Sympathetic Denervation Blocks Blood Pressure Elevation in Episodic Hypoxia

Eugene C. Fletcher, Joachim Lesske, Juraj Culman, Charles C. Miller, and Thomas Unger

We have previously described a rat model that responds to repetitive episodic hypoxia (FiO₂ nadir 3–5% for 12 seconds every 30 seconds for 7 hr/day for 35 days) with chronic increase in arterial blood pressure. The purpose of the current study was to determine if peripheral sympathetic nervous system denervation blocks this persistent blood pressure elevation. Chemical sympathetic denervation was achieved and maintained by three intraperitoneal injections (100 mg/kg 6-hydroxydopamine) on days 1, 3, and 27 of a 47-day experiment in two groups of rats. One denervated group was subjected to episodic hypoxia for 40 consecutive days beginning on day 7 and the other remained unhandled in their usual cages. A third group was injected with vehicle only and subjected to the same episodic hypoxia while a fourth group remained unhandled for 40 days. The vehicle-treated, episodic hypoxia–exposed group showed a 7.7 mm Hg increase in mean arterial blood pressure (conscious, unrestrained) over the 40-day period, whereas all other groups showed a decrease in mean arterial pressure. The left ventricle and septum/whole body weight ratio was higher in both episodic hypoxia–exposed groups at the end of the study. Plasma epinephrine in both groups administered 6-hydroxydopamine was higher on day 6 than in the vehicle-injected rats. Measurement of catecholamines in cardiac muscle homogenate confirmed denervation in 6-hydroxydopamine animals. These results indicate that the peripheral sympathetic nervous system is necessary for the persistent increase in blood pressure in response to repetitive episodic hypoxia. (Hypertension 1992;20:612–619)

Key Words: • apnea • sleep apnea syndromes • anoxia • anoxemia • hypertension, essential • blood pressure, high • sympathetic nervous system

Obstructive sleep apnea with transient hypoxemia is associated with acute elevation of blood pressure, and in up to 50% of patients with chronic apnea, persistent elevation of daytime blood pressure is seen.1–3 Repetitive acute hypoxia with heightened sympathetic nerve activity could play an important role in the persistent elevation of blood pressure seen in some patients.

With some species variability, acute hypoxemia causes increased heart rate, variable changes in arterial pressure, and increased cardiac contractility and output through stimulation of central and peripheral chemoreceptors and perhaps other local effects.4–6 The effector arm of this reflex involves increased sympathetic discharge. Plasma norepinephrine is elevated in acute hypoxia,7,8 and urinary catecholamine elevation in patients with severe sleep apnea is reversed by tracheostomy.9 Acute hypoxia induces elevated blood pressure and increased microneurographic amplitude, implying increased postganglionic sympathetic activity.10,11 In spontaneously hypertensive rats (SHR)12 and in humans with borderline hypertension, the sympathetic response to acute hypoxia is exaggerated.13 The response is further exaggerated in borderline hypertensive humans when apnea is added to acute hypoxia.14 Finally, muscle nerve sympathetic activity recorded during apnea in humans shows a progressive increase throughout the apnea followed by an abrupt reduction at apnea termination.15

A key concept in the above hypothesis is how the hypoxic occurs: chronic, recurrent, acute dips versus chronic continuous exposure. Many studies have shown a blood pressure-lowering effect of continuously applied hypoxia. Residents at high altitudes have a lower blood pressure and lower incidence of hypertension than sea level control subjects.16 Several authors have reported blunting of the usual rise in blood pressure when SHR17–19 or renal hypertensive rats20 are exposed to chronic, continuous high-altitude hypoxia. Lund and Tomane21 reported a reduction in blood pressure in rats exposed to daily continuous hypoxia for 6 weeks. Even acute hemodynamic changes vary with the duration of the hypoxic stimulus. Marshall and Metcalfe22 report a fall in blood pressure with tachycardia in rats exposed to 3-minute, graded hypoxia of 12%, 8%, and 6%. However, they often observed a rise in pulse and blood pressure in the first 60–90 seconds of hypoxia. Cardenas and Zapata23 created atmospheric hypoxia (100% nitrogen) in rats for periods of 5, 10, and 15 seconds and observed tachycardia with increased blood pressure during the first 10 seconds of hypoxia.

It appears that the duration of acute hypoxia or the manner by which chronic hypoxia is administered plays...
a key role in systemic hemodynamic response. We have described a rat model that responds to 35 days (7 hr/day) of repetitive, acute (12-second stimulus) hypoxia, simulating the pattern of episodic hypoxia of sleep apnea in humans by showing a persistent elevation of daytime mean arterial blood pressure (±13.7 mm Hg) and an increase in left ventricular mass. Further work with that model has demonstrated that removal of peripheral chemoreceptor feedback by sectioning of the carotid sinus nerve eliminates the persistent elevation of blood pressure. Since the efferent loop of the proposed recurrent acute hypoxia-chemoreceptor-blood pressure elevation arc involves increased activity of the sympathetic nervous system, we hypothesized that ablation of peripheral sympathetic nerves with multiple injections of 6-hydroxydopamine (6-OH dopamine) might block the chronic elevation of blood pressure in this model.

Methods

Forty-five male (300–450 g) Wistar rats (Dr. K. Thomae GmbH, Biberach, Germany) were used in this experiment. They were housed in designated animal facilities and provided with standard rat chow and water ad libitum. This protocol was reviewed and approved by the Regierungspräsidium Karlsruhe, Abteilung Tier- schutz (Animal Protection Committee, State of Baden Württemberg).

Hemodynamic Measurements

After intraperitoneal injection of chlorohydrate (400 mg/kg), rats were instrumented with a catheter placed in the abdominal aorta (PP-10 in PP-50, Portex Corp., Hythe, UK) via the right femoral artery and a catheter (PP-25) placed in the inferior vena cava via the right femoral vein on day 0 of the experiment. Both catheters were exteriorized at the nape of the neck for recording blood pressure, sampling blood, or administering drug therapy, always with the animals breathing room air. Because of a previously noted tendency for abdominal aortic aneurysms to develop at the tip of indwelling catheters within 2 weeks, both catheters were surgically removed on day 6 after blood pressure measurements were taken. The insertion procedure was repeated on day 46 using the left femoral artery and vein. Ampicillin (20 mg/kg) was administered intraperitoneally immediately after and 24 hours after catheter insertion, and any animal showing evidence of limb ischemia or infection 24 hours after surgery was euthanatized. Upon arterial catheter insertion, 0.2 ml whole blood was removed for hematocrit and hemoglobin determination (Coulter Counter, Coulter Elect., Hialeah, Fla.). The rats were allowed at least 20 hours for recovery before hemodynamic measurements were taken, and no measurement was made if a rat appeared to be in pain or had obvious ischemia of the extremity operated on. On the day of blood pressure recording (room air), catheters were attached to Statham P23Db pressure transducers with signal amplification by a Gould Brush pressure computer (Gould Inc., Oxnard, Calif.). The first 30 minutes of recording was not used to allow the animals time for adjustment from handling. Continuous mean blood pressure and heart rate were recorded for 2–3 hours, with periodic recording of pulse pressure. The lowest stable mean blood pressure recorded continuously for 10 minutes or more and the nearest coinciding pulse pressure were used to define mean, systolic, and diastolic blood pressure for the recording session.

Experimental Groups

After baseline blood pressure recording (day 1) in all 45 rats, 13 rats remained in their usual cages for 40 days, serving as unhandled, untreated controls (group 1). Nineteen rats were injected intraperitoneally with 100 mg/kg 6-OH dopamine (2,4,5-trihydroxyphenethylamine hydrobromide; Sigma Chemical Co, Stuttgart, FRG) dissolved in 0.9% NaCl and 1% ascorbic acid (vehicle) on days 1, 3, and 27 of the experimental period. Their blood pressure was recorded again on day 6. Subsequently, eight of these rats (group 3) were placed in Plexiglas chambers and subjected to episodic hypoxia (3–5% nadir ambient oxygen) every 30 seconds (see below) for 40 days. The remaining 11 rats were placed in their usual cages for 40 days, serving as unhandled, 6-OH dopamine-treated controls (group 4).

After a second blood pressure recording on day 6, 13 vehicle-only–treated rats (group 2) were placed in Plexiglas chambers and exposed daily to the same level of episodic hypoxia as group 3.

Hypoxic Chambers

During daily episodic hypoxia, animals were housed in 20 identical cylindrical Plexiglas chambers (length 28 cm, diameter 10 cm, volume 2.4 l) with tight-fitting lids. Using a timed solenoid valve, nitrogen (20%) was distributed to each chamber for 12 seconds at a flow that was adjusted to reduce the ambient fractional concentration of oxygen (FiO2) to 3–5% for approximately 3–6 seconds. This was followed by infusion of compressed air, allowing gradual return (over 15–18 seconds) of ambient air to FiO2 20.9%. The cycle was repeated twice per minute during the day for 6–8 hours on consecutive days. Multiple serial arterial blood samples in three nonprotocol rats during episodic hypoxia showed the average nadir level of SaO2 in this system to be 70% (range, 60%–80%). At the same time nitrogen was being distributed to hypoxic chambers, compressed air at approximately the same nitrogen flow rate was distributed to the sham cages simulating the same noise and air disturbance. A dampening device at the air/nitrogen end of the chamber was used to dissipate the air stream so that no direct jets of gas disturbed the animal.

Plasma and Myocardial Catecholamine Measurement

On days 1, 6, and 47 during blood pressure–measuring sessions in conscious, unrestrained animals, 0.5–1.0 ml whole blood was rapidly withdrawn from the arterial catheter while an equal volume of normal saline was instilled through the venous catheter. Using this sample, plasma epinephrine, norepinephrine, and dopamine were determined by radioenzymatic assay on the majority of the animals in each group (see Table 2). In group 1 animals, catecholamines were drawn only at the final day 47 session. Total body weight was recorded on day 1 and day 46 just before catheter placement. After blood pressure was measured on day 47, the rats were anesthetized and rapidly exsanguinated, and the heart was removed. The atria and great vessels were dissected away, the right ventricle was separated from the left
ventricle and septum, and the two muscles were weighed separately. Small sections of the muscle of the right and left ventricle were weighed, frozen on dry ice and stored at −80°C for assay of cardiac muscle catecholamines. The specimens were later homogenized in ice-cold 0.1 M HCl (0.1 mg tissue in 20 μl HCl). Aliquots of the homogenate were taken for protein determination, and the rest was centrifuged 30 minutes at 12,000 rpm at 4°C. This supernatant was assayed for norepinephrine and epinephrine content by reverse-phase high-performance liquid chromatography with electrochemical detection (HPLC-EC). The mobile phase was delivered by a pump at a flow rate of 0.6 ml/min onto a 20 cm × 4 mm chromatograph column with Nucleosil 100–5 C18 packing (Macherey Nagel, Duren, Germany). Identification and quantification of samples was achieved by measurement of elution time and peak size. Detection was performed with a glassy carbon electrode, and applied potential was set at 0.6 V versus Ag/AgCl reference electrode.28,29

**Statistical Analysis**

All morphometric and blood pressure measurements made at baseline were compared across groups by analysis of variance (ANOVA) using Tukey's multiple-comparison tests.30 Comparisons of the change in parameters over time between each episodic hypoxia-exposed group and its respective normoxic unhandled control (groups 1 and 2 versus 3 and 4) were done using ANOVA with simultaneous linear contrast. Differences between baseline and day 47 values either expressed as absolute or percentage of baseline within each group were tested using Wilcoxon signed rank comparison. The null hypothesis was rejected at p<0.05. Throughout the article and in the tables, deviation from the mean is reported as ±1 SEM.

**Results**

There were no significant differences in weight, hematocrit, hemoglobin, heart rate, or systolic, diastolic, and mean blood pressures among the four groups at baseline (Table 1). On the sixth day after baseline measurements and after the first two intraperitoneal injections of 6-OH dopamine, the mean blood pressure in both 6-OH dopamine–injected groups was significantly lower than their respective day 1 values (Figure 1). The heart rates were also significantly higher than

**TABLE 1. Baseline and Follow-up Change in Morphometric, Blood, and Blood Pressure Data**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1 vehicle unhandled (n=13)</th>
<th>Group 2 vehicle &amp; hypoxia (n=13)</th>
<th>Group 3 6OH-DOP &amp; hypoxia (n=8)</th>
<th>Group 4 6OH-DOP unhandled (n=11)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>368 (8)</td>
<td>372 (7)</td>
<td>382 (13)</td>
<td>370 (12)</td>
<td>NS</td>
</tr>
<tr>
<td>Change of base (%)</td>
<td>+26* (3)</td>
<td>+12 (1)</td>
<td>..NS..</td>
<td>3 (3)</td>
<td>21* (4)</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>44.1 (0.6)</td>
<td>43.5 (0.6)</td>
<td>44.4 (0.5)</td>
<td>44.1 (1.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Absolute change</td>
<td>−0.8 (0.7)</td>
<td>+6.1* (1.1)</td>
<td>..NS..</td>
<td>+2.7 (1.1)</td>
<td>−2.9 (1.8)</td>
</tr>
<tr>
<td>Hemoglobin (g%)</td>
<td>14.2 (0.2)</td>
<td>15.1 (0.2)</td>
<td>14.4 (0.4)</td>
<td>14.8 (0.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Absolute change</td>
<td>+0.1 (0.2)</td>
<td>+1.0 (0.5)</td>
<td>..NS..</td>
<td>+0.6 (0.6)</td>
<td>−1.7 (0.8)</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>365 (9)</td>
<td>335 (11)</td>
<td>364 (12)</td>
<td>351 (12)</td>
<td>NS</td>
</tr>
<tr>
<td>Change of base (%)</td>
<td>−8 (4)</td>
<td>−3 (4)</td>
<td>..NS..</td>
<td>−5 (5)</td>
<td>−7 (3)</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>116 (3)</td>
<td>119 (2)</td>
<td>124 (4)</td>
<td>120 (3)</td>
<td>NS</td>
</tr>
<tr>
<td>Change of base (%)</td>
<td>−3 (2)</td>
<td>+5 (4)</td>
<td>..NS..</td>
<td>−10 (6)</td>
<td>−8 (3)</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>79 (3)</td>
<td>80 (2)</td>
<td>84 (1)</td>
<td>80 (3)</td>
<td>NS</td>
</tr>
<tr>
<td>Change of base (%)</td>
<td>0 (4)</td>
<td>+8* (3)</td>
<td>..&lt;0.05..</td>
<td>−13 (6)</td>
<td>−3 (4)</td>
</tr>
<tr>
<td>Mean BP (mm Hg)</td>
<td>89.3 (2.6)</td>
<td>89.7 (1.6)</td>
<td>93.8 (1.7)</td>
<td>89.8 (3.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Change of base (%)</td>
<td>−1.7 (2.8)</td>
<td>+8.5* (3.6)</td>
<td>..&lt;0.04..</td>
<td>−9.1 (5.5)</td>
<td>−4.7 (3.6)</td>
</tr>
</tbody>
</table>

6OH-DOP, 6-hydroxydopamine; bpm, beats per minute; BP, blood pressure. Unbracketed values are group means, and values in parentheses are SEM. NS in far right column for a given parameter indicates lack of statistical significance between the means of the four groups at baseline using analysis of variance (ANOVA) (Tukey's multiple comparison test). The NS or p values bracketed by braces indicate results of statistical comparisons of the differences between each study group and its respective unhandled control compared with the differences between the other two groups (groups 1 and 2 versus 3 and 4) using ANOVA with simultaneous linear contrast.

*pDifferences between baseline and day 47 value either expressed as absolute or percent of baseline, significant at p<0.05 using the Wilcoxon signed rank comparison.
FIGURE 2. Line graph shows mean arterial blood pressure in all four groups of rats. There was an 8.5% increase in blood pressure for the vehicle-injected, episodic hypoxia–exposed rats versus no increase or a fall in blood pressure in the other groups (analysis of variance with simultaneous linear contrast, p<0.04). The period of episodic hypoxia, days of 6-hydroxydopamine (6OH-DOP) injection, and mean arterial pressure measurements (MAP) are displayed at the bottom of the graph.

baseline at day 6 in the rats injected with 6-OH dopamine (group 3, 364±12 versus 381±46 bpm, p<0.05; group 4, 351±12 versus 392±16, p<0.05; days 1 and 6, respectively) but not in the untreated rats.

Both groups treated with episodic hypoxia showed less body weight gain over the 46-day period of the experiment than either of their respective unhandled controls (Table 1). In addition, group 2 showed a significant increase in hematocrit. All groups except the vehicle-treated, episodic hypoxia–exposed rats showed a downward trend in their systolic, mean, and diastolic blood pressures over the 46-day period. The mean blood pressure of the vehicle-injected, episodic hypoxia–treated group increased significantly compared with its unhandled controls, whereas the 6-OH dopamine–injected, episodic hypoxia–treated versus the 6-OH dopamine–injected, unhandled group showed no differences in their blood pressure trend from day 1 to day 47 (p<0.04) (Figure 2).

At termination of the study, there were no significant differences in right ventricular weights among the four groups. However, both groups exposed to episodic hypoxia (irrespective of 6-OH dopamine) showed increased left ventricle/body weight ratios compared with those of their respective controls (Figure 3).

Plasma catecholamine levels did not vary among groups at baseline, but mean norepinephrine level in

the vehicle-injected, episodic hypoxia–exposed rats was higher compared with that in the unhandled controls at final determination (Table 2). On day 6 (72 hours after the second dose of 6-OH dopamine but before hypoxia exposure), epinephrine levels in both 6-OH dopamine–injected groups were higher than those in the vehicle-treated group. These values returned to control levels by the end of experiment. The ventricular catecholamine levels collected on day 47 after completion of the episodic hypoxia confirmed catecholamine depletion in the 6-OH dopamine–treated animals. Right and left ventricular epinephrine and norepinephrine concentrations and contents were significantly lower in both 6-OH dopamine–injected groups compared with those in the nontreated groups (Table 3). Right ventricle epinephrine and norepinephrine levels were significantly higher in the vehicle-treated, hypoxia-exposed rats than those in their unhandled controls.

Discussion

Several previous publications have shown that acute blood pressure elevation in relation to apnea and hypoxemia may be related to increased sympathetic nervous system activity. We postulated that persistent elevation of blood pressure in response to chronic recurrent episodic hypoxia might also be mediated through the sympathetic nervous system. We tested this hypothesis with an animal model of chronically elevated blood pressure in response to episodic hypoxia that was developed in our laboratory. Important new findings of the present study are 1) animals treated with 6-OH dopamine (causing chemical sympathectomy) and exposed to recurrent episodic hypoxia for 40 days do not show chronic elevation of daytime blood pressure, 2) left ventricular weight increased in all animals exposed to episodic hypoxia despite changes in blood pressure, and 3) hematocrit increased in the episodic hypoxia–exposed, vehicle-treated animals.

It is important to understand which components of the sympathetic nervous system 6-OH dopamine affects when injected systemically. Shortly after the injection of
a large dose, 6-OH dopamine accumulates by active uptake in catecholamine-containing neurons and is transported to intraneuronal sites where it displaces norepinephrine. There, a destructive process begins where energy-producing cytochromes or related elements of the respiratory transport chain are destroyed. The nerve synapses lose their ability to conduct, and depending on the dose, there may be destruction or alteration of the postsynaptic receptor site. If nerve terminals are completely destroyed, there is marked reduction of norepinephrine, tyrosine hydroxylase activity, and monoamine uptake capacity. This phenomenon is termed a “chemical sympathectomy” and is relatively selective, with cholinergic neurons, Schwann cells, glia, endothelial, and other cells remaining unaffected at the ultrastructural level. The drug has no effect on adrenal catecholamines. Pretreatment "chemical sympathectomy" and is relatively selective, with cholinergic neurons, Schwann cells, glia, endothelial, and other cells remaining unaffected at the ultrastructural level. The drug has no effect on adrenal catecholamines. Pretreatment with 6-OH dopamine has been used to prevent development of hypertension in certain rat models, including SHR and adrenocorticotrophic hormone–induced hypertension. However, some results concerning the antihypertensive effect of 6-OH dopamine in SHR are contradictory, indicating that the effect may be transient or depend on which portion of the denervated adrenergic vasculature controls hypertension.

The success of this experiment depended on the injection of enough 6-OH dopamine to maintain chemical sympathectomy while avoiding the direct acute blood pressure–lowering effects of the drug. Studies comparing the injection of 6-OH dopamine into adult versus neonatal rats indicate that peripheral sympathetic denervation may be complete and permanent when injected at an early age. Studies in adult rats require multiple injections of the drug to maintain chemical sympathectomy. The frequency of injections needed to maintain sympathetic blockade varies according to the parameter monitored. Previous publications indicate that blood pressure lowered by 6-OH dopamine injection returns to basal levels as early as 3–5 days or as long as 8 days after injection. Recovery of adrenergic nerve function in vascular smooth muscle occurs in 7 days; however, the blood pressure response to electrical stimulation of the spinal cord in pithed rats reaches only 50% of that of controls by day 14 and returns completely to baseline by day 28. Norepinephrine levels in the heart tissue decrease by 95% 1 day after injection and remain less than 20% of control levels at 21 days and 30% of control by 28 days. Thus, depending on the site and mechanism whereby episodic hypoxia affects chronic blood pressure changes, the blocking effect of 6-OH dopamine may vary. Based on the above data, we chose day 20 to reinject the animals, allowing a 20-day recovery period before final blood pressure measurement to minimize any chance of an acute hypertensive effect of the drug.

The schedule followed in the present study allowed the blood pressure to return to preinjection levels by the

<table>
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<th>Group 1 vehicle unhandled (n=13)</th>
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<th>Group 4 6OH-DOP unhandled (n=11)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epinephrine (nmol/l)</td>
<td>0.49 (0.11)</td>
<td>0.42 (0.11)</td>
<td>0.39 (0.10)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Norepinephrine (nmol/l)</td>
<td>0.75 (0.11)</td>
<td>0.72 (0.12)</td>
<td>0.70 (0.16)*</td>
<td>0.81 (0.13)*</td>
<td></td>
</tr>
<tr>
<td>Dopamine (nmol/l)</td>
<td>0.77 (0.13)</td>
<td>0.63 (0.17)</td>
<td>0.83 (0.14)</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Far right column indicates statistical differences between group means (*) using Tukey's multiple comparison tests. The final norepinephrine value (†) for group 2 varied significantly from the final one for group 1, unhandled controls. Both 6-hydroxydopamine (6OH-DOP) groups had significantly higher epinephrine levels at 6 days.

**Table 3. Myocardial Catecholamine Levels**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1 vehicle unhandled (n=8)</th>
<th>Group 2 vehicle &amp; hypoxia (n=9)</th>
<th>Group 3 6OH-DOP &amp; hypoxia (n=7)</th>
<th>Group 4 6OH-DOP unhandled (n=10)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epinephrine</td>
<td>15.8 (2.7)</td>
<td>30.1 (3.3)*</td>
<td>8.2 (1.6)</td>
<td>12.1 (2.3)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>18.1 (3.1)*</td>
<td>24.0 (3.2)*</td>
<td>5.7 (1.1)</td>
<td>9.3 (2.7)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Epinephrine and norepinephrine values in the myocardium of the vehicle-injected animals differed significantly from those in the 6-hydroxodopamine (6OH-DOP)–injected animals using Tukey's multiple-comparison tests.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1 vehicle unhandled (n=8)</th>
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<th>Group 3 6OH-DOP &amp; hypoxia (n=7)</th>
<th>Group 4 6OH-DOP unhandled (n=10)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epinephrine</td>
<td>965 (104)*</td>
<td>1,535 (89)*</td>
<td>226 (38)</td>
<td>140 (26)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>1,004 (73)*</td>
<td>988 (62)*</td>
<td>174 (30)</td>
<td>141 (17)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Catecholamine values are expressed in terms of picograms per gram of actual muscle weight.

*Epinephrine and norepinephrine values in the myocardium of the vehicle-injected animals differed significantly from those in the 6-hydroxodopamine (6OH-DOP)–injected animals using Tukey's multiple-comparison tests.

†Group 2 values also varied from group 1 by p<0.05.
time of measurement but with sympathetic blockade of the vasculature and myocardium still in effect. First, myocardial catecholamine levels in both the episodic hypoxia and handled, 6-OH dopamine-treated animals remained one-half to one-third of the values in vehicle-treated and unhandled animals, indicating depression of myocardial sympathetic nerve terminal function without complete suppression. Second, plasma norepinephrine between baseline and end of study remained the same in 6-OH dopamine-treated and control rats, indicating potential return of some peripheral sympathetic function. Blood pressure in both 6-OH dopamine-treated animals remained at or below preinjection levels, whereas in episodic hypoxia-exposed animals, blood pressure climbed significantly in comparison with baseline values within the study group and compared with unhandled control values.

By depleting norepinephrine stores, 6-OH dopamine may cause “denervation hypersensitivity” either because of increased numbers or increased affinity of remaining α-receptors.21 Denervation hypersensitivity would tend to preserve the blood pressure response to a given stimulus such as episodic acute hypoxia through stimulation of α-receptors by circulating (e.g., adrenal medulla) norepinephrine. Hypersensitivity could only have influenced the results of the present study if blood pressure in the 6-OH dopamine, hypoxia-treated animals had increased during the 40-day trial, reflecting a preserved response to vascular or baroreceptor sympathetic stimulation. Instead, blood pressure in both 6-OH dopamine groups actually showed a slight decrease. This, along with myocardial catecholamine levels, confirms that 6-OH dopamine was effective in partially paralyzing the sympathetic nervous system, and denervation hypersensitivity could not have affected outcome.

Plasma epinephrine was elevated in the 6-OH dopamine-treated animals at day 6 compared with vehicle-injected controls (Table 2). These levels were taken 3 days after the second injection of 6-OH dopamine, presumably at a point of maximal peripheral sympathetic destruction and before regeneration of nerve terminals. Since 6-OH dopamine damaged homestatic mechanisms and blood pressure in these animals was low at this time (Figure 1), we presume that the adrenal medulla was attempting to compensate for the relative hypotension/underperfusion by excreting catecholamines with epinephrine predominating at approximately an 8/1 ratio. Day 47 epinephrine levels (20 days after the last dose of 6-OH dopamine) were not elevated in the 6-OH dopamine-treated rats presumably because of partial regeneration of the baroreceptor and vascular sympathetic terminals with decreased adrenal gland stimulation. There was no correlation between day 6 plasma epinephrine levels and blood pressure or corrected ventricular weights. These results were not unexpected but were ancillary to the purpose of the study. Of further note is the elevation of norepinephrine in the vehicle and hypoxia-treated group at day 47. There was no correlation between these elevated levels and the change in mean blood pressure or left ventricular weight in this group. Since the elevation was not uniform in the group 2 animals, a much larger group would be needed to verify or refute this finding. We examined norepinephrine levels after 35 days of episodic hypoxia in a previous study and found no elevation (n=8), suggesting that this data may be spurious.28

Control values for norepinephrine levels in the cardiac ventricles have been reported at 500–950 pg/mg,29-40 which is consistent with the value of 965 pg/mg in our unhandled controls (Table 3). The right ventricular epinephrine and norepinephrine values in our animals and hypoxia-exposed animals were one and one half and two times that of unhandled controls, respectively, whereas the levels in the left ventricle were not different. Since the methods of analysis were identical and the samples were run concurrently, one cannot ignore the elevated catecholamines in the right ventricle. Acute hypoxia has been shown to increase myocardial norepinephrine turnover in rats41,42 and guinea pigs,43 but chronically increased sympathetic activity is usually associated with no change or a decrease in tissue catecholamine levels.3 After chronically administrating intermittent hypoxia (4 continuous hours per day, 24–75 exposures) is a known potent stimulus to pulmonary artery vasoconstriction and right ventricular stress and hypertrophy,44-47 one could speculate that sympathetic activity to the right heart might be differentially increased over that of the left in the present model. The fact that right ventricular hypertrophy was not seen in the current study is consistent with results in our previous studies.24,25 Only those animals with daytime hypoxia (brought about by carotid body denervation) and episodic hypoxia develop such hypertrophy, indicating that right ventricular hypertrophy is probably proportional to a certain cumulative effect of hypoxia. Certainly, kinetic studies of catecholamine turnover are more accurate for determining myocardial sympathetic activity than absolute levels and would be needed to prove an elevated level of sympathetic activity in the right ventricle of this model.

The vehicle-injected (group 2) rats showed a small but significant increase in left ventricle/body weight ratio that could be attributed to the 7.7 mm Hg persistent increase in blood pressure in response to episodic hypoxia. However, the 6-OH dopamine-injected, episodic hypoxia-exposed (group 3) rats also showed an increase in left ventricle weight despite the absence of a daytime increase in systemic blood pressure. This may in part be related to the very small body weight gain in group 3. However, such an increase in left ventricular weight in response to hypoxia but without elevation in systemic blood pressure has been reported previously.21,46,49 We have also seen this in carotid body–denervated rats exposed to episodic hypoxia that did not develop increased systemic blood pressure.25 One possible cause of this could be pulmonary hypertension–induced right ventricle–septal hypertrophy. Since we did not weigh the septa separately, we cannot completely rule this out. However, right ventricular hypertrophy did not occur in any of the hypoxia-exposed rats, making this possibility unlikely. Ou and Smith49 have demonstrated increased left ventricle/body weight ratio exclusive of septal hypertrophy in hypoxia-exposed rats without “systemic hypertension.” Lund and Tomanek21 have shown increased left ventricle/body weight ratio and a 20% increase in myocyte diameter in Wistar-Kyoto rats exposed to continuous hypobaric hypoxia equivalent to 6,100 m for 6 weeks with an actual decrease in systolic blood pressure compared with baseline. This suggests that chronic hypoxia exposure in the form of continuous high
altitude or episodic hypoxia as in our model may have an
effect on left ventricular mass independent of blood
pressure.

In summary, the present study shows that the periph-

eral (nonadrenal) sympathetic nervous system is neces-
sary for the development of persistent elevation of blood
pressure in response to 40 days of episodic hypoxemia for
approximately 7 hours per day in rats. The association of
sleep apnea and hypertension has led to much speculation
about the need to examine aging hypertensive populations
for occult sleep apnea at potentially great expense. Hope-

fully, further studies in this model may allow greater
insight into the relation of these two diseases.

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