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.

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In a previous study, Vaziri and Wang1 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.1 In a subsequent study, Ni et al2 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 maintained at 390 mm Hg using a continuous vacuum pump and an adjustable inflow valve. 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 appro-
priate. 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 sphygmomanom-
eter (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 intraperi-
toneal thiobutabarbital) animals were euthanized by ex-
sanguination 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 mea-
ured by Western blot analysis using an antibody pur-
chased 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 endo-
thelial (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 aprotinin at 0°C 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 precipi-
tating 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 ex-
tracts (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 trans-
ferred onto hybond-ECL membrane (Amersham Life Sci-
ence Inc., Arlington Heights, IL) at 400 mA for 120 min
with the Novex transfer system. In preliminary exper-
iments, 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 de-
veloped 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
autoluminoography for 10 sec. The autoluminograms were
scanned with a laser densitometer (model PD1211, Mo-
lecular Dynamics, Sunnyvale, CA) to determine the rela-
tive optical densities of the bands. In all instances, the
membranes were stained with Ponceau stain, which veri-
fi ed 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 pres-
sure steadily increased in the animals subjected to hypo-
baric 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 sig-
nificantly less in animals subjected to hypobaric hypoxia

![FIG. 1. Longitudinal measurements of systolic arterial pressure in rats exposed to hypobaric (390 mm Hg) hypoxia for 21 days. n = 6 animals at each time point; *p < .05, **p < .01 v baseline.](https://academic.oup.com/ajh/article-abstract/16/12/1043/157335/1044)
(final weight, $312 \pm 5\, g$) than that seen in the control group ($353 \pm 6\, g$; $P < .01$, $P < .01$).

**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. This supposition is supported by our earlier studies, which showed that administration of L-arginine prevents hypertension and restores urinary NOx excretion 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 counterregulatory factor opposing vasoconstrictor effects of en-
dothelin, angiotensin II, and renal sympathetic nerve activity. 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. This may be, in part, mediated by NO-induced increase in medullary blood flow leading to natriuresis by increasing the medullary interstitial pressure. Furthermore, NO generated by nNOS in the macula densa attenuates tubuloglomerular feedback-mediated afferent arteriolar vasoconstriction in response to luminal sodium concentration and, as such, facilitates natriuresis. 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. 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.

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

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. Upregulation of brain nNOS seen in rats with hypoxia-induced hypertension is also seen in other models of hypertension, including salt-sensitive Dahl rats, spontaneously hypertensive rats, rats with abdominal aorta coarctation, lead-induced hypertension, oxidative stress-induced hypertension, and uremic hypertension. In 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. 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 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.

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References


