Effects of Atrial Natriuretic Factor in Chronic Hypoxic Spontaneously Hypertensive Rats

Chau-Fong Chen, Chiang-Ting Chien, Hwai-Sze Fang, and Ing-Sh Chiu

The present study was designed first to investigate the pulmonary hypertensive effects of chronic hypoxia in spontaneously hypertensive rats and second to compare the cardiovascular effects of atrial natriuretic factor on rats exposed to hypoxia and on control rats kept at sea level. Catheters were placed in the femoral and pulmonary arteries for measurement of mean systemic arterial pressure and mean pulmonary arterial pressure. The cardiac output was measured by thermodilution method. It was found that 4 weeks of simulated 18,000-foot hypoxia led to polycythemia, right ventricular hypertrophy, and pulmonary hypertension, which resulted from an increased pulmonary vascular resistance. However, systemic arterial pressure was not significantly different between the two groups of rats. Atrial natriuretic factor administration decreased systemic arterial pressure and pulmonary arterial pressure to a lesser extent in the hypoxic group compared with the sea level control group. It is concluded that these animals showed an impaired response to atrial natriuretic factor after long-term exposure to hypoxia. (Hypertension 1991;18:355-359)

Atrial natriuretic factor (ANF) possesses potent vasorelaxant properties in blood vessels and natriuretic and diuretic effects in the kidney after exogenous administration as well as endogenous release.1 Changes in plasma ANF concentration or the sensitivity to ANF have been shown to be associated with alterations of body fluid and especially hypertension. Tikkannen et al9 showed that NaCl and deoxycorticosterone acetate–NaCl loading increased plasma ANF levels and that elevated blood pressure did not stimulate ANF release further. Sonnenberg et al10 reported a decreased atrial content of ANF in the spontaneously hypertensive rat (SHR), but Petersson et al11 showed that basal plasma ANF levels were similar in the SHR and Wistar-Kyoto rats. Nevertheless, most reports agreed that the increase in plasma ANF was attenuated during acute volume expansion in SHR rats.2-4 The sensitivity of blood pressure drop after exogenously administered ANF in the normotensive and hypertensive rats is also controversial.3,11,12 ANF may play an important role in the pulmonary hypertension induced by hypoxia.13 Acute hypoxia stimulates the release of ANF from isolated hearts and increases plasma ANF in anesthetized rabbits and pigs.15 In the chronic hypoxia of widespread lung disease or high altitude residents or animals pulmonary hypertension, ventricular hypertrophy, and polycythemia may develop.17 In rats exposed to 21 days of hypoxia, the left atrial ANF content was significantly increased, whereas right atrial ANF content was significantly reduced, and plasma ANF concentration was elevated compared with air control animals.18

ANF has a direct effect on the pulmonary vasculature, causing relaxation of isolated segments of pulmonary artery in vitro19 and lowered pulmonary arterial pressure in hypoxic humans and rats.20,21 Vachiery et al22 showed ANF did not inhibit canine hypoxic pulmonary vasoconstriction. Jin et al23 showed ANF has an enhanced direct vasodilator effect on the pulmonary vasculature in hypoxia-induced pulmonary hypertension in normotensive rats; they also found ANF attenuated the development of pulmonary hypertension in rats adapted to chronic hypoxia.24

Guazzi et al25 observed that patients with systemic hypertension have a greater pulmonary vasoreactivity to acute alveolar hypoxia; the mechanism of action is interpreted to be mediated by an increased availability of calcium ions for the contractile elements of the vessels, which is facilitated with systemic hypertension. However, there are no reports on the incidence and severity of pulmonary hypertension after chronic hypoxia in systemic hypertensive subjects.
The current report is the first experiment designed to study the cardiovascular changes of chronic hypoxia in SHR and the response to ANF administration.

Methods

Male SHR weighing 200–250 g (more than 12 weeks old) were exposed to an altitude chamber (HA) at constant temperature and light cycle, and control rats were maintained at sea level (SL). The level of 18,000 foot (380 torr) was selected because it represents the maximal altitude to which most rats can successfully adapt. The animals were returned to SL pressure for 30 minutes every day to replenish food, water supplies, and animal beds. The body weight of the animals was measured once a week. Food and water were freely available to the animals at all times.

After 4 weeks, the animals were anesthetized with pentobarbital sodium. The trachea was intubated to keep the airway patent, the right external jugular vein was isolated, and through a small incision, an introducer and Silastic catheter were inserted. With the aid of a pressure transducer and a recorder the catheter and introducer were advanced into the right ventricle, and the catheter alone was inserted into the pulmonary artery. This method has been described by Rabinovitch et al.17 The cardiac output was measured with a Cardiomax 11 (model 85, Columbus Instruments, Columbus, Ohio). A thermodilution microprobe (1.5 F) was placed in the left carotid artery. Cold saline (0.15 ml) was injected into the heart through a cannula in the left jugular vein. The cardiac output was calculated in the computer, and the thermodilution curve was also recorded in a chart recorder to ascertain the accuracy of the measurement. The femoral artery and vein were also cannulated for arterial blood pressure measurement and ANF administration. All the experiments were performed under normoxic conditions. In the first experiment, bolus intravenous injections of ANF (rat ANF, Peninsula Lab) in a volume of 100 µl were given at 10-minute intervals in the amounts of 1, 3, and 10 µg/100 g body wt. Changes in mean arterial pressure (Psa), and pulmonary arterial pressure (Ppa) were monitored continuously. In the second experiment, changes of cardiac output, heart rate, cardiac index (cardiac output/kg body wt), Psa, Ppa, systemic resistance (Rs), and pulmonary resistance (Rp) were calculated before and after a 10-minute infusion of ANF (0.1 µg/min/kg). All the effects after ANF administration were expressed in percent of change. The hematocrit was determined with a Triac centrifuge (Clay-Adams, Parsippany, N.J.).

The rats were killed by overdose of pentobarbital. The heart and lungs were removed en bloc, weighed, and fixed with formalin. The right ventricular free wall was then completely separated and removed. The left ventricle and septum were thereafter removed together according to the method of Fulton et al.26

The results are expressed as mean±SEM. Differences between groups were evaluated by the unpaired t test. Changes after ANF administration were analyzed by paired t test.

Results

All the animals were in apparently good health after 4 weeks of continuous exposure to simulated 18,000-foot hypoxia, although the final mean body weight in HA rats was significantly less than that of the SL rats. The hematocrit was significantly higher in HA rats compared with that of SL rats (Table 1).

The changes in ventricular weights are also shown in Table 1. The right ventricle was increased in absolute weight (61%) or corrected by body weight (109%) in hypoxic SHR. The absolute left ventricular plus septum (LV+S) weight showed no significant difference in two groups of rats, but after correction for body weight, the hypoxic rats due to their lower body weight showed a significant increase in ratio. There was a 60% increase in the right ventricular (RV)/LV+S ratio, also demonstrating right ventricular hypertrophy in HA rats.

The basal absolute cardiac output was lower (but not significantly) in hypoxic than in control SHR (62.8±6.44 ml/min versus 76.9±4.89 ml/min), which was mostly due to a smaller stroke volume. However, after correction for body weight, the hypoxic rats due to their lower body weight showed a significant increase in ratio. Figure 1 shows that ANF injections produced significant dose-dependent reductions (from p<0.05 to 0.001) in Psa and Ppa in both groups of animals. The depressor effect of Psa was significantly reduced in HA rats. The hypotensive effect of ANF on Ppa

<table>
<thead>
<tr>
<th>Group</th>
<th>Hct (%)</th>
<th>RV (mg)</th>
<th>RV/BW (mg/100g)</th>
<th>(LV+S)/BW (mg/100g)</th>
<th>RV/(LV+S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SL (n=8)</td>
<td>48.9±0.73</td>
<td>166±12</td>
<td>848±25</td>
<td>54±4</td>
<td>0.198±0.01</td>
</tr>
<tr>
<td>HA (n=7)</td>
<td>76.2±1.47*</td>
<td>267±26*</td>
<td>879±45</td>
<td>113±9*</td>
<td>0.316±0.017*</td>
</tr>
</tbody>
</table>

Hct, hematocrit; RV, right ventricular weight; LV+S, weight of left ventricle plus septum; BW, body weight; SL, sea level; HA, high altitude.

*p<0.001 between control or SL and hypoxic or HA rats.

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was also lower in the HA group but at the largest dose (10 μg/kg), this difference was not statistically significant.

The drop of Psa and Ppa after ANF (0.1 μg/min/kg) infusion was less intense in hypoxic rats as well. Both the systemic and pulmonary vascular resistances did not significantly change after ANF infusion (0.1 μg/min/kg) in SL and HA rats. ANF significantly decreased stroke volume, heart rate, and cardiac output in SL rats. However, ANF reduced heart rate but did not significantly alter stroke volume and cardiac output in HA rats (Table 2).

Thus, the attenuated response of blood pressure to ANF in HA rats is mainly due to the reduced response of the heart to ANF.

**Discussion**

Acclimatization to HA hypoxia is characterized by a number of adaptive responses to maintain homeostasis with minimal energy expenditure.27 Chronic hypoxia stimulates cardiopulmonary acclimatization, the magnitude of which depends on the intensity and duration of the hypoxic stimulus. It is generally agreed that failure to adapt to chronic hypoxia is associated with the development of severe pulmonary hypertension and right ventricular hypertrophy and failure. On the other hand, successfully adapting species invariably develop only mild pulmonary hypertension and right ventricular hypertrophy.28

From the hemodynamic point of view, pulmonary arterial pressure was reported to be normal29 or enhanced30 in hypertensive patients with no obvious signs of cardiac decompensation. However, Guazzi et al31 showed a lower threshold and an enhanced acute hypoxic pulmonary vasoconstriction in hypertensive patients and suggested that abnormalities in sodium transport in hypertension would lead to an increase in intracellular calcium and contractility, which might facilitate the increase in the cytosolic free calcium concentration during alveolar hypoxia and thus augment the vasoconstricting potency. In the current study, exposure of SHR to an 18,000-foot simulated altitude for 4 weeks led to pulmonary hypertension but did not alter systemic arterial pressure compared with the SL control SHR. It is difficult to compare the severity of pulmonary hypertension with other reports since the methods and duration of hypoxia, the methods of arterial pressure measurement, and the conditions of the animals were different. However, judged from the reduced body weight gain, the hematocrit increase, the degree of right ventricular hypertrophy as measured from the percentage increase of right ventricular weight or the RV/LV+S ratio, and the hemodynamic parameters after chronic hypoxia of SHR were comparable to what happened in normotensive rats.17 An increase in LV+S was observed after hypoxia in agreement with some reports on normotensive rats,31 but the cause is not clear.

A deficiency in the ANF system could be involved in the pathogenesis of hypertension, so the depressor action of ANF had been studied extensively in SHR. Injection or sustained infusion of ANF showed a greater11 or no3 difference in systemic blood pressure drop in SHR, but there have been no reports on the response of pulmonary arterial pressure to ANF in SHR. Jin et al12 showed the pulmonary depressor effect of ANF was significantly greater in hypoxia
than in SL control Sprague-Dawley rats; however, there was no significant difference between experimental and control rats in the reduction of systemic arterial pressure. The response of SHR is quite different in the present study. Our results showed an attenuated action of systemic vessels and pulmonary vessels to ANF after long-term hypoxia compared with the air control group in SHR.

From the blood pressure and cardiac output, we determined that ANF caused an insignificant change in vascular resistance. We are planning to study the roles that viscosity and vascular geometry might play after administration of ANF by using intravital microscopy. The depressor action of ANF in this experiment is clearly due to the reduction of cardiac output. The reduction of cardiac output was less prominent in HA rats. The reasons for the attenuated cardiac effect of ANF in hypoxic SHR cannot be deduced from these experiments. However, some hypotheses can be suggested. Chronic hypoxia caused cardiac hypertrophy and polycythemia. Polycythemia due to primary and secondary erythrocytosis could be associated with hypervolemia. The hypervolemia may represent an important determinant of the structural alteration of the cardiovascular system, including the heart. The structural change may result, by some mechanism, in a system less responsive to the action of ANF.

Physical distention of the atrial walls may provide a strong stimulus for the release of ANF, as shown by Dietz, who demonstrated increased release of ANF from the hearts of rats with atria distended by increased venous return pressure. The increase of atrial pressure after pulmonary hypertension induced by 21 days of chronic hypoxia or 14 days after a single dose of monocrotaline injection, resulted in a significant increase of plasma ANF and decrease in the right atrial ANF content. So the contribution of injected or infused ANF to increase the plasma ANF is less in HA rats than in SL rats. Furthermore, long-term changes in the concentration of plasma ANF have been shown to regulate ANF receptor number. Thus, the alterations in the number of affinity of vascular ANF receptors may also relate to the response to ANF observed in the current study. Another possibility is that chronic hypoxia may activate the autonomic system and the secretion of some hormones, which may offset the action of ANF. Further studies will be needed in this area.

In summary, the results of the current study demonstrated that hypertensive rats can adapt to chronic hypoxia and almost the same severity of pulmonary hypertension is produced; however, the hypotensive effect of ANF was attenuated in the pulmonary hypertensive SHR.

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References


**KEY WORDS** • atrial natriuretic factor • hypoxia • pulmonary hypertension • spontaneously hypertensive rat
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