Reduced Oxygen Tension Increases Atrial Natriuretic Peptide Release from Atrial Cardiocytes

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To test the hypothesis that reduced oxygen tension stimulates cardiac atrial natriuretic peptide (ANP) secretion, we measured ANP release and expression in neonatal rat atrial and ventricular cardiac myocytes exposed to 45 min and 3, 6, and 24 hr of 3% or 21% oxygen. In atrial cardiocytes, the percentage of increase in culture media ANP concentration from baseline was greater in cells exposed to 3% than in cells exposed to 21% oxygen after 3 hr (814% ± 52% vs. 567% ± 33%, $P < 0.05$) and 6 hr of exposure (1639% ± 91% vs. 1155% ± 73%, $P < 0.05$). No differences in the percentage of increase in culture media ANP concentration was seen at 45 min (284% ± 27% vs. 201% ± 16%, P = NS) or 24 hr (2499% ± 250% vs. 2426% ± 195%). There was a significant increase in cellular ANP content between 3 and 24 hr in atrial cardiocytes exposed to 21% oxygen (105% ± 40% vs. 296% ± 60%, $P < 0.05$), but not in atrial cardiocytes exposed to 3% oxygen (118% ± 20% vs. 180% ± 26%, P = NS). Steady-state ANP mRNA levels in atrial cardiocytes were not affected by oxygen tension. In ventricular cardiocytes, oxygen tension did not affect ANP secretion, cellular ANP content, or steady-state ANP mRNA levels. We conclude that reduced oxygen tension increases release of ANP from atrial, but not ventricular cardiocytes and that this mechanism may contribute to the elevation in plasma ANP seen during acute hypoxia.

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Atrial natriuretic peptide (ANP) is a cardiac hormone that has potent diuretic, antihypertensive, and antimitogenic effects and appears to play an important role in modulating intravascular volume homeostasis and cardiopulmonary hemodynamics (1, 2). Circulating ANP levels rise in response to acute hypoxia (3) and remain elevated during exposure to chronic hypoxia (4). This occurs because of an increase in cardiac ANP synthesis and a decrease in pulmonary ANP clearance (3, 4, 10–12). Recent studies (5–9) suggest that hypoxia-associated increases in circulating ANP levels and cardiac ANP expression play critical roles in blunting the development of pulmonary hypertension and right ventricular hypertrophy.

Despite the important role of endogenous ANP in protecting against the development of hypoxic pulmonary hypertension, the mechanism(s) responsible for hypoxia-induced cardiac ANP secretion is (are) not well understood. Originally, hypoxia-induced secretion of ANP from the heart was attributed to increased right atrial pressure secondary to hypoxic pulmonary hypertension (4). Studies (3, 11) showing a close correlation between the increase in pulmonary artery pressure and plasma ANP levels during acute hypoxia supported this hypothesis. However, hypoxia is a greater stimulus for ANP release in intact animals than volume loading, despite a smaller associated increase in right atrial pressure (11). Furthermore, hypoxia increases ANP release in the isolated perfused heart where the atria are empty and perfusion pressure and ventricular volume are kept constant (13). These studies suggest that hypoxia may be able to stimulate cardiac ANP release directly, independent of its effect on right heart afterload.

In the present study we hypothesized that if hypoxia has a direct stimulatory effect on cardiac ANP release that is independent of its pulmonary hypertensive effects, then cultured cardiac myocytes exposed to reduced oxygen tension should secrete more ANP than cardiac myocytes kept under normoxic conditions. In order to determine if changes in cardiac myocyte ANP secretion are due to increased ANP synthesis, increased release of stored ANP, or both, we measured ANP concentration in the culture media and ANP

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concentration and steady-state ANP mRNA levels in cell lysates. Atrial and ventricular cardiocytes were studied separately because atrial cardiocytes release stored ANP *via* a regulated as well as a constitutive pathway, whereas ventricular cardiocytes secrete ANP mostly *via* a constitutive pathway (14, 15).

Materials and Methods

Cell Cultures. All studies were approved by the Animal Welfare Committee at Rhode Island Hospital. Sprague-Dawley rat pups, 1 to 3 days old, were anesthetized by hypothermia and were then decapitated. Hearts were removed intact through a sternal thoracotomy and then rinsed in 1× ADS buffer (116 mM NaCl, 20 mM HEPES, 10 mM NaH_2PO_4, 5.5 mM glucose, 5.4 mM KCl, and 0.8 mM $MgSO_4$). The atrial appendages and the apical portion of the ventricles were removed and placed into separate petri dishes with fresh 1× ADS. Tissues were minced with a scalpel blade, washed three times in 1× ADS, and transferred to a 100-mm tissue culture dish. They were digested with 10 mg/ml Collagenase Type II and 0.6 mg/ml Pancreatin (Gibco-BRL Life Technologies, Gaithersburg, MD). The tissue was disaggregated by triturating with a siliconized Pasteur pipette over the course of 2 hr. Digestion was stopped by washing tissues twice with a solution of 1× ADS and 10% horse serum.

Cells were incubated in 1× ADS with 10% horse serum at 37°C for 45 min to allow for fibroblast adhesion. Larger tissue pieces were removed by passing the cell suspension through a Cellector Tissue Sieve with a 40-mesh screen (Bellco Glass Inc., Vineland, NJ), washed in 1× ADS, and resuspended in 1.5 ml of 1× ADS per 7 to 10 hearts. Cardiac myocytes were further enriched using a Percoll density dual gradient (1.059 and 1.082 g/ml, Amersham Pharmacia Biotech, Piscataway, NJ) and were then centrifuged for 30 min at 1800*g*. Myocytes were isolated from the interface of the density gradient, washed with 1× ADS, and incubated overnight in Dulbeccos' modified Eagle's medium (DMEM) with 10% fetal calf serum and 10 μM arabinoside. Unless noted otherwise, cells were kept in a standard water-jacketed incubator gassed with 21% O_2, 5% CO_2, and balance N_2 at 37°C.

After 24 hr, cells were washed and incubated in growth media (MEM with 10% horse serum and 5% Chick Embryo Extract (Gibco-BRL Life Technologies). Greater than 90% of cells isolated in this manner stained positive for tropomyosin. Cell viability was assessed by exclusion of trypan blue and changes in the secretion of creatinine kinase. Cells were examined daily for adherence to the cell culture plate and spontaneous beating. Culture media was replaced every 48 hr and cells were allowed to grow for 4 days prior to experimentation to ensure a stable state.

Reduced Oxygen Tension. To simulate hypoxic conditions, cells were given culture media that had been equilibrated with 3 % O_2, 5% CO_2, balance N_2, and then placed in a custom made plexiglass chamber that was flushed with the same gas mixture. The chamber had armholes that allowed the investigators to obtain samples of culture media and cells without exposing them to room air. The chamber was kept at 37°C and the O_2 concentration was monitored continuously with an oximeter. For exposures greater than 6 hr, culture plates were placed in a modular incubator chamber (Billups-Rothernberg, Del Mar, CA), flushed with 3% O_2, 5% CO_2, and balance N_2 for 5 min, sealed, and then placed in a conventional incubator overnight. Control cells were kept in 21% O_2, 5% CO_2, and balance N_2 in a standard incubator. The degree of hypoxia produced by these techniques was characterized by measuring pO_2, pCO_2, and pH in culture media alone at 15, 45, and 90 min and at 3, 6, and 24 hr. The pO_2 in culture media exposed to 21% oxygen was approximately 150 Torr and remained constant for up to 24 hr. The pO_2 of the culture media exposed to 3% oxygen dropped within 15 min, reached a nadir of approximately 45 Torr at 90 min, and then remained constant for the next 24 hr.

Experimental Protocol. Immediately prior to beginning each experiment, growth media was removed and cells were washed three times with release media (DMEM-F12 with 2.5 mg/ml bovine serum albumin [BSA]). Culture media was changed in order to avoid the confounding affect of serum on ANP release. Baseline samples of culture media were collected 45 min after switching to release media. Cells were then exposed to 3% or 21% oxygen as described above. In preliminary experiments (16), we found an increase in ANP release from atrial cardiocytes after 3 hr, but not after 15, 45, or 90 min of 3% oxygen. In the present study, samples of cell culture media from atrial and ventricular cardiocytes exposed to 3% and 21% oxygen were collected at 45 min and 3, 6, and 24 hr. Samples were assayed for creatine phosphokinase (CPK) and ANP concentration. Cell lysates were collected at 3, 6, and 24 hr for measurement of cellular ANP content and steady-state ANP mRNA levels. Samples of culture media were also obtained for measurement of pH, pO_2, and pCO_2 at the time that cell lysates were collected. Cellular ANP content and steady-state ANP mRNA levels were not measured at the 45 min time point because previous studies (17) in cultured cardiocytes found that ANP expression was not increased until after 3 hr of stimulation.

Measurement of Cellular Protein, CPK, and ANP. Total protein was isolated from cell lysates using Tri-Reagent (Sigma Chemical Co., St. Louis, MO). Briefly, cells were lysed and scraped free from culture plates using a pipette tip and 1 cc of Tri-Reagent per well. Cell lysates were transferred to 1.5-ml microcentrifuge tubes and mixed with 100 μl/ml of 1-bromo-3-chloro-propane. Tubes were centrifuged at 15,000*g* and the aqueous and organic phases were separated for RNA and protein extraction, respectively. Isolated protein was resuspended in 1% SDS for analysis. Protein levels were measured using Bio-Rad D_C Protein Assay (Bio Rad, Hercules, CA) with BSA as a standard. The CPK concentration was measured by a kit from

Sigma Diagnostics (Sigma Chemical Co.). Samples of cell culture media and cell lysates were analyzed for ANP concentration using an enzyme-linked immunoassay (Caymen Chemical, Ann Arbor, MI). Samples of culture media were run undiluted, and samples of cell lysates were diluted 1/25 in assay buffer before analysis. Serial measurements of ANP concentration in the cell culture media were expressed as the percentage of change from baseline level. Cellular ANP content was expressed as nanograms per milligram of cellular protein.

Northern Analysis. Total RNA was isolated from cardiocytes by using Tri-Reagent (Sigma Chemical Co.) as described above. The isolated RNA was resuspended in TE buffer (0.05 mM Tris-HCl, 0.2 μM EDTA). For Northern blots, 10 μg of total RNA was loaded and electrophoresed on 1% agarose gels with 1× MOPS running buffer (20 mM 3-(N-morpholino) propanesultonic acid, 5 mM sodium acetate). The RNA was passively transferred via Turboblotter (Schleicher & Schuell, Kenne, NH) to Nytran-N nylon membranes (Schleicher & Schuell). Blots were probed with a ^{32}P-labeled single-strand cDNA probe for rat proANP, courtesy of Dr. Christine Seidmann (18), and then ligated to a pBluescript II phagemid vector (Stratagene, La Jolla, CA). Blots were then stripped and reprobed for 18S mRNA. Audioradiographs of Northern blots of ANP and 18S mRNA were analyzed by densitometry using an Epson Expression 636 scanner (Seiko Epson Corp., Torrance, CA) and image analysis was done by Adobe Photoshop (Miocrosoft Corp., Seattle, WA). The ANP hybridization signals were normalized to hybridization signals for 18S mRNA.

Statistical Analysis. To standardize basal secretion between individual experiments, changes in culture media ANP concentration were analyzed as the percentage of change from baseline. Values shown are mean ± SE. Differences between mean values of cells exposed to 3% and 21% oxygen were compared by one-way analysis of variance (ANOVA) using computer software (SigmaStat, SPSS, Chicago, IL). Where significant differences were found, pairwise comparisons were made using Fisher's least significant difference test. Differences were considered significant at $P < 0.05$.

Results

Effect of Reduced Oxygen Tension on ANP Secretion in Atrial Cardiocytes. Culture media pO_2 remained constant between 3 and 24 hr of exposure in atrial and ventricular cardiocytes exposed to 3% and 21% oxygen (Table I). The pO_2 in the culture media of atrial and ventricular cardiocytes exposed to 3% oxygen was approximately one-third that of cells exposed to 21% oxygen at each time point measured (Table I). Culture media pH and pCO_2 were similar in cardiocytes exposed to 3% and 21% oxygen, except that at the 6-hr time point, pCO_2 was lower and pH was higher in cells exposed to 3% oxygen (Table I). Reduced oxygen tension did not affect CPK release in atrial or ventricular cardiocytes at any time point (Table II).

Culture media ANP concentration, expressed as the percentage of change from baseline, increased linearly during the first 6 hr of exposure in both groups of atrial cardiocytes, suggesting a constant rate of ANP secretion. The percentage of increase in ANP secretion was approximately 50% greater in atrial cardiocytes exposed to 3% oxygen than in those exposed to 21% oxygen at 3 and 6 hr of exposure (Fig. 1A). After 24 hr, the percentage of increase in culture media ANP concentration was the same in atrial cardiocytes exposed to 3% and 21% oxygen (Fig. 1A).

Effect of Reduced Oxygen Tension on ANP Secretion in Ventricular Cardiocytes. Baseline secretion of ANP in ventricular cardiocytes was approximately 60% of atrial cardiocytes. Culture media ANP concentration increased linearly during the first 6 hr of exposure to 3% and 21% oxygen, but then fell slightly (Fig. 1B). Reduced oxygen tension had no effect on ANP secretion from ventricular cardiocytes at any time point measured (Fig. 1B).

Effect of Reduced Oxygen Tension on ANP Synthesis in Atrial and Ventricular Cardiocytes. To determine if the increase in ANP release from atrial cardiocytes exposed to reduced oxygen tension was caused by increased secretion of stored ANP or increased synthesis of new ANP, cellular ANP content and steady-state ANP mRNA levels were measured in cell lysates. There was an increase in mean cellular ANP levels between 3 and 24 hr in atrial cardiocytes exposed to 21% oxygen (Fig. 2A). No

Table I. Effect of Reduced Oxygen Tension on pH and Partial Pressures of Oxygen and Carbon Dioxide in the Culture Media of Atrial and Ventricular Cardiocytes

	pH		pO_2 (Torr)		pCO_2 (Torr)	
	3% Oxygen	21% Oxygen	3% Oxygen	21% Oxygen	3% Oxygen	21% Oxygen
Atrial cardiocytes						
3 hr	7.32 ± 0.05	7.34 ± 0.05	51.3 ± 3.4[a]	151.0 ± 5.6	28.4 ± 1.0	27.5 ± 1.5
6 hr	7.25 ± 0.02[a]	7.16 ± 0.02	49.0 ± 1.9[a]	145.5 ± 2.8	27.2 ± 2.1[a]	36.4 ± 1.0
24 hr	7.19 ± 0.03	7.18 ± 0.01	59.5 ± 2.5[a]	153.8 ± 4.4	30.9 ± 0.7	33.7 ± 1.2
Ventricular cardiocytes						
3 hr	7.31 ± 0.06	7.36 ± 0.06	46.5 ± 2.3[a]	141.7 ± 5.2	28.5 ± 1.8	26.4 ± 1.8
6 hr	7.08 ± 0.04	7.17 ± 0.01	47.9 ± 2.2[a]	122.1 ± 3.7	27.4 ± 1.0	31.8 ± 3.0
24 hr	6.97 ± 0.08	7.18 ± 0.01	54.3 ± 3.8[a]	148.9 ± 6.6	28.7 ± 1.2	32.6 ± 1.6

Note. Values are mean ± SE, n = 6–9.
[a] $P < 0.05$ vs normoxia.

Table II. Effect of Reduced Oxygen Tension on CPK Levels in the Culture Media of Atrial and Ventricular Cardiocytes

	CPK (IU/ml)	
	3% Oxygen	21% Oxygen
Atrial cardiocytes		
Baseline	2.07 ± 0.22	2.11 ± 0.09
45 min	2.08 ± 0.15	2.17 ± 0.12
3 hr	2.17 ± 0.15	2.08 ± 0.11
6 hr	2.21 ± 0.20	2.10 ± 0.08
24 hr	2.54 ± 0.21	2.08 ± 0.08
Ventricular cardiocytes		
Baseline	2.10 ± 0.10	2.02 ± 0.02
45 min	2.13 ± 0.14	2.09 ± 0.11
3 hr	2.20 ± 0.10	2.09 ± 0.03
6 hr	1.98 ± 0.11	2.08 ± 0.08
24 hr	2.13 ± 0.14	1.99 ± 0.04

Note. Values are mean ± SE, $n = 6$ for atrial cardiocytes and 3 for ventricular cardiocytes.

significant increase in ANP levels was seen over time in atrial cardiocytes exposed to 3% oxygen. There was a trend toward higher ANP level in atrial cardiocytes exposed to 21% than in cells exposed to 3% oxygen at 24 hr, but the difference did not reach statistical significance ($P = 0.124$). The cellular content of ANP in ventricular cardiocytes was approximately 30% of atrial cardiocytes and no differences in cellular ANP content were seen between ventricular cardiocytes exposed to 3% and 21% oxygen at any time point (Fig. 2B).

Steady-state ANP mRNA levels were 2- to 3-fold higher in atrial than in ventricular cardiocytes (Fig. 3). No significant differences were seen between cells exposed to 3% and 21% oxygen in atrial or ventricular cardiocytes at any of the time points measured.

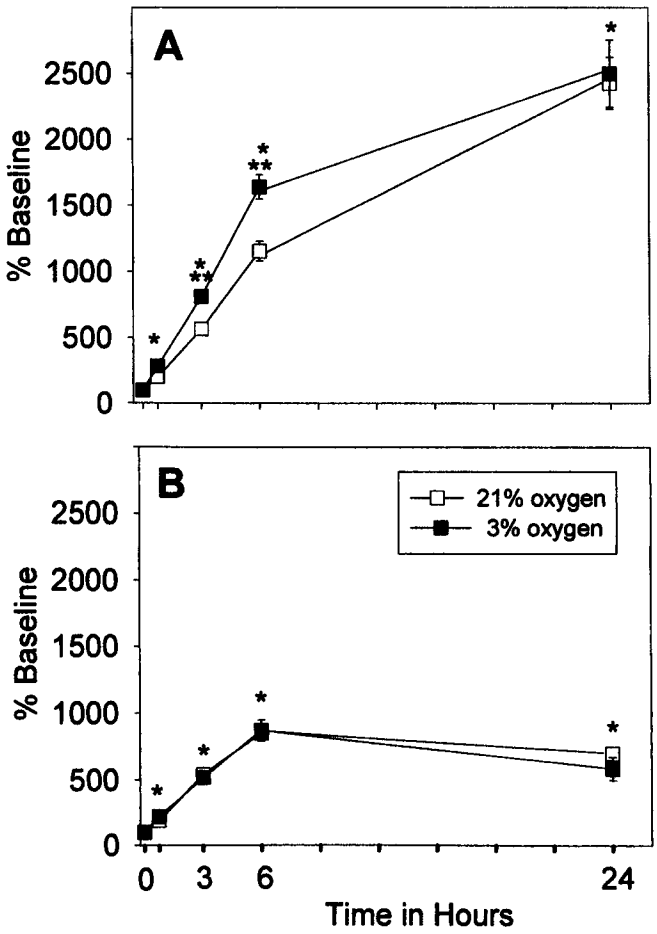

Figure 1. Change in ANP concentration in the culture media of atrial (A) and ventricular (B) cardiac myocytes exposed to 45 min and 3, 6, and 24 hr of 21% (normoxia) or 3% oxygen (hypoxia). Data are expressed as the percentage of baseline ANP culture media concentration. Values are mean ± SE. For atrial cardiocytes, $n = 24$ to 76 for each time point except 45 min, where $n = 6$. For ventricular cardiocytes, $n = 21$ to 99. * $P < 0.05$ normoxic and hypoxic cells versus their respective baseline values, ** $P < 0.05$ hypoxic cells versus normoxic cells.

Discussion

In the present study we sought to determine if hypoxia simulates ANP release from atrial and ventricular cardiocytes *in vitro*. Culture media pO_2 in cells exposed to 3% oxygen was intermittent between that of arterial and venous oxygen tensions experienced by cardiocytes under hypoxic conditions *in vivo*, such as ascent to high altitude or in patients with chronic lung disease. These are clinical conditions that have been associated with increased plasma ANP levels. However, it is uncertain if the lower oxygen tension used in the present study is equivalent to hypoxic conditions experienced by cardiocytes *in vivo*. Oxygen delivery in cultured cells may be aided by better perfusion because cells are less densely populated and are grown in monolayers. On the other hand, cells *in vivo* benefit from a renewable supply of oxygenated blood and the increased oxygen carrying capacity of hemoglobin. Because we cannot be sure that the reduced oxygen tension of our *in vitro* environment is the same as that experienced by cardiocytes in intact animals exposed to hypoxia, we have chosen to describe our experimental conditions as reduced oxygen tension relative to controls.

In intact animals, the primary stimulus of hypoxia-induced ANP release is thought to be right atrial stretch secondary to hypoxic pulmonary vasoconstriction (3, 4). However, the demonstration that hypoxia increases ANP release in the isolated perfused heart in the absence of any change in right atrial volume or pressure suggests that other factors contribute to hypoxia-induced ANP secretion. Numerous nonhemodynamic mechanisms have been proposed, including a pulmonary neural reflex (19), increases in adrenergic activity (20), and increased circulating levels of adenosine (21), arginine vasopressin (22), and endothelin-1 (ET-1) (12). The isolated perfused heart model eliminates autonomic reflexes and permits control of hemodynamic factors, but some of the paracrine mechanisms that have been proposed to contribute to hypoxia-induced ANP release may remain intact in this preparation. For example,

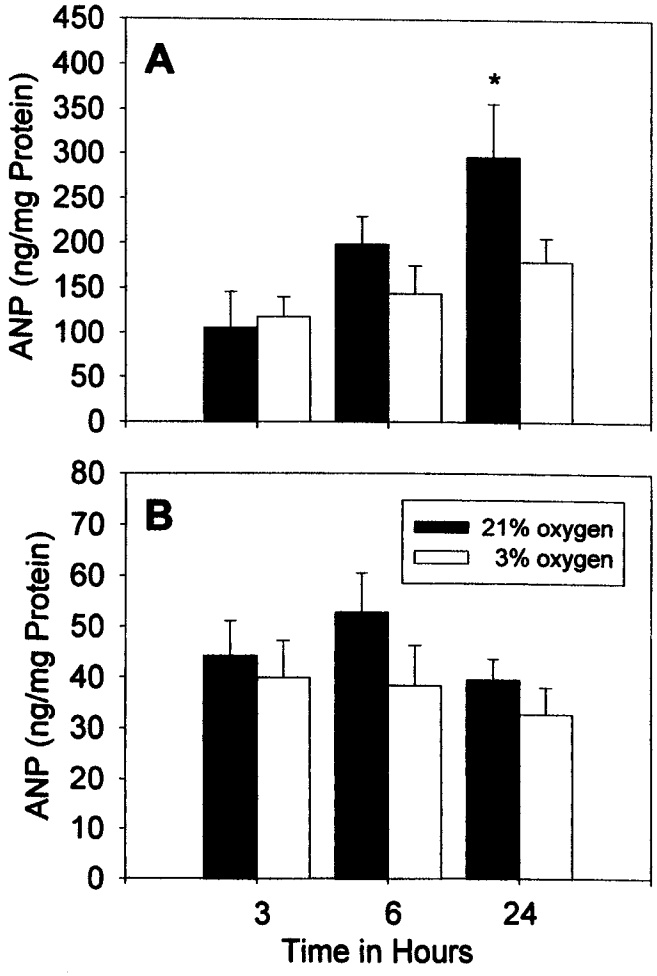

Figure 2. Change in ANP concentration in the cell lysates of atrial (A) and ventricular (B) cardiac myocytes exposed to 3, 6, and 24 hr of 21% or 3% oxygen. Data are normalized to total cell protein. Values are mean ± SE. For atrial cardiocytes, $n = 22$ to 31 for each group. For ventricular cardiocytes, $n = 17$ to 28 for each group. * $P < 0.05$ versus 3-hr time point.

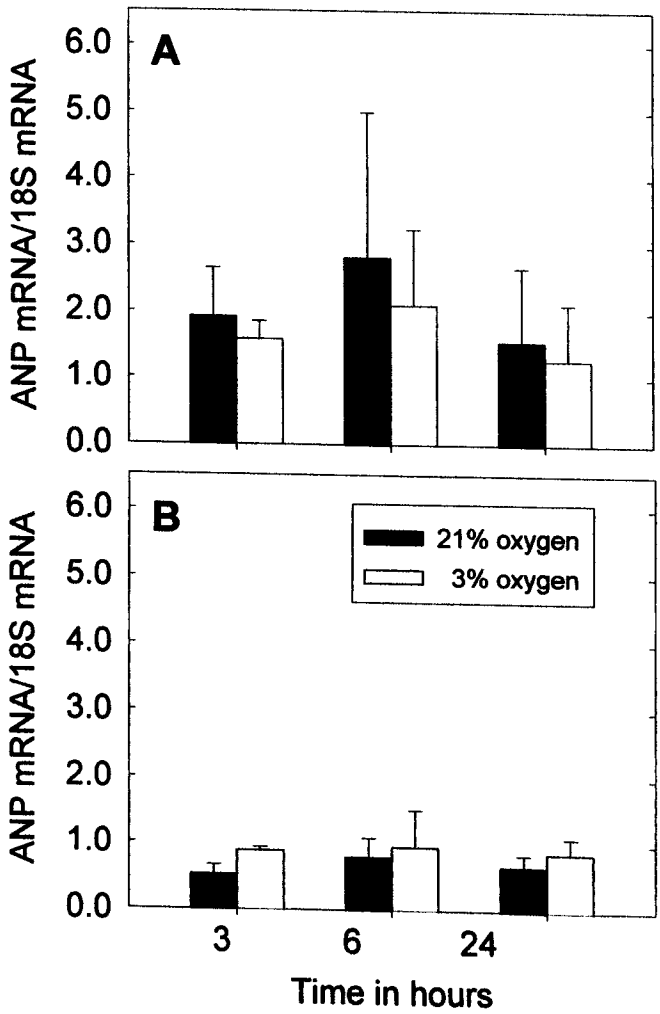

Figure 3. Steady-state ANP mRNA levels in atrial (A) and ventricular (B) cardiac myocytes exposed to 3, 6, and 24 hr of 21% or 3% oxygen. Data are expressed as the ratio of ANP hybridization signal normalized to 18S ribosomal mRNA. Values are mean ± SE, $n = 3$ to 4 experiments per group.

Skvorak *et al.* (12) found that an ET_A receptor antagonist suppressed hypoxia-induced ANP release from the isolated perfused heart and hypothesized that endothelial cells in the endocardium and coronary vessels secreted ET-1 in response to hypoxia. Our finding in the present study that reduced oxygen tension increases ANP release from primary cultures of atrial cardiocytes lends additional support to the hypothesis that hypoxia has a direct stimulatory effect on ANP release that is independent of hemodynamic or paracrine factors.

There are several potential mechanisms by which reduced oxygen tension could stimulate ANP release from atrial cardiocytes. Anaerobic metabolism in hypoxic cardiocytes could lead to intracellular acidosis. Cardiac myocytes protect against increases in intracellular hydrogen ion concentration $[H^+]$ by exchanging H^+ for Na^+, and then exchanging Na^+ for Ca^{++} (23, 24). Influx of extracellular Na^+ and Na^+-Ca^{++} exchange modulate ANP secretion induced by hyperosmolar conditions (25) and could play a similar role in hypoxia-induced ANP secretion. Another mechanism that may play a role in hypoxia-induced ANP secretion is the opening of ATP-sensitive potassium channels. Xu *et al.* (26) showed that ATP-sensitive potassium channels modulate hypoxia- and stretch-induced ANP release in the isolated perfused heart. Hypoxia has been shown to activate ATP-sensitive potassium currents in rabbit papillary muscle channels (27) and may have the same effect in cultured cardiocytes. Finally, recent studies (28) have shown that hypoxia selectively activates PKC isoforms in cultured neonatal rat cardiocytes by a phospholipase C-dependent pathway. Because PKC activation has been shown to stimulate ANP release in cultured atrial myocytes (29), it is possible that hypoxia-induced ANP release may be mediated *via* this mechanism.

The relative contribution of atrial and ventricular ANP release during hypoxia has not been defined. In a previous study, Arad *et al.* (23) found that 90% of the increase in ANP release stimulated by ischemia-reperfusion in isolated perfused rat hearts was of atrial origin. In preliminary studies (30) we have found that nearly all of the hypoxia-induced increase in ANP release from isolated perfused rat

hearts is derived from the atria. The findings of the present study extends this observation to cultured cardiac myocytes and lends further support to the hypothesis that the atria are the major source of hypoxia-induced ANP release.

Despite a significant increase in ANP secretion in atrial cardiocytes exposed to 3% oxygen, there was no evidence of increased ANP synthesis, suggesting that the increased ANP release must have derived from previously synthesized, stored ANP. The increase in the amount of released ANP, as a fraction of the quantity of stored ANP, was probably insufficient to cause a significant decrease in atrial ANP content compared with atrial cardiocytes exposed to 21% oxygen. In fact, McKenzie *et al.* (4) found no significant change in right atrial ANP levels in intact rats after 3 days of hypoxia, but found a significant decrease after 21 days. Although there was no significant difference between ANP content of atrial cardiocytes exposed to 3% and 21% oxygen, ANP content increased significantly between 3 and 24 hr in atrial cardiocytes exposed to 21% oxygen, but not in atrial cardiocytes exposed to 3% oxygen. It is possible that the increased rate of ANP secretion in atrial cardiocytes grown under conditions of reduced oxygen tension prevented the increase in stored ANP between 3 and 24 hr that was observed in cells exposed to 21% oxygen.

The hypothesis that acute hypoxia causes release of previously synthesized, stored ANP is consistent with the lack of an increase in ANP release that was observed in ventricular cardiocytes exposed to reduced oxygen tension in the present study. Ventricular cardiocytes secrete ANP by a constitutive pathway and store only about 10% of newly synthesized ANP, whereas atrial cardiocytes store approximately 50% of newly synthesized ANP (14, 15). Thus, the lack of a large pool of stored ANP may prevent ventricular cardiocytes from increasing ANP secretion in response to reduced oxygen tension.

Whether or not hypoxia has a stimulatory effect on ANP synthesis in cardiocytes is unclear. In intact rats, chronic hypoxia has little affect on ANP mRNA levels in the atria, but increases ANP expression dramatically in the ventricles (31, 32). In the present study we found no change in ANP expression in atrial or ventricular cardiocytes after 24 hr of reduced oxygen tension, suggesting that hypoxia is not an independent stimulus of ANP gene expression in these cells. However, Chen *et al.* (33) found an increase in steady-state ANP mRNA levels after 48 hr of exposure to 1% oxygen in atrial tumor cells. Although the use of immortalized, proliferating cells makes it difficult to extrapolate their findings to normal cardiocytes, we cannot exclude the possibility that a longer period of reduced oxygen tension may have stimulated ANP synthesis in our cells. Alternatively, it is possible that factors such as increased right ventricular afterload or increased cardiac ET-1 levels are responsible for increasing ventricular ANP expression during chronic hypoxia.

The increase in ANP release from cells exposed to reduced oxygen tension in our study does not appear to be the result of cell injury. We saw no microscopic evidence of cell injury during the 24-hr exposure period. Cells continued to beat spontaneously, remained attached to culture plates, and had no increase in CPK release. The oxygen tension that we achieved in our cell culture system was less than one-third that of normoxic cells, but was greater than that used by Chen *et al.* (33) for up to 7 days with no sign of cell injury.

The findings of this study are limited to rat neonatal cardiac myocytes. Atrial expression of ANP increases rapidly during the first few days of birth and ventricular expression of ANP decreases. Although our data are consistent with hypoxia-induced ANP release in adult animals, it is possible that altered oxygen tension may affect atrial ANP release differently in atrial cardiocytes obtained from neonatal and adult rats. Further studies may be needed to determine if our findings can be duplicated in adult atrial and ventricular cardiocytes.

In summary, the findings of this study suggest that reduced oxygen tension has a direct stimulatory effect on ANP release from atrial cardiocytes that is independent of its antihypertensive effect on the pulmonary and systemic circulation. Increased cardiac secretion of ANP plays an important role in limiting the severity of pulmonary hypertension and right ventricular hypertrophy that develop during exposure to hypoxia (5–9) and may blunt the development of pulmonary edema during ascent to high altitude (34). Defining cellular mechanisms by which hypoxia induces atrial ANP release could lead to new therapies that mitigate symptoms of acute mountain sickness or the development of cor pulmonale in patients with chronic lung disease.

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