Pulmonary Vasodilatory Action of Endogenous Atrial Natriuretic Factor in Rats With Hypoxic Pulmonary Hypertension
Effects of Monoclonal Atrial Natriuretic Factor Antibody

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We administered ascitic fluid containing atrial natriuretic factor (ANF) monoclonal antibody to rats after 3 weeks of exposure to hypoxia while the rats were still hypoxic. In additional chronically hypoxic rats, we infused synthetic rat ANF. In conscious chronically instrumented rats, after a bolus dose of 5 μg i.v. ANF, pulmonary arterial pressure fell significantly from 26.5±2 to 21±2 mm Hg (p<0.01), reaching its nadir at 5 minutes without change of systemic arterial pressure, cardiac output, or heart rate. Pulmonary arterial pressure increased gradually from 26±4 to 34±4 mm Hg within 30 minutes (p<0.05) after acute administration of ANF monoclonal antibody and decreased transiently to return to baseline within 15 minutes after infusion of control ascitic fluid containing monoclonal antibody against an apolipoprotein. Cardiac output and heart rate remained unchanged after both ANF monoclonal antibody and control ascitic fluid. In normoxic rats, acute administration of ANF monoclonal antibody did not cause significant changes in pulmonary arterial pressure, cardiac output, or heart rate. Rats receiving weekly intravenous injections of ANF monoclonal antibody that were started before initiation of exposure to hypoxia experienced significantly aggravated pulmonary hypertension and right ventricular hypertrophy compared with rats receiving repeated infusions of control ascitic fluid. However, there was no significant difference in small pulmonary arterial wall thickness or percentage of muscularized arteries at the alveolar duct level. These results suggest that endogenous ANF attenuates hypoxic pulmonary hypertension by decreasing pulmonary vascular tone. (Circulation Research 1992;70:184–192)

Several laboratories, including ours, have documented increased plasma levels of atrial natriuretic factor (ANF) in animals exposed to chronic hypoxia1–3 as well as humans exposed to high altitude4 or patients suffering from chronic obstructive lung disease.5 In a previous study, we found that gene expression for ANF is enhanced in both right and left ventricles of chronically hypoxic rats, suggesting that during chronic hypoxia, production of ANF by the ventricles may contribute to the high levels of circulating ANF.1

Several lines of evidence suggest that ANF may play a compensatory role in the pathophysiology of hypoxic pulmonary hypertension. ANF has been shown to be a potent vasorelaxant of the pulmonary circulation when its tone has been increased by hypoxia or various constrictor agonists. It relaxes precontracted isolated pulmonary vessels of guinea pigs, pigs, and humans.6,7 Administration of ANF blunts pulmonary vasoconstriction induced by acute hypoxia in conscious rats and in anesthetized rats, dogs, and pigs.8–11 Experiments using the isolated lung of chronically hypoxic rats have demonstrated significant vasorelaxant effects of ANF.12 Acute administration of synthetic ANF in patients with chronic obstructive lung disease and pulmonary hypertension causes pulmonary vasodilation, increase of cardiac output (CO) and ventilation, and suppression of aldosterone secretion.13 Moreover, in a recent study Jin et al14 reported that, in rats developing pulmonary hypertension after exposure to chronic hypoxia, continuous infusion of ANF attenuates pulmonary hypertension and right ventricular hypertrophy and decreases the wall thickness of small pulmonary arteries.
Hypoxic pulmonary hypertension is the consequence of increased smooth muscle tone and structural remodeling of the pulmonary arteries. Indeed, increase of pulmonary arterial pressure (PAP) during chronic hypoxia is associated with hypertrophy and hyperplasia of smooth muscle cells of normally muscularized arteries and appearance of new smooth muscle in nonmuscular and partially muscular segments of the intra-acinar circulation. In a recent study, Itoh et al demonstrated that ANF is an inhibitor of growth and proliferation of cultured rat aortic smooth muscle cells and suggested that ANF may inhibit effects of various growth factors on vascular smooth muscle. These observations led us to hypothesize that increased secretion of ANF may have a compensatory role in hypoxic pulmonary hypertension and that endogenous ANF, by decreasing tone and attenuating structural remodeling, may have a protective effect relative to the development of hypoxic pulmonary hypertension.

Since there is no pharmacological antagonist of ANF, passive immunization with monoclonal antibody (MAB) against ANF has been previously used as a tool to elucidate the role of endogenous ANF in various experimental and pathophysiological situations such as blood volume expansion, cardiac failure, and systemic hypertension. In the present study, we used MAB against rat ANF to assess the effect of acute as well as chronic blockade of endogenous ANF on the pulmonary vascular bed during exposure of rats to chronic hypoxia.

Materials and Methods

Chronic Hypoxia

Male Wistar rats weighing 250–300 g at the start of the experiment were exposed to chronic hypoxia (10–12% O2) in a 500-l ventilated chamber (France, Cachan, France) as previously described. To establish the hypoxic environment, the chamber was flushed with a mixture of room air and nitrogen, and gas was recirculated. The chamber environment was monitored with an oxygen analyzer (Servomex OA150, Crowborough, England). Carbon dioxide was removed by soda lime granules, and excess humidity was prevented by cooling of the recirculation circuit. Chamber temperature remained at 22–24°C. The chamber was opened every other day for ~2 hours to clean the cages and replenish food and water. Normoxic rats were kept in the same room, with the same light–dark cycle. Rat chow and tap water were provided ad libitum.

Hemodynamic Measurements in Conscious Rats

Rats were anesthetized with an intramuscular injection of ketamine (20 mg) and xylazine (1 mg). The right jugular vein was exposed, and a polyvinyl catheter was inserted and manipulated through the right ventricle into the pulmonary artery. Location of the catheter was assessed from the shape of the pressure tracing. A polyethylene catheter (PE-50) was inserted into the right carotid artery. A silastic catheter was inserted into the left jugular vein for return of blood to the rat during subsequent cardiac output measurements. A second polyvinyl catheter was also inserted into the left jugular vein to allow injection of drugs and indocyanine green dye. The catheters were filled with heparinized saline, sealed, and tunneled under the skin to the back of the neck, where they were exteriorized, secured, and protected in a small plastic container. Hypoxia-adapted rats were returned to the hypoxic chamber after recovery from anesthesia.

Measurements were made 1 day after surgery while the rat breathed the same gas mixture as that to which it had been chronically exposed. That is to say, those rats exposed to hypoxia for 3 weeks were placed in a small Plexiglas box flushed with 10–12% O2–88% N2. The control rats were studied in the same Plexiglas box flushed with room air. PAP and systemic arterial pressure (SAP) were measured using Gould P 23 ID transducers, coupled to pressure modules and a Gould TA 550 multichannel recorder (Gould, Ballainvilliers, France). Heart rate was computed from the SAP recording. CO was measured by a dye dilution technique. A 50-µg bolus of indocyanine green dye (1 mg/ml) was injected into the jugular vein. Blood (1 ml/min) was withdrawn from the carotid artery through a densitometer cuvette (Waters) and returned to the rat through the left jugular vein. CO was calculated from the dye dilution curve area after exponential extrapolation of the down slope. Calibration was performed at the end of each study with a known concentration of green dye (5 µg/ml rat blood). Each CO value was the mean of at least three determinations. Total pulmonary resistance was calculated as mean PAP divided by CO. Total systemic vascular resistance was calculated as mean SAP divided by CO. Percent change of hemodynamic value was calculated as (change/baseline) × 100.

Preparation and Administration of MAB Against ANF

The preparation of MAB against ANF (MAB-ANF) was performed by a standard method as described previously in detail. In brief, female 8-week-old BALB/c mice were successively injected at 3-week intervals with two subcutaneous injections (25 µg) and one intravenous injection (10 µg) of rat synthetic ANF-(101–126), which had been covalently coupled to bovine thyroglobulin by the carbodiimide method. The molar ANF to thyroglobulin ratio was 30:1. Cell fusion used spleen cells taken from the mice 3 days after the intravenous injection. After the fusion, cells were distributed in 600 microculture wells, and supernatants were screened by a solid-phase radioimmunoassay for the presence of antibodies reactive with both the free peptide ANF-(101–126) and ANF-(101–126) coupled to bovine serum albumin. Cells in positive wells were recloned at least twice by limiting dilution. BALB/c mice were injected intraperitoneally with cultured hybridoma cells. When sufficient ascitic fluid had accumulated,
the mice were killed, and the ascitic fluid was collected. The fluid, used as a source of MAb, was stored at \(-80^\circ\)C and thawed the day of the experiment. In a previous study, the ability of MAb-ANF to react with native ANF was demonstrated: the MAb was shown to neutralize the diuretic activity of rat atrial extracts when injected in vivo in rats. Moreover, Figure 5 demonstrates the ability of the vial of MAb-ANF ascitic fluid we used in the current study to bind with synthetic rat ANF. Specificity of the MAb-ANF has been previously described. In brief, the two aminoterminal residues of rat ANF are necessary for ANF to react with MAb-ANF. The MAb showed little cross-reactivity with extracts of rabbit atri and human synthetic ANF but recognized ANF-related peptides in mouse and hamster atrial extracts. Affinity chromatography of the ascitic fluid showed that the MAb secreted is of the immunoglobulin G1 subclass. Protein concentration in ascitic fluid was 70 mg/ml (Lowry method). Ascitic fluid containing MAb against a human apolipoprotein B (MAb-ALPB) was used as a control.

**Acute Hemodynamic Studies After Chronic Exposure to Hypoxia**

Acute hemodynamic studies were performed in conscious rats after 3 weeks of exposure to hypoxia, while the rats remained in the hypoxic environment.

**Effects of synthetic rat ANF.** Seven rats were injected with an intravenous bolus of 0.5 \(\mu\)g synthetic rat ANF. Five of these seven rats received a second bolus of 5 \(\mu\)g ANF 30 minutes later. Mean PAP and SAP were continuously monitored, and CO was measured before and 5, 15, and 30 minutes after each bolus.

**Effects of MAb-ANF.** Nine rats were injected with an intravenous bolus of ascitic fluid (300 \(\mu\)l). In five rats, the injectant contained MAb-ANF. In four rats, MAb-ALPB was used to define a control hypoxic sample. Mean PAP and SAP were continuously monitored, and CO was measured before and 15 and 30 minutes after the bolus.

An additional group of five rats that had not been previously exposed to hypoxia and had been breathing room air at the time of the study received an intravenous bolus of ascitic fluid containing MAb-ANF and was similarly studied.

**Chronic Administration of MAb-ANF During Exposure to Hypoxia**

In 18 rats, intravenous administrations of ascitic fluid (300-\(\mu\)l bolus through the tail vein) were repeated three times at 1-week intervals during chronic exposure to hypoxia. The first injection was performed immediately before starting exposure to hypoxia. Rats were randomly divided into two groups: one group (\(n=9\)) received injections of ascitic fluid containing MAb-ANF, and the other group (\(n=9\)) received the ascitic fluid containing MAb-ALPB and served as a control. Before each injection, body weight was measured, and venous blood was sampled for hematocrit determination. At the end of the third week of exposure to hypoxia, 1 week after the last injection of ascitic fluid, the rats were anesthetized with ketamine and xylazine. Immediately after insertion of catheters in pulmonary and carotid arteries, PAP and SAP were measured. Blood was collected from the carotid artery catheter for measurements of plasma sodium, chlorine, proteins, creatinine, urea, hematocrit, and MAb titer. Finally, after an intraperitoneal injection of sodium pentobarbital (20 mg/kg), the thorax was opened, and the heart was quickly removed. After freezing, the heart was sectioned halfway between the apex and the atrioventricular groove. The ratio of right ventricular free wall thickness to the sum of septal plus left ventricular free wall thickness was microscopically determined after coloration with hematoxylin phloxine saffron and used as an index of right ventricular hypertrophy.

The lungs were fixed in the distended state by infusion of 4% aqueous buffered formalin into the trachea at 30-cm \(H_2O\) pressure. The entire specimen was placed in a bath of the same fixative for a week. Midsagittal slices 4 mm thick were taken from both lungs. Sections 5 \(\mu\)m thick were cut for light microscopy and stained with hematoxylin phloxine saffron and orcein-picroindigo-carmine. In each rat, the median wall thickness of at least 20 muscular or partially muscular consecutive arteries (range, 50–100 \(\mu\)m) was determined under \(\times312\) magnification and related to the external diameter as a percent wall thickness (% WT) according to the formula:

\[
\%WT = \frac{2 \times \text{medial thickness} \times 100}{\text{external diameter}}
\]

For each artery, the structure of the accompanying airway was identified as precinar airway, terminal bronchiolus, respiratory bronchiolus, or alveolar duct. Percentage of muscularized and partially muscularized arteries at the alveolar duct level was determined as well as the median wall thickness of these arteries.

**Titer of Administered MAb in Rats In Vivo**

Blood was collected at the end of the hemodynamic measurement, 1 week after the last and third injection of ascitic fluid. Diluted plasma (50 \(\mu\)l) was incubated with 50 \(\mu\)l assay buffer containing 32 PM of a 3-[\(^{125}\)]iodotyrosyl derivative of ANF (74TBq/mmol, Amersham International, Amersham, Buckinghamshire, UK). Free and bound peptide were separated by precipitation with globulin (0.25% [wt/vol]) and polyethylene glycol (PEG 4000, 25% [wt/vol]). Specific binding of [\(^{125}\)]rat ANF was determined by measuring total binding and subtracting nonspecific binding with diluted plasma of the control rats having received MAb-ALPB.

**Statistical Analysis**

Results are expressed as mean±SEM. Student’s t test for comparisons of percentage values between
two groups was performed after arcsine transformation of individual values. A two-way analysis of variance (ANOVA) was used to evaluate the acute hemodynamic effects of exogenous ANF or MAb administration; the two factors in the analysis were time and animal. Comparison between values at different times after the bolus injection and preinjection value was carried out with a modified t test using the residual value to compute the t value.

Results

Acute Hemodynamic Effects of Synthetic Rat ANF in Chronically Hypoxic Rats

Intravenous bolus administration of rat synthetic ANF (0.5 μg) was followed by a significant fall in PAP from 25±2 to 22±2 mm Hg at 5 minutes with a return to baseline within 15 minutes. CO, heart rate, and SAP remained unchanged. Despite the significant decrease of PAP, pulmonary vascular resistance did not change significantly, nor did systemic vascular resistance.

In five of the seven rats receiving a second bolus of 5 μg rat ANF, PAP decreased significantly at 5 minutes and returned to baseline within 30 minutes (Figure 1). The magnitude of the PAP response was dose dependent, with the drop of PAP being more profound after 5 μg than after 0.5 μg rat ANF (p<0.05). However, CO, heart rate, and SAP remained unchanged. Although two-way ANOVA did not yield overall significant change of pulmonary vascular resistance over time, this calculated value decreased at 5 minutes compared with baseline in every rat (−18±6%).

Acute Hemodynamic Effects of MAb

In the five normoxic rats receiving ascitic fluid with MAb-ANF, PAP, CO, and heart rate remained unchanged as did pulmonary vascular resistance (Fig-
ure 2). Increase of SAP and systemic vascular resistance did not reach statistical significance.

The intravenous bolus of ascitic fluid with MAb-ANF in five chronically hypoxic rats was followed by a gradual increase of PAP, which was significantly different from baseline value at 15 and 30 minutes (Figure 3). Although heart rate and CO remained unchanged, there was a gradual increase of SAP also significantly different from baseline at 15 and 30 minutes. Pulmonary vascular resistance was significantly increased at 15 and 30 minutes, but increase of systemic vascular resistance did not reach statistical significance.

In contrast, in four chronically hypoxic rats, bolus injection of ascitic fluid with MAb-ALPB was followed by a transient and significant fall in PAP at 5 minutes, with a return to baseline within 15 minutes (Figure 3). CO and heart rate remained unchanged, but SAP increased gradually and was significantly greater than baseline at 30 minutes.

**Chronic Administration of MAb-ANF During Exposure to Hypoxia**

Increase of body weight during exposure to hypoxia was similar in the rats receiving MAb-ANF and control rats injected with the MAb-ALPB (Table 1). Hematocrit was maximal at the end of the first week of hypoxia and remained stable and similar in the two groups. At the end of 3 weeks of exposure to hypoxia, plasma concentrations of sodium, chloride, creatinine, proteins, and urea were similar and within normal limits in both groups (Table 2).

SAP and heart rate measured in the anesthetized rats were similar in both groups, but the PAP of rats receiving MAb-ANF was significantly higher than that of control rats (Table 3). The ratio of right ventricular wall thickness to septal plus left ventricular wall thickness was also higher in the group with blockade of ANF than in the control group (Figure 4). In contrast, medial wall thickness of small pulmo-
nary arteries (50–100 μm) as well as percentage of muscularized and partially muscularized arteries at the alveolar duct level did not differ significantly between the two treatments. In addition, medial wall thickness of muscularized arteries at the alveolar duct level was not significantly different between the group receiving MAb-ANF (42.1±3.1%) and the group receiving MAb-ALPB (37.3±2.0%).

Figure 5 illustrates specific binding of [125I]ANF with various dilutions of plasma obtained 1 week after the last administration of MAb-ANF (n=6). Nonspecific binding obtained with plasma of control

<table>
<thead>
<tr>
<th>Table 1. Body Weight and Hematocrit During Exposure to Hypoxia in Rats Receiving Weekly Intravenous Monoclonal Antibody</th>
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<tbody>
<tr>
<td>Rat group</td>
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<tr>
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<tr>
<td></td>
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<tr>
<td>MAb-ANF (n=9)</td>
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<tr>
<td>MAb-ALPB (n=9)</td>
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</tbody>
</table>

Values are mean±SEM. BW, body weight; Ht, hematocrit; MAb-ANF, monoclonal antibody against atrial natriuretic factor; MAb-ALPB, monoclonal antibody against human apolipoprotein B. There were no significant differences between the two groups (two-way analysis of variance).
rats receiving ascitic fluid with MAb-ALPB (n=5) remained constant below 4% with various dilutions. The binding ability of administered MAb-ANF was therefore well retained 1 week after the last injection.

**Discussion**

This study shows that in rats chronically exposed to hypoxia, acute administration of exogenous ANF (5 μg) induced a selective decrease in PAP without altering CO and systemic hemodynamics. Acute blockade of endogenous ANF with MAb-ANF caused an increase in pulmonary vascular tone. Chronic blockade of endogenous ANF with weekly administration of MAb-ANF aggravated development of pulmonary hypertension and right ventricular hypertrophy but did not significantly alter small pulmonary arterial wall thickness or extension of muscle into peripheral arteries. In contrast, MAb-ANF had no significant effect on pulmonary hemodynamics of normoxic rats.

Jin et al.\(^{12}\) showed that acute administration of ANF may reduce PAP in conscious or anesthetized chronically hypoxic rats. They studied rats after return to normoxia and observed a concomitant decrease of CO in anesthetized open-chest rats. In the present study, rats were studied while conscious and hypoxic. Under these conditions, we did not observe significant changes in CO or heart rate. Administration of ANF was associated with a selective decrease of pulmonary vascular resistance, whereas SAP and systemic vascular resistance remained unchanged. These results differ from those obtained in conscious normoxic humans\(^{25}\) and animals,\(^{26}\) in which decreased CO was observed after acute administration of ANF. In our study, the pulmonary vasodilatory effect of ANF may have offset the effect of decreased venous return and maintained CO at its baseline value. Although systemic vasodilation in response to ANF has not been consistently demonstrated, pulmonary vasodilation has uniformly been observed in humans,\(^{5}\) intact animals,\(^{8,11}\) and isolated lung preparations,\(^{8,12}\) with increased pulmonary vascular tone induced by hypoxia or various constrictor agonists.

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**TABLE 2.** Plasma Electrolytes, Urea, Creatinine, and Protein Concentrations in Rats Receiving Weekly Intravenous Monoclonal Antibody

<table>
<thead>
<tr>
<th>Rat group</th>
<th>Sodium (meq/l)</th>
<th>Chlorine (meq/l)</th>
<th>Urea (mmol/l)</th>
<th>Creatinine (μmol/l)</th>
<th>Proteins (meq/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAb-ANF (n=8)</td>
<td>139±0.7</td>
<td>108±1.3</td>
<td>7.6±0.3</td>
<td>53±3.4</td>
<td>12.4±0.2</td>
</tr>
<tr>
<td>MAb-ALPB (n=9)</td>
<td>138±0.9</td>
<td>106±0.7</td>
<td>7.5±0.4</td>
<td>50±3.3</td>
<td>12.5±0.2</td>
</tr>
</tbody>
</table>

Values are mean±SEM at the end of the 3-week exposure to hypoxia. MAb-ANF, monoclonal antibody against atrial natriuretic factor; MAb-ALPB, monoclonal antibody against human apolipoprotein B. There were no significant differences between the two groups (nonparametric test).

**TABLE 3.** Hemodynamic Values in Rats Receiving Weekly Intravenous Monoclonal Antibody

<table>
<thead>
<tr>
<th>Rat group</th>
<th>PAP (mm Hg)</th>
<th>SAP (mm Hg)</th>
<th>HR (bpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAb-ANF (n=6)</td>
<td>39±1*</td>
<td>102±5</td>
<td>290±17</td>
</tr>
<tr>
<td>MAb-ALPB (n=8)</td>
<td>33±2</td>
<td>107±4</td>
<td>292±14</td>
</tr>
</tbody>
</table>

Values are mean±SEM at the end of a 3-week exposure to hypoxia.

PAP, pulmonary arterial pressure; SAP, systemic arterial pressure; HR, heart rate; bpm, beats per minute; MAb-ANF, monoclonal antibody against atrial natriuretic factor; MAb-ALPB, monoclonal antibody against human apolipoprotein B. *p<0.05 compared with MAb-ALPB (nonparametric test).

**FIGURE 4.** Bar graphs showing right ventricular/left ventricular wall plus septal thickness (RV/LV+S), percentage of muscularized and partially muscularized arteries at the alveolar duct level, and wall thickness of small (50–100 μm) pulmonary arteries after chronic administration of monoclonal antibody against atrial natriuretic factor (MAb ANF) or monoclonal antibody against human apolipoprotein B (MAb APLB) during exposure to hypoxia. Results are mean±SEM; the numbers in parentheses indicate the number of rats per group. *p<0.05 between the two groups.
The pulmonary vasodilatory effect of ANF is consistent with previous studies demonstrating a large number of specific binding sites for ANF in the lung. Sustained increase in plasma levels of ANF during chronic hypoxia may induce some downregulation of the ANF receptors. Despite this, the present results and those of Jin et al.12 with isolated lungs imply that a sufficient number of guanylate cyclase–coupled receptors persist on pulmonary smooth muscle for moderate doses of exogenous ANF to exert a significant pulmonary vasodilatory effect.

Definite conclusions regarding the physiological role of ANF cannot be drawn from studies of effects of exogenous ANF. Therefore, we used MAb-ANF to block endogenous ANF. Acute administration of MAb-ANF to chronically hypoxic rats induced a sustained increase of PAP. Since CO remained unchanged, increase of PAP may be ascribed to an increase of pulmonary vascular tone. This suggests that endogenous secretion of ANF attenuates the increase of pulmonary vascular tone during chronic hypoxia. In normoxic rats, injection of MAb-ANF was not followed by an increase of PAP and pulmonary resistance, suggesting that ANF does not contribute to maintenance of low pulmonary vascular tone in normoxia.

We believe that the pulmonary hemodynamic effects of MAb-ANF in the present study were related to blockade of endogenous ANF. The MAb we used has previously been shown to neutralize the diuretic activity of rat atrial extracts. We may exclude nonspecific effects of ascitic fluid on pulmonary hemodynamics, because a decrease of pulmonary vascular resistance was observed in control rats receiving ascitic fluid with MAb-ALPB. Failure of MAb-ANF to alter pulmonary hemodynamics in normoxic rats also argues against a nonspecific effect of ascitic fluid on the pulmonary vascular bed.

The increase in SAP with MAb-ANF may not be related to blockade of endogenous ANF, and the sustained increase in SAP observed after administration of MAb-ANF or MAb-ALPB suggests a nonspecific effect of the ascitic fluid. In a previous study, nonspecific vasoconstrictor effects with reduction of renal blood flow were observed when ascitic fluid instead of purified MAb was administered in rats with chronic heart failure.19

The development and maintenance of pulmonary hypertension during chronic hypoxia is the result of long-term alterations in pulmonary vascular tone and erythropoiesis as well as structural vascular changes. Accordingly, we administered MAb-ANF repetitively over 3 weeks of exposure to hypoxia to observe effects of chronic blockade of endogenous ANF on pulmonary hemodynamics, vascular remodeling, and polycythemia. Passive immunization with MAb-ANF aggravated pulmonary hypertension and right ventricular hypertrophy.

At the time of hemodynamic and histological study, 1 week after the last injection of ascitic fluid, plasma retained significant binding ability for [125I]ANF. Therefore, if antibodies against ascitic fluid, plasma retained significant binding ability for [125I]ANF. Therefore, if antibodies against MAb were produced after repetitive administration, we can infer that such antibodies do not interfere with their ANF-binding ability. Our results are in accordance with those of Itoh et al., who documented in rats sustained ability of serum to bind ANF after similar repeated in vivo administrations of MAb-ANF.

After repetitive administration of ascitic fluid, plasma creatinine and urea remained constant and within the normal range. Thus, we may rule out renal failure caused by a nonspecific effect of MAb or chronic blockade of endogenous ANF. We did not measure natriuresis or diuresis. However, both groups of rats showed similar increases of body weight and hematocrit during exposure to hypoxia, as well as normal plasma sodium, at the time of the hemodynamic study. This suggests that chronic blockade of endogenous ANF did not cause gross sodium or water retention or affect pulmonary vascular resistance via effects on polycythemia and blood viscosity.

Despite more severe pulmonary hypertension and right ventricular hypertrophy, thickness of small pulmonary arteries and percentage of muscularized arteries at the alveolar duct level was not significantly altered by chronic blockade of endogenous ANF. This suggests that the main effect of increased levels of endogenous ANF during chronic hypoxia is to decrease pulmonary vascular tone. However, we cannot exclude that endogenous ANF attenuates smooth muscle proliferation in small pulmonary arteries. A cGMP analogue, 8-bromo-cGMP, and, more recently, ANF were shown to reduce mitogenesis of cultured rat aortic smooth muscle. Jin et al.14 observed that chronic infusion of ANF during hypoxic exposure significantly decreased wall thickness of small pulmonary arteries. However, these authors used a different method, as in the present study of preparing the lung. In addition to injection of formal in the trachea, they fixed pulmonary arteries with formalin under high pressure. Whereas plasma levels of ANF increased by 230%, reduction of PAP and right ventricular hypertrophy averaged 27% and
22%, respectively, but wall thickness of small pulmonary arteries was reduced by only 10%. In the present study during blockade of endogenous ANF, we observed an increase of wall thickness of small pulmonary arteries averaging 10%. However, there was a wide range in the degree of pulmonary vascular remodeling from one rat to another.

In summary, the present study demonstrates that ANF is a selective vasorelaxant of the pulmonary circulation during chronic hypoxia. Blockade of endogenous ANF with administration of MAb-ANF aggravated pulmonary hypertension and right ventricular hypertrophy of rats exposed to chronic hypoxia. These findings support a compensatory role for augmented secretion of ANF in this model of pulmonary hypertension.

Acknowledgments

The authors thank Mrs. Françoise Meyer for technical assistance and Dr. Michael Goldman for critical reading of the manuscript.

References


**KEY WORDS** hypoxia • atrial natriuretic factor • monoclonal atrial natriuretic factor antibody • pulmonary hypertension
Pulmonary vasodilatory action of endogenous atrial natriuretic factor in rats with hypoxic pulmonary hypertension. Effects of monoclonal atrial natriuretic factor antibody.
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Circ Res. 1992;70:184-192
doi: 10.1161/01.RES.70.1.184

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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