Blood pressure response to chronic episodic hypoxia: the renin-angiotensin system

EUGENE C. FLETCHER,1 NATALIA OROLINOVA,1 AND MICHAEL BADER2

1Departments of Medicine and Respiratory Disease, University of Louisville, Louisville, Kentucky 40292; and 2The Max Delbruck Center for Molecular Medicine, Humboldt University, D-13092 Berlin-Buch, Germany

Received 13 February 2001; accepted in final form 27 September 2001

Fletcher, Eugene C., Natalia Orolinova, and Michael Bader. Blood pressure response to chronic episodic hypoxia: the renin-angiotensin system. J Appl Physiol 92: 627–633, 2002; 10.1152/japplphysiol.000152.2001.—By using an inspired oxygen fraction that produces oxyhemoglobin desaturation equivalent to that seen in human sleep apnea, we have demonstrated that 35 days of recurrent episodic hypoxia (every 30 s for 7 h/day) results in an 8–13 mmHg persistent increase in diurnal systemic mean arterial blood pressure (MAP) in rats. Blockade of angiotensin II receptors (AT1a) eliminates this response. Separate groups of male Sprague-Dawley rats were fed high-salt (8%), ad libitum-salt, or low-salt (0.1%) diets for 7 wk: 2 wk of wash-in for baseline blood pressure measurement and 5 wk of experimental conditions. Rats in each salt group were subjected to episodic hypoxia whereas controls remained unhandled under normoxic conditions. MAP remained at basal levels in all nonepisodic hypoxia controls as well as high-salt-diet episodic hypoxia-exposed rats. Ad lib and low-salt episodic hypoxia rats showed an increase in MAP from 106 and 104 mmHg at baseline to 112 and 113 mmHg, respectively (P < 0.05). Whole kidney renin mRNA was suppressed in high-salt controls and episodic hypoxia rats, whereas kidney AT1a mRNA showed opposite changes. Suppression of the renin-angiotensin system with a high-salt diet blocks the increase in MAP in episodic hypoxia-challenged rats, in part by suppressing local tissue renin levels. Upregulation of the tissue angiotensin II system appears to be necessary for the chronic blood pressure changes that occur from episodic hypoxia.

We have developed a normobaric rat preparation that mimics the hypoxic changes seen in sleep apnea patients. With the use of individual cylindrical cages with a rapid (12 s) exchange of the inspired concentration of oxygen (FiO2) to as low as 2–3%, blood oxygen saturation acutely falls to levels around 70–75%. Such episodic hypoxia, when administered repetitively in 30-s cycles for 7 h/day for 35 days, increases resting unstimulated diurnal mean arterial blood pressure (MAP) by 8–13 mmHg (8). Chemoreceptor denervation as well as chemical sympathectomy blocks the increase in MAP after episodic hypoxia, suggesting that chemoreflex-activated, sympathetic nervous system activity plays an important role in sustained elevation of blood pressure in this setting (3, 11). Acute episodic hypoxia in these rats is associated with increased splanchnic nerve sympathetic activity (10). Bilateral renal nerve ablation, removal of the adrenal medulla, and angiotensin II-receptor (AT1a) blockade also blunt the blood pressure response to episodic hypoxia (2,9). In the latter two studies, plasma renin activity was elevated in episodic hypoxia groups compared with controls, implying that increased activity of the renin-angiotensin system might be integral in converting recurrent acute hypoxia during sleep into awake, sustained elevated blood pressure.

Thus both renal nerve sympathetic efferent and systemic renin-angiotensin system activity appear important in the pathogenesis of elevated blood pressure in response to episodic hypoxia (2,9). Salt loading is known to suppress the renin-angiotensin system and renal nerve sympathetic activity (5). Yet earlier studies in salt-sensitive spontaneously hypertensive rats show that hypertension is worsened in response to stressful stimuli (i.e., air jet) in the presence of a salt load (5). These seemingly contradictory studies prompted us to examine what salt loading and salt depletion would do in the face of another serious stress: intermittent or episodic hypoxemia. We hypothesized that chronic dietary salt loading would suppress the renin-angiotensin system to the point of preventing the elevated blood pressure response to chronic episodic hypoxia. We ex-

Address for reprint requests and other correspondence: E. C. Fletcher, Division of Respiratory Medicine, Univ. of Louisville, 530 South Jackson St., Louisville, KY 40222 (E-mail: ecflet01@guise.louisville.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
amined renal tissue renin, AT1α, and endothelial nitric oxide synthase (eNOS) mRNA in response to episodic hypoxia to confirm the effect of salt loading and depletion on sympathetic mechanisms in the kidney. We wished to establish which is the more powerful mechanism in episodic hypoxia with salt loading: central sympathetic renal nerve activation (hypoxia) or volume expansion with renal nerve suppression.

**METHODS**

**Surgery.** Fifty-seven 12- to 14-wk-old (300 ± 50 g) Sprague-Dawley rats were purchased from Harlan Sprague Dawley (Indianapolis, IN). Anesthesia was achieved by intraperitoneal injection of 0.5–1 ml of a mixture of ketamine (37.5 mg/ml) and xylazine (5 mg/ml). In 43 rats, 3 wk before experimental conditions were begun, the catheter portion of a radiotelemetry probe (Data Sciences, St. Paul, MN) was introduced transmurally 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. Twenty-one rats served as unhandled controls, receiving comparable diets to the episodic hypoxia rats but without exposure to episodic hypoxia. Because of the limited number of telemetry devices, high- and low-salt unhandled control rats (n = 14) had femoral artery catheters placed for their baseline and follow-up blood pressure measurement after 2 wk on their respective diets and at the end of the study. With the use of similar anesthesia as above, abdominal aorta catheters (Silastic, 0.05 mm ID; Dow Corning, Midland, MI) were placed via the right femoral artery and exteriorized at the nape of the neck for recording heart rate and blood pressure.

**Study groups.** Rats were divided into six groups. Telemetry rats consisted of the following: 12 high-salt diet with episodic hypoxia (EH-High), 12 ad libitum-salt diet with episodic hypoxia (EH-AdLib), 12 low-salt diet with episodic hypoxia (EH-Low), and 7 ad libitum-salt diet with no exposure to episodic hypoxia (Con-AdLib); all telemetry rats were given their respective diets for 50 days beginning on day −15 of the study. The high-salt diet consisted of 6% NaCl (no. 100078, Dyets, Bethlehem, PA), the low-salt diet consisted of 0.1% NaCl (no. 113764, Dyets), and the ad libitum diet consisted of standard rat chow (150 meq NaCl/kg). Exposure to episodic hypoxia began after measurement of baseline blood pressure at day 0 (day 15 on their respective diets) and continued for 35 days. The seven Con-AdLib and 14 nontelemetry rats [7 high-salt diet (Con-High) and 7 low-salt diet (Con-Low) rats] served as unhandled, salt diet controls. These rats likewise began their respective diets on day −15 but were not exposed to episodic hypoxia at any time during the study.

**Hemodynamic measurements.** Telemetry-probe rats exposed to episodic hypoxia as well as Con-AdLib rats were placed in their chambers on the mornings of days 0, 8, 14, 20, 26, 32, and 37 for measurement of resting (nonhypoxia stimulated) blood pressure. On these days, from 0900 to 1200, episodic hypoxia was withheld for 3 h while MAP and pulse data were collected continuously and averaged. In the diet control rats (high- and low-salt diets) without telemetry devices, blood pressure was measured instead with an abdominal aorta catheter within 24 h preceding day 0 and again within 48 h after day 35 of the study period under resting, unrestrained, and unanesthetized conditions. Catheters were attached to Statham P23Db pressure transducers with signal amplification (Hewlett Packard 7858B, Andover, MA), and heart rate and MAP were measured over a 2- to 3-h time period corresponding to the time period of that in the episodic hypoxia telemetry rats. The day 37 blood pressure required new femoral artery catheters to be placed in the limb opposite the site of the original catheter. The lowest stable MAP, which was recorded continuously for 10 min or more, was taken as the value for the recording session.

**Hypoxic chambers.** Episodic hypoxia animals were housed in identical cylindrical Plexiglas chambers (length = 28 cm, diameter = 10 cm, volume = 2.4 liters) with snug-fitting lids (8). With the use of a timed solenoid valve, pure nitrogen was distributed to each chamber for 12 s at a flow that was adjusted to reduce ambient FIO2 to 2–3% for ~3–6 s. This was followed by an infusion of compressed air, allowing gradual return (over 15–18 s) of ambient air to a FIO2 of 20.9%. The cycle was repeated twice per minute for 6–8 h on 35 consecutive days. A dampening device at the air/nitrogen end of the chamber was used to dissipate the airstream so that no direct jets of gas disturbed the animal. Each day of the 35-day experiment, rats were placed in the same chamber in the morning, and nitrogen flow was adjusted to reach the above-specified concentrations. Minimal FIO2 in each chamber was assessed at least twice daily (and adjusted) throughout the 35-day exposure period by sampling ambient nadir oxygen (MiniOX I, Catalyst Research, Owings Mills, MD). Mean daily nadir FIO2 was calculated by cage.

**Terminal blood and morphometric studies.** Total body weight was recorded at baseline and after the 35-day study period. The thorax and abdomen were opened, a large-bore catheter was inserted into the lower thoracic aorta, the aorta was clamped below the renal arteries, and the kidneys were flushed with 50 ml of iced physiological saline or until blanched. The kidneys were removed and flash frozen in liquid nitrogen. Tissue was stored at −70°C until assay. The heart was removed, and the atria and great vessels were dissected, weighed, and also flash frozen. Heart weight was normalized to body weight.

**RNase protection assays.** Probes were labeled by linearization and transcription of plasmids by using previously described techniques (1,18). Linearization was achieved by overnight incubation at 37°C with the appropriated restriction enzyme. The cDNA sequences used as probes for the mRNA of renin, AT1α, and eNOS were obtained by RT-PCR and cloned into vectors (Table 1) (1). Labeled RNA probes were synthesized in the presence of [32P]UTP by using an RNA transcription kit (Stratagene, Heidelberg, Germany) and purified on a 5% acrylamide-8 M urea gel.

Total RNA was isolated from kidney tissue by using the TRIzol reagent (Life Technologies, Eggenstein, Germany) followed by chloroform-isopropanol extraction. RNA was measured by spectrophotometry, and samples were diluted to reach the same end concentrations of total RNA. Identification of mRNA specific for rat AT1α, renin, and eNOS was done with the use of an Ambion RNase protection assay II kit (AMS Biotechnology, Witney, UK). Twenty-five micrograms of total RNA per sample were hybridized with ~30,000 cpm of the radiolabeled antisense probe. A β-actin probe was used in all experiments as an internal control.

<table>
<thead>
<tr>
<th>AT1α</th>
<th>Renin</th>
<th>eNOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>299 bp</td>
<td>300 bp</td>
<td>221 bp</td>
</tr>
</tbody>
</table>

**Table 1. mRNA protection assay methods**

- AT1α, angiotensin II receptor; eNOS, endothelial nitric oxide synthase; bp, base pair.
as a control for the amount of RNA used for the RNase protection assay. Hybridized fragments protected from RNase A and RNase T1 digestion were separated by electrophoresis on a denaturing gel (5% polyacrylamide, 8 M urea) and analyzed by using a FUJIX BAS 2000 phospho-imager system (Fuji, Dusseldorf, Germany). This system directly measures the radioactivity in a band on a RNase protection assay gel and allows the quantification over a linear range covering seven orders of magnitude.1 If this linear range is exceeded, the system gives a warning by showing a red band on the digitally generated image of the gel. Final activity counts of the gels were counted for renin, AT1a, eNOS, and β-actin, and the ratio was taken as activity for that probe in arbitrary units.

Statistical methods. All portions of this protocol were approved by the animal studies committee of the University of Louisville School of Medicine. End-of-study heart weights, body weights, and heart-to-body weight ratios were compared by paired t-tests. Weekly MAP values from the telemetry-probe rats were compared by one-way ANOVA for repeated measures with post hoc Fisher’s and Student’s t-tests when appropriate. Comparisons between groups were performed by ANOVA for multiple groups with post hoc Fisher’s exact test where appropriate. Null hypothesis was rejected at P < 0.05. Values are reported as means ± SE.

RESULTS

There was no significant difference in end-of-study body or heart weights or heart-to-body weight ratios between groups (Table 2). Six of the 57 rats either expired or had transducers that malfunctioned before final end-of-study blood pressure measurement. The final number of rats in each group appears in Table 2.

Baseline MAP was not different between the six groups (Fig. 1). There was no significant change in MAP for the three control groups nor for the EH-High group from baseline to the end of study. Both EH-Low and EH-AdLib groups showed a significant increase in MAP by the end of week 5 of the episodic hypoxia period (Fig. 1). For EH-AdLib rats, MAP was greater at days 26 and 37 than at days 0, 8, and 20. For EH-Low rats, MAP was greater at days 26 and 37 than at day 0.

Kidney renin mRNA in EH-High and Con-High rats was fourfold lower than those in EH-AdLib and EH-Low rats (Fig. 2). The differences between episodic hypoxia rats and respective diet control groups were not significant. The rat kidney AT1a mRNA normalized to β-actin was significantly lower in EH-Low and Con-Low rats compared with their respective diet control groups (Fig. 3). There were no significant differences in renal tissue eNOS mRNA normalized to β-actin between all of the groups studied (Table 3).

DISCUSSION

The present study was undertaken to further define the role of the renin-angiotensin system in the diurnal blood pressure elevation that results from 35 days of repetitive, episodic hypoxia in rats. The important findings of the current study are that 1) a high-salt diet suppresses the blood pressure elevation in response to episodic hypoxia, 2) tissue renin mRNA is suppressed by a high-salt diet and remains suppressed despite the added stimulus of episodic hypoxia, and 3) tissue AT1a mRNA was influenced by a low-salt diet but did not appear to be affected by episodic hypoxia.

DiBona and Sawin (5) have shown in non-salt-sensitive rats that renal sympathetic efferent activity during acute saline volume expansion is enhanced under conditions of chronic salt depletion (low-salt diet) and lasix volume contraction and is diminished in the face of salt loading. Right atrial pressure and renal sodium clearance are directly proportional, whereas renal nerve activity is inversely proportional, to dietary sodium intake. In other words, the volume contraction brought about by a low-sodium diet (high renin state) increases renal sympathetic activity whereas the opposite is true for a high-sodium diet. On the other hand, in studies on systemic hypertensive rats (known to be salt sensitive), these authors demonstrate that hypertension is worsened in response to stressful stimuli (i.e., air jet) in the presence of a salt load (15). Thus, in dietary salt load states, blood pressure is under opposing peripheral and central influences (i.e., decreased renal nerve activity in non-salt-sensitive rats with suppression of the renin-angiotensin system but aggravation of hypertension in salt-sensitive rats when other stresses are applied). Our data in non-salt-sensitive Sprague-Dawley rats show that salt loading did not aggravate the blood pressure elevation induced by the stress of chronic episodic hypoxia but did just the opposite, suppressed it. Although the direct effect of a high-salt diet on renal sympathetics was not measured, the blood pressure appeared to follow a behavior appropriate to renin-angiotensin system suppression and thus would suggest that renal sympathetic activity in this model is suppressed. It would appear that part of the answer to this dilemma might lie in what is going on with the local renin-angiotensin system in the kidney.

Our results showed that kidney tissue renin mRNA was about fourfold lower in EH-High and Con-High animals compared with both low and ad libitum salt

---

Holmer et al. (12), using 0.02% (low), 0.6% (normal), and 4% (high) salt diets in rats for 20 days, showed that plasma renin activity increased transiently in low-salt rats about threefold compared with controls and then returned to baseline levels by day 20. The kidney renin mRNA paralleled this. In high-salt rats, kidney renin mRNA decreased and remained at 50% of control values. If we consider the Con-AdLib group to have normal kidney mRNA levels, then Con-Low is near normal (7 wk on the diet) but both high-salt groups are below control levels. The combination of episodic hypoxia and low salt was not additive; it did not accentuate the blood pressure change nor kidney renin mRNA beyond the effect of a normal salt diet. Perhaps some insight concerning this is evident from the study by Holmer et al. (12). Unilateral renal denervation in low salt rats (removal of sympathetic input) still allowed renin mRNA to rise to the same level as in the innervated kidney. Kidney renin mRNA in high-salt rats was further depressed by renal denervation. This implies that the systemic volume stimulus from a low- or high-salt diet appears to supercede the sympathetic signal via the renal nerves to increase or decrease renal sympathetic output. In our model, episodic hypoxia normally increases sympathetic signaling to the kidneys, but the high-salt volume suppressed this effect, appearing to be a stronger stimulus to the kidney than hypoxia. It was surprising that the effect of low salt and episodic hypoxia on blood pressure and kidney renin mRNA were not additive, but this may reflect the tendency for renin mRNA to return to baseline levels after 20 days in low-salt animals (12).

There was a lowering effect of low-salt diet on AT1a mRNA in both the episodic hypoxia and control groups (Fig. 3). Previous studies are somewhat conflicting as to the effect of a high-salt diet on the level of AT1a in
the kidney; different studies showed no effect (23), elevation (24), and lowering (6) of AT1a receptor levels. In most studies, however, low-salt diet is associated with a decrease in AT1a mRNA [e.g., by a factor of 1.6 over a 20-day period (23)]. We examined only AT1a mRNA, but this is the predominant isoform found in the kidney, with a ratio of AT1a-to-AT1b of about 3:1, and is the isoform most affected by a low-salt diet (23,24). The mechanism for the change in AT1a mRNA to diet is unknown. Our interest in kidney AT1a mRNA was to see whether episodic hypoxia had a differential effect on it since our previous data indicate that the elevated blood pressure of episodic hypoxia is AT1a dependent (Losartan blocks it). There was no significant difference in any of the AT1a mRNA levels between respective control and hypoxia groups. Despite evidence of high renin-angiotensin system activity at 5 wk in episodic hypoxia, kidney AT1a does not appear to be changed significantly. This is compatible with studies showing that circulating levels of angiotensin II do not appear to moderate AT1a mRNA since both 7- (24) and 14-day (13) infusions of angiotensin II do not modify kidney AT1a mRNA.

Several reports of altered vascular reactivity in humans with obstructive sleep apnea have recently appeared. Kraiczi et al. (16) infused angiotensin II into 10 men with sleep apnea and 10 controls and found that forearm vascular conductance (venous occlusion plethysmography) was roughly 40% lower in sleep apnea patients vs. controls. The authors suggest that one of three mechanisms could account for this observation: 1) vascular remodeling (increased wall-to-lumen ratio) with enhanced resistance response, 2) augmented presynaptic effects of angiotensin II on catecholamine release and reuptake in sympathetic nerves, and 3) reduced coactivation of endothelial NO synthesis or increased NO degradation. The latter mechanism is particularly relevant in light of other studies. With the use of similar techniques in humans, two studies have shown decreased NO-dependent vasodilation in patients with obstructive sleep apnea (4,7); in one of these studies, treatment with nasal continuous positive airway pressure appeared to reverse this phenomenon (7). We have also demonstrated that the cremaster arterioles from rats exposed to 35 days of episodic hypoxia show a decreased responsiveness to acetylcholine vasodilatation, which is NO dependent (26). Because the rate-limiting enzyme in endothelial NO production is eNOS (type III NOS), we examined eNOS in whole kidney tissue but were unable to see differences between

Table 3. eNOS mRNA-to-β-actin ratio for each group

<table>
<thead>
<tr>
<th></th>
<th>EH-High</th>
<th>EH-AdLib</th>
<th>EH-Low</th>
<th>Con-High</th>
<th>Con-AdLib</th>
<th>Con-Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>eNOS mRNA</td>
<td>0.650 ± 0.090</td>
<td>0.853 ± 0.050</td>
<td>0.798 ± 0.109</td>
<td>0.697 ± 0.104</td>
<td>0.774 ± 0.092</td>
<td>0.705 ± 0.098</td>
</tr>
</tbody>
</table>

Values are means ± SE.
between salt-depleted and salt-loaded animals and between episodic hypoxia and nonepisodic hypoxia animals (Table 3). Singh et al. (25) examined inducible NOS (iNOS), eNOS, and neuronal NOS in salt-loaded and salt-deprived rats and found that eNOS and iNOS mRNA are present in the renal cortex but that neither were altered by high- or low-salt diets. Even in micro-dissected glomeruli in which eNOS receptors are regularly seen, there was no effect from salt loading or depletion. In view of this, it is possible that we missed changes in eNOS from episodic hypoxia because we examined whole kidney extracts, and eNOS may be more concentrated in the highly vascular renal cortex.

In summary, we have shown that a high-salt diet, which suppresses the renin-angiotensin system both systemically and in many organs, suppresses the development of elevated blood pressure in response to 35 days of episodic hypoxia. This was accompanied by a suppression of kidney renin mRNA despite what should be excessive renal nerve sympathetic activity due to episodic hypoxia. Both of these pieces of information indicate that volume factors in response to dietary salt alteration seem to supercede renal nerve activity in causing chronic blood pressure changes from stressful stimuli such as recurrent hypoxemia. The same can be said for kidney AT1a mRNA, which again did not appear to be influenced by chronic episodic hypoxia. These data suggest that, although the effect of episodic hypoxia on the kidney via renal sympathetics may in part contribute to systemic blood pressure elevation, the local tissue renin-angiotensin system may play a more primary role.

What relationship could this experiment have to human hypertension in the setting of recurrent hypoxemia from obstructive sleep apnea? The aim of this study was not to suggest that a high-salt diet should be protective for hypertension in sleep apnea patients. However, occasional reports of salt loading in humans with renovascular hypertension show amelioration of malignant blood pressure elevation in this setting (14). Studies in one-clip two-kidney rats demonstrate that this is probably due to blood pressure reaching a threshold level high enough to cause renal salt wasting. This results in total body sodium loss, volume contraction, and marked activation of the renin-angiotensin system with further progression to severe hypertension (19). This exact situation is unlikely to be applicable to obstructive sleep apnea. However, it has been repeatedly demonstrated that a night of obstructive apnea is associated with salt and water diuresis, presumably secondary to the effect of markedly negative intrathoracic pressure on the atria, causing over expression of atrial natriuretic peptide. Were this the etiology of hypertension in sleep apnea, there might be demonstration of associated elevation of plasma renin, which thus far has not been the case. But the findings of this study suggest the possibility that the tissue renin-angiotensin system might be a contributor to hypertension in humans with sleep apnea.

This study was supported in part by a grant from the Jewish Hospital Foundation, Louisville, Kentucky.

REFERENCES


