perivascular mast cells were defined as those in and immediately surrounding the adventitia of both pulmonary arteries and veins.
Mast cells in the perivascular space were included. Peribronchial mast cells were defined as those in and immediately surrounding the peribronchial connective tissue. Alveolar septal mast cells were those in the alveolar septa. The numbers of perivascular, peribronchial, and alveolar septal mast cells and the total number of mast cells were divided by the total number of graticule unit fields counted. These ratios of mast cells per unit area are henceforth referred to as 'lung mast cell densities'. The mean lung mast cell densities of each rat were obtained from the number of lung sections counted, and subsequently the average mean perivascular, peribronchial, alveolar septal and total mean lung mast cell densities were calculated for each experimental group.

CAROTID BODY MAST COUNTS
The total number of mast cells within both carotid body sections from each rat were counted at a magnification of ×120. An estimate of the area of the carotid body cross-section was obtained by counting the number of unit graticule areas at a microscope magnification of ×480 required to cover the surface area of the carotid bodies when the microscope stage was moved horizontally and vertically in a systematic manner. At the magnification of ×480 the area of the section bounded by the graticule was 0.0259 mm². The carotid body mast cell count was then divided by the estimate of carotid body area, and the ratio is henceforth called the 'carotid body mast cell density'. The carotid body mast cell density unit differs from the lung mast cell density as the areas used to calculate the ratios differ. The mean carotid body mast cell density in each rat was obtained and the average mean carotid body mast cell density and average mean carotid body mast cell count were calculated for each of the three experimental groups (hypoxic, recovery, and control).

DATA ANALYSIS
Differences between lung mast cell densities were compared using the Student's t test. Where more than two means required comparison the analysis of variance technique was used. Correlations between the lung mast cell density on the one hand and the right ventricular weight and medial thickness of the muscular pulmonary arteries on the other were sought using linear regression analysis.

Results

RATS EXPOSED TO SEVERE ACUTE HYPOXIA
The perivascular, peribronchial, alveolar septal, and total lung mast cell densities of the hypoxic and control rats are shown in Table 1. There was no significant difference in their values between
hypoxic and control rats. No differences in the histological appearance of the pulmonary mast cell from hypoxic and control rats were found. However, cell counts of granulated and ungranulated mast cells were not carried out.

RATS EXPOSED TO CHRONIC HYPOXIA

Tissues from the recovery study of Heath et al. (1973)
The lungs of one of the control rats showed histological evidence of severe chronic inflammation and were, therefore, excluded from the study. The lung mast cell densities of the hypoxic, recovery, and control groups of rats are shown in Table 2. In the hypoxic rats the perivascular, alveolar septal, and total lung mast cell densities were significantly greater than the corresponding values in the control and recovery groups (Fig. 2). There was no significant difference between these values in the control and recovery groups. No significant difference was found between the peribronchial mast cell density in the three experimental groups.

There was no significant difference in the carotid body mast cell density of the control, recovery, and hypoxic groups (Table 3). However, the total carotid body mast cell count was greater in the hypoxic rats than in the control and recovery rats.

The hypoxic animals showed right ventricular hypertrophy, medial hypertrophy of the pulmonary trunk, and enlargement of the carotid body (Heath et al., 1973). In the recovery group these changes had regressed. The group mean value of the medial thickness of the 'muscular pulmonary arteries' in the hypoxic animals (4.3%) showed a statistically significant increase compared to the control (3.4%) and recovery (2.5%) group mean.

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Table 1  Average* lung mast cell density in rats exposed for 8 hours to severe acute hypoxia

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Average mast cell density</th>
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<tbody>
<tr>
<td></td>
<td>Perivascular</td>
</tr>
<tr>
<td>Test (n=5)</td>
<td>0.70(±0.27)</td>
</tr>
<tr>
<td>Control (n=5)</td>
<td>0.47(±0.14)</td>
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</tbody>
</table>

*Statistical comparison: t = 1.73, t = 1.91, t = 0.81, t = 1.85

The figures in parentheses in Tables 1 to 4 represent ± one standard deviation.
*For each rat five lung sections were counted, the mean of the five counts was obtained, and the group average of these means is quoted.
†No significant difference between test and control values.
Graticule area = 0.392 mm². To convert number of mast cells per graticule area to number per mm², multiply by 2.55.

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Table 2  Average lung mast cell density in rats exposed chronically to simulated high altitude and recovering from such exposure

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Average mast cell density</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Perivascular*</td>
</tr>
<tr>
<td>Control (n=9)</td>
<td>0.79(±0.34)</td>
</tr>
<tr>
<td>Simulated high altitude (n=10)</td>
<td>1.93(±0.55)</td>
</tr>
<tr>
<td>Recovery (n=10)</td>
<td>1.05(±0.40)</td>
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* Group means differ significantly all p < 0.001 (analysis of variance).
† Significantly different from control value t = 5.40, p < 0.001
‡ Significantly different from recovery value t = 3.56, p < 0.005
§ Significantly different from control value t = 3.05, p < 0.001
¶ Significantly different from recovery value t = 3.05, p < 0.001
** Significantly different from control value t = 3.05, p < 0.001
*** Significantly different from recovery value t = 3.05, p < 0.001

For each rat two lung sections were counted, the mean of the two counts was obtained, the group average of these means is quoted.
Graticule area = 0.392 mm². To convert number of mast cells per graticule area to number per mm², multiply by 2.55.
values. However, obvious medial hypertrophy of these arteries was not apparent on examination of the sections. There was a significant correlation between the logarithm of the perivascular lung mast cell density and the logarithm of the right ventricular weight \((r=0.6179; \ p<0.001)\) in the control, hypoxic, and recovery groups of rats.

Tissues from the age and sex study of Smith et al. (1974)

The perivascular, peribronchial, alveolar septal, and total lung mast cell densities are shown in Table 4.

A three-factor analysis of variance (testing the experimental factors age, sex, and exposure to simulated high altitude and their interactions) showed:

1 a significantly greater perivascular \((p<0.01)\), alveolar septal \((p<0.05)\), and total \((p<0.01)\) lung mast cell density in hypoxic compared to control rats; and

2 a significantly greater perivascular \((p<0.01)\), alveolar septal \((p<0.05)\), and total \((p<0.01)\) lung mast cell density in adult hypoxic compared to young hypoxic rats (both male and female).

Peribronchial mast cell densities were not significantly different in the hypoxic compared to the control rats.

Each of the four hypoxic groups showed right ventricular hypertrophy and medial hypertrophy of the pulmonary trunks (Smith et al., 1974). Medial hypertrophy of the small pulmonary arteries was found only in the adult female group. Again the mean values of the medial thickness of the 'muscular pulmonary arteries' of each group of test animals showed a statistically significant increase compared to the control groups. Only four hypoxic rats had values of medial thick-
it is more pronounced in adult animals. In the chronically hypoxic rats in the age and sex series the perivascular lung mast cell density correlates with the medial thickness of the muscular pulmonary arteries, suggesting that the thickest muscular arteries have the most mast cells in their adventitial coats. Acute hypoxia for eight hours does not induce a proliferation of mast cells in the lungs.

In this study we made no attempt to distinguish between periartrial and perivenous mast cells. In retrospect this was a mistake for it is likely that additional data would have been obtained, especially with respect to the rôle of the pulmonary mast cell in differential constriction of pulmonary arteries and veins in states of chronic hypoxia. We recommend that further histological studies in this field should attempt to make this distinction.

The lack of change in the number of mast cells in the carotid bodies in response to chronic hypoxia suggests that the mast cell hyperplasia seen in the lung is not a general phenomenon within the body tissues, which accords with previous observations (Kay and Grover, 1975). The increase in the total number of mast cells in the carotid bodies of the hypoxic rats merely reflects the increased size of the carotid bodies in these animals, since no difference is seen in the carotid body mast cell count per unit area.

The functional significance of the proliferation of mast cells in the lungs of rats exposed to chronic hypoxia is still not clear. Recently the view has gained ground that in some way they are concerned with the pulmonary vasoconstriction induced by hypoxia. The concept of such an intermediary agent is based on the premise that alveolar hypoxia is much more potent as a pulmonary vasoconstrictor than hypoxaemia, but even this basic premise has recently been challenged by Fishman (1976). If one accepts the idea, however, it is clear that the periarterial mast cell is in a most strategic position to act as an intermediary, being stimulated by the hypoxic air in the adjacent alveolar spaces and liberating some humoral agent from its electron-dense secretory granules (Fig. 3) to affect the media of the muscular pulmonary arteries. Certainly mast cells contain a number of vasoactive substances, including 5-hydroxytryptamine and histamine (Selye, 1965). All the evidence suggests that the former is not involved in the hypoxic response (Lloyd, 1964; Bergofsky, 1974; Harris and Heath, 1977). Hence the favoured chemical mediator is histamine. In the isolated perfused rat lung large doses of antihistamine and histamine-depleters diminish the pulmonary pres-
Fig. 3 A perivascular mast cell as seen under the electron microscope. It contains a large central nucleus (N) and numerous electron-dense secretory granules (G) which in favourable sections can be seen to be bounded by a single limiting membrane. It is these granules which are believed to contain vasoactive substances. The mast cell is surrounded by collagen fibrils (C) and fibrocytes (F) of the adventitia of the vessel. Electron micrograph ×18,750.

$H_1$ receptors were stimulated alone (by selectively blockading the $H_2$ receptors) there was an abrupt pulmonary pressor response which was sustained for the duration of the histamine infusion. When both $H_1$ and $H_2$ receptors were stimulated simultaneously, the net result was a transient decrease
in pulmonary vascular resistance followed by a progressive increase. The pulmonary vascular bed behaved as though there was an initial predominance of stimulation of H2 receptors followed by either withdrawal of stimulation of H2 receptors or by a relative increase in stimulation of H2 receptors. Thus, in the dog, histamine administration stimulates opposing pulmonary vascular responses which exhibit differential activation. The two histamine actions may affect different vessels or the same vessel according to its state of tone (Barer et al., 1976). In such circumstances it is hardly surprising that investigators using different methods and species have reported conflicting pulmonary vascular responses to histamine infusion.

Since clinicians are likely to be concerned mainly with the human pulmonary circulation it is worthy of note that in an extensive review of the literature on the pharmacology of the pulmonary circulation we found that in man the effects of exogenous histamine on the pulmonary circulation are vasodilator (Harris and Heath, 1977). Thus intravenous injections of histamine in a dose ranging from 0·1 to 0·45 μg kg⁻¹ min⁻¹ in five normal women caused pulmonary vascular resistance to fall on an average from 86 to 46 dyn s⁻¹ cm⁻² and in six normal men from 57 to 34 dyn s⁻¹ cm⁻² (Bjure et al., 1967). In eight subjects with mitral stenosis an intravenous dose of 0·1 to 0·3 μg kg⁻¹ min⁻¹ caused a fall of pulmonary vascular resistance of 221 to 114 dyn s⁻¹ cm⁻² (Lindell et al., 1964). Falls of comparable magnitude in pulmonary vascular resistance were reported in four subjects with constrictive pericarditis by Lindell et al. (1963) and in two normal subjects and in two patients with pulmonary disease by Bjure et al. (1966).

There is evidence that chronic interstitial pulmonary oedema may be associated with proliferation of mast cells in the alveolar walls. Thus the administration of hydrazine sulphate and monocrotaline to rats leads to the development of pulmonary oedema associated with mast cell proliferation (Kay et al., 1967). Mast cell proliferation has been observed in the lungs of patients suffering from mitral stenosis and chronic left ventricular failure and oedema of pulmonary alveolar walls (Heath et al., 1969). Sudden ascent to altitudes over 3000 m above sea level carries the risk of development of high altitude pulmonary oedema in susceptible human subjects (Heath and Williams, 1977). Accordingly, it is conceivable that in chronically hypoxic rats, simulating high altitude conditions, the lung mast cell hyperplasia might be due to chronic pulmonary oedema. However, such rats show no histological evidence of pulmonary oedema (Kay et al., 1974). Furthermore, if chronic hypoxia did produce chronic interstitial pulmonary oedema, then eventually this would surely lead to interstitial pulmonary fibrosis such as occurs in mitral stenosis and chronic left ventricular failure. We have not seen interstitial pulmonary fibrosis in rats kept at a simulated altitude of 4300 m for periods in excess of 400 days, and interstitial pulmonary fibrosis is not a feature of the lungs of high altitude dwellers.

The observations that the lung mast cell density increases with the right ventricular weight and medial thickness of the muscular pulmonary arteries in hypoxic rats, and that the mast cell hyperplasia regresses when the hypoxic stimulus is removed, suggest that the proliferation is associated in some way with the onset and regression of pulmonary hypertension. Mungall (1976) showed that in chronically hypoxic rats, the mast cells did not proliferate until after 21 days of exposure, whereas right ventricular hypertrophy was apparent after only 14 days. The fact that right ventricular hypertrophy apparently precedes lung mast cell hyperplasia makes it unlikely that it is lung mast cell hyperplasia which causes the pulmonary hypertension. Such an observation is in fact more consistent with the concept that pulmonary hypertension causes the mast cell hyperplasia. It is even conceivable that this hyperplasia of mast cells in the lungs represents a defensive mechanism which limits the severity of hypoxic pulmonary constriction and hypertension rather than mediates it, as has been suggested in the past.

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References


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