Chronic hypercapnia downregulates arginase expression and activity and increases pulmonary arterial smooth muscle relaxation in the newborn rat

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Belik J, Stevens D, Pan J, Shehnaz D, Ibrahim C, Kantores C, Ivanovska J, Grassemann H, Jankov RP. Chronic hypercapnia downregulates arginase expression and activity and increases pulmonary arterial smooth muscle relaxation in the newborn rat. Am J Physiol Lung Cell Mol Physiol 297: L777–L784, 2009. First published August 7, 2009; doi:10.1152/ajplung.00047.2009.—In rats, chronic hypercapnia has been reported to ameliorate hypoxia-induced pulmonary hypertension in newborn and adult and to enhance endothelium-dependent vasorelaxation in adult pulmonary arteries. The underlying mechanisms accounting for chronic hypercapnia-induced improvements in pulmonary vascular function are not understood. Hypothesizing that downregulation of arginase activity may be contributory, we examined relaxation responses and arginase activity and expression in pulmonary arteries from newborn rats that were exposed (from birth to 14 days) to either mild-to-moderate (5.5% inhaled CO2) or severe (10% CO2) hypercapnia with either normoxia or hypoxia (13% O2). Pulmonary arteries from pups exposed to normoxia and chronic hypercapnia (5.5 or 10% CO2) contracted less in response to a thromboxane A2 analog, U-46619, and showed enhanced endothelium-dependent (but not independent) relaxation compared with arteries from normocapnic pups (P < 0.01). Parallel with these changes, arginase activity and arginase I (but not II) expression in lung and pulmonary arterial tissue were significantly decreased (P < 0.05). Exposure to 10% CO2 significantly increased (P < 0.01) pulmonary arterial tissue nitric oxide (nitrite) generation. In pups chronically exposed to hypoxia (13% O2), severe hypercapnia (10% CO2) significantly (P < 0.05) enhanced endothelium-dependent relaxation, increased nitric oxide generation, and decreased arginase activity but not expression. We conclude that chronic hypercapnia-induced downregulation of lung arginase expression and/or activity may reduce pulmonary vascular resistance by enhancing nitric oxide generation and thus endothelium-dependent relaxation. This mechanism may explain some of the beneficial effects of chronic hypercapnia on experimental pulmonary hypertension.

permissive hypercapnia, a commonly employed clinical strategy in the treatment of neonatal respiratory disease (17), is characterized by adjustment of ventilatory support parameters to maintain an arterial PCO2 (PaCO2) higher than physiological levels, generally in the range of 50–70 mmHg. This approach has been shown to reduce the severity of chronic lung disease in neonates (17).

The mechanisms accounting for the beneficial effects of permissive hypercapnia in the treatment of neonatal lung dis-

ease are unclear. The reduction in ventilator-induced lung injury achieved by the use of a lower minute ventilation and airway pressures is likely an important contributor to this positive effect. CO2, however, is also well-described to provide protective effect. CO2, however, is also well-described to provide pulmonary vascular resistance, and thus endothelium-dependent vasorelaxation in adult pulmonary arteries. This mechanism may enhance positive effect. CO2, however, is also well-described to provide permissive hypercapnia in the treatment of neonatal lung dis-

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Animal exposures. On their anticipated day of delivery, pathogen-free pregnant Sprague-Dawley dams (Taconic, Germantown, NY) were placed in sealed 80 × 60 × 50 cm exposure Plexiglas chambers (BioSpherix, Redfield, NY) under 12:12-h light-dark cycles, with the temperature maintained at 25 ± 1°C and humidity at 50%. Each litter, maintained at 10–12 pups to control for nutritional effects, was nursed in one of the following gas concentrations from birth until 14 days: 1) normoxia (21% O2) and normocapnia (<0.5% CO2); 2) normoxia and hypercapnia (5.5 or 10% CO2); 3) hypoxia (13% O2) and normocapnia; or 4) hypoxia and hypercapnia (10% O2). O2 and CO2 concentrations, temperature, and humidity were continuously monitored, recorded, and regulated by computer using customized hardware (OxyCycler model A84XOC; BioSpherix) and software (AnaWin2 Run-Time, version 2.2.18; Watlow Anafaze, St. Louis, MO). O2 and CO2 sensors were calibrated weekly.

Dams were exchanged daily between paired normoxia and hypoxia chambers to prevent any maternal toxicity and consequent nutritional effects on the pups. At the end of each exposure period, pups were either killed by pentobarbital sodium overdose or exsanguinated after anesthesia.

Preparation of tissue extracts. Tissue extracts were made in lysis buffer consisting of 50 mM HEPES buffered with sodium hydroxide to pH 7.4, 150 mM sodium chloride, 1% Triton X-100, 10% glycerol, and 1:1,000, respectively. After washing with TBS-T for 30 min, the transblots were incubated with IgG conjugated with horseradish peroxidase (1:20,000 dilution in TBS-T containing 5% milk) for 60 min and washed again with TBS-T for 40 min at room temperature. Subsequently, treatment of the membranes with enhanced chemiluminescence (ECL; PerkinElmer, Shelton, CT) reagent produced the signal that was transferred on to the Kodak Scientific Imaging film. The membrane blots were stripped and then exposed to β-actin (dilution 1:40,000; Sigma-Aldrich, Oakville, Ontario, Canada). The arginase I and II bands on imaging film were quantified by chemiluminescence (FluorChem FC2; Alpha Innotech, San Leandro, CA) and expressed as a ratio of the corresponding β-actin densities.

Nitr· measurements. The nitrite content of bronchoalveolar lavage (BAL) fluid and pulmonary arterial tissue nitrite generation was measured following 14 days of exposure to air or hypoxia in the absence or presence of 10% CO2. For that, the newborn animals were anesthetized intraperitoneally with ketamine (80 mg/kg) and xylazine (20 mg/kg). BAL was obtained by cannulating the trachea and repeatedly injecting 1 ml of 0.9% saline followed by its aspiration until the lavage fluid became opaque. Following measurement of protein, the samples were frozen, lyophilized, and resuspended for nitrite determination. The BAL fluid protein concentration was measured using a Qubit fluorometer and Quant-iT Protein Assay Kit.

To measure NO generation, third-to-fourth generation pulmonary arteries, similar to the vessels used for the organ bath studies, were dissected immediately after the animals’ death. Given their small size, two pulmonary arteries were dissected from each animal, and four vessels were combined to provide enough tissue for each analyzed sample. The pulmonary arterial tissue was placed in an air-tight, rubber-sealed vial and incubated in 800 μl of Krebs-Henseleit solution with L-arginine (10–5 M) at 37°C for 60 min, as proposed by others (7). An aliquot of 100 μl of buffer was taken at the end of the incubation period, and the nitrite content was measured in triplicate. The pulmonary arterial tissue protein content was extracted and measured by the Bradford method (6).

The BAL fluid and pulmonary arterial tissue-incubated buffer samples were treated with nitrate reductase to convert nitrate to nitrite (Nitrate/Nitrite Colorimetric Assay Kit; Cayman Chemical, Ann Arbor, MI). NO was liberated from samples, or sodium nitrite standards, by adding to a solution of 100 μM H2SO4 + 100 μM KI and detected using an electrochemical probe (ISO-NOP) and Apollo 4000 Free Radical Analyzer according to the manufacturer’s instructions (World Precision Instruments, Sarasota, FL). The nitrite concentration is expressed as nanomoles per milligram of protein.

Organ bath studies. Third generation lung intralobar pulmonary artery ring segments (average diameter = 80–100 μm; length = 2 mm) were dissected free and mounted in a wire myograph (Danish Myo Technology A/S, Aarhus, Denmark). Isometric changes were digitized and recorded online (Myodaq; Danish Myo Technology A/S). Tissues were bathed in Krebs-Henseleit buffer (115 mM NaCl, 25 mM NaHCO3, 1 mM MgSO4·7H2O, 1.91 mM CaCl2, 5.56 mM dextrose) bubbled with air-6% CO2 and maintained at 37°C. After 1 h of equilibration, the optimal tissue resting tension was determined by repeated stimulation with 128 mM KCl until maximum active tension was reached. All subsequent force measurements were obtained at optimal resting tension.

Pulmonary vascular muscle force generation was evaluated by stimulating with the thromboxane A2–mimetic U-46619. Contractile responses were normalized to the tissue cross-sectional area as fol-
lows: (width × diameter) × 2 and expressed as mN/mm². Relaxation was induced with the acetylcholine and sodium nitroprusside of the endothelium-dependent and -independent agonists, respectively, following precontraction with U-46619 at concentrations equivalent to the EC₇₅. The arginase inhibitor nor-NOHA (Calbiochem) was added to the muscle bath 20 min before the initiation of the acetylcholine dose-responses at a 10⁻⁵ M concentration.

Drugs. Unless otherwise indicated, all drugs were obtained from Sigma-Aldrich.

Data analysis. Data were evaluated by one- or two-way ANOVA with multiple comparisons obtained by the Tukey-Kramer test or unpaired Student’s t-test when appropriate. Statistical significance was accepted at *P < 0.05. All statistical analyses were performed with the Number Cruncher Statistical System (NCSS, Kaysville, UT). Data are presented as means ± SE.

RESULTS

Effects of chronic CO₂ in normoxia-exposed animals. Chronic CO₂ exposure had a significant effect on the pulmonary arterial smooth muscle force and relaxation potential. Compared with control animals, a significant reduction in the thromboxane A₂ analog (U-46619)-induced muscle force development was observed in the arteries of 5.5 and 10% CO₂-exposed animals (Fig. 1). In U-46619-precontracted pulmonary arteries, exposure to either CO₂ concentration significantly (*P < 0.01) enhanced the endothelium-dependent (>10⁻⁶ M acetylcholine-induced) relaxation response compared with normocapnia-exposed controls (Fig. 2). No significant difference in the endothelium-independent (sodium nitroprusside-induced) relaxant response was observed among the three groups (Fig. 2).

To determine whether hypercapnia-induced enhanced pulmonary vasorelaxation was related to changes in arginases, we measured total arginase activity and isoform expression in lung tissue. As shown in Fig. 3, chronic CO₂ exposure at either concentration significantly reduced (*P < 0.01) lung arginase activity compared with tissue from control animals. A significant reduction (*P < 0.01) in arginase activity was also documented when pulmonary arterial and bronchial tissue from 10% CO₂-exposed animals were examined individually (Fig. 3). For the 5.5% CO₂-exposed animals, a significant decrease in arginase activity (*P < 0.01) was observed in bronchial tissue, whereas a nonsignificant downward trend was observed in pulmonary arterial tissue compared with normocapnia-exposed pups.

We further evaluated arginase I and II isoform expression in lung tissue from control and 5.5% and 10% CO₂-exposed animals. As shown in Fig. 4, compared with control lung tissue, 10% CO₂ exposure significantly (*P < 0.01) reduced arginase I, but not II, isoform expression. No effects were observed on lung tissue eNOS protein expression (Fig. 4).

Effects of chronic CO₂ in hypoxia-exposed animals. We (20) have previously shown that chronic exposure to 10% CO₂ normalized right ventricular function and attenuated vascular remodeling induced by chronic exposure of neonatal rats to hypoxia. To further evaluate mechanisms responsible for such improvements, and given our observations in normoxia-exposed animals, we studied the effects of hypercapnia on pulmonary arterial endothelial-dependent vasorelaxation in these animals (Fig. 5). CO₂ exposure normalized attenuated acetylcholine-induced pulmonary arterial vasorelaxation, observed in normocapnic hypoxia-exposed animals (Fig. 5). In addition, 10% CO₂ abrogated a chronic hypoxia-induced reduction in pulmonary arterial smooth muscle force (Fig. 5).

We comparatively evaluated the lung arginase activity of control and chronic hypoxia-treated normocapnic and hypercapnic animals (Fig. 6). Compared with unexposed chronically hypoxic animals, the lung arginase activity of 10% CO₂-treated pups was significantly reduced (*P < 0.01), whereas the ex-
pression of its two isoforms was unaltered. eNOS expression was not altered by chronic hypoxia.

To obtain further evidence that the enhanced endothelium-dependent relaxation was related to a hypercapnia-induced reduction in arginase activity, we measured the NO in BAL fluid and its generation in pulmonary arteries as well as the in vitro effect of an arginase inhibitor (nor-NOHA). Chronic exposure to 10% CO\textsubscript{2} significantly increased (P < 0.01) nitrite (NO surrogate marker) generation of air-treated pulmonary arteries and BAL fluid content (Fig. 7). Compared with hypoxia alone (2.1 ± 0.3 nm/mg protein), hypoxia + 10% CO\textsubscript{2} significantly increased (P < 0.05, unpaired Student’s t-test) the pulmonary arterial tissue nitrite generation (3.1 ± 0.3 nm/mg protein).

Nor-NOHA (10\textsuperscript{-5} M), by increasing eNOS l-arginine availability and NO production, significantly reduced the post-U-46619 stimulation (EC\textsubscript{75}) pulmonary arterial muscle force generation of air- and chronic hypoxia-exposed animals (Fig. 8). Although not significant, a similar trend was observed in the hypoxia + 10% CO\textsubscript{2}-treated animals’ vessels. In contrast to air-exposed control pulmonary arteries, in the presence of nor-NOHA, acetylcholine stimulation to induce eNOS-dependent NO release did not further decrease the force in 10% CO\textsubscript{2} air- and chronic hypoxia-exposed animals (Fig. 8).

These data corroborate the evidence for a reduced pulmonary arterial tissue arginase activity of CO\textsubscript{2}-exposed animals. It further confirms that the hypercapnia-dependent enhancement of pulmonary vasodilation is mediated via NO, since no further
relaxation with acetylcholine was observed following the arginase inhibition-induced increase in L-arginine availability to eNOS.

**DISCUSSION**

In the present study, we documented a significant increase in pulmonary arterial NO generation and endothelium-dependent vasorelaxation following chronic CO₂ exposure in newborn rats. This enhanced vasorelaxation in the chronic CO₂-exposed pups was associated with increased NO generation and likely secondary to a significant reduction in lung arginase activity and type I isoform expression. Decreased arginase activity and improved endothelium-dependent relaxation were also observed in newborn rats with chronic hypoxia-induced pulmonary hypertension.

The effects of chronic CO₂ exposure on the pulmonary vasculature have received limited attention despite the widespread clinical use of permissive hypercapnia as part of the mechanical ventilatory management of sick neonates. We (20) have previously shown that chronic exposure to 10% CO₂ (but not 5.5% CO₂) significantly reduces pulmonary vascular resistance and attenuates pulmonary vascular remodeling in newborn rats subjected to chronic hypoxia-induced pulmonary hypertension. A similar reduction in the severity of chronic hypoxia-induced pulmonary hypertension was also observed following chronic 10% CO₂ exposure in adult rats (31).

The mechanism accounting for the increased endothelium-dependent pulmonary vasorelaxation following chronic CO₂ exposure has not been previously investigated. Ooi et al. (31) were the first to document an increase in endothelium-dependent pulmonary arterial relaxation in vessels from chronically hypoxia-exposed animals treated with 10% CO₂. In the same study, chronic hypercapnia under normoxic conditions was not associated with a change in endothelium-dependent pulmonary vasorelaxation, in contrast to our current findings in neonatal rats. Yet chronic hypercapnia reduced the hypoxic pulmonary vasoconstriction response. Following NOS inhibition, vessels from both untreated and chronic hypercapnia-treated normoxic animals exhibited a similar hypoxic pulmonary vasoconstriction response (31). Taken together, the data of Ooi et al. (31) are in keeping with chronic CO₂ exposure enhancing NOS-dependent NO production. The present study provides a mechanistic explanation for this phenomenon by demonstrating that hypercapnia decreased arginase activity and/or expression.

**Fig. 6.** Lung tissue arginases activity (top) and arginase I and II isoform as well as eNOS expression (bottom) from control (n = 4), 13% O₂ (hypoxia; n = 4), and hypoxia + 10% CO₂-treated animals (n = 4). **P < 0.01 compared with hypoxia values by 1-way ANOVA and Tukey-Kramer multiple comparisons test.

**Fig. 7.** Top: 3rd-to-4th generation pulmonary arterial tissue in vitro nitrite generation from air (n = 9), air + 10% CO₂ (n = 9), 13% O₂ (hypoxia; n = 9), and hypoxia + 10% CO₂-treated animals (n = 7). Bottom: bronchoalveolar lavage fluid nitrite content from air (n = 5), air + 10% CO₂ (n = 3), 13% O₂ (hypoxia; n = 4), and hypoxia + 10% CO₂-treated animals (n = 3). **P < 0.01 compared with air-treated values by 1-way ANOVA and Tukey-Kramer multiple comparisons test.

**Fig. 8.** Endothelium-dependent (ACH) dose-response changes in U-46619-precontracted (EC₇₅) pulmonary arterial smooth muscle force generation obtained from control (air; n = 8), air + 10% CO₂ (n = 8), and hypoxia + 10% CO₂-treated animals (n = 4). **P < 0.01 compared with control values. ††P < 0.01 compared with pre-ACh stimulation (ACH “0” M) values by 2-way ANOVA and Tukey-Kramer multiple comparisons test.
Expression and activity of arginases in the lung are developmentally regulated (5) and increased in several lung diseases (1, 44) including pulmonary hypertension (26–29, 33, 43). In the present study, we showed that chronic CO₂ exposure reduces the newborn rat lung arginase activity likely via downregulation of its type I isoform. This reduction in arginase activity following chronic CO₂ exposure was observed in animals maintained under normoxic as well as hypoxic conditions. Yet only in the former was a reduction in lung arginase expression observed.

Pulmonary arteries acutely subjected in vitro to an increase in PCO₂ exhibit enhanced contraction (35–37) that appears to be endothelium-mediated (21). The mechanism responsible for this effect is not clear but is possibly related to Na⁺/HCO₃⁻ transport and activation of Ca²⁺/Cl⁻ channels (38). In the present study, we showed that chronic hypercapnia reduces the normoxia-treated newborn rat pulmonary arterial smooth muscle contraction to the thromboxane A₂ analog U-46619, likely because of enhanced basal NO generation. This was further corroborated by the increased NO generation documented in pulmonary arteries derived from 10% CO₂-exposed compared with the air-only-treated animals.

The pulmonary arteries from chronic hypoxia-treated animals showed decreased force generation dose-response to U-46619 in the present study. Chronic hypoxia has been previously shown to reduce the adult rat pulmonary arterial smooth muscle contraction in vitro in response to agonist stimulation (14). A similar reduction in pulmonary arterial muscle force generation in response to agonist stimulation has also been reported by us (39) in the chronic hypoxia-exposed chicken embryo. In addition, we (3) previously showed that the decreased pulmonary arterial muscle force generation in the duc tus arteriosus-ligated fetal sheep model of pulmonary hypertension was associated with smooth muscle phenotypic modulation. As such, pulmonary vascular smooth muscle phenotypic modulation from a contractile to a proliferative form is a hallmark of pulmonary vascular remodeling (14).

We (20) have previously shown that chronic hypoxia induces pulmonary vascular remodeling in newborn rats, which is significantly attenuated by exposure to 10% CO₂. In the present study, the increase in U-46619-induced pulmonary arterial muscle force generation in CO₂-exposed, compared with hypoxia-only-exposed, animals possibly relates to abrogation of the muscle phenotypic modulation. Further investigation on the effect of chronic CO₂ exposure on pulmonary arterial smooth muscle phenotypic modulation is warranted but beyond the scope of the present study. Aside from its effect on pulmonary arterial muscle force generation, we (16) have previously reported that chronic hypoxia in the newborn rat reduces the endothelium-dependent relaxation, an effect partially reversed by concomitant administration of allopurinol. A similar decrease in endothelium-dependent relaxation was observed in the chronically hypoxic animals in the present study. Likely, the hypercapnia-induced reduction in arginase activity, as documented in the present study, accounts for the greater magnitude of acetylcholine-induced relaxation in these compared with vessels from chronic hypoxia alone.

The evidence that hypercapnia-induced enhancement of endothelium-dependent relaxation is mediated via NO is supported by the following observations. First, we (4, 16) and others (34) have shown that in newborn rats, acetylcholine induces pulmonary arterial relaxation via eNOS stimulation and the consequent NO release. Second, there was no difference in the magnitude of endothelium-independent (sodium nitroprusside-induced) relaxation when comparing control and CO₂ (5.5 and 10% CO₂)-exposed animals, strongly suggesting that hypercapnia enhances vaso-dilation via eNOS-derived NO. Third, in the presence of nor-NOHA, the U-46619-induced pulmonary arterial muscle force generation of 10% CO₂-exposed animals was significantly reduced compared with vessels from air-treated controls. For this to occur, the chronic hypercapnia-treated animals’ vascular tissue arginase activity had to be lower than air-treated pups. Under these conditions, the increased L-arginine availability to eNOS (arginase inhibition) led to enhanced NO generation and the reduced agonist-stimulated force observed in the chronic hypercapnic compared with normocapnic animals.

In chronic lung disease, hypercapnia frequently coexists and is believed to worsen the severity of pulmonary hypertension in these subjects (15). Yet clinical evidence for such dogma is lacking. We propose, based on our current and previous (13) findings, that hypercapnia is a surrogate marker for worsening chronic lung disease and is associated with, but not causally linked to, the severity of pulmonary hypertension. In fact, mild hypercapnia in adults postheart surgery improves cardiac output and does not interfere with weaning from ventilatory support (2); thus hypercapnia in chronic lung disease may represent an adaptive or protective response rather than contributing to pathology.

Permissive hypercapnia is often used to prevent ventilator-induced lung injury and has been shown to reduce the severity of bronchopulmonary dysplasia (BPD) in neonates requiring mechanical ventilation (24). Although considered to be a safe clinical practice (24), an association between high PaCO₂ and intraventricular hemorrhage has been reported in very premature infants (19). Although considered to be a safe clinical practice (24), an association between high PaCO₂ and intraventricular hemorrhage has been reported in very premature infants (19). Yet, large clinical trials involving premature infants with BPD subjected to mild hypercapnia did not show an increased prevalence of this condition (17).

The extent to which maintaining a higher-than-physiological PaCO₂ results in a reduction in pulmonary vascular resistance and/or prevention of pulmonary arterial hypertension is unclear and, to the best of our knowledge, has not been previously investigated. Following chronic hypoxia-induced pulmonary hypertension, we (20) have also reported a significant reduction in right ventricular hypertrophy in 10% CO₂-treated newborn rats. Thus, to the extent that the newborn rat data can be extrapolated to humans, chronic hypercapnia reduces pulmonary vascular resistance and abrogates the development of chronic hypoxia-induced pulmonary hypertension. Further human studies addressing the effect of permissive hypercapnia and/or inhaled CO₂ are required before any conclusions about the clinical benefit and safety of their use in neonates can be derived.

The mechanism responsible for the 10% CO₂-induced reduction in arginase activity without changes in enzyme expres-
sion in the chronic hypoxic animals requires further investigation. Hibernation in hamsters is associated with hypercapnia (22), and in rats exposure to much higher CO₂ levels than used in this study induces anesthesia (25) and hibernation (23). Interestingly and potentially pertinent to the present study findings, liver arginase activity is reduced during hibernation (41). The reduced arginase activity during hibernation is associated with decreased protein metabolism and rapidly increases during arousal, implying changes in enzyme activity but not expression (41). We speculate that hypercapnia-induced reduction in arginase activity is directly responsible for abrogating the pulmonary vascular remodeling associated with chronic hypoxia. BPD in neonates is a chronic inflammatory process associated with airway and vascular remodeling. Arginases mediate the conversion of L-arginine to urea and ornithine. The latter is associated with decreased protein metabolism and rapidly increases in arginase activity is directly responsible for abrogating the pulmonary vasculature, there is evidence that the upregulation of arginase expression is mediated via a transforming growth factor-β₁ (TGF-β₁) pathway (10). An increase in pulmonary TGF-β signaling occurs with disruption of the terminal stage of lung development, and anti-TGF-β antibodies reduced extra-cellular matrix deposition in a murine model of BPD (30). We speculate that a reduction in arginase activity induced by maintenance of a high PaCO₂ may partly explain the putative benefits of permissive hypercapnia.

In summary, we demonstrate that chronic hypercapnia downregulates lung arginase expression and activity, resulting in enhanced endothelium-dependent pulmonary vasorelaxation in the newborn rat. These findings suggest a mechanism for the clinical observation of a reduced severity of chronic lung disease when permissive hypercapnia is employed.

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REFERENCES


