C-type natriuretic peptide (CNP), the third member of the natriuretic peptide family, has a 22-amino acid structure that closely resembles that of atrial and brain natriuretic peptides (ANP and BNP, respectively). First isolated from porcine brain tissue (30), CNP was originally thought to be a neurotransmitter expressed primarily in the central nervous system (18, 30). More recently, CNP has been found in tissues throughout the body including colon, lung, heart, and kidney (24). Although the physiological functions of the natriuretic peptides are not completely understood, all three compounds possess vasorelaxant and antiproliferative properties and appear to mediate vascular tone and remodeling primarily via particulate guanylate cyclase-linked receptors.

Despite structural similarity of CNP to the other natriuretic peptides, considerable evidence suggests that it plays a unique role in circulatory physiology. Whereas ANP and BNP are synthesized and secreted by the heart and interact primarily with the A-type particulate guanylate cyclase receptor, natriuretic peptide receptor-A (NPR-A) (19), CNP is expressed predominantly in the central nervous system and in vascular endothelial cells (31, 32) and exerts its vasoactive effects via the B-type particulate guanylate cyclase receptor, natriuretic peptide receptor-B (NPR-B) (8). The distribution of NPR-A and NPR-B is such that CNP is less potent than ANP and BNP at increasing intracellular cGMP levels in vascular endothelial cells but is up to 10-fold more potent than ANP and BNP at increasing intracellular cGMP levels in vascular smooth muscle (27). In a study on anesthetized dogs (6), CNP had a greater effect on reducing blood pressure than ANP and little, if any, diuretic effect. Furthermore, CNP synthesis in vascular endothelial cells is markedly upregulated by transforming growth factor-β, one of the key growth factors involved in vascular remodeling (22), suggesting that CNP may act as a counterregulatory factor in vascular mitogenesis. Taken together, these findings suggest that unlike ANP and BNP, which appear to act in an endocrine fashion as cardiac hormones, CNP may act in a paracrine fashion to modulate vascular tone and remodeling.

Considerable evidence suggests that ANP and BNP help to mitigate the severity of pulmonary hypertension and right ventricular hypertrophy that develop during exposure to chronic hypoxia. Both peptides are potent pulmonary vasodilators (2, 10) and inhibit proliferation of vascular smooth muscle in vitro (1, 3, 12). Right ventricular expression and circulating levels of both peptides are elevated in hypoxia-adapted rats (10, 33), and continuous infusion of ANP during chronic hypoxia has been shown to blunt pulmonary hypertension, muscularization of peripheral pulmonary vessels, and right ventricular hypertrophy (14). Finally, anti-ANP antibodies have been shown to worsen hypoxic pulmonary hypertension subacutely (13) and chronically (28). Moreover, preliminary studies on ANP-deficient
gene-targeted mice demonstrate higher right ventricular pressures and greater right ventricular mass under both normoxic and chronically hypoxic conditions than are found in mice with normal ANP expression (17). The role of CNP in modulating pulmonary circulatory responses remains to be elucidated. In a preliminary study, Hill et al. (11) found that CNP was a less potent inhibitor of hypoxic pulmonary vasoconstriction in isolated perfused rat lungs than ANP. However, a more recent study (3) suggests that CNP is a more potent antiproliferative agent for rat pulmonary arterial smooth muscle cells. Plasma CNP levels are an order of magnitude lower than ANP levels (6, 32). However, because CNP is synthesized by pulmonary vascular endothelial cells, the concentration of CNP adjacent to pulmonary vascular smooth muscle may be greater than circulating CNP levels. Furthermore, steady-state mRNA levels of the functional CNP receptor NPR-B increase in the lungs during hypoxia (21). If this upregulation of gene expression is associated with higher NPR-B concentrations in pulmonary vascular smooth muscle, then the pulmonary vasodilatory effects of CNP could be potentiated during hypoxia.

The purpose of this study was to explore the potential role of CNP in modulating the pulmonary hypertensive response to hypoxia. We aimed to determine the pulmonary vasodilator potency of CNP relative to ANP and to determine whether the pulmonary vasodilator potency of CNP increases during chronic hypoxia. Furthermore, we hypothesized that if CNP helps to mitigate the development of hypoxic pulmonary hypertension, then circulating CNP levels and pulmonary CNP expression would increase during chronic hypoxia.

METHODS

Hypoxic exposure. Male Sprague-Dawley rats (Charles River Laboratory) weighing between 200 and 300 g were exposed to 3 wk of hypoxia with the use of hypobaric chambers (0.5 atm). Normoxic control animals were housed in identical cages adjacent to the hypobaric chambers. Cages were removed three times weekly for cleaning and replenishment of food and water. Rats were given standard rat chow and water ad libitum.

Isolated vessel studies. After anesthesia with pentobarbital sodium (100 mg/kg ip), rats underwent a median sternotomy and the heart and lungs were removed en bloc. The thoracic aorta and proximal intrapulmonary artery were carefully dissected out and cut into 2- to 3-mm rings. The rings were suspended between tungsten wires (32 µm) in a vessel bath containing Earle’s balanced salt solution (Sigma, St. Louis, MO) at 37°C and aerated with 95% O2-5% CO2. The vessels were hung at tensions of 0.5 g for pulmonary arterial (PA) rings and 1.0 g for thoracic aortic (TA) rings. This has been shown to be optimal for active tension generation in a preliminary study. Vessels were tested for viability and intact endothelium by preconstriction with 10−6 M phenylephrine and demonstration of dose-dependent relaxation to acetylcholine. The baths were then flushed, and dose-response curves were constructed as previously described (10). Briefly, the PA cannula was injected with a bolus of angiotensin II (0.25 ng in 0.1 ml of saline) and then preconstricted with 10−6 M phenylephrine. The rings were then exposed to 10−5 to 10−4 M concentrations of ANP or CNP in saline vehicle or 100 µl of saline alone. Dose-response curves to natriuretic peptides were constructed as previously described (10). Briefly, the PA cannula was injected with a bolus of CNP that contained 10−3 to 10−7 M ANP or CNP in saline vehicle or 100 µl of saline alone. Dose-response curves were constructed as previously described (10).

Measurements in intact animals. After 3 wk of hypoxia or normoxia (control), animals were anesthetized with ketamine (60 mg/kg im) and pentobarbital sodium (20 mg/kg ip). A catheter of V3 tubing (Biolabs, Lake Havasu City, AZ) was inserted into the right jugular vein and advanced into the right ventricle. Right ventricular systolic pressure was measured as the mean systolic pressure over 30 s. Another catheter was placed in the right carotid artery for measurement of mean arterial pressure. Blood (0.3 ml) was removed from the arterial catheter for measurement of hematocrit. Blood for CNP determination was obtained from the inferior vena cava via an abdominal incision. The heart, lungs, and brain were removed and dissected. The heart was separated into right and left atria, right ventricle (RV), and left ventricle plus interventricular septum (LV+S) for measurement of RV-to-body weight (RV/BW), LV+S weight-to-BW [(LV+S)/BW], and RV-to-(LV+S) weight ratio (RV/(LV+S)). Right-sided pressure lines were used for CNP determination and RT-PCR. The left lung was dried at 40°C for 1 wk and reweighed for calculation of lung dry weight-to-body weight ratio (LDW/BW).

Determination of ANP and CNP levels. Blood was collected in chilled syringes with 1 mg/ml of EDTA and 500 kallikrein inhibitory units/ml of aprotinin, and plasma was separated by centrifugation at 4°C. Excised tissues were placed immediately in ice-cold Chomczynski-Sacchi reagent (3.68 M guanidine isothiocyanate, 0.016 M sarkosyl, 0.023 M sodium citrate, 0.18 M sodium acetate, and 0.96 M β-mercaptoethanol plus an equal volume of water-saturated phenol) and homogenized for 15 s at speed 6 with a Brinkmann homog...
Plasma samples and tissue homogenates were stored at −70°C and assayed within 1 mo of collection. Samples were acidified with an equal volume of 1% trifluoroacetic acid (TFA) and loaded onto 200-µg Sep-Pak C18 cartridges (Waters, Milford, MA) that had been conditioned with 1 ml of 1% TFA and three washes of 3 ml of 60% acetonitrile. Columns were then washed twice with 3 ml of 1% TFA, and samples were eluted with 3 ml of 60% acetonitrile, dried by vacuum centrifugation, reconstituted in assay buffer, and run in duplicate. The CNP concentration was measured by RIA with a kit from Phoenix Pharmaceuticals (Mountain View, CA). No cross-reactivity was seen to rat ANP or rat BNP and run in duplicate. The CNP concentration was measured by enzyme-linked immunooassay with a kit from Cayman Chemical (Ann Arbor, MI). Interassay variability was 10% for the CNP RIA and 10–15% for the ANP immunoassay. Protein content of tissue homogenates was measured by colorimetric assay (Bio-Rad, Richmond, CA) with bovine albumin as a standard. Plasma and tissue natriuretic peptide levels are expressed in picograms per milliliter and picograms per milligram of protein, respectively.

RT-PCR. Tissue homogenates were thawed on ice and centrifuged at 3,000 rpm for 15 min at 4°C to remove particulate matter. Total RNA was isolated as previously described (5) by extraction with 0.1 volume of chloroform and precipitation in an equal volume of isopropanol at −20°C overnight. Precipitated RNA was washed once with 70% ethanol, evaporated to dryness in a vacuum centrifuge, and reconstituted in 100 µl of Tris-EDTA buffer. After removal of contaminating genomic DNA by digestion with DNase (MessageClean kit, GenHunter), the final RNA preparation was reconstituted in diethyl pyrocarbonate-treated water, and the concentration was measured by spectrophotometry. Tissue RNA samples (1 µg) were transcribed into cDNA with oligo(dT)15 primers and a first-strand cDNA synthesis kit (Boehringer Mannheim, Indianapolis, IN). For PCR reactions, oligonucleotide primers that were chosen spanned a 444-bp intron and corresponded to bases 151–169 and 379–397 of rat CNP cDNA, as previously described (7). These primers delinated regions of the CNP gene with low homology to ANP and BNP.

Amplification of CNP transcripts was performed with a modification of the protocol by Dean et al. (7). Briefly, 5 µl of the cDNA reaction were incubated with 10 µM Tris, 50 mM KCl (pH 8.3), 1.5 mM MgCl2, 2 mM 3′-deoxythymidine 5′-triphosphates (dNTPs), 1 µl of each primer, and 0.25 µl of Taq polymerase in a total volume of 50 µl. A thermocycler was used to achieve the following temperature protocol: dissociation at 96°C for 1 min, annealing at 56°C for 1 min, and extension at 74°C for 1 min. This was repeated for 35 cycles followed by a 10-min final extension at 72°C. The PCR products were identified by electrophoresis on a 3% agarose gel (2:1 NuSieve to agarose) in 40 mM Tris, 20 mM acetic acid, 2 mM EDTA, pH 8.0, 0.25 µg/ml of ethidium bromide, and a 123-bp ladder (GIBCO) as a molecular-weight standard. A single band was identified migrating at 246 bp, consistent with the 247-bp CNP fragment delineated by the primers. To quantify the abundance of CNP transcripts in tissue samples, relative RT-PCR was employed with β-actin as an endogenous standard. Trial runs were performed to find the optimal number of cycles that would encompass the linear part of the amplification curve for both sets of primers. Template amplification by RT-PCR reached a plateau between 30 and 40 cycles when CNP primers were used and between 20 and 25 cycles when β-actin primers were used. Serial dilutions of reverse-transcribed RNA from each organ were made to yield 1:1, 1:10, and 1:100 concentrations. Additional RT-PCR reactions were performed as described above with the use of both sets of primers on all three concentrations of reverse-transcribed RNA from each group of organs. Reactions were stopped after 20 cycles to measure amplification of PCR products by β-actin primers and after 40 cycles to measure amplification of template by CNP primers. To ensure that no amplification of genomic DNA occurred during PCR, DNase-treated RNA that had not been reverse transcribed was used as an internal control. Amplified CNP and β-actin transcripts were identified by electrophoresis on 3 and 2% agarose gels (for CNP and β-actin primer products, respectively) as described above and subsequently photographed under ultraviolet light. Steady-state CNP transcript levels were quantified relative to β-actin transcripts by determining the concentration of template DNA at which RT-PCR product was first visible. Relative RT-PCR was done on individual samples from the organs of three rats in each experimental group.

Data analysis. Values shown are means ± SE. For isolated vessel and lung experiments, differences between means were tested by two-way ANOVA with repeated measures with a computer software program (SigmaStat; Jandel Scientific Software, San Rafael, CA). All other comparisons were made by paired t-test with the same software program. Differences were considered significant at P < 0.05.

RESULTS

Isolated vessel studies. The vasodilator potencies of ANP and CNP on preconstricted TA and PA rings are shown in Fig. 1. ANP was a slightly more potent vasodilator than CNP in TA and PA rings isolated from normoxic and hypoxia-adapted rats. Exposure to chronic hypoxia did not affect the vasodilator potencies of ANP and CNP on TA rings (Fig. 1A). There was a trend toward a decreased vasodilator effect of ANP and CNP on PA rings isolated from hypoxia-adapted rats compared with normoxic rats, but the differences did not reach significance (P = 0.09 and 0.21 for ANP and CNP, respectively; Fig. 1B).

Isolated lung studies. To determine the relative potencies of ANP and CNP on hypoxic pulmonary vasoconstriction, dose-relaxation curves were constructed for both peptides with the use of isolated intact lungs obtained from normoxic and hypoxia-adapted rats (Fig. 2). Acute hypoxia increased PA perfusion pressure from 12.2 ± 0.8 to 18.0 ± 0.6 mmHg in isolated lungs from normoxic rats and from 18.1 ± 0.9 to 21.1 ± 1.1 mmHg in lungs from hypoxia-adapted rats. The pulmonary presor response to hypoxia was stable in normoxic and hypoxia-adapted lungs. Both ANP and CNP blunted hypoxic pulmonary vasoconstriction in isolated lungs obtained from normoxic rats, but ANP was more potent than CNP. At the maximum dose tested (10−8 mol), ANP completely reversed hypoxic pulmonary vasoconstriction, whereas CNP reduced peak PA pressure only ~30%. In isolated lungs obtained from hypoxia-adapted rats, ANP, but not CNP, blunted hypoxic pulmonary vasoconstriction. The vasodilator potencies of both ANP and CNP were less in hypoxia-adapted rat lungs than in lungs obtained from normoxic rats.

Cardiopulmonary responses to hypoxia. As shown in Table 1, rats exposed to 3 wk of hypoxia had lower body weights and higher hematocrits than normoxic rats.
There was no difference in mean systemic arterial blood pressure between the two groups, but, as anticipated, hypoxia-adapted rats had higher PA pressures than normoxic rats as evidenced by more than a twofold increase in right ventricular systolic pressure. The development of pulmonary hypertension in the hypoxia-adapted rats was accompanied by severe right ventricular hypertrophy. Right ventricular mass referenced either to body weight (RV/BW) or to LV+S [RV/(LV+S)] nearly doubled in hypoxia-adapted rats, although the difference did not reach significance. This may have been related to the lower body weight compared with normoxic rats, as opposed to an actual increase in left ventricular mass. Hypoxic rats had higher LDW/BW values than normoxic rats, consistent with increased lung mass related in part to pulmonary vascular remodeling.

Plasma and tissue levels of CNP and ANP. Circulating CNP levels were within the range previously reported (6) and were 2.5 times higher in hypoxia-adapted rats than in normoxic control rats (15 ± 2 vs. 6 ± 1 pg/ml; P < 0.01). Figure 3 shows normoxic and hypoxic rat CNP and ANP levels normalized to tissue protein concentrations in the brain, right heart, and lung. In comparison to ANP, CNP levels in normoxic rats were approximately 30-fold higher in brain, 3-fold higher in lung, 50-fold lower in the right atrium, and nearly the same in the right ventricle. As reported previously, exposure to chronic hypoxia increased right ventricular ANP markedly (Fig. 3). There was a trend toward lower right atrial ANP levels in hypoxia-adapted rats, but the difference did not reach significance. Chronic hypoxia had little effect on ANP levels in the lung and brain. The effect of chronic hypoxia on tissue CNP content was most pronounced in lung where it fell nearly 75% (Fig. 3). This marked decrease could not be attributed to higher protein levels because hypoxia-adapted rat lung protein concentrations were not significantly greater than those from normoxic rats (11.9 ± 1.2 vs. 9.7 ± 1.1 µg/ml; P = 0.178). Exposure to chronic hypoxia also decreased right atrial CNP levels but had no effect on CNP concentrations in the right ventricle or brain (Fig. 3).

Steady-state CNP transcript levels. Because expression of the CNP gene in the lungs of normoxic and hypoxia-adapted rats could not be detected by Northern blot analysis, we measured steady-state CNP transcript levels by RT-PCR (Fig. 4). In concordance with tissue CNP concentrations, a greater degree of CNP gene expression was seen in the brain than in the right heart or lung. With the use of β-actin as an endogenous standard, right atrial CNP gene expression in hypoxia-adapted rats was higher than in normoxic rats, as evidenced by a greater than twofold increase in CNP gene expression (Fig. 4).

Fig. 1. Percent relaxation of isolated thoracic aortic rings (A) and isolated pulmonary arterial rings (B) obtained from normoxic (N) and hypoxia-adapted (H) rats in response to increasing concentrations of atrial and C-type natriuretic peptides (ANP and CNP, respectively) or to repeated dosing of vessel bath with same volume of normal saline (control). Vessels were preconstricted with phenylephrine (10−6 M). Values are means ± SE; n = 6–12 vessels/group. *P < 0.05 vs. baseline. †P < 0.05 vs. ANP.

Fig. 2. Percent reduction in pulmonary arterial perfusion pressure (PAP) of isolated lungs ventilated with 5% CO2-95% N2 in response to increasing concentrations of ANP and CNP. Lungs were obtained from N and H rats. Values are means ± SE; n = 6 vessels/group. *P < 0.05 vs. control. †P < 0.05 vs. ANP. ‡P < 0.05 vs. normoxia.

Fig. 3. CNP and ANP levels in normoxic and hypoxic rats.
adapted rats was decreased compared with that in normoxic rats (Fig. 4), consistent with the finding of lower CNP levels (Fig. 3). No differences in CNP transcripts were detected between normoxic and hypoxia-adapted rats in the brain, right ventricle, or lung.

DISCUSSION

We had anticipated that the vasodilator potency of CNP would be increased in the pulmonary circulation of hypoxia-adapted rats because the genetic expression of the guanylate cyclase-linked receptor for CNP (NPR-B) is upregulated in the lung during hypoxia (21). However, we found no evidence to support this hypothesis. The vasodilator potency of CNP on PA rings from normoxic rats was slightly less than that of ANP and did not change significantly in hypoxia-adapted rats. Furthermore, CNP was a considerably less potent inhibitor of hypoxic pulmonary vasoconstriction than ANP in isolated perfused lungs from normoxic rats and had no detectable effect on hypoxic pulmonary vasoconstriction in isolated lungs from hypoxia-adapted rats. It is possible that the posttranscriptional rate of NPR-B expression is not enhanced during hypoxia despite the increase in steady-state mRNA levels. Alternatively, NPR-B expression may be increased elsewhere in the lung besides the pulmonary vascular bed, or hypoxia may decrease the responsiveness of NPR-B to CNP.

Our finding of a reduced pulmonary vasodilator effect of ANP in hypoxia-adapted animals conflicts with a previous study by Jin et al. (15), who reported that ANP caused a greater reduction in PA pressure in hypoxia-adapted rats than in normoxic control rats with the use of both intact animals and isolated lung preparations. However, Chen et al. (4) reported that ANP has less of a pulmonary vasodilator effect in hypoxia-adapted animals than in normoxic control animals. Furthermore, the studies both by Jin et al. (15) and Chen et al. (4) measured the effect of ANP on baseline perfusion pressure, as opposed to the present study in which we measured the ability of ANP to reverse hypoxic pulmonary vasoconstriction. Because the hypoxia-induced increase in PA perfusion pressure was only about one-half as great in hypoxia-adapted lungs as in normoxic lungs, it is possible that the reduced vasodilator effect of ANP in hypoxia-adapted lungs was due to lower pulmonary vascular tone rather than decreased responsiveness to ANP. Recently, ANP was shown to be less effective at reversing pulmonary pressor responses to the endoperoxide analog U-46619 in isolated lungs obtained from hypoxia-adapted vs. normoxic newborn piglets (26), consistent with the findings in our study. Regardless of the effect of chronic hypoxia on ANP vasodilator potency, our data show that CNP is a weaker pulmonary vasodilator than ANP in normoxic

Table 1. Effects of chronic hypoxia on BW, RVSP, MAP, Hct, heart weights, and LDW

<table>
<thead>
<tr>
<th></th>
<th>BW, g</th>
<th>Hct, %</th>
<th>MAP, mmHg</th>
<th>RVSP, mmHg</th>
<th>RV/BW, mg/g</th>
<th>RV/(LV+S), mg/g</th>
<th>(LV+S)/BW, mg/g</th>
<th>LDW/BW, mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoxia</td>
<td>404 ± 7</td>
<td>41 ± 0.5</td>
<td>110 ± 5</td>
<td>32 ± 2</td>
<td>0.56 ± 0.02</td>
<td>0.27 ± 0.01</td>
<td>2.06 ± 0.05</td>
<td>0.24 ± 0.02</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>300 ± 3*</td>
<td>77 ± 1.5*</td>
<td>123 ± 9</td>
<td>77 ± 3*</td>
<td>1.30 ± 0.04*</td>
<td>0.51 ± 0.02*</td>
<td>2.60 ± 0.08</td>
<td>0.40 ± 0.01*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6–16 rats. BW, body weight; RVSP, right ventricular systolic pressure; MAP, mean arterial pressure; Hct, hematocrit; RV, right ventricle; LV+S, left ventricle and septum; LDW, lung dry weight. *P < 0.01 vs. normoxia.

Fig. 3. ANP and CNP levels in brain, right (R) atria, right ventricle, and lung of normoxic and hypoxia-adapted rats. Values are means ± SE; n = 8–15 rats. *P < 0.05 vs. normoxic rats.
and hypoxia-adapted rats and argue against a role for CNP in protecting against hypoxic pulmonary vasoconstriction.

It is possible that CNP could act to blunt hypoxic pulmonary hypertension via other mechanisms such as inhibition of pulmonary vascular remodeling. In a recent study, Furaya et al. (9) demonstrated a pronounced antiproliferative effect of CNP in an in vivo model of carotid artery intimal thickening and found that the antiproliferative effect of CNP was more potent than that of ANP in systemic vascular smooth muscle cells. If CNP has a similar effect on pulmonary vascular smooth muscle, elevated levels of CNP in the plasma or pulmonary vascular tissue during chronic hypoxia could blunt pulmonary vascular remodeling.

In our study, circulating CNP levels were within the range previously reported (6) and rose two- to threefold during hypoxia, as we previously observed for both ANP and BNP (10). However, in contrast to the increase in right heart ANP and BNP synthesis that develops in the right ventricles of hypoxia-adapted rats (10, 33), we saw no evidence of increased CNP synthesis in the heart, lung, or brain after chronic hypoxia. In fact, CNP concentrations fell in the right atria and lungs of hypoxia-adapted rats compared with normoxic control rats. In an earlier study (23), a drop in right atrial ANP concentration during hypoxia was interpreted as increased ANP secretion, suggesting that the right atrium is an important source of increased plasma ANP levels during hypoxia. However, it is unlikely that the drop in right atrial and pulmonary CNP concentrations observed in the present study represents increased CNP synthesis by these organs or that these organs produce enough CNP to raise circulating levels. In fact, CNP concentrations fell in the right atria and lungs of hypoxia-adapted rats compared with normoxic control rats. In an earlier study (23), a drop in right atrial ANP concentration during hypoxia was interpreted as increased ANP secretion, suggesting that the right atrium is an important source of increased plasma ANP levels during hypoxia. However, it is unlikely that the drop in right atrial and pulmonary CNP concentrations observed in the present study represents increased CNP synthesis by these organs or that these organs produce enough CNP to raise circulating levels.

In our study, circulating CNP levels were within the range previously reported (6) and rose two- to threefold during hypoxia, as we previously observed for both ANP and BNP (10). However, in contrast to the increase in right heart ANP and BNP synthesis that develops in the right ventricles of hypoxia-adapted rats (10, 33), we saw no evidence of increased CNP synthesis in the heart, lung, or brain after chronic hypoxia. In fact, CNP concentrations fell in the right atria and lungs of hypoxia-adapted rats compared with normoxic control rats. In an earlier study (23), a drop in right atrial ANP concentration during hypoxia was interpreted as increased ANP secretion, suggesting that the right atrium is an important source of increased plasma ANP levels during hypoxia. However, it is unlikely that the drop in right atrial and pulmonary CNP concentrations observed in the present study represents increased CNP synthesis by these organs or that these organs produce enough CNP to raise circulating levels. In fact, CNP concentrations fell in the right atria and lungs of hypoxia-adapted rats compared with normoxic control rats.

In view of the lack of any discernible increase in CNP expression in the heart, lung, or brain, it is unlikely that the increase in plasma CNP levels in hypoxia-adapted rats was the result of increased CNP secretion. An alternative mechanism for the increase in circulating CNP levels may be a reduction in plasma CNP clearance. One of the primary routes of CNP elimination from the circulation is binding to the natriuretic peptide clearance receptor (NPR-C) followed by internalization and degradation of the peptide. Although clearance of CNP from the circulation has not been examined in hypoxia-adapted animals, Klinger et al. (16) have previously shown that the concentration of pulmonary NPR-C binding sites markedly decreases during hypoxia and that ANP clearance from the circulation is reduced. Other investigators (21) have shown a downregulation of steady-state NPR-C mRNA levels in the lungs of hypoxia-adapted rats. Because CNP and ANP have similar binding affinities for NPR-C, it is likely that the hypoxia-induced decrease in pulmonary NPR-C concentration slows pulmonary clearance of CNP from the circulation and contributes to elevated plasma CNP levels.

Although CNP was initially thought to be localized to the central nervous system and vascular endothelial cells, our finding of significant amounts of CNP in the heart and lungs is consistent with more recent reports (7, 24) of CNP mRNA transcripts in numerous organs, including colon, kidney, and heart. The presence of CNP in the heart and lungs is not related to cross-

![Fig. 4. Relative RT-PCR comparison of CNP and β-actin transcripts in organs obtained from normoxic and hypoxia-adapted rats. Representative blots are from 1 of 3 experiments. RT-PCR reactions were stopped after 20 cycles for β-actin products and after 40 cycles for CNP. Lanes in each blot (left to right) are nontranscribed RNA (negative control), cDNA diluted 1:100, cDNA diluted 1:10, and cDNA undiluted.](http://ajplung.physiology.org/)
reactivity with other natriuretic peptides because we found no cross-reactivity of the anti-CNP antibody used in this study with ANP or BNP, even at concentrations well above those found in these tissues. It is also unlikely that the isolation of CNP transcripts in the heart and lungs could be attributed solely to vascular endothelial cells within the organ. If the presence of CNP in the heart and lungs were limited to the vascular endothelium, we would have expected to find the greatest concentration of CNP in the lungs because of its greater endothelial cell surface. The fourfold higher concentration of CNP in the atria than in the lungs suggests that either CNP expression in the heart is not limited to the endothelium or the endothelial concentration of CNP is greater in the heart. In either event, our findings indicate that the atria may serve as sites of synthesis for circulating CNP that are downregulated during hypoxia.

Additional bands larger than the true CNP product were noted in some of the PCR reactions. Contamination of the reaction with genomic DNA was excluded by the lack of a PCR product in control lanes that contained RNA from the same samples that had not been reverse transcribed. It is possible that posttranscriptional processing of CNP results in multiple transcript sizes in some tissues.

In the present study, we found that CNP synthesis in the heart and lung is unchanged or reduced during hypoxia, whereas circulating CNP levels increase. The low circulating levels of CNP (2 orders of magnitude lower than ANP) under normoxic and hypoxic conditions suggest that circulating CNP is unlikely to play an important role in modulating pulmonary vascular tone relative to ANP. However, CNP levels in whole lung homogenates and in the systemic circulation may not reflect CNP activity in the pulmonary vascular bed. Recent studies (20, 31) suggest a paracrine role for CNP whereby it is synthesized and secreted in endothelial cells adjacent to vascular smooth muscle and then acts locally to inhibit vasoconstriction and vascular mitogenesis. Additional studies are needed to determine whether CNP expression in pulmonary vascular endothelial cells is augmented during chronic hypoxia in vivo. In the absence of such studies, our findings suggest that CNP plays a minor role compared with that of the other natriuretic peptides in protecting against the development of hypoxic pulmonary hypertension. Alternatively, it is possible that a hypoxia-related reduction in CNP synthesis in pulmonary vascular endothelial cells could lead to disinhibition of vascular smooth muscle growth (20) and thus be a contributing factor in the development of hypoxic pulmonary hypertension.

This work was supported by a development grant from Rhode Island Hospital and National Heart, Lung, and Blood Institute Grants HL-02613 (to J. R. Klinger) and HL-45050 (to N. S. Hill).

Address for reprint requests: J. R. Klinger, Division of Pulmonary, Sleep, and Critical Care Medicine, Rhode Island Hospital, SWP Rm 420, Providence, RI 02903.

Received 17 February 1998; accepted in final form 4 June 1998.

REFERENCES


