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Density and ultrastructure of mast cells in lung vessels of aging rats exposed to and recovering from chronic hypoxia*

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Summary. A decrease in pulmonary vascular responsiveness in aging animals during exposure to chronic hypoxia has been previously reported; however, morphological documentation is lacking. Lungs from young (3–5 months) and aging (12–14 months) Sprague-Dawley rats, exposed to and recovering from chronic hypoxia, were morphometrically analyzed at the light-microscopic level for changes in perivascular mast cells, and at the electron-microscopic level for cellular alterations. While young rat lungs showed proliferation of mast cells around elastic and muscular pulmonary arteries and arterioles, perivascular mast cell density in lungs of aging rats was significantly greater than in young rat lungs. At the ultrastructural level, perivascular mast cells in aging hypoxic rats showed numerous profiles of cellular extensions that contained remnants of discharged secretory vesicles. The results suggest that increased proliferation of perivascular mast cells as well as increased secretory activity of vasoactive substances in aging animals might represent a humoral determinant of the hyporesponsiveness of pulmonary vessels that occurs with increasing age during chronic hypoxia.

Key words: Lung – Mast cells – Hypoxia – Aging – Histamine

Aging alters the responsiveness of systemic vascular smooth muscle to mechanical stimulation (Mackay et al. 1978) and vasoactive agents (Toda and Hayashi 1979; Owen 1980). This altered vascular responsiveness has been associated with changes in structure (Toda et al. 1980) and enzymatic content (Ericsson and Lundholm 1975). Numerous studies on isolated smooth muscle of the systemic vasculature have shown that, in addition to impaired

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β-adrenoceptor activity (Fleisch et al. 1970), increasing age decreased contractile responsiveness to serotonin and KCl (Cohen and Berkowitz 1976), as well as to norepinephrine (Tuttle 1966). Likewise, isoproterenol-induced relaxation of rabbit and rat aortae was notably decreased with age (Fleisch and Hooker 1976).

Although several studies concern the effects of aging on the systemic vasculature, the literature dealing with the functional and morphological parameters of aging in the pulmonary vasculature is deficient. While Fleisch and Hooker (1976) demonstrated decreased relaxation of vascular smooth muscle in rabbit and rat pulmonary arteries with increasing age, Park et al. (1976) showed increased maturation of β-adrenoceptor activity in 4- to 5-month-old animals. Tucker et al. (1982) have recently demonstrated decreased reactivity of pulmonary vessels to several vasoactive agents in 12- to 14-month-old rats. Since mast cells (MC) and histamine have been shown to alter pulmonary hemodynamics (Mungall et al. 1975; Tucker et al. 1977), the present study was designed to morphometrically assess perivascular MC density as well as possible ultrastructural changes of MC in aging and young rats exposed to chronic hypoxia, a stress that is known to alter MC density (Mungall and Barrer 1975; Tucker et al. 1977; Williams et al. 1977).

Materials and methods

In order to test our hypothesis that decreased pulmonary vasoreactivity in aging rats is due to increased MC density and possible subsequent release of histamine, 56 young adult (3–5 months of age) male Sprague-Dawley rats (Charles River), weighing 300–400 g, and 34 healthy aging male rats (12–14 months of age), weighing 500–600 g, were exposed to and allowed to recover from simulated high altitude. The rats were given food and water ad libitum and were maintained on a 12 h light/dark cycle. The animals were housed in a hypobaric chamber at a barometric pressure of 380 mm Hg (equivalent to an altitude of 18000 ft) for six weeks. During the initial 24 h the barometric pressure was reduced gradually to 380 mm Hg and was then maintained at that level for the remainder of the exposure. Sufficient air flow through the chamber was maintained to prevent CO_2 accumulation. The rats were returned to normal barometric pressure (635 mm Hg) for 15 min every 3rd day so that the cages could be changed, and food and water replenished. During the six week recovery period the rats were housed in the same surroundings as the normoxic control rats.

Light microscopy

Twenty-eight hypoxic animals were killed at 3 and 7 days, and at weekly intervals thereafter for six weeks during the hypoxic exposure period and the following normoxic recovery period. Each rat was anesthetized with sodium pentobarbital (30 mg/Kg i.p.) and the heart and lungs were cannulated and isolated as previously described (Tucker et al. 1982). At the termination of the lung perfusion, which lasted approximately 100 min, intratracheal fixation with a 10% buffered formalin solution was conducted. The lungs were further fixed in 10% buffered formalin overnight, and cross sections were processed routinely for light microscopy. Sections of 5 μm were stained with toluidine blue for evaluation of mast cell distribution. Mast cells were identified by the characteristic metachromatic staining of secretory granules by toluidine blue. Two blocks representing the right and left lungs were sectioned for each rat; 2 sections per block as well as 2 fields per section were used for study. An average of 5–7 elastic and muscular arteries (outer diameter >150 μm) per lung and an average of 10–12 arterioles and small muscular arteries (outer diameter <150 μm) per lung were evaluated in each animal.

Round as well as ellipsoid profiles of vessels, showing an internal elastic lamina, and located adjacent to bronchioles were used for study. The cross sectional area of the vessel wall was determined by subtracting the luminal area from the cross sectional area of the vessel. The perivascular mast cell density was calculated as the number of mast cells per unit area (μm^2) of vessel wall.

Electron microscopy

Thirty-four normoxic and hypoxic rats were anesthetized with sodium pentobarbital (30 mg/Kg i.p.). The trachea was exposed and cannulated, and the rat was mechanically ventilated with room air by use of a respirator (inspiratory pressure, 10 cm H_2O; expiratory pressure, 3 cm H_2O). The chest was opened to expose the heart and lungs, and heparin (1000 units) was injected into the right ventricle. The right ventricle was pierced with an 18 gauge needle attached to saline-filled polyethylene tubing and a calibrated Statham pressure transducer, which was connected to a Gilson recorder (model ICT-2H). A fixative solution of 3% glutaraldehyde in 0.2 M cacodylate buffer (390 mosm) was introduced in the right ventricle at a pressure of 15–20 mm Hg and perfused through the lungs. The left ventricle was incised to facilitate outflow. Following fixation, the pulmonary trunk and hilar region of the lungs (where muscular arteries and arterioles are located) were dissected, minced, and further fixed in the same fixative for 2 h. Following overnight rinsing in buffer, the tissues were postfixed in 1% buffered OsO_4 for 2 h, dehydrated in a graded series of ethanols, embedded in Spurr's epoxy resin, and polymerized for 24 h. Thick sections (1.0 μm) were stained with toluidine blue for light microscopic survey. Thin sections of selected vessels were mounted on 300 mesh copper grids, and stained with uranyl acetate and lead citrate. The sections were examined in a Philips EM 400 transmission electron microscope with an accelerating voltage of 60 kV.

Results

Light-microscopic morphometric analysis

Histologic preparations of young and aging rat lungs showed a normal distribution of vascular and airway-conducting elements. The lungs of normoxic and hypoxic rats were devoid of any pathological lymphocytic infiltrations. The density of perivascular MC associated with elastic and muscular arteries, as well as arterioles, in normoxic aging rats was significantly greater than in normoxic young rats (Table 1). The density of perivascular MC associated with elastic and muscular arteries (diameter >150 μm), increased in young rats during the 6 weeks of the hypoxic exposure (Fig. 1). MC density associated with pulmonary elastic and muscular arteries (diameter >150 μm) also increased in aging animals throughout the exposure period

Table 1. Normoxic young and aging vascular MC density (MC/μm^2)

Group	Elastic muscular arteries	Arterioles
Normoxic young (10)	$1.16 \times 10^{-4} \pm 0.56$	$2.40 \times 10^{-4} \pm 0.28$
Normoxic aging (7)	$2.49 \times 10^{-4} \pm 0.26$	$3.45 \times 10^{-4} \pm 0.81$[a]

Values given as mean ± S.E.M.
Numbers in parentheses represent numbers of animals sampled

[a] Significantly different from normoxic young at $p < 0.001$

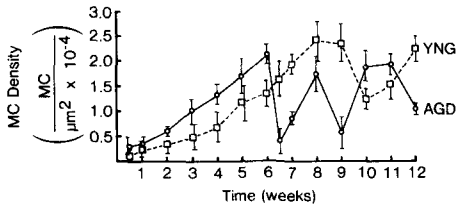

Fig. 1. Mast cell density in pulmonary elastic and muscular arteries [diameter >150 μm] of young (*YNG*) and Aging (*AGD*) rats exposed to 18000 ft for 6 weeks, and subsequently recovering at 5000 ft for 6 weeks

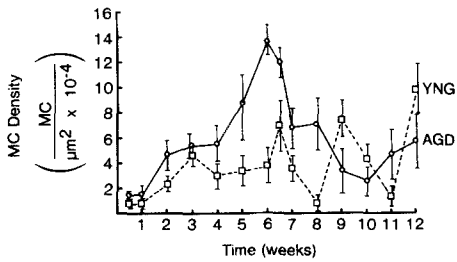

Fig. 2. Mast cell density in pulmonary small muscular arteries and arterioles [diameter <150 μm] of young (*YNG*) and Aging (*AGD*) rats exposed to 18000 ft for 6 weeks, and subsequently recovering at 5000 ft for 6 weeks

(Fig. 1), and was greater than in the young rats. MC density around small pulmonary muscular arteries and arterioles (diameter <150 μm) showed a progressive rise during the first three weeks in young rats. There was a significant decrease during the fourth week (Fig. 2). Throughout the remainder of the exposure period, the density of perivascular MC continued to increase, but to a lesser degree than that of the MC in aging animals (Fig. 2). The density of MC around the arterioles and small muscular arteries of aging animals registered a significant progressive increase throughout the hypoxic exposure period (Fig. 2). During the recovery period the density of MC associated with elastic and large muscular arteries (diameter >150 μm) in lungs of young animals continued to increase until the 21st recovery day when a significant decrease was observed. However, MC density in the aging rat lungs showed a precipitous decline during the initial recovery phase and, although MC density increased during the remainder of the recovery period, it was noticeably lower than the corresponding values in young rats (Fig. 1). MC density around small muscular arteries (diameter <150 μm) and arterioles in lungs of aging animals decreased throughout the recovery period until the fifth week when a noticeable increase was observed. The corresponding values of MC density in young rats were considerably lower than those in aging animals (Fig. 2).

Electron microscopy

At the ultrastructural level pulmonary perivascular MC of young and aging normoxic rats were similar in appearance (Fig. 3). Perivascular MC of young rats did not show any significant changes following exposure to high altitude; however, numerous alterations were noted in perivascular MC of aging rats. These alterations included a proliferation of the rough endoplasmic reticulum, a noticeable decrease in secretory granules, as well as several

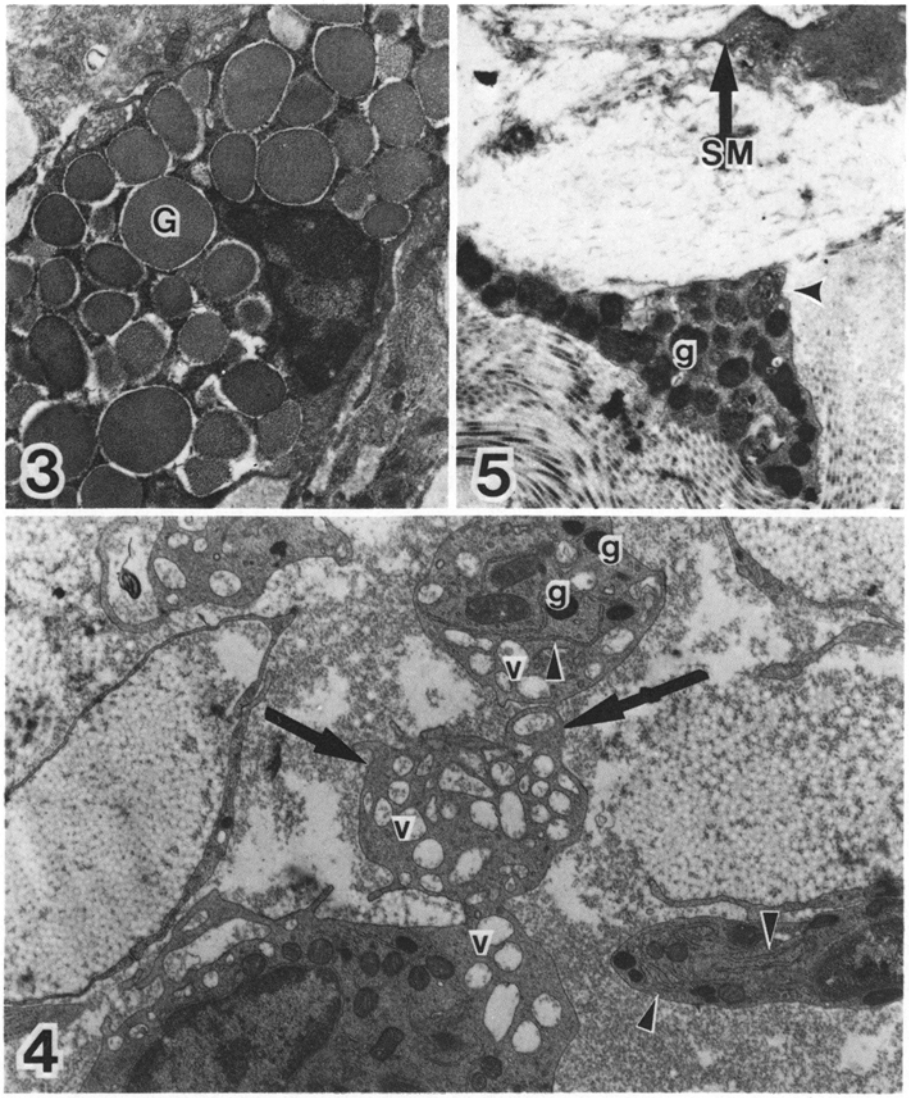

Fig. 3. Perivascular mast cell in lung of aging normoxic rat. Note abundance of secretory granules (*G*). ×10000

Fig. 4. Perivascular mast cells in 4-week altitude-exposed aging rat. Note decreased granular content (*g*), cellular extensions (*arrows*), RER (*arrowheads*), and membranous remnants of discharged secretory vesicles (*V*). ×11350

Fig. 5. Mast cell extension containing secretory granules (*g*) near smooth muscle (*SMarrow*) in hypoxic aging rat. Granules being extruded (*arrowheads*). ×9000

profiles of cellular extensions which contained membranous remnants of discharged secretory vesicles (Fig. 4). Secretory granules, presumably containing vasoactive substances, were usually noted in close proximity to smooth muscle cells (Fig. 5). During the recovery phase the fine structure of pulmonary perivascular MC of the young recovering control animals was similar to that in the young control rats. In the aging hypoxic recovery rats, the MC ultrastructure resembled that of the aged hypoxic rats described earlier.

Discussion

Studies dealing with pulmonary vascular alterations with increasing age have concentrated on the normoxic state (Smith and Heath 1980) but few concern the morphology of the aging pulmonary vasculature during chronic hypoxia (Williams et al. 1977). Chronic alveolar hypoxia has been shown to stimulate the proliferation of perivascular MC which are strategically located in the connective tissue between the airways and small pulmonary vessels (Haas and Bergofsky 1972). Lung MC contain vasoactive agents such as histamine which affects vascular tone through the stimulation of H_1- and H_2 receptors that mediate pulmonary vasoconstriction and vasodilation, respectively (Silove and Simcha 1973; Turker 1973; Flynn and Owen 1974; Tucker et al. 1975; Barer et al. 1978). In the present study, MC density in small muscular arteries and arterioles was notably greater than that associated with elastic and large muscular arteries in both normoxic and hypoxic aging and young rats. This is similar to the findings of Newman et al. (1980). Pulmonary arterioles and small muscular arteries, which are the primary regulators of increased blood pressure during chronic hypoxia (Wagenvoort and Wagenvoort 1977), have been shown to possess exceptional sensitivity to histamine (Holl et al. 1980). In the present study, the greater proliferation of MC around arterioles and small muscular arteries in aging compared to young rats suggests that histamine released by these perivascular elements might interact with H_2 receptors inducing pulmonary vasodilation (Turker 1973), and inhibiting vasoconstriction (Chand and Altura 1980). This could be a humoral determinant for the decreased vascular responsiveness to vasoactive agents observed with increasing age (Tucker et al. 1982). During the recovery phase, continued higher density of periarteriolar MC in aging than in young rat lungs suggested that reversal of MC hyperplasia, following removal of the hypoxic stimulus, proceeds at a slower rate with increasing age. Regression of MC density to control values in lungs of aging rats during the recovery period agrees with that reported in previous studies (Williams et al. 1977). Although the present morphometric findings seem to suggest an increase in the density of MC in lungs of aging animals during chronic hypoxia, the type of density calculated in this study (number of MC per unit area) need not intimate that there is a difference in the number of MC per unit volume. Consequently if the diameter of the mast cells increased by a factor of 2 then the number

of mast cell profiles per unit area would be doubled but the number of mast cells per unit volume would remain unchanged.

The fine structural appearance of decreased granular content, increased cellular extensions, and membranous remnants of discharged secretory vesicles in perivascular MC of aging hypoxic rats strongly suggests secretory release of presumably vasoactive agents by the MC. Also, proliferation of the rough endoplasmic reticulum indicates increased protein synthetic activity, which is usually noted following release of cellular contents (Padawer 1979). These ultrastructural observations of increased activity of perivascular MC associated with vascular hyporesponsiveness in aging rats exposed to chronic hypoxia is consistent with the hypothesis that MC hyperplasia, and the release of histamine, could be a protective mechanism which blunts, rather than mediates, the hypoxic hypertensive response (Howard et al. 1975; Tucker et al. 1976; Williams et al. 1977; Martin et al. 1978).

Although modulatory alterations in H_1- and H_2 pulmonary vascular receptors have been demonstrated in normoxic and hypoxic neonatal lambs (Goetzman and Milstein 1980) the effect of aging on H_1- and H_2 pulmonary vascular receptors and their role in hypoxic pulmonary vascular responsiveness remains to be elucidated.

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