Role of the Mast Cell in the Pulmonary Pressor Response to Hypoxia

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A B S T R A C T This study investigated the role of the mast cell in the pulmonary arterial pressor response to hypoxia. We found that pulmonary arteries 50–500 μ in diameter have a predictable distribution of perivascular mast cells; that such pulmonary mast cells are degranulated in vivo during alveolar hypoxia; that hypoxia releases histamine from mast cells isolated from the peritoneal cavity without apparent injury to the cells; and that histamine is released from the lung of intact guinea pigs during alveolar hypoxia, with the rise in pulmonary vascular resistance during this period proportional to the amount of histamine released. These data point to the perivascular pulmonary mast cell in the rat and guinea pig as an important structure in the mediation of the pulmonary pressor response to hypoxia, even though the responsible humoral vasoconstrictor released from such a cell may not be histamine, or histamine alone.

INTRODUCTION

The long-standing search for the mechanism whereby hypoxia elicits pulmonary vasoconstriction has recently focused on local events at the site of the small pulmonary resistance vessel and its smooth muscle cell (1). Some of the mechanisms put forth have included: (*a*) depolarization of the muscle membrane (2), and (*b*) release of active humoral agents from surrounding tissue (3). With respect to the latter, many agents, such as catecholamines, serotonin, prostaglandins, and, in particular, histamine, have been considered (4). Recent evidence has shown that depletion of histamine, or the use of antihistamines, will diminish or abolish the pulmonary response to hypoxia (5, 6). The drawback to these studies, however, is that the antagonists or depleters used may be nonspecific blockers of pulmonary vasoconstriction or may even be vasodilators.

The present study tests the hypothesis of locally released humoral agents through an appraisal of the role of the most obvious source of such agents, i.e., the mast cell (7). This form of investigation avoids the use of nonspecific pharmacological agents which modify the response to hypoxia and examines a different step in what may be a sequence of events leading to pulmonary vasoconstriction. The techniques involved: (*a*) a histological study of the distribution of mast cells around pulmonary blood vessels and the effect of hypoxia on the degree of degranulation; (*b*) the direct measurement of humoral release from mast cells harvested from the peritoneal cavity; and (*c*) an appraisal of the effect of hypoxia on the release of isotopically labeled histamine from the lungs of intact animals.

METHODS

Distribution of pulmonary mast cells and effect of hypoxia on degranulation. A double blind counting technique was used to determine the arrangement and degree of degranulation of pulmonary perivascular mast cells in rats made hypoxic by breathing an artificial gas mixture.

In each experiment two Wistar rats were used, each in a separate isolation cage ventilated from a tank of air. After 30 min the ventilating mixture of one cage was switched to an average of 12.5% O_2, balance N_2, with flow and noise levels remaining constant. Although the onset of the pressor response to acute alveolar hypoxia is fairly rapid, the use of such moderate degrees of hypoxia suggested the use of a longer period of exposure (60–90 min) to assure histologically visible degranulation of mast cells. Arterial blood samples at this time by cardiac puncture revealed, in the animals breathing air, a pH of 7.33–7.42, P_{CO_2} of 32–40 mm Hg and a P_{O_2} of 85–95 mm Hg. In hypoxic animals, the pH ranged from 7.34–7.36, the P_{CO_2} from 30 to 37, and the P_{O_2} from 50 to 60 mm Hg; these P_{O_2} values yielded an average arterial O_2 saturation of 82%, a level in our experience usually producing pulmonary vasoconstriction. The differences in pH and P_{CO_2} between the groups were not significant. After the blood samples, while still in their respective atmospheres, each rat was anesthetized with ether, the chest opened, and catheters inserted into the trachea and the pulmonary artery so that 3% formalin could be flushed through before immersing the whole lung in formalin. This
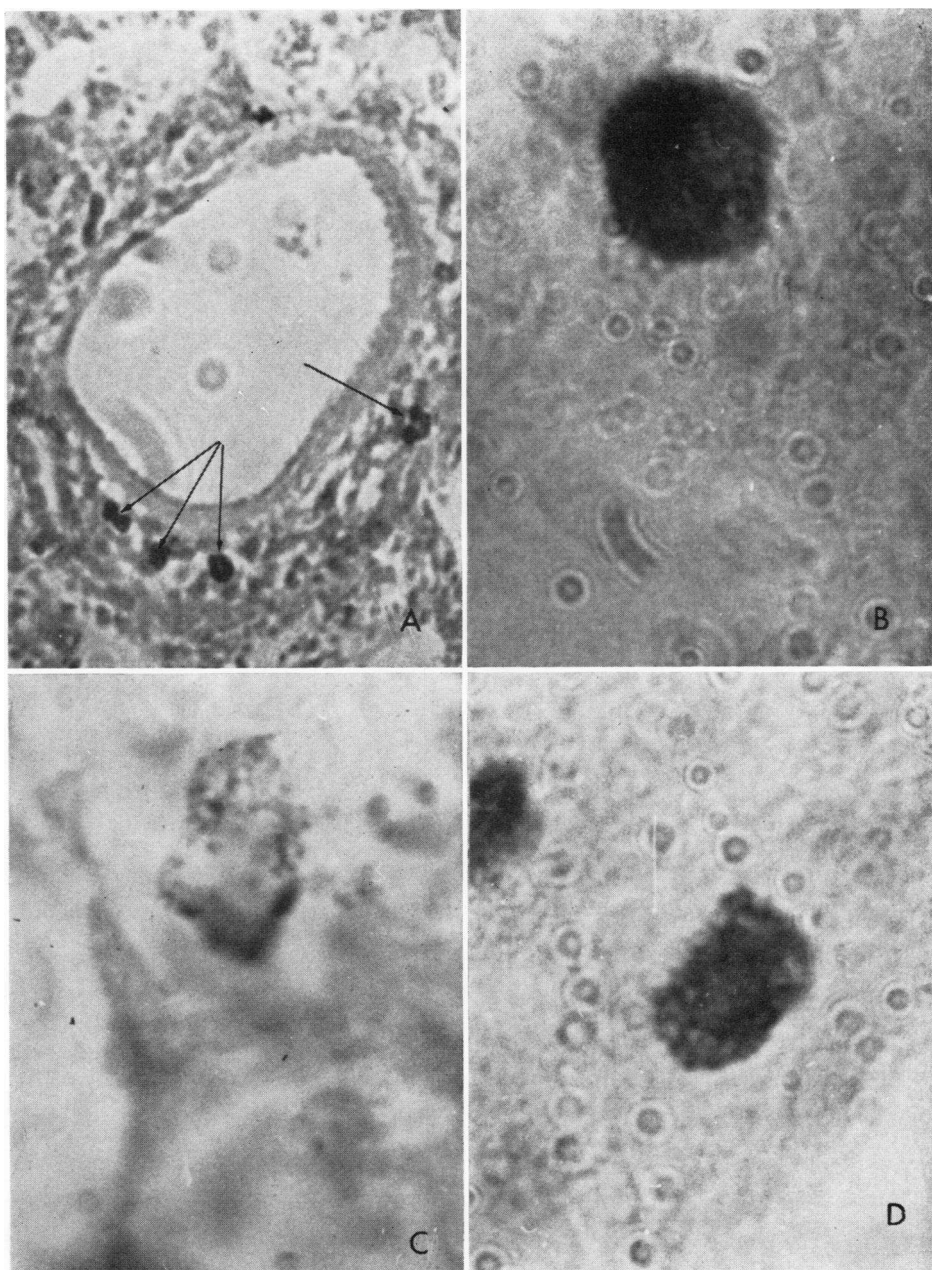
FIGURE 1 (A) A section through the lung of a normal Wistar rat showing the relationship between perivascular mast cells (arrows) and a blood vessel of 250μ diameter (Unna's stain, $\times 150$). (B) A heavily granulated perivascular mast cell (grade 1) from a normal lung ($\times 1000$); the cytoplasm of the mast cell stains lightly; hence, the outline of the cell body is taken from the heavily staining granular aggregates. (C) A mast cell from the lung of a hypoxic rat showing advanced degranulation (grade 3) ($\times 1000$). (D) A mast cell showing only a moderate amount of degranulation (grade 2) ($\times 1000$).

procedure, always accomplished within 5 min, assured rapid and uniform fixation and the removal of the cellular elements of the blood in the pulmonary vasculature.

At least four sections (8μ thick) were made from the tissue blocks taken from each lobe of the lungs to insure a uniform survey. They were stained with "Unna's" stain,

a methylene blue polychrome stain for mast-cell granules. 96 sections from the lungs of four control animals, and an equal number from four hypoxic animals, were analyzed for the number of perivascular mast cells (Fig. 1 A), the per cent of vessels surrounded by mast cells (grouped into vessels larger than 500μ and smaller than 500μ), and the

TABLE I

Effects of Hypoxia on Distribution and Granulation of Mast Cells in 192 Rat Lung Microsections

	Normoxia	Hypoxia
>500-μ arteries/section		
With mast cells	16±2	13±2
Without mast cells	12±3	9±1
% with mast cells	54±4	59±3
Mast cells/artery with mast cells	3.0±0.2	3.2±0.1
<500-μ arteries/section		
With mast cells	37±5	42±7
Without mast cells	112±13	92±13
% with mast cells	28±2	33±4
Mast cells/artery with mast cells	1.4±0.1	1.6±0.1
Degranulation index		
Arteries >500 μ	1.5±0.1	2.6±0.1*
Arteries <500 μ	1.4±0.1	2.5±0.1*
Maximal degranulation, %		
Arteries >500 μ	38±5	57±7*
Arteries <500 μ	31±3	53±5*

* The difference in values between control and hypoxia is significant; $P < 0.05$.

degree of degranulation (judged from 1 to 3 using the criteria illustrated in Figs. 1 B and C). The investigator delivered unlabeled, but numbered, specimens to a colleague for microscopic review. This colleague tabulated findings without knowledge of each specimen's source (i.e., hypoxic or control lungs), while a third investigator collated the final results.

Effect of hypoxia on discharge of histamine from peritoneal mast cells. This approach measured the histamine released from mast cells harvested from the rat peritoneal cavity when suspended in Ringer's solution and aerated with hypoxic gas mixtures. Each experiment used six Wistar rats (200–250 g) which were decapitated and drained of blood in order to minimize the contamination of peritoneal fluid and the associated clotting which entraps mast cells (8). 7 ml of heparinized Ringer's solution (50 U/ml) was injected over the intestinal surface and 5 ml of solution was recovered, containing on the average 3×10^5 mast cell/ml. The suspensions from the six rats were pooled and divided in four 6-ml portions. The following measurements were taken: (*a*) total histamine in fresh specimens; (*b*) ratio of histamine released to that retained in mast cells; (*c*) the same ratio after swirl-equilibration with 95% O_2, 5% CO_2; and (*d*) the same ratio after swirl-equilibration with hypoxic gas (i.e., 3% O_2, 5% CO_2, 92% N_2).[1] The imposed P_{O_2} of 23

[1] The 3% O_2 imposed on the solution yielded a P_{O_2} of 23 mm Hg at the surface of the mast cells and was therefore considered a degree of hypoxia similar to that imposed on the pulmonary arterial mast cell of the present group of intact guinea pigs and rats by the following considerations: (*a*) the alveolar P_{O_2} to which periarterial mast cells are at least partly exposed, with inspired O_2 levels of 9 and 12.5%, ranges from 33 to 52 mm Hg; (*b*) the pulmonary arterial (mixed venous) P_{O_2} to which these cells are also exposed

mm Hg during hypoxia was thus considerably lower than the normal *in situ* ambient peritoneal P_{O_2} of 52–60 mm Hg, as estimated by lymph (9). The first portion was hemolyzed with 4 ml distilled water, the content deproteinized by addition of 0.05 ml of 70% $HClO_4$ and centrifuged for 10 min at 2500 rpm, and the supernate was analyzed for histamine. The second portion was immediately centrifuged and the cellular fraction resuspended, hemolyzed, and deproteinized. The supernate was diluted by the same volume of distilled H_2O and $HClO_4$ and both fractions analyzed for histamine content. The two remaining portions were placed into swirl-equilibration flasks and handled thereafter as was the second portion.

To determine whether the histamine found during hypoxia was due to active release or to release caused by cell injury or death, the O_2 consumption of the mast cell was measured under conditions identical with those outlined above. 100-μl samples of a suspension of mast cells were taken periodically from the tonometer flask after equilibration with both high and low O_2 gas mixtures. These samples were then placed in a sealed 70 μl cuvette into which was inserted a radiometer Clark microelectrode. The rate of fall of P_{O_2} was measured for 5 min and O_2 consumption calculated. The entire experiment measuring O_2 consumption at both low and high P_{O_2} was performed on each of four harvests of mast cells. The suspension of cells was kept homogenous by the agitation of the swirl-equilibration flask before withdrawal of samples used to measure the rate of fall in P_{O_2}. The O_2 electrode was calibrated by two gases (and two solutions of Ringer's solution equilibrated therewith) at 150 and 21 mm Hg. Measurements of O_2 loss by electrode utilization were made with Ringer's solution equilibrated at a P_{O_2} of 150 and 21 mm Hg. This rate was subtracted from the far greater rate of loss in suspensions of O_2 consuming cells. The general method of harvesting peritoneal cells produced yields that contained 75% mast cells, the remainder being mostly red blood cells with somewhat lower metabolic rates and some leukocytes; thus, the large alterations in O_2 consumption in such a solution during hypoxia, to be described in Results, is due largely to the mast cells.

Histamine was determined by Shore's technique (10), condensing histamine with *o*-phthaldehyde to yield a fluorescent produce. The fluorescence (at 450 mμ wavelength; excitation at 360 mμ) was measured against standard 10 mg/100 ml histamine stock solutions diluted to give solutions of 0.1, 0.05, and 0.02 μg/ml, and read on a Beckman ratio fluorometer (model 77205, Beckman Instruments, Inc., Fullerton, Calif.). Recovery of histamine from known samples was 80% efficient; the data have been corrected for this loss. Derivatives of histidine, of histamine, and of other vasoactive amines do not produce fluorescence at this wavelength or are discarded during extraction by *n*-butanol from an alkaline solution.

Hypoxia-induced histamine-^{14}C release by the guinea pig lung. The experiments to correlate the concentration of histamine released from the lung with the rise in pulmonary vascular resistance during alveolar hypoxia utilized a counting technique for isotopically labeled histamine rather than direct chemical analysis. This increased accuracy required

would, with an arteriovenous O_2 difference in the present study of 30%, range from 18 to 27 mm Hg; (*c*) the average alveolar-pulmonary arterial P_{O_2} is therefore 25 and 38 mm Hg, respectively; with further decreases in P_{O_2} due to diffusion gradients from the sources of O_2 in the artery and alveolus to the mast cell, the ambient P_{O_2} at this cell would be expected to be in the vicinity of 23 mm Hg.

that small animals be used to minimize the labeled histidine precursor. The guinea pig was chosen because it can maintain more stable levels of blood pressure and cardiac output, even after blood loss, than can the rat of equal size. In order to obtain rigorous hemodynamic studies of cardiac output and histamine release from animals having small blood volumes, two separate groups of animal were required. The first group was used to measure pulmonary arterial, left ventricular, and aortic pressures and arteriovenous O_2 concentration differences ($C_{(a-v)O_2}$) as an index of cardiac output, in order to assess the effect of alveolar hypoxia on pulmonary vascular resistance in the species. The second group was used to correlate the changes in pulmonary vascular resistance with the release of histamine during alveolar hypoxia.

For the first group, guinea pigs weighing 300–400 g were anesthetized with sodium pentobarbital (60 mg/kg intraperitoneally), and ventilated by a positive pressure respirator through a tracheostomy. A polyethylene tube (PE 60) was threaded from the left carotid artery into either the left ventricle or the aorta. The chest was opened and a polyethylene tube (PE 90) introduced into the right ventricle and advanced into the pulmonary artery. Pressures were measured by Statham strain gauges (model P23, Statham Instruments, Inc., Oxnard, Calif.) and a Grass polygraph (Grass Instrument Co., Quincy, Mass.). After a steady state of blood pressure and arteriovenous O_2 differences in room air was achieved, the animals were exposed to 8-min periods of breathing 9% O_2, balance N_2, delivered by the piston respirator. Pulmonary arterial and aortic or ventricular pressures were measured continuously except for the last minutes of this period when blood was again sampled.

The $C_{(a-v)O_2}$ was calculated by measuring P_{O_2} and pH by the Clark microelectrode described above. Control values for P_{O_2} in mm Hg and pH were 85±7 and 7.33±0.05, respectively. Their average Wintrobe hematocrit was normal for the guinea pig, i.e., 38.5±4 ml/100 ml blood (11). Oxgen saturation of the blood was calculated from the pH and P_{O_2} using the guinea pig O_2 dissociation curve (12). From the O_2 saturation and the hemoglobin concentration (as derived from the hematocrit) the O_2 content of hemoglobin was calculated. Since the animals were kept under constant ventilation and since O_2 uptake remains unchanged during moderate hypoxia (13, 14), an index of cardiac output was obtained directly from $C_{(a-v)O_2}$. To support this contention on O_2 uptake in the guinea pig, a subgroup of this group was similarly anesthetized, tracheotomized, and mechanically ventilated under identical conditions in order to measure O_2 uptake, CO_2 production, and respiratory exchange ratio during hypoxia. The O_2 uptake during normoxia averaged 2.7 ml/min±0.2 SE and during hypoxia, 2.6 ml/min±0.2 SE; respiratory exchange ratios were 1.04 and 1.03, respectively ($n = 8$).

This subgroup was also used to measure the pressure-volume characteristics of the guinea pig lung during hypoxia. Utilizing tracheal pressure during mechanical ventilation and tidal volume, pressure volume diagrams were examined under identical conditions of normoxia and hypoxia. Hypoxia caused no alteration of airway resistance and a slight increase in total thoracic compliance, i.e., from 0.52 to 0.57 ml/cm H_2O ($n = 15$).

The identical surgical preparation was used for the second group, which was prepared 24 hr beforehand by injecting 5–10 μCi of histidine-^{14}C (Nuclear-Chicago Corp., Des Plaines, Ill.: universally labeled) which could be expected to be decarboxylated to histamine-L-^{14}C (15). Arterial and

TABLE II

Effect of Hypoxia on Histamine Concentration of Rat Peritoneal Mast Cells, In Vitro

Specimen	Histamine	% in supernate
	$\mu g/ml$	%
Nonaerated suspensions		
Freshly extracted ($n = 9$)	0.94±0.18	—
Freshly spun ($n = 8$)		
cells	0.70±0.33	
supernate	0.31±0.07	32
total	1.01±0.39	
Aerated suspensions		
95% O_2, 5% CO_2 ($n = 8$)		
cells	0.55±0.16	
supernate	0.29±0.16	34*
total	0.82±0.22	
3% O_2, 5% CO_2 ($n = 8$)		
cells	0.38±0.15	
supernate	0.48±0.14	53*
total	0.87±0.27	

* The difference between the two values is significant, $P < 0.05$.

venous samples (0.25–0.40 ml) were taken during the control periods and at the end of the hypoxic periods. The cellular elements of these samples were separated by centrifuge, the supernate deproteinized, alkalinized, and extracted with *n*-butanol. Duplicate 0.4-ml fractions were plated on gelatin-based copper planchetes and counted for 20 min on a Nuclear-Chicago model 1105 Spectro/Shield Automatic Low Background Planchete Sample Changer and 8703 Series Decade Scaler.

RESULTS

Effect of hypoxia on distribution and granulation of mast cells. The effect of alveolar hypoxia on the distribution and granulation of pulmonary mast cells is shown in Table I. Among vessels of more than 500 μ, more than 50% demonstrated clusters of perivascular mast cells. Hypoxia did not affect this distribution; in fact, the average number of mast cells in each cluster about a vessel was almost identical under control and hypoxic conditions (3.0 and 3.2, respectively). With respect to vessels of less than 500 μ diameter, larger numbers of vessels and cells were counted per section; however, the ratio of vessels with mast cells to total vessels was significantly smaller (28%) than that of the larger vessels. Nonetheless, hypoxia did not change the distribution of mast cells; the number of mast cells in each cluster about a vessel was 1.4 in control animals and 1.6 in hypoxic animals. Although hypoxia did not alter the total number of visible mast cells, the degree
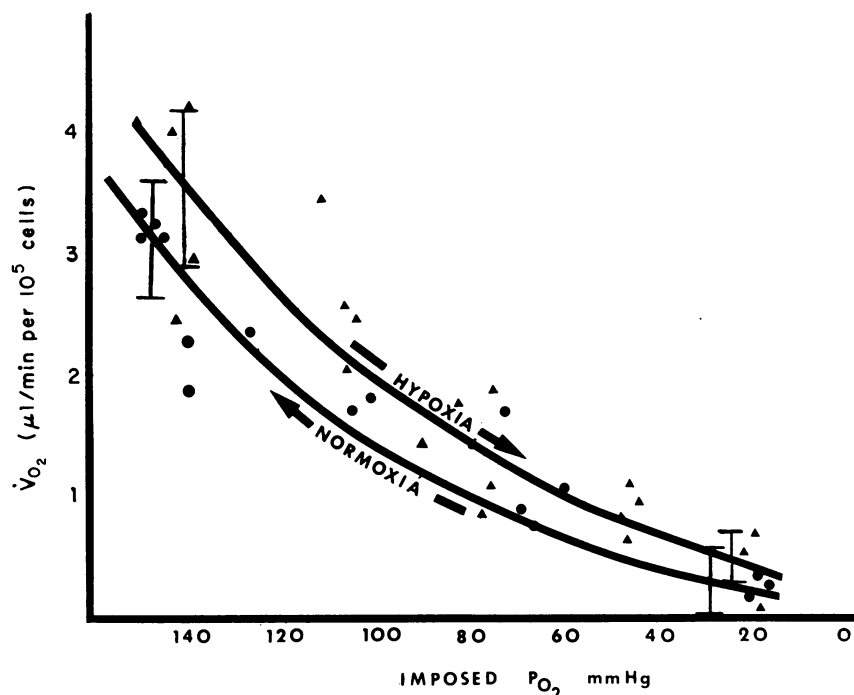
FIGURE 2 The relation between O_2 consumption and the imposed ambient P_{O_2} of rat peritoneal mast cells, in vitro. Points labeled ▲ are values for O_2 consumption during continuous decreases in ambient P_{O_2} toward hypoxia, whereas ● are values during the subsequent increase in P_{O_2} toward normal. No statistical difference is evident between the two curves, which show average values and standard deviations at P_{O_2} values of 130 and 30 mm Hg.

of granulation was markedly affected. In both groups of vessels (> 500 μ, < 500 μ) the degranulation index during hypoxia exceeded that during control by 73 and 70%, respectively. This observation is supported (Table I) by a significant rise in the number of maximally degranulated cells during hypoxia: for vessels of more than 500 μ diameter, the percentage of maximally degranulated cells rose from 38% in the control lungs to

TABLE III

Effects of Alveolar Hypoxia on the Dynamics of the Pulmonary Circulation

Animal No. and run	$C_{(a-v)O_2}$		Pulmonary arterial pressure		Resistance index ($P_{PA} \times C_{(a-v)O_2}$)	
	Normoxia	Hypoxia	Normoxia	Hypoxia	Normoxia	Hypoxia
	ml/100 ml		*mm Hg*			
1	8.6	6.2	5.0	8.0	43.0	49.0
2	6.8	6.4	6.5	9.5	44.0	60.8
3a	3.0	6.4	8.5	9.5	25.5	60.8
3b	5.2	6.8	8.0	8.5	41.6	57.8
3c	4.4	5.8	6.5	7.5	28.6	43.5
4a	4.6	8.4	8.5	9.5	39.0	79.8
4b	7.4	9.0	7.0	7.5	51.0	67.5
5	4.4	4.2	9.0	12.0	39.6	50.4
6a	5.8	6.8	8.5	10.0	49.3	68.0
6b	8.2	8.2	6.5	8.5	53.3	69.7
M±SE	5.8±0.6	6.8±0.4	7.4±0.4	9.1±0.4	41.5±2.7	60.7±3.3
P (paired Student's t test)	NS		<0.005		<0.001	
P (nonparametric sign test)	NS		<0.001		<0.001	

57% in hypoxic lungs; in the smaller vessels, the increase was from 31% during control to 53% during hypoxia.

The relatively high values of 38 and 31% for maximal degranulation in the control groups are consistent with variations in the plane of sections and the fixation by formalin, as previously observed (16, 17), and do not necessarily mean that considerable degranulation of cells is normally present.

Effect of hypoxia on rat peritoneal mast cells. The upper portion of Table II (nonaerated suspensions) compares the histamine content of freshly extracted and centrifuged specimens of rat peritoneal mast cells. As indicated, a freshly extracted suspension contains no more histamine (0.94 ± 0.18 µg/ml) than the sum of both sedimented cell and supernate after a 10 min centrifugation (1.01 ± 0.39 µg/ml). Within the centrifugate, the cells contain about two-thirds of the total histamine present. Aeration did not significantly reduce the total histamine in specimens swirled in tonometry flasks, nor was the distribution of histamine between cells and
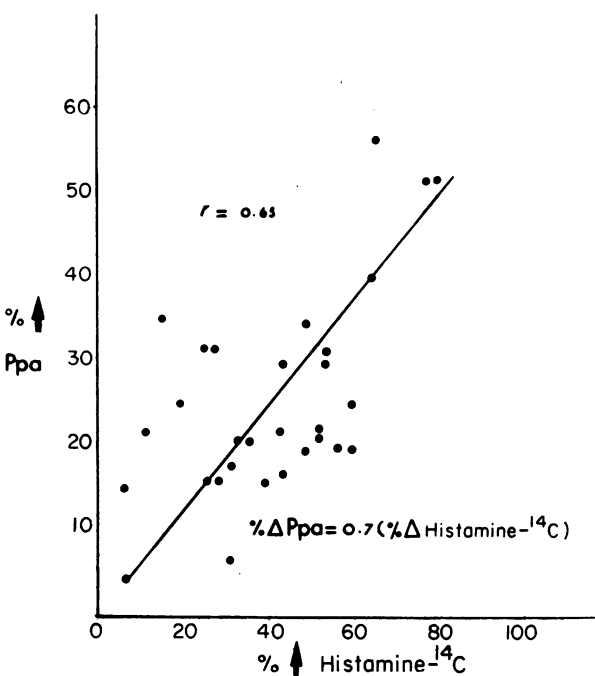

FIGURE 3 The relation between the percentile increases in pulmonary arterial pressure and the percentile increase in histamine-^{14}C in left ventricular blood during alveolar hypoxia in the guinea pig. Each point represents data from an experimental period of steady-state alveolar hypoxia.

TABLE IV

The Effect of Hypoxia on the Histamine-^{14}C Content of Right and Left Ventricular Blood of Guinea Pigs

Experiment	Normoxia histamine-^{14}C		Hypoxia histamine-^{14}C	
	Right ventricle	Left ventricle	Right ventricle	Left ventricle
	cpm		*cpm*	
1	60	75	65	95
2	95	86	100	130
3	300	343	400	537
4	98	104	103	151
5	50	54	55	71
6	150	164	170	390
7	145	176	160	218
8	60	70	70	104
9	75	69	75	78
10	350	354	375	523
M±SE	138±31	150±34	157±38	230±55

(1) A rise in left ventricular histamine-^{14}C from normoxic to hypoxic periods is significant by nonparametric sign test ($P < 0.001$) and the difference of 80 cpm between the two periods is significant by paired Student's t test ($P < 0.02$). (2) The histamine-^{14}C cpm during hypoxia was significantly higher in left ventricle compared with right ventricle by nonparametric sign test ($P < 0.001$) and the difference of 73 cpm was significant by paired Student's t test ($P < 0.02$). (3) Right ventricular histamine-^{14}C during hypoxia was significantly higher than during normoxia by nonparametric sign test ($P < 0.001$). Left ventricular histamine-^{14}C is not significantly higher than right ventricular values during normoxia by nonparametric sign test.

supernate affected. However, equilibration with hypoxic gas produced a marked and significant redistribution of histamine from cells into supernate; the histamine concentration during hypoxia averaged 53% in the supernate compared with 34% during control conditions. The O_2 uptake of the isolated mast cells as a function of the various imposed O_2 tensions is shown in Fig. 2. The data indicate that O_2 uptake is directly related to P_{O_2} (between P_{O_2} of 20–140 mm Hg) and approaches zero as the P_{O_2} approaches 0 mm Hg. It is thus similar to previously described behavior of mast cells (18). The viability of the cells after exposure to a low ambient P_{O_2} is supported by the increasing O_2 consumption when the same cell population is re-equilibrated with an increasing P_{O_2}.

Histamine release from the lung of hypoxic guinea pigs. The results of the separate hemodynamic studies determining the effects of alveolar hypoxia in the guinea pig are shown in Table III. Hypoxia caused an increase in pulmonary artery pressure in all cases while decreasing cardiac output (inverse of $C_{(a-v)O_2}$) in 6 of the 10 experiments. These increases in mean pulmonary artery pressure (averaging 1.7 mm Hg) were converted to an index of pulmonary vascular resistance by utilizing the inverse of the $C_{(a-v)O_2}$; the average increase in this index with hypoxia was 45%. Table IV com-

pares left and right ventricular differences in hista-mine-^{14}C before and during hypoxia. A wide difference in control values for labeled histamine is present be-cause of the deliberate use of varied doses of precursor histidine-^{14}C. During room air breathing, no significant difference occurred between the average histamine con-tent in left (150 cpm) and right (138 cpm) ventricular blood. During hypoxia, the histamine-^{14}C levels in left ventricular blood emanating from the lung increased significantly over control levels (150 to 230 cpm, $P < 0.02$ by paired Students t test); the histamine-^{14}C levels of the right ventricular blood draining the systemic cir-culation rose also during hypoxia (138 to 157 cpm, $P < 0.001$ by nonparametric sign test).

Not shown in Table IV is the relation between hista-mine-^{14}C and total ^{14}C radioactivity in the acid soluble fraction of blood (i.e., before extraction by n-butanol). The histamine-^{14}C accounted for 52% of the total ^{14}C radioactivity during the control period and 82% during the hypoxic periods, indicating that the bulk of the radioactivity increase in the acid-soluble fraction of blood during hypoxia was due to histamine release.

The relationship between the release of histamine-^{14}C from the lung and the rise in pulmonary artery pressure during hypoxia in the guinea pig is shown in Fig. 3. Each point relates the percentile increase in pulmonary arterial pressure to the percentile increase in left ven-tricular histamine-^{14}C content during one or more hy-poxic periods in 10 animals. The average increase in histamine-^{14}C was 45±6% and the accompanying aver-age percentile increase in pulmonary arterial pressure was 26.5±3%. These increases were significant at P levels of 0.05 and 0.02, respectively. A linear relation-ship is apparent between the two variables, with a cor-relation coefficient of 0.65 ($P < 0.01$).

DISCUSSION

The present study on rats and guinea pigs has demon-strated that: (a) pulmonary vessels of 50–500 μ diam-eter with a well-defined muscular media have a pre-dictable distribution of mast cells within the adventitia; (b) such pulmonary mast cells are degranulated in vivo during alveolar hypoxia; (c) hypoxia causes mast cells isolated from the peritoneal cavity to release histamine and possibly other humoral agents in an in vitro situa-tion; and (d) histamine is released by the lung of in-tact guinea pigs during alveolar hypoxia, with the rise in pulmonary arterial pressure during this period pro-portional to the amount of histamine released. These data deserve discussion from several points of view.

The localized clustering of mast cells around pulmo-nary vessels has been noted (19). The present study analyzed their distribution with respect to vascular di-ameter and smooth muscle thickness; in accordance

with the definition of von Hayeck (20) that venous segments below a diameter of 500 μ do not contain a well-defined muscle coat, the mast cells in the present study seem almost entirely distributed around pulmonary arteries. Although only an average of one-third of the arteries smaller than 500 μ diameter had mast cells in any single histological section, every artery of each generation from 50 to 500 μ may have adjacent mast cells in the neighboring sections. This conclusion stems from the probability that, when one-third of the arteries have mast cells in histological sections 8 μ thick, the other two-thirds of the arteries will have mast cells in the next serial sections of 8 μ each. Thus, for a single artery of less than 500 μ diameter, 1.4 mast cells may be expected every 24 μ of length. For vessels greater than 500 μ diameter, since 50% of these showed mast cell clustering in any section, there would be 3.0 cells for every 16 μ of vessel length. Moreover, utilizing a diameter-to-length ratio of 1:3 for pulmonary arteries of less than 500 μ (footnote 2) in conjunction with the data in Table I, it can be calculated that 90, 55, 33, 16, and 9 mast cells will be found per vessel of 500, 300, 175, 90, and 50 μ diameter.

These data permit a calculation of the amount of hista-mine available from mast cells during hypoxia. Assuming a value of 10^{-12} g/mast cell (21) and three other factors, i.e., the number of arterial segments per given genera-tion $= 2^n$ (footnote 2) the estimates above of the number of mast cells per segment, and an approximate value of 50% for the amount of histamine released per cell (based on the present in vitro peritoneal mast cell results), it is possible to estimate that 10^{-5} g of histamine are released by arterial segments 16 through 20 (60–500 μ) of a whole lung during a hypoxic period. This amount of histamine has been found sufficient to increase pulmonary vascular resistance by 30% when infused into the cat (22); and such increases in pulmonary vascular resistance are simi-lar to those spontaneously observed in the present group of animals during alveolar hypoxia.

The present data indicate that histamine is released during hypoxia from mast cells harvested from the peri-toneal cavity of rats and which are therefore independent of all other neural or humoral influences. Moreover, as indicated by the oxygen consumption data, the cells survive even after the release of histamine. These data are consistent with the release of histamine and other vasoactive agents as an energy-requiring step, which can be inhibited by total lack of oxygen (23) but carried on at environmental O_2 tension as low as 21 mm Hg (18, 24). Thus, from these data, the mast cell of the lung seems to fall within the general concept of a chemore-ceptor cell, like that of the carotid body cells, in that: (a) it releases an active transmitter substance when the

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[2] Domingo Gomez, personal communication.

environmental P_{O_2} falls (25), and (b) its drop in O_2 consumption during mild hypoxia indicates a high critical P_{O_2}, which would result in alterations of intracellular metabolism and consequent release of a transmitter in some proportion to the degree of hypoxia.

Although release of histamine or other substances from the lung has been noted during hypoxia previously (26), the present isotopic-labeled techniques permitted a more precise analysis of the amount released and the establishment of a relationship between the amount of histamine found in the left ventricular blood and the associated rise in pulmonary vascular resistance. The smaller increase during hypoxia of labeled histamine in the mixed venous blood, even though mast cells are widely distributed in the tissue of the guinea pig (7), may be a result either of (a) rapid degradation of both pulmonary and systemically released histamine (27) or (b) a lower capacity of tissues other than the lung to release histamine (28).

Mast cells contain a large number of vasoactive agents (7), of which histamine is but one major constituent; in view of this, histamine was chosen as an indicator of mast cell degranulation and release of humoral agents with the reservation that it need not be the mediator, or the only mediator, of the pulmonary vasoconstrictor response to hypoxia. The role of histamine and of other possible pulmonary vasoconstrictors contained in the mast cell in the continuation of the pulmonary pressor response to hypoxia long after it has begun is uncertain from the present study. The maintenance of this response may involve an increase in the rate of synthesis of these substances by the partially degranulated mast cells so that a new steady state of increased synthesis, decreased storage pool in cellular granules, and increased release of vasoconstrictor substances is established during hypoxia.

The failure to observe bronchoconstriction as a counterpart to the vasoconstriction during alveolar hypoxia is attributable in part to the far fewer numbers of mast cells seen around the periphery of bronchi in the present histological sections. Bronchoconstriction might also be prevented if the peribronchial mast cell enjoyed higher environmental O_2 tensions than the perivascular mast cell: the former has access to O_2 sources composed of inspired air, alveolar air, and bronchial (systemic) arterial blood, all of which give an average P_{O_2} higher than is likely near the mast cell around the pulmonary arteries, which sees O_2 sources only in the alveolus on one hand, and within the pulmonary artery (mixed venous), on the other.

ACKNOWLEDGMENTS

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