Brain natriuretic peptide inhibits hypoxic pulmonary hypertension in rats

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Brain natriuretic peptide inhibits hypoxic pulmonary hypertension in rats. J. Appl. Physiol. 84(5): 1646–1652, 1998.—Brain natriuretic peptide (BNP) is a pulmonary vasodilator that is elevated in the right heart and plasma of hypoxia-adapted rats. To test the hypothesis that BNP protects against hypoxic pulmonary hypertension, we measured right ventricular systolic pressure (RVSP), right ventricle (RV) weight-to-body weight (BW) ratio (RV/BW), and percent muscularization of peripheral pulmonary vessels (%MPPV) in rats given an intravenous infusion of BNP, atrial natriuretic peptide (ANP), or saline alone after 2 wk of normoxia or hypobaric hypoxia (0.5 atm). Hypoxia-adapted rats had higher hematocrits, RVSP, RV/BW, and %MPPV than did normoxic controls. Under normoxic conditions, BNP infusion (0.2 and 1.4 µg/h) increased plasma BNP but had no effect on RVSP, RV/BW, or %MPPV. Under hypoxic conditions, low-rate BNP infusion (0.2 µg/h) had no effect on plasma BNP or on severity of pulmonary hypertension. However, high-rate BNP infusion (1.4 µg/h) increased plasma BNP (69 ± 8 vs. 35 ± 4 pg/ml, P < 0.05), lowered RV/BW (0.87 ± 0.05 vs. 1.02 ± 0.04, P < 0.05), and decreased %MPPV (60 vs. 74%, P < 0.05). There was also a trend toward lower RVSP (55 ± 3 vs. 64 ± 2, P = not significant). Infusion of ANP at 1.4µg/h increased plasma ANP in hypoxic rats (759 ± 153 vs. 393 ± 54 pg/ml, P < 0.05) but had no effect on RVSP, RV/BW, or %MPPV. We conclude that BNP may regulate pulmonary vascular responses to hypoxia and, at the doses used in this study, is more effective than ANP at blunting pulmonary hypertension during the first 2 wk of hypoxia.

pulmonary circulation; anoxia; atrial natriuretic peptide; brain natriuretic peptide

ATRIAL AND BRAIN NATRIURETIC PEPTIDES (ANP and BNP, respectively) are potent vasodilator and natriuretic agents that are thought to modulate vascular tone and intravascular volume (5). Although discovered in porcine brain, BNP, like ANP, is found in greatest concentration in the heart. Both peptides have a 17-amino-acid loop that interacts with a particulate guanylate cyclase-linked receptor [natriuretic peptide receptor-A (NPR-A)] to raise intracellular guanosine 3',5'-cyclic monophosphate that is thought to mediate their biological actions (14).

Despite these similarities, recent findings suggest differences in the physiological roles of these two peptides. Under normoxic resting conditions, plasma ANP levels and atrial ANP content are an order of magnitude greater than those for BNP (9, 23). Ventricular concentrations of the two peptides are comparable (23). Up to 95% of cardiac ANP secretion derives from the atria, whereas 60% of BNP release comes from the ventricles (23). In hypertrophic disease states, ventricular expression of both peptides is markedly increased and, in some cases of severe congestive heart failure, plasma BNP levels are higher than those of ANP (20). Also, in response to injury, such as acute myocardial infarction, genetic expression of BNP is upregulated in the ventricles more quickly than is that of ANP (8, 21). Thus BNP appears to be primarily a ventricular natriuretic peptide, whereas ANP, as its name implies, is more atrial in derivation. In addition, the half-life of BNP in plasma is longer than that of ANP (20), suggesting a different rate of metabolism. Finally, at equimolar infusion rates, ANP and BNP have similar effects on natriuresis, contraction of plasma volume, and inhibition of aldosterone release, but the increase in plasma guanosine 3',5'-cyclic monophosphate is fourfold higher for ANP (11), suggesting differences in signal transduction. The above differences suggest that ANP and BNP may be part of a dual-peptide system with differing roles in the modulation of vascular responses and intravascular fluid homeostasis (18, 20).

Substantial evidence suggests that ANP mitigates the pulmonary hypertensive and cardiac hypertrophic response to hypoxia and, thereby, protects the right ventricle (RV) from pressure overload and the development of cor pulmonale. Plasma ANP levels rise in response to acute (2) and chronic hypoxia (15) and right ventricular ANP expression increases during chronic hypoxic exposure (9, 16, 25). Exogenous ANP blunts acute hypoxic pulmonary vasoconstriction (2) and attenuates right ventricular hypertrophy and pulmonary vascular remodeling in rats exposed to chronic hypoxia (14), whereas neutralization of circulating ANP by monoclonal antibodies worsens hypoxic pulmonary hypertension (24).

Much less is known about the effect of BNP on pulmonary vascular responses to hypoxia. Like ANP, BNP relaxes preconstricted isolated pulmonary arteries and blunts hypoxic pressor responses in isolated perfused rat lungs (9, 28). Furthermore, the percent increase in plasma BNP levels and right heart BNP expression during exposure to chronic hypoxia is similar to that of ANP (9). BNP also has antiproliferative effects on pulmonary vascular smooth muscle cells in culture (3) and thus may play a role in inhibiting pulmonary vascular remodeling. These data suggest that BNP, in concert with ANP, could play a role in modulating pulmonary hypertensive responses and protecting the right heart from the development of hypoxic pulmonary hypertension. The purpose of this study was to test the feasibility of this hypothesis by determining whether the administration of exogenous BNP inhibits hypoxic pulmonary hypertension and to compare its pulmonary antihypertensive effect to a similar dose of ANP. To accomplish these aims, we
measured right ventricular pressure, right ventricular hypertrophy, and the muscularization of pulmonary vessels in normoxic and chronically hypoxic rats after 2 wk of continuous infusions of BNP, ANP, or saline alone.

**METHODS**

Animals and exposures. Male Sprague-Dawley rats (250–300 g) were obtained from Charles River Breeding Laboratories (Wilmington, MA). Hypoxic rats were housed in hypobaric chambers (0.5 atm). Air intake was regulated to achieve 10–15 volume changes per hour to prevent the accumulation of CO₂, NH₃, or excessive humidity. Rats were given standard rat chow and water ad libitum and were exposed to 12:12-h light-dark cycles. Chambers were opened briefly three times weekly for cleaning and to replenish food and water. Normoxic rats were kept adjacent to the hypoxic chambers in identical cages.

Experimental protocol. Initially, four groups of eight to nine rats were studied. Group 1: normoxia + saline alone, group 2: normoxia + BNP (0.2 µg/h), group 3: hypoxia + saline alone, and group 4: hypoxia + BNP (0.2 µg/h). The rate of BNP infusion was chosen based on the results of a previous study of ANP infusion (15) and on preliminary experiments in our laboratory, demonstrating that this rate of BNP infusion increased plasma BNP levels compared with saline controls under normoxic conditions (76 ± 32 vs. 8 ± 1 pg/ml, P < 0.05). However, no increase in plasma BNP was seen when the rate of infusion was used in rats exposed to chronic hypoxia (10 ± 2 vs. 16 ± 6 pg/ml, BNP infusion vs. saline alone, P = not significant). Also, no changes in pulmonary hemodynamics, right ventricular hypertrophy, or muscularization of peripheral pulmonary vessels were seen in rats given this rate of BNP infusion during chronic hypoxia, compared with hypoxic rats that received saline alone. To study the effects of increased circulating BNP levels during chronic hypoxia, additional studies were done with a higher rate of BNP infusion (1.4 µg/h) under hypoxic conditions (group 5), along with an additional hypoxic saline control (group 6). Simultaneously, another group of rats was given the same dose of ANP (3.4 µg/h) during chronic hypoxia (group 7); these served as positive controls, on the basis of the results of the previous study (12). An additional high-dose BNP group was then studied under normoxic conditions, along with another group of normoxic saline controls (groups 8 and 9).

Animals were anesthetized with ketamine (60 mg/kg im) and pentobarbital sodium (20 mg/kg ip). Miniosmotic pumps (model 2ML2, Alzet, Palo Alto, CA) were filled with ANP or BNP (Peptides International, Louisville, KY) dissolved in a sterile, 0.9% sodium chloride, 10–15 volume changes per hour to prevent the accumulation of CO₂, NH₃, or excessive humidity. Rats were given standard rat chow and water ad libitum and were exposed to 12:12-h light-dark cycles. Chambers were opened briefly three times weekly for cleaning and to replenish food and water. Normoxic rats were kept adjacent to the hypoxic chambers in identical cages.

**Physiological measurements.** After 2 wk of normoxia or hypobaric hypoxia, rats were weighed and anesthetized as described above. A catheter (vinyl tubing, size V3; Biolab Products, Lake Havasu City, AZ) was inserted into the right jugular vein and advanced into the RV. Right ventricular systolic pressure (RVSP) was measured as the mean over 5 min. A second catheter was placed in the right carotid artery. An aliquot of blood (2 ml) was removed for measurement of hematocrit (Hct) and natriuretic peptide concentrations and replaced with an equal volume of saline. Mean systemic arterial pressure (MAP) was measured in the carotid artery over 5 min. The carotid artery catheter was then replaced with a 3-Fr thermistor, which was advanced to 2–3 mm above the aortic valve. Cardiac outputs were measured by a thermodilution computer (Cardiotherm 500, Columbus Instruments, Columbus, OH) after injection of 0.2 ml normal saline into the right ventricular catheter. Cardiac index (CI) was determined as the mean of three cardiac output measurements divided by body weight (BW).

After hemodynamic measurements were completed, the abdomen was opened, and the animal was exsanguinated via the inferior vena cava. The heart and lungs were removed en bloc and weighed after dissection. The RV was separated from the left ventricle (LV) plus interventricular septum (LV+S) for determination of RV/BW, RV/(LV+S), and (LV+S)/BW ratios.

Histology. Lung sections were prepared for histological analysis by using a modification of the technique described by Meyrick and Reid (17). Briefly, cannulas were placed in the trachea and pulmonary artery in situ. Lungs were then removed from the chest and infused with normal buffered Formalin. Intratracheal pressure was fixed at 23 cmH₂O and pulmonary artery pressure at 60 cmH₂O. Transverse sections were cut from the left lower lobe (5 µm) and stained with trichrome. For each animal examined, 35 consecutive vessels with external diameters between 25 and 100 µm were counted. The extent of muscularization of the media interna of each vessel was categorized as nonmuscularized (no muscularization seen), partially muscularized (<3/4 of the media interna), or completely muscularized (>3/4 of the media interna). The percentage of vessels in each category of muscularization was determined by dividing the number of vessels in that category by the total number of vessels counted. Four lungs from each group were examined by two investigators who were blinded to the experimental groups.

**Measurement of ANP and BNP levels.** Arterial blood for ANP and BNP measurements was collected in chilled EDTA tubes and centrifuged immediately. The plasma was frozen in polypropylene tubes at −80°C and assayed within 1 mo of collection. Plasma was acidified with three parts 4% acetic acid and loaded onto Prep-Sep C18 extraction columns (Fisher Scientific, Pittsburgh, PA) that had been prepared with 10 ml methanol and 10 ml deionized water. Samples were eluted with 2 ml 86% ethanol and 4% acetic acid and evaporated by vacuum centrifugation. For ANP, samples were reconstituted in 125 µl of assay buffer and assayed in duplicate by using an enzyme-linked immunoassay (Cayman Chemical, Ann Arbor, MI). Sample immunoreactivity was compared with an unextracted standard curve of rat atriopeptin III [ANP(5–28)]. This assay has 100% cross-reactivity with rat α-ANP and 0% cross-reactivity with rat BNP. For measurement of BNP, extracted plasma samples were reconstituted in 250 µl of assay buffer and assayed in duplicate by using a radioimmunoassay (Peninsula Laboratories, Belmont, CA). Immunoreactivity of plasma samples was compared with an unextracted curve of rat BNP-32. This assay has 100% cross-reactivity with rat BNP-45 and 0% cross-reactivity with ANP. Both assays showed <10% intra-assay variability.

**Statistical analysis.** Data from all experimental and control groups were pooled for statistical comparisons and are expressed as means ± SE. Except where noted otherwise, differences between group means were tested by using one-way ANOVA (Sigma Stat, Jandel Scientific, San Rafael, CA). Where statistically significant differences were found, pairwise comparisons were made with the use of the Student-Newman-Keuls test. Histology readings were analyzed by proportion comparison and Yates correction. Differences were considered significant at P < 0.05.
RESULTS

Chronic hypoxia. Pulmonary hypertension developed in all groups of hypoxic rats, as evidenced by increases in RV/(LV+S), RVSP, and RV/BW compared with normoxic saline controls (Table 1, Figs. 1 and 2). Rats exposed to chronic hypoxia had lower BW and higher Hct than normoxic saline controls (Table 1). Hypoxia had no effect on MAP, CI, or (LV+S)/BW.

Effects of BNP and ANP infusions on BW, Hct, hemodynamics, and ventricular mass. Under normoxic conditions, BNP infusion at 0.2 and at 1.4 µg/h had no effect on BW, Hct, MAP, CI, RV/(LV+S), or (LV+S)/BW (Table 1). Neither rate of BNP infusion affected RVSP or RV/BW (Figs. 1 and 2). Under chronically hypoxic conditions, rats given low dose BNP infusion (0.2 µg/h) had lower BW and higher MAP than rats given saline alone (Table 1), but no differences were seen in Hct, CI, or (LV+S)/BW. There was a trend toward lower RV/(LV+S), but the difference did not reach statistical significance. Low-dose BNP infusion (0.2 µg/h) did not affect RVSP or RV/BW in chronically hypoxic rats (Figs. 1 and 2). In hypoxic rats given high-dose BNP or ANP (1.4 µg/h), no differences were observed in BW, Hct, MAP, CI, RV/(LV+S), or (LV+S)/BW, compared with hypoxic rats given saline alone. High-dose BNP, but not ANP, infusion attenuated the development of RV hypertrophy, as shown by a reduction in RV/BW compared with hypoxic rats given saline alone (Fig. 1). There was also a strong trend toward lower RVSP in hypoxic rats given high-dose BNP, compared with hypoxic rats given saline alone (55 ± 2 vs. 64 ± 3 mmHg), although the difference did not reach statistical significance (Fig. 2).

Muscularization of pulmonary vessels. As shown in Fig. 3, normoxic rats given low and high dose BNP had less percent muscularization of peripheral pulmonary vessels (%MPPV) than rats given saline alone. This effect was evident only in the larger (50–100 µm) sized pulmonary vessels, which showed a decrease in the percentage of fully muscularized vessels (Fig. 3B). Chronic hypoxia caused muscularization of the walls of peripheral pulmonary vessels (Fig. 4) and increased the percentage of fully muscularized vessels in all groups of rats (Fig. 3). Hypoxic rats given high-dose BNP infusion (1.4 µg/h) had less %MPPV in both large and small vessels than did hypoxic rats given either the same rate of ANP infusion or saline alone (Figs. 3 and 4).

Table 1. Effects of brain natriuretic peptide, atrial natriuretic peptide, and saline infusion on body weight, hemodynamics, hematocrit, and ventricular hypertrophy in normoxic and hypoxic rats

<table>
<thead>
<tr>
<th>Condition</th>
<th>BW, g</th>
<th>Hct, %</th>
<th>MAP, mmHg</th>
<th>CI, ml·min⁻¹·kg⁻¹</th>
<th>RV/(LV+S), mg/mg</th>
<th>(LV+S)/BW, mg/g</th>
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<tbody>
<tr>
<td>Normoxic saline</td>
<td>386 ± 8</td>
<td>44 ± 1</td>
<td>109 ± 6</td>
<td>186 ± 18</td>
<td>0.281 ± 0.008</td>
<td>0.197 ± 0.004</td>
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<tr>
<td>Normoxic BNP (0.2 µg/h)</td>
<td>363 ± 9</td>
<td>46 ± 1</td>
<td>93 ± 5</td>
<td>195 ± 25</td>
<td>0.284 ± 0.015</td>
<td>0.201 ± 0.007</td>
</tr>
<tr>
<td>Normoxic BNP (1.4 µg/h)</td>
<td>382 ± 13</td>
<td>42 ± 1*</td>
<td>112 ± 5</td>
<td>255 ± 13</td>
<td>0.496 ± 0.015*</td>
<td>0.208 ± 0.008</td>
</tr>
<tr>
<td>Hypoxic saline</td>
<td>300 ± 13*</td>
<td>67 ± 2*</td>
<td>137 ± 8*</td>
<td>229 ± 12</td>
<td>0.433 ± 0.028*</td>
<td>0.224 ± 0.018</td>
</tr>
<tr>
<td>Hypoxic BNP (0.2 µg/h)</td>
<td>257 ± 13*+</td>
<td>72 ± 2*</td>
<td>108 ± 7</td>
<td>237 ± 32</td>
<td>0.450 ± 0.025*</td>
<td>0.195 ± 0.008</td>
</tr>
<tr>
<td>Hypoxic BNP (1.4 µg/h)</td>
<td>283 ± 10*</td>
<td>61 ± 3*</td>
<td>114 ± 9</td>
<td>194 ± 29</td>
<td>0.505 ± 0.021*</td>
<td>0.202 ± 0.008</td>
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Values are means ± SE; n = 8–12 subjects, except hypoxic saline, where n = 16. BW, body weight; Hct, hematocrit; MAP, mean arterial pressure; CI, cardiac index; RV/(LV+S), right ventricle/(left ventricle + septum); (LV+S)/BW, (left ventricle + septum)/body weight; BNP, brain natriuretic peptide; ANP, atrial natriuretic peptide. *P < 0.05 vs. normoxic saline; †P < 0.05 vs. hypoxic saline.
Plasma BNP and ANP levels. Plasma levels of BNP and ANP in hypoxic rats given saline or high-dose (1.4 µg/h) BNP or ANP infusion are shown in Fig. 5. High-dose BNP infusion increased plasma BNP levels, compared with hypoxic rats given saline alone, but had no effect on plasma ANP levels as expected. Similarly, ANP infusion at the same rate (1.4 µg/h) increased plasma ANP levels, compared with hypoxic rats given saline alone, but had no effect on plasma BNP.

DISCUSSION

The finding that the lower rate of BNP infusion (0.2 µg/h) increased plasma levels under normoxic, but not hypoxic, conditions suggests that hypoxia-adapted rats have a higher rate of BNP metabolism or plasma clearance than do normoxic rats. This is contrary to our expectations, because our earlier studies have shown that pulmonary expression of the natriuretic peptide clearance receptor and plasma clearance of ANP are decreased in hypoxia-adapted rats. Furthermore, Jin et al. (12) were able to raise circulating ANP levels in hypoxia-adapted rats with a much lower rate of infusion. Additional studies are needed to determine whether hypoxia affects the plasma clearance of BNP differently than that of ANP.

The attenuation of hypoxic pulmonary hypertension in the rats given high-dose BNP could have been due to a blunting of hypoxic pulmonary vasoconstriction, an inhibition of pulmonary vascular remodeling, or, more likely, a combination of both. This study was not designed to determine to what extent each of these possibilities was involved. However, we have previously shown that BNP relaxes preconstricted pulmonary rings with the same potency as ANP and that BNP reverses hypoxic pulmonary vasoconstriction in isolated lung preparations (9). In the present study, we have shown that the decrease in RVSP and the lower

| Fig. 2. Right ventricular systolic pressure (RVSP) in rats receiving a continuous infusion of saline, BNP (0.2 or 1.4 µg/h), or ANP (1.4 µg/h) after 2 wk of normoxia (A) or hypobaric hypoxia (B). All pressures were measured in anesthetized rats spontaneously breathing room air. Values are means ± SE; *P < 0.05 vs. normoxic saline.

| Fig. 3. Percent muscularization of small (A; 25–50 µm in diameter) and mid-size (B; 50–100 µm in diameter) peripheral pulmonary vessels in rats receiving a continuous infusion of saline, BNP (0.2 or 1.4 µg/h), or ANP (1.4 µg/h) after 2wk of normoxia or hypobaric hypoxia. Values are means ± SE; *P < 0.05 vs. normoxic or hypoxic saline; †P < 0.05 vs. hypoxic saline; ‡P < 0.05 vs. hypoxic ANP.
RV mass in the BNP-infused rats did not occur as a result of a fall in cardiac output, suggesting that BNP reduced pulmonary vascular resistance. This measurement of cardiac output is particularly important in studies that examine the pulmonary antihypertensive effect of the natriuretic peptides, because ANP has previously been shown to decrease cardiac output (27). Although the changes in RVSP and RV mass seen in this study could also be due to an inhibitory effect of BNP on pulmonary vascular remodeling, the findings of reduced pulmonary hypertension without a change in CI are consistent with a pulmonary vasodilator effect of BNP acting to blunt hypoxic pulmonary hypertension in vivo.

Evidence for elevated plasma BNP levels reducing pulmonary vascular remodeling derives from the finding that BNP infusion reduced %MPPV not only in hypoxia-adapted rats but also in rats kept in normoxic conditions (Fig. 3B). Because BNP did not affect RVSP or CI in normoxic rats, the decrease in %MPPV was likely due to an independent inhibitory effect of BNP on pulmonary vascular smooth muscle growth. A recent report (3) that BNP inhibits proliferation of pulmonary vascular smooth muscle cells in vitro also supports the hypothesis that BNP has a direct antimitogenic effect on the pulmonary circulation.

Our finding that a continuous infusion of ANP during chronic hypoxia had no inhibitory effect on the develop-
BNP INHIBITS HYPOXIC PULMONARY HYPERTENSION

Increased ventricular BNP synthesis and release may contribute to the development of pulmonary hypertension. In hypoxia-adapted rats, the pulmonary antihypertensive effects of BNP could be mediated by increased release of BNP from pulmonary vascular endothelial cells (19). Although ventricular BNP synthesis and release may precede the development of pulmonary hypertension, the role of BNP in modulating pulmonary hypertension remains to be determined.

REFERENCES


