Blood Pressure Response to Hypoxia: Role of Nitric Oxide Synthase

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**Background:** Chronic exposure to hypobaric hypoxia has been shown to increase arterial pressure in genetically normal rats. The associated increase in blood pressure is unrelated to the hypoxia-induced erythrocytosis and persists indefinitely after restoration of normoxia. It is accompanied by a marked reduction in urinary excretion of nitric oxide metabolites (NOx) and is ameliorated by L-arginine supplementation. In view of the latter observations, we hypothesized that hypoxia-induced hypertension may be associated with downregulation of NO synthase (NOS).

**Methods:** Male Sprague Dawley rats were randomized to the hypoxic and control groups. Rats assigned to the hypoxic group were placed in chambers with air pressure maintained at 390 mm Hg. Animals assigned to the control group were kept in the chamber at 760 mm Hg air pressure. Animals were kept in their respective conditions for up to 21 days. Group of animals were tested at days 2, 3, 7, and 21.

**Results:** The hypoxic group exhibited a steady increase in arterial pressure beginning at day 3. This was accompanied by a transient increase followed by a significant decline in kidney NOS-I, NOS-II, and NOS-III abundance. A similar biphasic change was observed with NOS-II and NOS-III in the cardiac and vascular tissues. The changes in NOS abundance in the given tissues were associated with parallel changes in nitrotyrosine abundance, which reflects local NO production. The latter finding provides functional evidence for the changes observed in NOS abundance.

**Conclusions:** Chronic hypoxia-induced hypertension in rats is associated with marked downregulation of NOS isotypes, which can, in part, account for the previously reported L-arginine-responsive hypertension in this model. Am J Hypertens 2003;16:1043–1048 © 2003 American Journal of Hypertension, Ltd.

**Key Words:** Hypertension, hypoxia, nitric oxide synthase.

In a previous study, Vaziri and Wang reported that extended exposure to hypobaric hypoxia can cause sustained arterial hypertension in genetically normotensive Sprague-Dawley rats. The observed hypertension was not due to the associated erythrocytosis, as prevention of erythrocytosis by either iron depletion or regular phlebotomies failed to prevent the increase in arterial pressure. In a subsequent study, Ni et al reported that the increase in blood pressure (BP) was accompanied by a marked reduction in urinary excretion of nitric oxide metabolites (NOX) in this model. They further showed that hypoxia-induced hypertension and the associated decrease in urinary NOX could be prevented by L-arginine supplementation. On the basis of these observations, they concluded that hypertension induced by chronic exposure to hypobaric hypoxia in the rat is associated with and, at least in part, due to decreased NO availability. The present study was undertaken to test the hypothesis that the reduction in NO availability in this model is due to downregulation of NO synthase (NOS).

**Methods**

Male Sprague-Dawley rats (Charles River, Wilmington, MA), weighing 270 to 300 g, were used in this study. They were allowed free access to food (regular rat chow; Purina Mills Inc., Brentwood, MO) and water throughout the study period. The animals were randomly assigned to the hypoxic and sham-treated control groups. Animals assigned to the hypoxic group were placed in a hypobaric chamber in which the air pressure was kept at 390 mm Hg using a continuous vacuum pump and an adjustable inflow valve. The interior of the chamber was maintained at the ambient temperature. A normal interior light cycle was accommodated through the glass windows in the chamber’s structure.

The hypoxic group was kept under hypobaric condition
for up to 21 days. The chamber was briefly opened three times weekly for routine animal care, measurement of arterial BP, and procurement of blood samples, as appropriate. Animals randomized to the sham-treated control group were handled in an identical manner except for the normal air pressure within the chamber.

Arterial BP was measured using a tail sphygmomanometer (Harvard Apparatus, South Natick, MA) at baseline (day 0) and on days 1, 3, 7, 14, and 21. Urine collections were obtained periodically by use of individual metabolic cages placed within the chamber. Blood samples were obtained by orbital sinus puncture under light anesthesia at appropriate intervals. At the conclusion of the observation period, under general anesthesia (100 mg/kg of intraperitoneal thiobutabarbital) animals were euthanized by exsanguination and brain, kidney, thoracic aorta, and heart were harvested. The tissues were immediately cleaned, then frozen in liquid nitrogen and stored at −70°C until processed. Groups of animals were euthanized at days 2, 3, 7, and 21. Six animals were used at each time point.

Measurement of Nitrotyrosine

Nitrotyrosine abundance in the given tissues was measured by Western blot analysis using an antibody purchased from Upstate Biotechnology Inc. (Lake Placid, NY) as described in our earlier studies.³

Measurements of Tissue NOS Isoforms

Frozen tissues were processed for determination of endothelial (eNOS or NOS-III), inducible (iNOS or NOS-II), and neuronal (nNOS or NOS-I) NOS protein abundance using anti-eNOS, anti-iNOS, and anti-nNOS monoclonal antibodies (Transduction Laboratories, Lexington, KY) as described in our previous studies.⁴ Briefly, thoracic aorta, kidney, left ventricle, and brain were homogenized (25% wt/vol) in 10 mmol/L HEPES buffer, pH 7.4, containing 320 mmol/L sucrose, 1 mmol/L EDTA, 1 mmol/L DTT, 10 mg/mL leupeptin, and 2 mg/mL apro tinin at 0° to 4°C with a tissue grinder fitted with a motor-driven ground glass pestle. Homogenates were centrifuged at 12,000 g for 5 min at 4°C to remove tissue debris without precipitating plasma membrane fragments. The supernatant was used for determination of NOS proteins. Total protein concentration was determined with the use of a Bio-Rad kit (Bio-Rad Laboratories, Hercules, CA). The tissue extracts (50 μg of protein for aorta and heart and 100 μg protein for kidney and brain) were size-fractionated on 4% to 12% Tris-glycine gel (Novex, Inc., San Diego, CA) at 120 V for 3 h. After electrophoresis, proteins were transferred onto hybond-ECL membrane (Amersham Life Science Inc., Arlington Heights, IL) at 400 mA for 120 min with the Novex transfer system. In preliminary experiments, we had found that the given protein concentrations were within the linear range of detection for our Western blot technique. The membrane was prehybridized in 10 μL of buffer A (10 mmol/L Tris-HCl, pH 7.5, 100 mmol/L NaCl, 0.1% Tween 20, and 10% nonfat milk powder) for 1 h and then hybridized for an additional 1-h period in the same buffer containing 10 μL of the given anti-NOS monoclonal antibody (1:1000). The membrane was then washed for 30 min in a shaking bath, and the wash buffer (buffer A without nonfat milk) was changed every 5 min before 1 h of incubation in buffer A plus goat antimouse IgG/horseradish peroxidase at the final titer of 1:1000. Experiments were performed at room temperature. The washes were repeated before the membrane was developed with a light-emitting nonradioactive method with the use of ECL Western blot detection reagent (Amersham Life Science Inc). The membrane was then subjected to autoluminography for 10 sec. The autoluminographs were scanned with a laser densitometer (model PD1211, Molecular Dynamics, Sunnyvale, CA) to determine the relative optical densities of the bands. In all instances, the membranes were stained with Ponceau stain, which verified the uniformity of protein load and transfer efficiency across the test samples.

Data Analysis

Data are expressed as mean ± SEM. Analysis of variance (ANOVA), multiple range test, and Student t test were used as appropriate. P values less than .05 were considered significant.

Results

General Data

Longitudinal monitoring of arterial pressure revealed no significant change in BP during the initial 48 to 72 h after exposure to hypobaric hypoxia. Thereafter, arterial pressure steadily increased in the animals subjected to hypobaric hypoxia (Fig. 1). In contrast, BP remained virtually unchanged in the control animals, which were kept in the chamber at normal atmospheric pressure. Body weight significantly increased in both groups during the study period. However, the magnitude of weight gain was significantly less in animals subjected to hypobaric hypoxia.
(final weight, 312 ± 5 g) than that seen in the control group (353 ± 6 g; P < .01, P < .01).

**Kidney NOS and Nitrotyrosine**

Data are illustrated in Fig. 2. Compared with the control group, the hypoxic group exhibited an initial increase in kidney tissue eNOS and iNOS proteins on day 2, followed by a significant decline to subnormal values during the 21-day observation period. Similarly, renal tissue nNOS abundance significantly decreased during the observation period. The transient increase and the subsequent decrease in immunodetectable NOS isoforms in the kidney was accompanied by parallel changes in nitrotyrosine abundance in this tissue.

**Cardiac Tissue NOS Data**

Data are illustrated in Fig. 3. Cardiac tissue eNOS and iNOS abundance increased early in the course of hypobaric hypoxia. This was followed by a decline to the control levels in the chronic phase. As with the NOS isoforms, a transient increase followed by a decline toward baseline level was observed in the heart nitrotyrosine abundance.

**Aorta NOS Data**

Data are shown in Fig. 4. As with the kidney, aorta eNOS and iNOS abundance increased significantly on days 2 and 3 of exposure to hypobaric hypoxia. Thereafter, both eNOS and iNOS abundance declined to values that were slightly higher than in the control group. Nitrotyrosine abundance in the aorta increased significantly on day 3 followed by a significant decline thereafter.

**Brain nNOS Data**

Data are given in Fig. 5. Brain tissue nNOS and nitrotyrosine abundance increased significantly within 48 h after exposure to hypobaric hypoxia and remained elevated throughout the study period.

**Discussion**

Chronic exposure to hypobaric hypoxia resulted in a significant increase in arterial pressure in animals used in the present study, confirming the results of our earlier studies. The study revealed a steady increase in arterial pressure beginning 3 days after exposure to hypobaric
hypoxia, with virtually no change during the first 48 to 72 h. Sequential measurements of NOS isoforms in groups of animals studied at different time points revealed a significant but transient increase in eNOS and iNOS in the kidney, thoracic aorta, and left ventricle within 48 to 72 h of exposure to hypobaric hypoxia. This was followed by a steady decline in eNOS and iNOS abundance toward the control values in the aorta and left ventricle and to significantly below the control levels in the kidney of hypoxic animals. In addition, kidney nNOS abundance was significantly reduced in hypoxic rats as compared to the control group.

These data demonstrate a consistent pattern of early increase followed by a steady decline in eNOS and iNOS in the kidney and cardiovascular tissues during the exposure to hypobaric hypoxia. Thus, maintenance of normal BP during the initial phase of exposure to hypoxia was accompanied by an increasing eNOS and iNOS in the aorta, heart, and kidney. Moreover, subsequent development and progression of hypertension was associated with declining tissue eNOS, iNOS, and nNOS in the hypoxic animals. The transient increase and the subsequent decline in NOS isoforms in the kidney, cardiac, and vascular tissues was associated with parallel changes in nitrotyrosine abundance. As a byproduct of tyrosine nitration, nitrotyrosine abundance can parallel production of NO in the tissues. Thus, the sustained reduction of nitrotyrosine abundance in kidney, heart, and aorta of rats exposed to chronic hypobaric hypoxia points to the reduction of NO production and its possible role in the genesis of hypertension in this model.

Under physiologic conditions, renal NO is mainly derived from constitutively expressed NOS isoforms. This includes eNOS, which is expressed in the renal vascular endothelium; a form of iNOS, which is constitutively expressed in the thick ascending limb of Henle’s loop and cortical collecting ducts; and nNOS, which is primarily expressed in the macula densa. Nitric oxide plays an important role in regulation of renal hemodynamics and arterial BP. In this regard NO produced in the vascular tissue serves as a potent vasodilator and a major counter-regulatory factor opposing vasoconstrictor effects of en-
dothelin, angiotensin II, and renal sympathetic nerve activity.\textsuperscript{5,8} Therefore, NO functions as a modulator of both preglomerular and postglomerular circulation. In addition, NO plays a prominent role in regulation of the pressure natriuresis.\textsuperscript{9–11} This may be, in part, mediated by NO-induced increase in medullary blood flow leading to natriuresis by increasing the medullary interstitial pressure.\textsuperscript{12,13} Furthermore, NO generated by nNOS in the macula densa attenuates tubuloglomerular feedback-mediated afferent anteriolar vasoconstriction in response to luminal sodium concentration and, as such, facilitates natriuresis.\textsuperscript{14} In fact, inhibition of renal nNOS activity results in salt-sensitive hypertension and an attenuated renal nNOS response to salt loading may contribute to hypertension in the Dahl salt-sensitive rat.\textsuperscript{15,16} Thus, progressive reductions of renal eNOS, iNOS, and nNOS in parallel with an increase in arterial pressure in the hypoxic animals points to a possible causal association. This supposition is supported by the favorable response to L-arginine administration in this model shown in our earlier study.\textsuperscript{2}

In addition to downregulation of renal NOS isoforms shown here, chronic hypobaric hypoxia can cause oxidative stress,\textsuperscript{17} which can aggravate hypertension through the inactivation of NO\textsuperscript{18} and generation of vasoconstrictive isoprostanes.\textsuperscript{19}

Our rats with hypoxia-induced hypertension exhibited a significant increase in brain tissue nNOS. Nitric oxide derived from nNOS in the brain attenuates central sympathetic activity.\textsuperscript{20,21} Upregulation of brain nNOS seen in rats with hypoxia-induced hypertension is also seen in other models of hypertension, including salt-sensitive Dahl rats,\textsuperscript{22} spontaneously hypertensive rats,\textsuperscript{23} rats with abdominal aorta coarctation,\textsuperscript{24} lead-induced hypertension,\textsuperscript{25,26} oxidative stress-induced hypertension,\textsuperscript{27} and uremic hypertension.\textsuperscript{18} In addition, isolated cerebral arterial pressure in the absence of systemic hypertension, such as that induced by chronic exposure to microgravity, results in upregulation of brain nNOS.\textsuperscript{28} It thus appears that upregulation of brain nNOS in rats with hypoxia-induced hypertension represents a compensatory response, which is common to other forms of hypertension.

It is not clear whether the increase in arterial pressure during extended exposure to hypobaric hypoxia represents an adaptive or maladaptive biological response. However, persistent hypertension after cessation of hypoxia in this model\textsuperscript{1,2} is clearly abnormal and is reminiscent of sleep apnea-associated hypertension in humans.

In conclusion, chronic hypoxia-induced hypertension in rats is associated with marked downregulation of NOS isoforms, which can, in part, account for depressed urinary NOx excretion and L-arginine-responsive hypertension in this model.

Acknowledgments

Dr. Ni and Dr. Barton have contributed equally to this project.

References