Renin Activity and Blood Pressure in Response to Chronic Episodic Hypoxia

Eugene C. Fletcher, Gang Bao, Rena Li

Abstract—Previous studies in several strains of rats have demonstrated that 35 days of recurrent episodic hypoxia (EH) (7 hours per day), with fractionation of inspired oxygen that produces desaturation equivalent to the recurrent hypoxemia of sleep apnea, results in an 8 to 13 mm Hg persistent increase in diurnal systemic blood pressure (BP). Carotid chemoreceptors and the sympathetic nervous system have been shown to be necessary for development of this BP increase. Both renal artery denervation and adrenomedullation block the BP response to chronic EH. The present study was undertaken to define further the role of the kidneys and the renin-angiotensin system in this BP increase. Separate groups of male Sprague-Dawley rats had either (1) bilateral renal artery denervation with EH, (2) sham surgery with EH, (3) sham surgery with sham EH (compressed air), (4) EH with losartan, (5) unhandled with losartan, or (6) unhandled. The experimental period lasted 35 days. Both renal-artery denervated and losartan-treated animals showed no BP change or a lowering of BP in response to EH, whereas the sham-operated EH animals showed a progressive, sustained increase in resting room air BP. BP remained at basal levels or fell in unhandled and unhandled losartan-treated animals. Plasma renin activity was elevated 4-fold versus basal levels in EH animals with renal nerves intact but remained at baseline levels in denervated animals. At the end of the experiment, renal tissue catecholamines confirmed renal denervation in those animals. In conclusion, EH causes a progressive increase in BP, mediated in part through renal sympathetic nerve activity that acts to increase renin-angiotensin system activity through angiotensin II type 1 receptors. (Hypertension. 1999;34:309-314.)

Key Words: hypoxia ■ blood pressure ■ denervation, renal ■ sympathetic nervous system ■ sleep apnea syndromes ■ hypertension, systemic ■ renin-angiotensin system

Recent publications have implicated increased sympathetic nervous system activity, driven by chemoreceptor response to progressive hypoxemia, as a contributing cause of acute elevation of blood pressure (BP) in patients with obstructive sleep apnea.1–3 The mechanism for sustained diurnal rise in BP after many years of repetitive apnea remains a mystery, but chronic stimuli, including recurrent episodic hypoxia (EH), repeated arousals, or repetitive Mueller maneuvers, may contribute to it.

We have developed a rat preparation that mimics the hypoxic changes seen in sleep apnea patients. With the use of individual cylindrical cages and a method for rapid exchange of inspired concentration of oxygen (FiO2) to as low as 2% to 3%, arterial oxyhemoglobin saturation falls acutely to levels of 70% to 75%. Such EH, when administered repetitively (every 30 seconds for 7 hours per day for 35 days), increases resting, diurnal mean arterial pressure (MAP) by 8 to 13 mm Hg.4 Chemoreceptor denervated5 and chemical sympathectomy rats (neurotoxin 6-OH dopamine)6 show no increase in MAP following EH, which suggests that chemoreflex-activated sympathetic nervous system activity plays an important role in sustained elevation of BP in this setting. Further investigation of sympathetic mechanisms in this model show that either ablation of renal artery nerves or removal of the adrenal medulla blunt the BP response to EH.7

Sympathetic innervation of the kidney by way of renal artery nerves has been implicated as an important mechanism of chronic BP regulation.8 The kidney contains both α- and β-adrenoceptors that respond to sympathetic stimulation with increased renin secretion.9 Hypertension in several animal models, including the spontaneously hypertensive rat (SHR), is believed to be related to increased adrenergic activity.10,11 Increased activity of both splanchnic and renal nerves has been reported in SHR versus normotensive Wistar-Kyoto control rats.12 Bilateral renal denervation in SHR will ameliorate or delay the usual chronic increase in systemic BP.12 The present study was undertaken to see whether renal denervation in the rat blocked the BP response to chronic EH through its effect on the renin-angiotensin system (RAS) and, if so, whether or not the angiotensin II (Ang II) type 1 receptor (AT1) was instrumental in this response.
Methods

Surgery
Thirty-eight, 12- to 20-week-old (300 to 350±50 g) Sprague-Dawley rats were purchased from Harlan Sprague Dawley (Indianapolis, Ind). Approximately 5 days before EH exposure began, the animals were anesthetized by intraperitoneal injection of 1 cc of a cocktail containing ketamine (37.5 mg/mL) and xylazine (5 mg/mL). Injections were repeated as needed to maintain deep anesthesia. Both renal arteries were exposed through an abdominal incision. The arteries were dissected free of connective tissue and painted with 20% phenol. Midway through the experiment, abdominal surgery was repeated; the renal arteries were again isolated and painted with 20% phenol.

During the initial surgery, the catheter portion of a radio-telemetry probe (Data Sciences) was introduced transcutaneously at the iliac bifurcation of the abdominal aorta, with the tip resting just distal to the renal arteries. The telemetry unit was attached to the anterior abdominal wall as the incision was closed. A similar approach was used to implant radio-telemetry probes in 21 additional rats in which the renal arteries remained undisturbed. In 10 rats, similar anesthesia was used to allow insertion of abdominal aorta catheters (Silastic, id 0.05 mm; Dow Corning) through the right femoral artery. The catheter tips were exteriorized at the nape of the neck for recording heart rate (HR) and BP.

Study Groups
Rats were divided into 6 groups (Table 1). Seven EH renal denervated telemetry rats (EH-DEN), 7 sham-operated EH telemetry rats (EH-SO), and 7 sham-operated telemetry rats treated with losartan (EH-LO) were exposed to EH for 35 days. Seven sham-operated telemetry rats were exposed to episodic compressed air for 35 days (SHAM). Five arterial-catheter rats remained unhandled (UNH) and UNH-LO, BP was measured 24 hours before or 48 hours after the 35-day study period, under resting, unrestrained, and unanaesthetized conditions. The catheters were attached to Statham P23Db pressure transducers with signal amplification (Hewlett Packard Co 7858B) and HR and MAP were measured over a corresponding 2- to 3-hour time period. In most cases, this required new femoral artery catheters to be placed in the limb opposite the site of the original catheter. The lowest stable MAP recorded continuously for ≥10 minutes was taken as the value for the recording session.13

Tissue Catecholamines
As confirmation of successful renal artery denervation, animals were euthanized at the end of the study and the kidneys were harvested and flash frozen in liquid nitrogen. Tissue was stored at −70°C until assay. Later, 100 mg of kidney tissue was sonicated in 0.5 mL of buffer (pH 4.0) containing 0.17 mol/L citrateacate and 10% methanol. The sonicate was centrifuged and the clear aspirate was subjected to microfiltration (Amicon, W.R. Grace) and high-performance liquid chromatography with electrochemical detection.14 Levels of tissue norepinephrine lower than SHAM controls indicated successful renal artery sympathetic denervation.

Plasma Renin Activity
At baseline and again at the end of the 35-day study period, 2 mL of blood yielding 0.7 to 1.0 mL of plasma was drawn, centrifuged, and frozen at −70°C. Later, after being warmed, the plasma was incubated at 37°C in the presence of protein inhibitors to prevent conversion of angiotensin I to Ang II. Measurements were made as described in published techniques.15,16 Plasma renin activity (PRA) is expressed as ng angiotensin I generated per mL of plasma per hour of incubation.

Terminal Morphometric Studies
Total body weight was recorded at baseline and after the 35-day study period. All aspects of the protocol were approved by the Animal Studies Subcommittee of the University of Louisville School of Medicine. Animals were housed in designated animal facilities and provided rat chow and water ad libitum.

Statistical Methods
Tissue catecholamine levels were compared between groups by unpaired t test. Baseline versus follow-up body weights, BP, and HR in the UNH and UNH-LO rats were compared by paired t tests. Weekly MAP values in the telemetry probe rats and basal versus end of study PRA were compared by 1-way ANOVA for repeated measures with post hoc Bonferroni and Student t tests, when

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Chamber Exposure</th>
<th>Surgery</th>
<th>BP Measurement Method</th>
<th>Drug Rx</th>
</tr>
</thead>
<tbody>
<tr>
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<td>7</td>
<td>Hypoxia</td>
<td>Abd incision</td>
<td>Telemetry</td>
<td>None</td>
</tr>
<tr>
<td>EH-DEN</td>
<td>7</td>
<td>Hypoxia</td>
<td>Denervation</td>
<td>Telemetry</td>
<td>None</td>
</tr>
<tr>
<td>SHAM</td>
<td>7</td>
<td>Compressed air</td>
<td>Abd incision</td>
<td>Telemetry</td>
<td>None</td>
</tr>
<tr>
<td>EH-LO</td>
<td>7</td>
<td>Hypoxia</td>
<td>Abd incision</td>
<td>Telemetry</td>
<td>Losartan</td>
</tr>
<tr>
<td>UNH</td>
<td>5</td>
<td>None</td>
<td>Art catheter</td>
<td>Direct</td>
<td>None</td>
</tr>
<tr>
<td>UNH-LO</td>
<td>5</td>
<td>None</td>
<td>Art catheter</td>
<td>Direct</td>
<td>Losartan</td>
</tr>
</tbody>
</table>

The 35-day exposure period by sampling ambient nadir oxygen (MiniOX I, Catalyst Research Corp). A mean daily nadir FiO2 was calculated for each cage.

Hemodynamic Measurements
The telemetry-probe rats exposed to EH were placed in their chambers in the morning of days 7, 14, 21, 28, and 35 for measurement of resting (non-hypoxia stimulated) BP. From 09:00 AM to noon, EH was withheld for 3 hours while MAP data were collected continuously and averaged. In catheterized rats (UNH and UNH-LO), BP was measured 24 hours before or 48 hours after the 35-day study period, under resting, unrestrained, and unanaesthetized conditions. The catheters were attached to Statham P23Db pressure transducers with signal amplification (Hewlett Packard Co 7858B) and HR and MAP were measured over a corresponding 2- to 3-hour time period. In most cases, this required new femoral artery catheters to be placed in the limb opposite the site of the original catheter. The lowest stable MAP recorded continuously for ≥10 minutes was taken as the value for the recording session.13

The cycle was repeated; the renal arteries were again isolated and painted with 20% phenol.
Results

EH-SO rats exhibited an overall 10.0±2.5 mm Hg increase in resting MAP over the 5-week period, whereas EH-DEN rats showed no significant change in BP (Table 2, Figure 1). Likewise, SHAM rats showed no significant change in MAP. EH-LO rats showed a significant fall in MAP from 98.2±1.7 mm Hg to 85.9±2.7 mm Hg (P<0.002). UNH-LO rats showed a similar decrease in MAP from 95.2±1.6 mm Hg to 82.3±1.8 mm Hg (P<0.001), whereas the UNH rats showed no significant change (Table 1, Figure 1). None of the groups showed any significant change in resting HR during any week of the study conditions (Figure 2).

Norepinephrine levels from the kidney homogenates of EH-DEN rats (113.2±27.6 pg/g) were significantly lower than homogenates of SHAM rats (441.4±24.5 pg/g), which served as controls (P<0.001) (Figure 3). Epinephrine levels from kidneys in the same groups at the end of the study were 106.5±7.5 pg/g and 127.0±35.1 pg/g, respectively (NS).

PRA was elevated ≈4-fold from baseline in EH-SO rats (1.7±0.2 versus 6.6±1.2 pg/mL) (Figure 4). Both the EH-LO and UNH-LO rats showed an expected, marked increase in PRA from baseline (EH-LO=2.1±0.5 versus 16.5±1.8 pg/mL; UNH-LO=1.7±1.0 versus 18.8±3.2 pg/mL). None of the other groups (UNH, EH-DEN, or SHAM) showed any significant change in PRA from baseline to follow-up at 5 weeks.

Baseline and follow-up body weights for each group of rats are shown in Table 3. All EH rat groups, regardless of treatment, failed to gain significant weight over the 5 weeks of EH. Only SHAM, UNH, and UNH-LO animals gained significant weight over the 5-week study period.

Discussion

The present study was undertaken to define further the role of renal sympathetic nerve activity and PRA in the diurnal BP response to chronic EH. The important findings of this study are (1) renal artery denervation as well as AT1 blockade eliminate the diurnal BP response during 35 days of EH, (2) resting PRA after 35 days of EH is elevated 4-fold versus baseline and this rise is blocked by renal denervation, and (3) losartan in unhandled and EH treated animals causes a further lowering of BP below baseline, with marked elevation of PRA, which implies that the RAS is in part responsible for maintenance of basal BP. These results support and expand upon the observations of our previous study that examined the role of the adrenal medulla and renal sympathetic nerve activity in EH. In that study, we found that both renal denervation and, separately, adrenal demedullation blocked the development of diurnal BP elevation in EH. Our wish to examine PRA activity and the effect of AT1 blockade in the

TABLE 2. Mean Arterial Pressures, in mm Hg

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
<th>Week 5</th>
</tr>
</thead>
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<tr>
<td>EH-SO</td>
<td>92.3±2.2</td>
<td>103.2±3.1</td>
<td>101.5±4.5</td>
<td>103.7±4.5</td>
<td>105.1±4.8</td>
<td>102.3±2.7</td>
</tr>
<tr>
<td>EH-DEN</td>
<td>93.1±2.7</td>
<td>93.6±5.0</td>
<td>88.9±3.6</td>
<td>86.7±3.3</td>
<td>91.6±2.8</td>
<td>91.9±4.1</td>
</tr>
<tr>
<td>SHAM</td>
<td>93.4±6.5</td>
<td>91.2±5.1</td>
<td>92.6±4.7</td>
<td>92.4±3.7</td>
<td>89.1±5.3</td>
<td>88.4±5.0</td>
</tr>
<tr>
<td>EH-LO</td>
<td>98.2±1.7</td>
<td>89.7±1.5</td>
<td>88.0±1.9</td>
<td>91.1±2.1</td>
<td>87.8±3.3</td>
<td>85.9±2.7</td>
</tr>
<tr>
<td>UNH</td>
<td>94.0±2.6</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>94.5±3.4</td>
</tr>
<tr>
<td>UNH-LO</td>
<td>95.2±1.6</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>82.3±1.8</td>
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</table>

Figure 1. Change in MAP over the 35-day period in all groups. Only the EH-SO rats showed significant elevation of BP vs baseline. Both losartan treated groups (EH-LO, UNH-LO) showed a significant fall in BP by day 35 of treatment. The o and ç represent UNH and UNH-LO, respectively. BP and HR were measured by indwelling arterial catheters in these 2 groups vs telemetry measurement in the other groups, thus making data available only on days before and after the 35-day exposure. EH-SO indicates sham-operated rats exposed to episodic hypoxia; EH-DEN, rats exposed to episodic hypoxia with bilateral renal artery denervation; SHAM, sham-operated rats exposed to episodic compressed air; EH-LO, rats exposed to episodic hypoxia with losartan; UNH, unhandled rats; and UNH-LO, unhandled rats given losartan. * indicates a significant change from baseline. P<0.05.

Figure 2. Change in mean HR over 35-day period in all groups. None of the rats showed significant change in HR vs baseline. Abbreviations as in Figure 1.
EH model prompted the present study. The results suggest that 8 hours per day of EH for 35 days induces diurnal BP increase through long-term elevation of renal sympathetic activity, in turn activating the renal RAS, which is evidenced by increased resting (non-hypoxia) renin activity, and elimination of the increased BP response by AT1 blockade.

The techniques used in the present study were identical to those used in our previous renal denervation study.7 As in that study, we recognized that renal artery sympathetic denervation is not permanent, and functional reinnervation might take place during the period of the trial.12,17 Successful maintenance of renal artery denervation requires repeat denervation at 3 weeks to keep renal tissue norepinephrine content ≤20% of SHAM rats. Our EH-DEN animals showed renal tissue norepinephrine levels (collected ∼4 days after the last hypoxic exposure) that were 4-fold lower than the levels of SHAM rats. This indicates that the renal artery denervation was successfully maintained until the end of the hypoxic exposure.

Studies have postulated that sympathetic overactivity could be causal in some forms of systemic hypertension. Most studies conclude that plasma norepinephrine is elevated in young hypertensive subjects18 and that these catecholamines emanate from sympathetic overactivity mainly in the heart and kidneys.19 It is believed that renal vascular resistance is increased in essential hypertension and mediated by heightened activity of renal sympathetic nerves. Augmented forearm vasodilatation in response to α-adrenergic blockade suggests that enhanced sympathetic vasoconstrictor tone exists in skeletal muscle of young, mildly hypertensive humans.20 Increased muscle nerve sympathetic activity has been demonstrated in mildly hypertensive humans versus normotensive age-matched controls.21 Increased muscle sympathetic nerve activity has also been demonstrated in renovascular hypertension that is associated with increased activity of the RAS; also, angiotensin has been shown to increase sympathetic activity through central nervous system action.22 Apparently, sympathetic overactivity occurs in early stages of hypertension but not in later stages because other mechanisms such as renal vascular disease or vascular remodeling evolve. Increased muscle nerve sympathetic activity has not been found uniformly in older, obese hypertensive individuals.23

Hypoxia-driven arterial chemoreceptors are potent stimulators of sympathetic activity. SHR exhibit exaggerated carotid sinus chemoreceptor discharge during hypoxia.24 Furthermore, sympathetic activity during hypoxia is accentuated by a breathhold, as is seen in sleep apnea.25 In humans, Hedner has demonstrated increased muscle nerve sympathetic activity in the waking state in apnea subjects both with and without hypertension.26 These data suggest that both acute and chronic hypertension associated with the hypoxemia of sleep apnea may result from heightened sympathetic activity. Hypoxia can also increase sympathetic activity by other mechanisms. Uremic toxins and ischemic metabolites (such as adenosine, produced during hypoxemia) injected into the kidney have been shown to reflexively increase efferent renal sympathetic nerve activity and BP through renal afferent activity.27,28 Furthermore, Ang II is known to act in the central nervous system, in autonomic ganglia, and at the neuroeffector junction to facilitate sympathetic activity.29 Thus, it seems that recurrent hypoxia could stimulate efferent renal nerve sympathetic activity directly through chemoreflex sympathetic activation, indirectly through the effect of circulating adenosine or other ischemic metabolites, and by RAS facilitation of central and peripheral nervous system sympathetic activity. Finally, hypoxia is also a potent stimulant of adrenal medullary activity. Epinephrine excreted by the adrenal medulla may accentuate sympathetic nerve activity.

<table>
<thead>
<tr>
<th>Table 3. Final Body Weights, All Animals</th>
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<tbody>
<tr>
<td>Group</td>
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<tr>
<td>-------</td>
</tr>
<tr>
<td>EH-SO</td>
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<tr>
<td>EH-DEN</td>
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<td>SHAM</td>
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<tr>
<td>EH-LO</td>
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<tr>
<td>UNH</td>
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<tr>
<td>UNH-LO</td>
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</table>

Change in body weight from baseline to follow-up for each group of rats. Only SHAM, UNH, and UNH-LO animals gained significant weight over the 5-week period of study. EH rats, regardless of treatment, failed to gain significant weight.

Figure 3. Renal tissue catecholamines in EH-DEN and SHAM rats at end of study. Norepinephrine of EH-DEN rats was approximately one fourth that of SHAM rats. * indicates a significant difference from SHAM rats at P<0.05. Abbreviations as in Figure 1.

Figure 4. PRA at baseline and 35 days in all study and control groups. PRA in EH group was elevated ∼3-fold vs baseline, whereas no change occurred in PRA in the EH-DEN, SHAM, or UNH groups. Both groups treated with Losartan (EH-LO, UNH-LO) showed marked increases in PRA as expected with AT1 blockade alone. * indicates a significant change from baseline at P<0.05. Abbreviations as in Figure 1.

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by several mechanisms suggested by Floras. These include (1) epinephrine-induced stimulation of prejunctional β-2 adrenoceptors that enhance norepinephrine release during sympathetic neural transmission, and (2) neuronal uptake of circulating epinephrine, which is then coreleased with norepinephrine to increase the neurotransmitter effectiveness. Our previous study demonstrates that adrenal medullectomy and, therefore, decreased epinephrine release, blocks the effect of EH on chronic BP response.

Acutely, increased renal sympathetic nerve activity promotes the release of renin, increased Ang II formation, antinaturesis, and renal vasoconstriction. The short-term effects of Ang II are (1) potentiation of peripheral sympathetic neurotransmission and end organ response to norepinephrine, (2) vasoconstriction, (3) increased HR and myocardial contractility, (4) increased vasopressin release, (5) increased drinking behavior and salt appetite, and (6) increased tubular reabsorption of water. Long-term vascular effects of Ang II are (1) hypertrophy/hyperplasia of cardiomyocytes and vascular smooth muscle cells and (2) activation of vascular growth factors. The SHR and the first generation cross between SHR and the Wistar-Kyoto rat respond to a salt load with increased sympathetic activity and decreased sodium and water excretion. Over the long term, the sympathetic nervous system promotes trophic effects on vascular smooth muscle and cardiac muscle growth independent of BP effects. Cardiac muscle growth is mediated by α-adrenergic receptors. Activation of cardiac myocyte α1-receptors induce selective increases in contractile protein gene transcription and hypertrophic myocyte growth. As stated above, sympathetic overactivity has been demonstrated to be greatest during the early stages of hypertension when vascular remodeling is most likely to change the structure and function of the heart and blood vessels (cardiac muscle hypertrophy, decreased lumen size), which influence long-term regulation of BP.

The HR changes of acute apnea are variable; they range from bradycardia to tachycardia. A higher resting HR has recently been reported in unmedicated patients with obstructive sleep apnea versus matched controls. Although one might expect a higher HR in resting non-hypoxic rats with elevated sympathetic activity, this was not seen in the present experiment. This might be because the rats were almost uniformly asleep during these resting measurements when HR would be lowest. This would minimize differences among the various groups.

The results of this present study and our previous renal denervation study suggest that chronic, recurrent EH may elevate BP in rats through peripheral neurogenic sympathetic vasoconstriction and adrenal medullary epinephrine release induced by hypoxic stimulation of chemoreceptors. Circulating epinephrine released by the adrenal medulla may be taken up by post-ganglionic nerves and released as a cotransmitter with norepinephrine. This would prolong and accentuate the vasoconstrictive signal to renal, mesenteric, and muscle vasculature beyond the period of EH. Beyond the acute response, increased sympathetic activity to the kidney could further contribute to diurnal BP increase by increasing renin production and converting angiotensin I to Ang II. Ang II is instrumental in the vascular remodeling associated with chronic hypertension. Other mechanisms under investigation for the EH effects on changes of BP include the possible role of endothelin.

Acknowledgments

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References


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