Pulmonary vascular response of the coati to chronic hypoxia

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Departments of 1Anesthesia, 2Physiology/Biophysics, and 3Pediatrics, Indiana University School of Medicine, Indianapolis, Indiana 46202; 4Cardiovascular Pulmonary Research Laboratory, University of Colorado Health Sciences Center, Denver, Colorado 80262; 5Biology Department, Eastern Washington University, Cheney, Washington 99004; 6Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada L8N 4A6; and 7Department of Chest Medicine, Chiba University School of Medicine, Chiba 260-8670, Japan

Hanson, Wendy L., Dona F. Boggs, J. Michael Kay, Stephen E. Hofmeister, Osamu Okada, and Wiltz W. Wagner, Jr. Pulmonary vascular response of the coati to chronic hypoxia. J. Appl. Physiol. 88: 981–986, 2000.—The unusually muscular pulmonary arteries normally present in cattle and swine residing at low altitude are associated with a rapid development of severe pulmonary hypertension when those animals are moved to high altitude. Because these species lack collateral ventilation, they appear to have an increased dependence on hypoxic vasoconstriction to maintain normal ventilation-perfusion balance, which, in turn, maintains thickened arterial walls. The only other species known to lack collateral ventilation is the coati, which, similarly, has thick-walled pulmonary arteries. We tested the hypothesis that coatis will develop severe high-altitude pulmonary hypertension by exposing six of these animals (Nasua narica) to a simulated altitude of 4,900 m for 6 wk. After the exposure, pulmonary arterial pressures were hardly elevated, right ventricular hypertrophy was minimal, there was no muscularization of pulmonary arterioles, and, most surprisingly of all, there was a decrease in medial thickness of muscular pulmonary arteries. These unexpected results break a consistent cross-species pattern in which animals with thick muscular pulmonary arteries at low altitude develop severe pulmonary hypertension at high altitude.

collateral ventilation; species variation to hypoxia; pulmonary hypertension; high altitude; distal arterial muscularization; medial thickness; vascular smooth muscle; Nasua narica

CATTLE AND SWINE CAN DEVELOP potentially fatal pulmonary hypertension at high altitude (7, 17, 30). Pulmonary arterial pressures in other species, however, become only modestly elevated, even after prolonged exposure to high-altitude hypoxia (6, 21–23, 29). Insight into this large species variation came from Tucker et al. (29), who found among eight species a strong positive correlation between the thickness of pulmonary arterial smooth muscle at low altitude and the development of pulmonary hypertension at high altitude. In that study, cattle and swine, which had the thickest pulmonary arterial smooth muscle among the species at low altitude, rapidly developed severe pulmonary hypertension with chronic hypoxia. In contrast, dogs and sheep, with thin-walled pulmonary arteries at low altitude, developed little or no pulmonary hypertension with chronic hypoxia. It has been suggested that cattle and swine have thick muscular pulmonary arteries at sea level because they lack collateral ventilation, causing them to depend more heavily on hypoxic vasoconstriction to regulate local ventilation-perfusion balance (14, 15). According to this concept, the added work for the arterial smooth muscle leads to and maintains medial hypertrophy. The only species other than cattle and swine known to lack collateral ventilation is the coati (5, 9). Because coatis have muscular pulmonary arteries with even thicker walls than those of cattle and swine (9), these animals provide an opportunity to test the hypothesis that animals with thick muscular pulmonary arteries at sea level develop severe pulmonary hypertension at high altitude. To investigate this idea, we exposed a group of coatis to simulated high altitude.

METHODS

Six adult coatis (Nasua narica), mean body weight 8.2 ± 1.5 (SD) kg, were sedated with intramuscular ketamine hydrochloride (22 mg/kg), anesthetized with intravenous pentobarbital sodium (12 mg/kg, supplemented as needed), and paralyzed with intravenous injections of pancuronium bromide (0.14 mg/kg). The animals were intubated and ventilated by a constant-volume respirator (model 646, Harvard). Under sterile conditions, polyethylene catheters were threaded percutaneously into the pulmonary artery via the jugular vein for pressure measurements and injection of indocyanine green dye for cardiac output determinations and into the aorta via a percutaneous cannulation of the femoral artery for withdrawal of arterial blood for cardiac output, blood-gas, and pressure measurements. Cardiac outputs (Waters D402A), heart rates, and mean pulmonary arterial and systemic arterial pressures (Statham P23 Db) were calculated on-line by a personal computer. Inhaled oxygen mixtures were varied to obtain a pulmonary arterial pressure dose response curve to hypoxia (35, 21, 14, 13, and 12% O2, 8–10 min for each treatment). We calculated total pulmonary vascular resistance, thereby neglecting downstream pressure.

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The coatis were exposed individually to a simulated altitude of 4,900 m (16,000 ft, barometric pressure = 430 mmHg) for 6 wk. The animals were returned to normobaric conditions (630 mmHg in Denver, CO) where they were studied promptly (usually ~1 h) with the same protocol. After the postaltitude physiological measurements, the animals were euthanized with pentobarbital sodium, and the lungs were fixed in situ by tracheal instillation of 10% buffered Formalin at 20 cmH2O pressure. The morphological data from control animals were taken from a previous study of the same species (Nasua narica) of similar size (9). The trachea was ligated, and the entire, unopened thorax was submerged in 10% buffered Formalin for 2 wk. After fixation, the free wall of the right ventricle was dissected from the interventricular septum, and the atria, great vessels, and valves were removed from both ventricles. The weight of the right ventricular free wall was expressed as a percentage of the total ventricular weight (right ventricular free wall weight/total ventricular weight × 100). In each animal, four sections of lung tissue were taken, two each from the right and left lower lobes. Sections (1–2 cm²) were cut parallel to the pleural surface, the first ~5 mm below the pleura and the second 10–15 mm deeper. The sections were stained with either Luna’s elastic stain or Gieson method. Photomicrographic prints were made of all histological sections of lung from each coati were systematically examined by using a x40 objective in a microscope fitted with a calibrated eyepiece grid. In each slide, we counted the first 100 blood vessels that were <50 µm in diameter. Such vessels possess a tunica media composed of a complete layer of circular smooth muscle sandwiched between the internal and external elastic laminae. The internal and external elastic laminae were taken to be the internal and external elastic laminae, respectively, of the media. Arteries were measured only if they met the following criteria. 1) They had to be circular, or, if oval in cross section, the major and minor axes had to be within 50% of each other. 2) If the vessels were oval, the location of the major and minor axes had to be obvious. 3) They had to have two different laminae around the entire circumference. 4) A number of vessels met criteria 1–3 but had laminae that were so crenated that reasonable measurements could not be made. If the vessels were only mildly crenated, the dimensions were estimated by averaging the inner and outer circumferences ascribed by the inner and outer peaks, respectively, of the serpentine lamina. The arteries were measured across their major axes to obtain internal and external diameters by using a digitizing pad (Houston Instruments), quantification software (Jandel Scientific), and a personal computer. The medial thickness (of one wall), expressed as a percentage of external diameter, was computed by

\[
\frac{\text{External diameter} - \text{Internal diameter}}{2 \times \text{External diameter}} \times 100
\]

The pulmonary artery in the coati is an arterial vessel <50 µm in diameter, the wall of which is normally devoid of smooth muscle except adjacent to its origin from a pulmonary artery. It consists of a single elastic lamina lined by endothelium (9). In chronic pulmonary hypertension, the pulmonary arterioles become muscularized with the development of a thick medial coat of circularly oriented smooth muscle bound by internal and external elastic laminae (12). We used the technique of Hunter et al. (11) and Kay et al. (13) to determine whether muscularization of the pulmonary arterioles had occurred in the coati exposed to chronic hypoxia. Four histological sections of lung from each coati were systematically examined by using a x40 objective in a microscope fitted with a calibrated eyepiece grid. In each slide, we counted the first 100 blood vessels that were <50 µm external diameter, had a continuous elastic lamina, and lay adjacent to alveolar ducts or alveolar spaces. The number of such small pulmonary vessels with two elastic laminae for >50% of their circumference was expressed as a percentage of the total number of vessels counted. We compared the quantitative morphological data from the hypoxic coatis with similarly obtained data from six coatis (also Nasua narica) that were not exposed to altitudes higher than Denver once they were obtained (9). The tissue was prepared and examined in an identical way.

Group means were compared by using the t-test, paired or unpaired, one- or two-tailed tests where appropriate. Unless otherwise specified, all values are means ± SE.

RESULTS

The pre- and postaltitude responses in individual animals are shown in Fig. 1. For the data shown in Fig. 1, we chose specific measurements in each animal in which there was the closest possible match, pre- and postaltitude, between arterial oxygen tension, carbon dioxide tension, body temperature, and pH, variables that are known to affect hypoxic vasoconstriction (pre- vs. postaltitude): pH = 7.40 ± 0.01 vs. 7.35 ± 0.2 (P = 0.2), arterial carbon dioxide tension = 25.6 ± 1.9 vs. 25.1 ± 2.8 Torr (P = 0.88), and body temperature = 37.0 ± 0.5 vs. 37.0 ± 0.7°C (P = 0.98). Group means are shown in Table 1. Pulmonary arterial pressure was somewhat increased during normoxia in the postaltitude measurements (prealtitude = 19 ± 1, postaltitude = 24 ± 2 mmHg; P = 0.05), but during hypoxia there was no consistent change between pre- vs. postaltitude pulmonary arterial pressure (Fig. 1; P = 0.44). Cardiac output and pulmonary vascular resistance were not altered from their prealtitude values by chronic hypoxia (Fig. 1, Table 1). Chronic hypoxia caused the expected rise in hematocrit (prealtitude = 34.0 ± 1.8, postaltitude = 44.0 ± 1.3%: P < 0.004). The animals tended to lose body weight at high altitude (prealtitude = 8.2 ± 1.5, postaltitude = 6.8 ± 1.0 kg; P < 0.07). The weight of the untreated control group was 6.8 ± 0.8 kg, which was not different from the experimental group before altitude exposure (P > 0.09). An average of 14 muscular pulmonary arteries in each animal met the morphological selection criteria. All of the muscular pulmonary arteries sampled from these animals had thick walls. The medial thickness in the low-altitude controls (n = 5 animals, 93 arteries) was 16.6 ± 0.4% (Table 2). The medial thickness of the postaltitude coatis (n = 6 animals, 76 arteries) was significantly lower (10.9 ± 0.6%; P < 0.0001). The frequency distributions of the medial thickness from low-altitude control and postaltitude animals are clearly different (Fig. 2). The percentage of small pulmonary vessels <50 µm in diameter with two elastic laminae was not different between the two groups (Table 2). The weight of the right ventricle expressed as a percentage of the total ventricular mass increased modestly in the high-altitude animals (Table 2).

DISCUSSION

We tested the hypothesis that animals with thick muscular pulmonary arteries at low altitude will develop considerable pulmonary hypertension when ex-
posed to chronic hypoxia. Coatis were used as the experimental animal because they have extremely thick muscular pulmonary arteries at low altitude. Their response to chronic hypoxia, however, was unknown. The following lines of evidence show that the response of the coati to chronic hypoxia does not fit the hypothesis.

1) With each animal acting as its own control, and with pre- and postaltitude arterial oxygen and carbon dioxide tensions, arterial pH, and body temperatures being matched, there was no difference between pre- and postaltitude pulmonary arterial pressures or resistances during hypoxia. 2) During postaltitude normoxia, pulmonary arterial pressures averaged only 5 mmHg higher than prealtitude, and total pulmonary vascular resistance was only marginally increased. 3) Only mild right ventricular hypertrophy was present postaltitude. 4) There was no evidence of muscularization of pulmonary arterioles 50 µm, vessels that usually become muscularized shortly after the onset of chronic hypoxia (20, 24, 26). 5) Postaltitude, there was a surprising decrease in medial thickness of muscular pulmonary arteries. These data are consistent with each other and provide substantial evidence that chronic hypoxia at a simulated altitude of 4,900 m for 6 wk in the coati did not produce significant pulmonary hypertension or cause alterations of pulmonary vascular morphology expected to occur with pulmonary hypertension.

The only evidence consistent with increased pulmonary arterial pressure at high altitude was mild right ventricular hypertrophy (Table 2). The ventricular weight ratio is an excellent method for estimating the chronic load on the right ventricle. In swine, for example, the correlation between mean pulmonary arterial pressures and ventricular weight ratios at low and high altitudes was $r = 0.97$ ($P < 0.001$) (17). From this

Table 1. Cardiopulmonary variables pre- and postaltitude in the same animals

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Prealtitude</th>
<th>Postaltitude</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoxic $P_{O_2}$, Torr</td>
<td>109 ± 13</td>
<td>108 ± 13</td>
<td>0.84</td>
</tr>
<tr>
<td>Normoxic $P_{pa}$, mmHg</td>
<td>19 ± 1</td>
<td>24 ± 2</td>
<td>0.05</td>
</tr>
<tr>
<td>Normoxic $CO$, ml/min</td>
<td>752 ± 57</td>
<td>701 ± 118</td>
<td>0.72</td>
</tr>
<tr>
<td>Normoxic $P_{VR}$, mmHg·l$^{-1}$·min</td>
<td>26 ± 2</td>
<td>37 ± 5</td>
<td>0.11</td>
</tr>
<tr>
<td>Hypoxic $P_{pa}$, mmHg</td>
<td>38 ± 2</td>
<td>38 ± 3</td>
<td>0.44</td>
</tr>
<tr>
<td>Hypoxic $CO$, ml/min</td>
<td>42 ± 4</td>
<td>40 ± 5</td>
<td>0.33</td>
</tr>
<tr>
<td>Hypoxic $P_{VR}$, mmHg·l$^{-1}$·min</td>
<td>575 ± 65</td>
<td>708 ± 172</td>
<td>0.52</td>
</tr>
<tr>
<td>Hypoxic $P_{VR}$, mmHg·l$^{-1}$·min</td>
<td>77 ± 9</td>
<td>64 ± 9</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Values are means ± SE for 6 animals. Normoxic measurements were made with 35% $O_2$; hypoxic measurements were made with 10 min of 12% $O_2$. $P_{pa}$, pulmonary arterial pressure; $CO$, cardiac output; $P_{VR}$, total pulmonary vascular resistance. Group means were compared by using the paired t-test, 1- or 2-tailed tests where appropriate.

Fig. 1. Response of individual animals to hypoxia pre- and postaltitude (each line is data from 1 animal). A: pulmonary arterial pressure. B: cardiac output. C: pulmonary vascular resistance. Specific measurements are compared in which there was closest possible match between arterial oxygen tension, carbon dioxide tension, pH, and body temperature. Pulmonary arterial pressures and resistances were slightly elevated during postaltitude normoxia; otherwise, there were no significant changes. Arrowheads indicate means.
and numerous other studies (2, 8, 21–23, 29), we think that this measurement indicates that the level of hypertension in the coatis was minimal and certainly unimpressive compared with that of cattle and swine (Fig. 3). It is possible that some of the right ventricular hypertrophy could have been accounted for by the polycythemia alone (27), although recent work by Petit et al. (19), using recombinant erythropoietin to produce polycythemia, showed there was no right ventricular hypertrophy during normoxia in either Hilltop or Madison Sprague-Dawley rats.

In the physiological studies, the animals served as their own controls. Utilizing historical controls for the morphological studies, however, has been a concern. This decision was made for several reasons. First, these animals are difficult to import into the United States. The Arizona coati population, representing the northernmost contingent of this Central American species, sometimes decreases because of disease such as distemper, which can cause significant and rapid attrition; this characteristic gives the animals an “endangered” label in the United States, even though they are a ready source of food in Mexico and elsewhere. The animals in our studies have, for this reason, been collected singly over a number of years. Because the morphological characteristics of the animals in our first study of coatis (9) were highly consistent both between and within animals, we elected to use the morphological measurements from these animals rather than attempting to collect a second group of controls solely for morphological data. We do not know the altitude at which each of these animals resided before being sent to Denver, although the usual location of these animals is near sea level. In any case, all animals were maintained in Denver for several weeks before the prealtitude studies, and the animals were assigned in random order to be used as low-altitude controls or as high-altitude experimental animals. This assigning was done to compensate for the effect of whatever altitude the animals were exposed to before we received them in Denver.

The lack of significant pulmonary hypertensive response of the coatis to high altitude was unexpected. On the basis of the highly correlated, cross-species relationship between arterial wall thickness at low altitude and the development of pulmonary hypertension at high altitude found by Tucker et al. (29), we anticipated that the coatis would develop significant pulmonary hypertension after 6 wk at 4,900 m. This relationship is so central to our hypothesis that, with the kind permission of Dr. Alan Tucker, we repeated the

![Table 2. Morphological characteristics of control and high-altitude coatis](image)

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Low Altitude n</th>
<th>High Altitude n</th>
<th>P Value</th>
</tr>
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<tbody>
<tr>
<td>Right ventricular weight ratio, %</td>
<td>22.8 ± 0.5</td>
<td>27.7 ± 1.6</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Medial thickness, %</td>
<td>16.6 ± 0.4</td>
<td>10.9 ± 0.6</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Small pulmonary vessels &lt; 50-µm</td>
<td>4.3 ± 0.9</td>
<td>3.5 ± 1.0</td>
<td>&gt; 0.1</td>
</tr>
<tr>
<td>diameter with 2 elastic laminae, %</td>
<td>5</td>
<td>6</td>
<td></td>
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Values are means ± SE; n, number of animals. Small pulmonary vessels < 50 µm with two elastic laminae are expressed as a percentage of total number of vessels < 50 µm (low altitude = 1,900 vessels in 5 animals, high altitude = 2,200 vessels in 6 animals). Right ventricular weight ratio is percentage of total ventricular mass occupied by the free wall of the right ventricle. P values from two-tailed t-test.

![Fig. 2. Frequency distribution of medial thickness of sampled muscular pulmonary arteries between 50 and 300 µm in diameter. Medial thickness of low-altitude controls averaged 16.6 ± 0.4% (n = 93). Postaltitude medial thickness was 10.9 ± 0.6% (n = 76), P < 0.0001.](image)

![Fig. 3. Comparison of right ventricular hypertrophy (and chronic pulmonary hypertension) developed by 3 species that lack collateral ventilation and have thick-walled pulmonary arteries at sea level. Cattle and swine develop severe high-altitude pulmonary hypertension as indicated by significant right ventricular hypertrophy [data from Tucker et al. (29)]. Coatis have a minimal response. Values are means ± SE. RV, right ventricular free wall weight; T, total ventricular weight. * P < 0.05 relative to control.](image)
measurements of medial hypertrophy in his original histological slides. Tucker et al. measured medial hypertrophy by projecting the magnified image of the pulmonary arteries onto a ground-glass screen and determining the vessel diameters and wall thicknesses with a transparent scale held against the screen. Using identical vessel selection procedures, we measured the pulmonary arteries using the photomicrographic technique and computer quantification method already described. The two measurement techniques produced very similar relationships. Given then that the relationship discovered by Tucker et al. is valid, we can add our measurements of coati vessels to the original graph of Tucker et al. The coatis are widely divergent (Fig. 4), a significant exception to this highly correlated, cross-species pattern.

Because we were convinced at the outset that the coatis would develop severe pulmonary hypertension during chronic hypoxia, our protocol was not designed to determine what mechanism the animals might employ to avoid pulmonary hypertension. Nevertheless, some potential mechanisms can be explored with our data. There are two general possibilities: either the theory is incorrect or the coatis have some method for avoiding pulmonary hypertension. Because the relationship shown in Fig. 4 successfully predicts the response of a wide variety of species, we presume that the theory is generally correct and that the response of the coatis is unusual.

One way that a species can limit its pulmonary arterial pressure at high altitude is to reduce cardiac output. That response occurred in the study of Rounds et al. (25) in which lambs were maintained at a simulated altitude of 4,573 m for 12 days. Pulmonary arterial pressure rose from 17 ± 1 to 25 ± 2 mmHg postaltitude, whereas cardiac output fell by 15%. Although the combination caused pulmonary vascular resistance to more than double, pulmonary arterial pressure remained relatively low. Similarly, in long-term human residents of Leadville, Colorado (altitude = 3,100 m), cardiac output at rest was 18% lower than at sea level (10). The coatis appear to respond differently with no evidence for a consistent reduction of cardiac output during post-altitude hypoxia (Table 1). In view of the extreme pulmonary hypertension expected from such thick-walled arteries, the required drop in cardiac output to offset the hypertension would have been sufficient to cause lethargy; such was not the case, for the animals were normally active when at high altitude. These observations argue against a drop in cardiac output preventing pulmonary hypertension.

Two other possibilities can be considered with our data. First, the unusually low arterial carbon dioxide tensions in the coatis (25.1 ± 2.8 Torr postaltitude) might reduce hypoxic vasoconstriction. Reduced carbon dioxide influences the hypoxic pressor response primarily through alkalosis (8). We think that alkalosis did not occur in these animals, because arterial pH was near 7.40 when arterial oxygen tensions were at normal values in prealtitude measurements. The low arterial carbon dioxide tension of our anesthetized coatis agrees with arterial carbon dioxide tensions of awake, spontaneously breathing coatis [23.4 ± 0.5 Torr (3)] and in anesthetized coatis (5, 9). Carbon dioxide tensions did not appear to drop further postaltitude. Reduced carbon dioxide tensions in the presence of normal pH values, therefore, seem an unlikely cause of suppressing pulmonary hypertension.

A second possibility that could account for the lack of pulmonary hypertension is that there might have been reduced vascular sensitivity to hypoxia at high altitude. Postaltitude, however, the animals had essentially the same response to hypoxia in terms of pulmonary arterial pressure, cardiac output, and pulmonary

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**Fig. 4.** Relationship between low-altitude medial thickness and high-altitude right ventricular hypertrophy. Coatis diverge widely from this pattern [data from Tucker et al. (29)].
vascular resistance (Fig. 1), thereby ruling against this mechanism. From these data, we are unable to explain the lack of response of the coati to high altitude.

These results add another species to the growing number of species (4, 7, 16, 28), confirming the idea proposed by Reeves et al. (21) that the acute pulmonary pressor response of a species to hypoxia does not predict the chronic response. It is only among individual cattle that there is a strong correlation between the change in pulmonary arterial pressure with acute hypoxia and chronic hypoxia \( r = 0.91, P < 0.001 (31) \).

In summary, the coati provides an interesting animal model for the further study of 1) the powerful acute pressor response to hypoxia, 2) the unusually low arterial carbon dioxide tension normally present, 3) the extraordinarily muscular pulmonary arteries present at sea level, and 4) the potentially important and at this time unknown mechanism by which these animals avoid developing severe pulmonary hypertension at high altitude.

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