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Effect of Chronic Hypoxia on Angiotensin-Induced Pulmonary Vasoconstriction and Converting Enzyme Activity in the Rat[1] (41568)

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Abstract. Rats were exposed to high-altitude (5500 m) hypoxia for 2 weeks. On examination 1–3 days after return to sea level and compared with control rats, they exhibited pulmonary hypertension, reduced angiotensin-converting enzyme activity, greater vascular responsiveness to angiotensin II (AII), and resistance to blockade of AII pulmonary pressor responses by the AII antagonist, saralasin.

High-altitude hypoxia leads to pulmonary hypertension in many species. The rise in pulmonary arterial pressure is approximately proportional to the decrease in barometric pressure and the resultant hypoxia (1, 2). This phenomenon has also been shown to occur in rats exposed to reduced ambient pressures in hypobaric chambers. Animals in such environments exhibit thickening of the pulmonary arterial wall and reduction in the number of small pulmonary arteries, hypertrophy of the right ventricle, and elevated hematocrit (3, 4). Although the adaptive need for these changes in maximizing blood–alveolar space contact is readily appreciated and the understanding of the mechanism(s) involved is growing, little is known about the ability of this physically altered vasculature to respond to and metabolize vasoactive agents. Some recent studies have indicated that acute alveolar hypoxia alters vascular metabolism. For example, in addition to producing pulmonary vasoconstriction, acute alveolar hypoxia has been demonstrated in dogs to reduce the ability of the pulmonary vasculature to convert angiotensin I (AI) to angiotensin II (AII) and to degrade bradykinin (5, 6). Similarly, reductions in converting activity have been observed in cultured pulmonary vascular endothelial cells subjected to reduced oxygen tension (7). However, it is not known how chronic exposure to hypoxia affects vascular converting enzyme activity.

The purpose of this study was to determine how 2 weeks at an altitude of 5500 m affects the vascular conversion of AI to AII, the pulmonary and systemic vascular responses to AII, and the degree of competitive blockade of vascular responses caused by an angiotensin antagonist, saralasin. It was found that rats, examined 1–3 days after return to sea level, exhibited less angiotensin-converting enzyme activity, greater vascular responsiveness to AII, and a lesser degree of blockade of AII pulmonary pressor responses by a given dose rate of saralasin infusion than control rats kept at sea level.

Methods. Male Sprague–Dawley rats weighing ca. 220 g were divided randomly into two groups. Group 2 (altitude) was placed in either of two hypobaric chambers, with sufficient food for 2½ weeks; water was piped into the chambers as needed (without recompressing). These rats gradually were exposed (over a 3-day period) to conditions simulating the PO_2 and barometric pressure of 5500 m and then maintained in this environment for 2 weeks. The construction and operation of the hypobaric chambers have been described by Blatteis and Tucker (8).

Group 1 (control) was placed in either of two cages maintained at 103 m (the altitude of Memphis–referred to as sea level) for the same duration as Group 2 in the chambers. Both habitats had wire-bottom cages (floor space of 36 × 18 in. for six rats) lined with absorbent paper sprinkled with sodium bicarbonate. Light was provided for 12 continuous hr per day.

After 2½ weeks at these exposures, rats were removed from their respective habitats in groups of three or four. They were examined

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TABLE I. EFFECTS OF A 2-WEEK EXPOSURE TO 5500 m ALTITUDE ON BODY WEIGHT, HEMATOCRIT, AND VENTRICULAR WEIGHTS OF RATS[a]

	Control (Group 1) (N = 8)	Altitude (Group 2) (N = 8)
Pulmonary arterial pressure (mm Hg)	16 ± 2[a]	34 ± 3*
Ventricular weight (mg)		
LV + S	796 ± 21	575 ± 17
RV	125 ± 9	324 ± 14
LV + S RV ratio	6.92 ± 0.23	1.77 ± 0.16*
Hematocrit (%)	46.4 ± 2.5	72.6 ± 3.2*

[a] Means ± SEM. LV, left ventricle; S, septum; RV, right ventricle.
* $P < 0.05$ compared to control value.

within 1–3 days for anatomical and physiological changes and for their reactions to pharmacological agents. For this purpose, 60 mg/kg of Na pentobarbital was injected ip into the rats to produce surgical anesthesia and a tracheostomy was performed. Because respiratory depression frequently is induced by this level of anesthesia, the rats immediately were placed on positive-pressure ventilation. This was provided by a Harvard Rodent Respirator; peak inspiratory pressure was kept at 10 cm of water with a stroke volume of 7 ml. Airway dead space was kept minimal by using a narrow, short tracheal tube. For monitoring systemic blood pressure, the right carotid artery was catheterized and connected to a Statham pressure transducer; for administering drugs, the jugular vein was catheterized to the level of the right atrium. The chest was then opened carefully by separating the sternal muscles and cutting across the caudal end of the sternum, followed by a cut up the center toward the clavicles; the ribs were gently spread with retractors. Finally, the bent tip of a 23-gauge needle was slipped into the pulmonary artery at the junction of the right ventricle and connected by a heparinized saline-filled catheter to a pressure transducer to measure pulmonary arterial pressure. Body temperatures were maintained at 37°C during the entire procedure with a control-heated surgery board. Survival rate for these preparations for the entire experimental period was 88%.

Following a 30-min postsurgery stabilization period, graded doses of AI and AII were injected into the right atrium and dose–arterial pressor response curves constructed for both groups of rats. Total vascular AI converting enzyme activity was estimated by determining the mean proportion of the magnitudes of the AI systemic pressor responses and comparing those to equimolar doses of AII at 0.1, 0.2, and 0.4 μmole/kg. Saralasin was then infused iv at a dose rate of 1 μg/kg/min and a flow rate of 0.25 ml/hr. A dose–response curve was again constructed for AII beginning 20 min after the start of the saralasin infusion. Test doses of AII given 25 min after the end of saralasin infusion confirmed return of pressor responses to near control values. Following the cardiovascular measurements, venous blood was sampled for determination of the hematocrit, and the rats were sacrificed. The heart ventricles were dissected and weighed, using the method of Fulton *et al.* (9), and the ratio of weights of left ventricle plus septum or right ventricle was estimated.

The data are presented as mean absolute values or percentage change from baseline. Data from the two experimental groups were compared statistically by the Student's t test; $P < 0.05$ was accepted as indicating statistically significant differences.

Results. The rats kept in the hypoxic high-altitude environment for 2 weeks (Group 2) exhibited right ventricular hypertrophy, elevated hematocrit, and elevated pulmonary arterial pressure (Table 1). Pulmonary arterial pressure measured 30 min after surgical preparation was 34 ± 3 mm Hg in Group 2, whereas that of the control rats (Group 1) was 16 ± 4 mm Hg. At this time systemic arterial pressures were the same in both groups, viz., 120 ± 2 and 125 ± 4 mm Hg, respectively.

The effects of AII on pulmonary arterial pressure before and during saralasin infusion are shown in Fig. 1. The pulmonary pressor responses to 0.2- and 0.4-μg/kg doses of AII were greater in the altitude rats (Group 2) than in the sea-level rats (Group 1). This occurred even though basal pulmonary pressure was higher in the former. During the infusion of saralasin, pressor responses of high-altitude rats to most doses of AII were greater than those of sea level rats. The degree of blockade

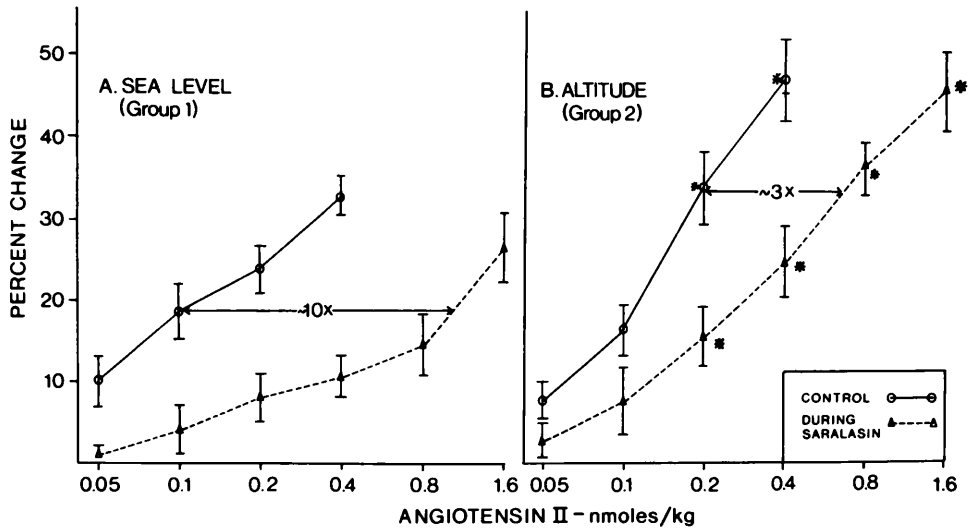

FIG. 1. Effect of angiotensin II given into right atrium on mean pulmonary arterial pressure before and during infusion of saralasin, 1 μg/kg/min. Brackets indicate ±1 SEM. *Values which are significantly greater than responses to that dose in control (sea level) rats.

of AII pressor responses produced by saralasin infusion was approximately 10-fold in the sea level rats (Group 1), compared to about 3-fold in the high-altitude rats (Group 2).

In contrast, there were no differences in the systemic vasopressor responses to AII of the two groups of rats: the control pressor curves to AII were similar in both groups (Fig. 2). Furthermore, the degree of blockade of systemic arterial pressor responses to AII by saralasin was similar in both groups of rats and matched the degree of antagonism for pulmonary responses within the control rats, i.e., about 10-fold. The altitude rats did, however, demonstrate less pulmonary pressor responses to AI than did the control rats. Total vas-

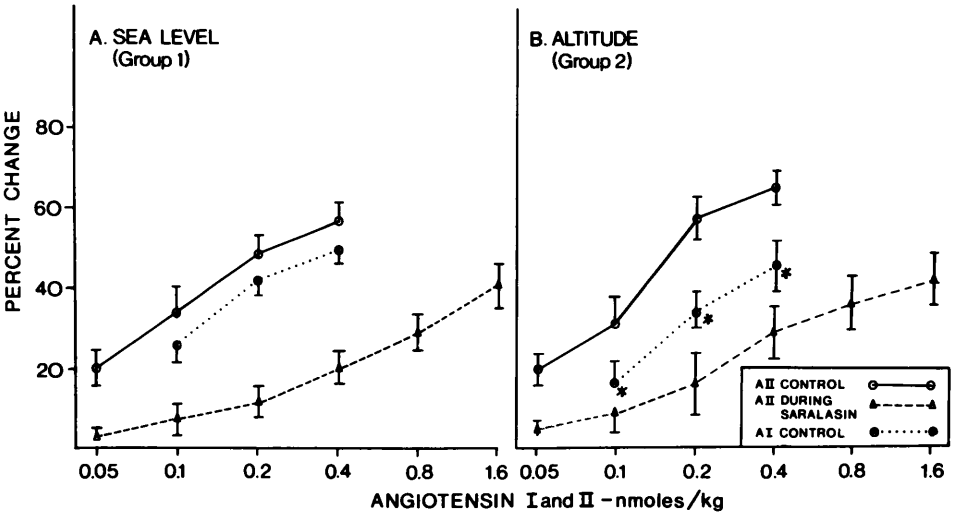

FIG. 2. Effect of angiotensin I or II on systemic arterial pressure before and during saralasin infusion, 1 μg/kg/min. Brackets indicate ±1 SEM. *Values for AI responses which are significantly less than molar equivalent dose of AII.

cular AI conversions, calculated as percentage of the responses to AI compared to responses to equimolar doses of AII, (0.1, 0.2, and 0.4 nmole/kg), were 55 ± 6 and 84 ± 4% for altitude and sea level rats, respectively.

Discussion. Exposure of these rats for 2 weeks to conditions simulating 5500 m altitude led to the expected pulmonary hypertension. It also induced a greater than expected pulmonary pressor response to AII, even in the presence of higher basal pulmonary artery pressures. By contrast, the systemic arterial pressor responses to AII were similar in both altitude and sea level groups. Total vascular conversion of AI to AII was lower in the hypobaria-exposed as compared with control rats. It should be noted that the surgery procedures in these experiments may have elevated plasma levels of catecholamines and angiotensin. However, rats in the sea level and altitude groups were handled identically, thus differences observed between the groups reflect changes induced by altitude exposure.

Acute hypoxia (up to 1 hr) has been shown to inhibit converting enzyme activity (5, 6) but this effect appears to be due to transient perturbations in the vascular endothelial membrane and is readily reversible. Our experiments suggested that conversion of AI to AII occurred readily in the normal rat, perhaps more effectively than in the dog (5); they indicated, furthermore, that converting enzyme amount or activity was markedly diminished during our chronic hypoxic treatment. In fact, apparent total vascular conversion of angiotensin I to AII was reduced in the altitude rats even 1 to 3 days after they were removed from the hypobaric chamber. The reason for residual depression in angiotensin-converting capacity is unknown and deserves study. It is known, however, that other cardiopulmonary changes induced by similar, chronic, hypoxic exposures, e.g., pulmonary vascular and right ventricular hypertrophy, persist for at least 2 weeks following removal from an hypoxic environment (10).

The increased pulmonary pressor responses of altitude rats to AII compared with those of the controls may be a consequence of: (1) an increase in the number of angiotensin-sensitive pressor receptors; (2) narrowed vascular lumina; (3) a reduced release or synthesis of attendant pulmonary vasodilator substances such as prostacyclin; (4) altered pulmonary vascular ionic composition; or (5) reduced metabolism of AII by angiotensinases. Depressed metabolism of AII by angiotensinases can be dismissed as a possibility, since such a phenomenon would be expected to allow additional accumulation of the antagonist, saralasin, which is a close structural analog and has a duration of action similar to that of AII.

A greater pulmonary vascular AII receptor number or density in the altitude-exposed rats is strongly supported by the fact that a given dose rate of saralasin was far more effective in blocking the AII pulmonary pressor response in the control group than in the altitude group; there were no differences in the degrees of blockade of AII systemic pressor responses between the two groups. Hypertrophy and hyperplasia occurring in the pulmonary vasculature at altitude may have produced attendent increases in AII receptor number. However, the second, third, and fourth possibilities cannot be dismissed and may also contribute to the enhanced pressor responses observed to AII. For example, if narrowing of the pulmonary vascular lumina occurred during hypoxic exposure (4), a given decrease in lumen radius might be expected to produce a greater pressure increase—via Poiseulle's Law. Also, AII is a well-known stimulator of the release of pulmonary vascular prostaglandins (11, 12); the release or synthesis of vasodilator prostaglandins may have been impaired by hypoxic exposure. Additionally, vascular responsiveness to AII is known to be enhanced when vascular Na^+ content is heightened, as when vascular Na^+-pump (Na^+, K^+-ATPase) activity is depressed (13, 14). The critical experimental determinants for these latter possibilities were not included.

Further study should examine these latter mechanisms. Direct measurement of angiotensin-converting enzyme activity, AII receptor binding, and circulating AI and AII levels would also be of benefit.

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