Blood pressure response to chronic episodic hypoxia: role of the sympathetic nervous system

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Bao, Gang, Naira Metreveli, Rena Li, Addison Taylor, and Eugene C. Fletcher. Blood pressure response to chronic episodic hypoxia: role of the sympathetic nervous system. J. Appl. Physiol. 83(1): 95–101, 1997.—Previous studies in several strains of rats have demonstrated that 35 consecutive days of recurrent episodic hypoxia (7 h/day) cause an 8- to 13-mmHg 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. The present study was undertaken to further define the role of renal artery sympathetic nerves and the adrenal medulla in this BP increase. Male Sprague-Dawley rats had either adrenal medullectomy, bilateral renal artery denervation, or sham surgery. Rats from each of these groups were subjected to episodic hypoxia for 35 days. Control groups received either compressed air or were left unhandled. Adrenal demedullation or renal artery denervation eliminated the chronic diurnal mean BP response (measured intra-arterially) to episodic hypoxia, whereas sham-operated controls continued to show persistent elevation of systemic BP. Plasma and renal tissue catecholamine levels at the end of the experiment confirmed successful adrenal demedullation or renal denervation in the respective animals. The chronic episodic hypoxia-mediated increase in diurnal BP requires both intact renal artery nerves as well as an intact adrenal medulla.

METHODS

Surgery. Seventy-five Sprague-Dawley rats (12–20 wk old; 280–530 ± 50 g) were purchased from Zivic-Miller (Zelienople, PA). Thirty-six underwent abdominal incision (by the investigators) only and served as sham-operated controls. Two weeks before use, adrenal demedullation (20-wk-old rats) was performed by the supplier in 22 rats. Demedullation was performed through an abdominal incision according to published techniques that have been shown not to damage adrenal cortex, verified by normal postoperative corticosterone and aldosterone levels (21). Through an abdominal incision, both renal arteries were exposed (by the investigators) in 19 other rats (12 wk old) ~5 days before the experiment. The renal arteries were dissected free of connective tissue and painted with 20% phenol. Midway through the 35-day experiment, the renal arteries were again isolated and painted with 20% phenol. The study groups are described in Study groups. At least 48 h before placement in the hypoxic chambers, the animals were anesthetized (ketamine, acepromazine, xylazine) and instrumented through the right femoral artery with abdominal aorta catheters (Silastic, 0.05-mm ID; Dow Corning, Midland, MI) that were exteriorized at the nape of the neck for recording heart rate (HR) and BP. Where appropriate, sham abdominal incision or renal artery dissections were combined with catheter-placement surgery. Rats were allowed at least 20 h of recovery before hemodynamic measurements, and if they appeared in pain or had obvious ischemia of the extremity they were killed. Abdominal aorta catheters were maintained chronically by flushing with a heparin solution.

Study groups. Adrenal study rats were divided into three groups. Twelve demedullated rats were exposed to episodic
hypoxia (nadir FIO2 = 2-3%) for 8 h/day for 35 days (Adr-Demed-Hypo). Seventeen sham-operated rats were concurrently exposed to the same level of episodic hypocapnic hypoxia (Sham-Oper-Hypo). Ten demedullated rats were left unhandled (Adr-Demed-Unh). Renal study rats were divided into five groups. Seven renal-denervated rats (Ren-Dener-Hypo) and 10 sham-operated rats (Sham-Oper-Hypo) were exposed to episodic hypoxia for 35 days. Seven renal-denervated (Ren-Dener-Air) and 9 sham-operated (Sham-Oper-Air) rats were exposed to episodic compressed-air infusions for 35 days, duplicating the noise and confinement of the episodic hypoxia group. Five renal-denervated (Ren-Dener-Unh) rats remained unhandled in their usual cages for 35 days. The purpose of unhandled controls in both arms of the experiment was to track BP, body weight, heart weight, and blood and tissue catecholamine levels in rats that were unhandled during the experimental periods.

Hypoxic chambers. Animals were housed in identical cylindrical Plexiglas chambers (length 28 cm, diameter 10 cm, volume 2.4 liters) with snug-fitting lids (12). With use of a timed solenoid valve, pure nitrogen was distributed to each chamber for 12 s at a flow that was reduced to adjust the ambient FIO2 to 2–3% for ~3.5 s. This was followed by an infusion of compressed air, allowing gradual return (over 15–18 s) of ambient air to FIO2 of 20.9%. The cycle was repeated twice per minute for 6–8 h on 35 consecutive days. At the same time nitrogen was being distributed to hypoxia chambers, compressed air at approximately the same flow was distributed to sham cages, simulating the same noise and air flow disturbance. A dampening device at the intake 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, the rats were placed in the same chamber in the morning, and nitrogen flow was adjusted to reach the above-specified concentrations. The minimal FIO2 in each chamber was assessed twice daily (and adjusted) throughout the 35-day exposure period by sampling ambient nadir oxygen (MiniOX I, Catalyst Research, Owings Mills, MD). A mean daily nadir FIO2 was calculated for each cage (Fig. 1).

Hemodynamic measurements. At 24 h preceding or 48 h after the 35-day study period, with the rats under resting and unrestrained conditions, the catheters were attached to Statham P23Db pressure transducers with signal amplification (model 7858B, Hewlett-Packard, Andover, MA), and HR and MAP were measured over 2–3 h. The lowest stable MAP recorded continuously for 10 min or more was taken as the value for the recording session (9). Peak MAP and HR were also measured in the adrenal-demedullated rats under acute episodic hypoxia conditions (nadir FIO2 = 3.5%) before and after chronic exposure to evaluate any effect that chronic exposure might have had on acute BP response to episodic hypoxia. For technical reasons in this experiment, acute hypoxia BP responses were not done before and after 35-day exposure in renal-denervated rats. All BP tracings were read unblinded but independently by two investigators. Disagreements were usually not >2 mmHg apart and were averaged. As proof of successful adrenal demedullation, 1 ml of blood was taken from each of the adrenal-demedullated study rats (unrestrained, behaviorally asleep) before chronic exposure and on the third day after 35-day exposure and assayed for plasma epinephrine (Epi) and norepinephrine (NE) by using high-performance liquid chromatography with electrochemical detection (16). Low or unmeasurable levels of Epi (<25 pg/ml) were indicative of successful demedullation. As proof of successful renal artery denervation, animals were killed 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 were sonicated in 0.5 ml of buffer (pH 4.0) containing 0.17 M citrate acetate and 10% 2–3 M. The sonicate was centrifuged, and the clear aspirate was subjected to microfiltration (Amicon W. R. Grace, Beverly, MA) and high-performance liquid chromatography with electrochemical detection (16). Low levels of tissue NE indicated successful renal artery sympathetic denervation.

Terminal blood and morphometric studies. Because we flushed the catheters with a mixture of normal saline and heparin about every 3 days, many catheters were patent after 35 days. In about one-half of the animals, however, the catheters had closed, and the same technique of arterial catheter placement was repeated. Total body weight (TBW) was recorded at baseline and after the 35-day study period. At termination of the studies, anesthetized rats were rapidly exsanguinated, the heart was removed, the atria and great vessels were dissected away, the right and left ventricles were separated, and the two muscles were weighed together (total heart-weight; THW) and separately. Heart chamber weight was normalized to body weight. All aspects of the protocol were approved by the Animal Studies Subcommittee. Animals were housed in designated animal facilities and provided rat chow and water ad libitum.

Statistical methods. Resting morphometric, BP, HR, and catecholamine measurements made at baseline, and changes from baseline to final were compared across all groups by
one-way analysis of variance for repeated measures with post hoc Bonferroni’s and Student’s t-tests when applicable. Acute MAP and HR response to hypoxia at baseline and post-35-day study conditions were compared within groups by t-test for paired data. The null hypothesis was rejected at $P < 0.05$. Deviation from mean is reported as ±SE.

RESULTS

Daily analysis of the mean nadir $F_{102}$ concentrations for Adr-Demed-Hypo and Sham-Oper-Hypo (Fig. 1A) and for Ren-Dener-Hypo and Sham-Oper-Hypo rats (Fig. 1B) showed identical hypoxia exposure levels throughout the 35-day period.

Sham-Oper-Hypo (adrenal study) rats exhibited an $8.1 \pm 2.1$-mmHg increase in resting MAP, whereas Adr-Demed-Hypo and Adr-Demed-Unh showed no significant change in MAP (Fig. 2). There was no significant difference in before vs. after HR among any of the adrenal groups (Fig. 2). The magnitude of change in MAP and HR response to acute phasic 3.5% hypoxia after the 35-day exposure to chronic episodic hypoxia remained the same as baseline in all three adrenal study groups (Fig. 3). The seven Sham-Oper-Hypo (renal control) rats showed a $9.0 \pm 2.4$-mmHg increase in MAP ($P < 0.05$), Ren-Dener-Hypo, Ren-Dener-Air, Sham-Oper-Air, and Ren-Dener-Unh rats showed no significant change in BP after the 35-day experimental vs. baseline period (Fig. 4). There was no difference in before vs. after HR among any of the renal-denervation groups (Fig. 4).

In the adrenal-demedullated groups, plasma levels of $Epi < 25$ pg/ml were not quantitated but for statistical comparison were assigned a value of 25 pg/ml (Fig. 5). Mean $Epi$ was $< 25$ pg/ml in Adr-Demed-Hypo and Adr-Demed-Unh rats both before and after the experimental conditions, but Sham-Oper-Hypo animals showed nearly a fourfold elevation in plasma $Epi$ after exposure. NE levels increased significantly in all adrenal study groups at 35-day follow-up but nearly tripled in the Sham-Oper-Hypo group. None of the between-group differences in post-35-day NE levels was significantly different.

NE levels from the kidney homogenates in Ren-Dener-Hypo, Ren-Dener-Air, and Ren-Dener-Unh were uniformly less ($\leq 53$ pg/mg) than homogenates of the Sham-Oper-Air animals, which were taken as control levels (Fig. 6). NE in kidney homogenates of the Sham-Oper-Hypo showed a sevenfold elevation compared with all renal-denervated groups and twofold
higher NE than the Sham-Oper-Air group. Renal homogenate Epi values were slightly higher in Ren-Dener-Hypo rats than in other groups (Fig. 6).

Adr-Demed-Unh animals showed a 192-g increase in body weight over the 35-day period, whereas hypoxia-stressed rats (Adr-Demed-Hypo and Sham-Oper-Hypo) showed no significant change over the same time period (Table 1). Ren-Dener-Unh, Sham-Oper-Air, and Ren-Dener-Air rats showed roughly a 200-g increase in body weight while hypoxia-stressed renal study rats (Ren-Dener-Hypo and Sham-Oper-Hypo) showed significantly (P < 0.05) less weight gain over the study period (Table 2). It must be noted that the adrenal study rats were larger at baseline than the renal study rats and would be expected to gain less weight under experimental conditions. These body weight changes are compatible with previous experiments in which hypoxia-exposed animals show less weight gain throughout the experimental period than do unhandled and compressed-air animals. The ratios of left ventricular (LV) to TBW, right ventricular (RV) to TBW, and THW to TBW were significantly higher in the hypoxia stimulated adrenal groups at the end of the study compared with unhandled controls (Table 1). Among the renal study rats, there were no significant differences in heart weight ratios between groups after the 35-day period (Table 2).

DISCUSSION

The present study was undertaken to further define the role of the SNS, the renal artery nerves, and the adrenal medulla in elevation of diurnal BP in response to chronic episodic hypoxia. The most important findings of this study are 1) both adrenal demedullation and renal artery denervation eliminate the chronic diurnal BP response to episodic hypoxia and 2) in adrenal-demedullated and sham-operated hypoxia-exposed rats, BP and HR responses to acute hypoxic challenge do not change with chronic exposure. This suggests that chronic repetitive episodic hypoxemia causes sustained increase in diurnal BP through SNS activity to the renal artery nerves and to the adrenal medulla.

In the present study, it appears that effective adrenal medulla ablation throughout the 35-day study period was achieved, in that both groups of adrenal medullaectomy rats (hypoxia exposed and unhandled) had “below detectable” levels of Epi (<25 pg/ml) at baseline and at the end of the study period. The sham-operated hypoxia exposed rats (Sham-Oper-Hypo), with a mean Epi level of 28.9 pg/ml at baseline, showed a nearly fourfold elevation in Epi levels at day 35, suggesting increased adrenal medullary activity in response to chronic episodic hypoxia. All three adrenal study groups had increased NE after 35 days of chronic hypoxia exposure. Although NE in the Sham-Oper-Hypo group appeared higher (194 pg/ml) than in the other groups, this difference was not significant. The increase during the study may reflect an increase in plasma catecholamine levels with aging, as noted in a previous study in...
normal weight and hypertensive humans (17). The implication of changes in plasma NE levels related to peripheral SNS activity must be interpreted with caution. Absolute levels of NE may not reflect true body and organ turnover or activity when release and clearance of NE are unknown. For example, it has been shown that hypoxia causes increased SNS activity (measured by muscle sympathetic nerve signals) with minimal change in plasma NE, which is explained by a significant increase in both NE release and clearance (25). Also, catecholamine values are somewhat labile, as judged by the wide standard error, because they are rapidly changed by movement or activity of the animal. The major reason for measuring catecholamine levels in this study was to confirm successful adrenal denervation and renal sympathetic denervation.

Similarly, it appears that successful renal artery denervation was achieved because renal tissue NE was low in all denervated animals at 35 days compared with Sham-Oper-Air controls. Renal artery sympathetic denervation is not permanent, and reinnervation may take place over time, allowing progression of systemic hypertension in spontaneously hypertensive rats (22).

Successful maintenance of renal artery denervation requires repeat denervation about every 3 wk to keep renal tissue NE content at or below 20% of sham-operated rats (29). Our Sham-Oper-Hypo animals showed renal tissue NE levels (collected 4 days after the last hypoxic exposure) that were twofold higher than those of Sham-Oper-Air rats. This suggests that episodic hypoxia may have caused increased renal SNS activity in these animals. Again, these results must be interpreted with caution because claims of increased SNS activity should be corroborated with measures of catecholamine turnover.

Despite the fact that the adrenal-demedullated rats did not show chronic BP elevation after 35 days of episodic hypoxia, they continued to show the same acute BP and HR response to episodic hypoxia at the end of the experimental period. One obvious explanation for this is that renal, cardiac, mesenteric, and other sympathetic beds remained intact to allow acute vasoressor response to episodic hypoxia, independent of Epi output by the adrenal medulla. This is not contradictory to the main outcome of the study (see below). It would be important to determine whether this same phenomenon occurred in the renal-denervated rats, which was planned for this study. Unfortunately, because of catheter closures in renal-denervated rats after chronic BP responses were taken, it was not possible to obtain meaningful data in the renal group.

Episodic hypoxia-exposed rats (Adr-Demed-Hypo, Ren-Dener-Hypo, and Sham-Oper-Hypo) showed no change or smaller increases in body weight throughout the study period compared with nonhypoxia controls. This has been a consistent finding throughout the series of studies involving chronic episodic hypoxia (10–12). We believe that this reflects increased metabolic (activity and distress) and handling stress in episodic hypoxia animals to which the controls are not subjected, as opposed to the stress of denervation or demedullation procedures themselves. Adrenal-demedullated and renal-denervated rats not exposed to hypoxia showed weight gains comparable to those of nonhypoxia animals.

THW/TBW ratios were compared at study termination. The Adr-Demed-Unh rats showed significantly lower ratios of cardiac chamber weight (RV and LV) to TBW ratios than did either of the renal artery control groups, findings comparable to the results of our previous studies (10–12). We are unable to explain why none of the chamber-to-TBW ratios was different in the renal study rats. In this and other studies, chamber hypertension appears to be related to hypoxia exposure rather than to increased ventricular afterload. In a previous study, sympathetic-denervated rats exposed to episodic hypoxia, where no BP (afterload) increase was seen, showed an increase in LV/TBW (11). The same occurred in carotid body-denervated rats that were exposed to episodic hypoxia but did not develop elevated BP (10). Past studies as well as the present data suggest that systemic hypoxemia alone may cause hypertrophy of cardiac muscle. Indeed, LV hypertrophy is associated with anomalous origin of the coronary artery from the pulmonary artery (hypoxic blood, no increased afterload) in humans, suggesting that hypoxemia directly stimulates myocardial growth (34).

Of the possible mechanisms for development of long-term systemic hypertension in the setting of sleep apnea, the effect of recurrent episodic hypoxia with stimulation of peripheral chemoreceptors and with increased sympathetic activity is the most attractive theory. Numerous publications have demonstrated increased SNS activity in humans with obstructive apnea.
by using percutaneous microneurography of the peroneal nerve (18, 27, 31, 32). With this technique, Somers et al. (31) demonstrated higher levels of sympathetic nerve activity in 10 patients with obstructive sleep apnea, both awake and during sleep apnea, compared with 10 age- and gender-matched nonapnea controls (31). In four of the apnea subjects, SNS activity returned toward control levels with the use of nasal continuous positive airway pressure to eliminate apnea (32). In another other study using microneurography, Somers et al. (32) showed that acute isocapnic vs. acute hypocapnic hypoxia was a stronger stimulus to muscle nerve sympathetic activity during induced hypoxia and that the response was accentuated when a simulated breath hold was added. While these studies address acute BP and SNS responses, Morgan et al. (27) demonstrated a more prolonged effect of intermittent asphyxia on SNS activity in awake humans. Muscle nerve sympathetic activity remained above baseline for at least 20 min beyond termination of the hypoxic period. But major questions remain. If increased SNS activity from repetitive episodic hypoxia eventually brings about the lasting, diurnal BP increase, how is such activity translated into fixed BP elevation? Can recurrent elevated SNS activity cause permanent changes in the resistance vasculature through sympathetic overactivity via renal sympathetic nerves and/or the adrenal medulla?

Hypoxia is a serious threat to mammals because it jeopardizes organ metabolism and function. It has long been known that Epi is excreted into adrenal venous blood during asphyxia (6), stimulating sympathetic-adrenal-renal discharge to counter the stress of the lack of oxygen (20, 23). NE is produced by both postganglionic neurons of the SNS and the adrenal medulla. The peripheral SNS and adrenal medulla act together to preserve homeostasis by increasing cardiac output, modifying blood flow distribution, and altering metabolism to improve oxygen delivery to vital tissues. In animals as well as humans, chronic hypoxia is associated with increased SNS activity. Adrenal medulla vs. peripheral SNS activity can be dissociated through the use of SNS toxins, adrenergic blockers, and adrenal medullectomy (1, 24, 30). In unanesthetized rabbits, sympathetic nerves play a more important part in the normal circulatory response to hypoxemia than does the adrenal medulla (23).

If renal sympathetic activity were the only mechanism modulating BP in episodic hypoxia, it would be difficult to explain why adrenal medullectomy eliminated the rise in BP after hypoxia exposure. The results of this study suggest that circulating Epi may also be important in regulating BP in the setting of episodic hypoxic stress. There are several ways that the combined action of adrenal Epi and renal artery sympathetic nerves may interact, explaining the findings of this study. One is that Epi enhances presynaptic NE release and facilitates neurogenic vasoconstriction. Another is that the renin-angiotensin system may participate in the diurnal BP increase and may be stimulated by both renal artery sympathetic nerves as well as circulating adrenal Epi.

While the local response of blood vessels to Epi is postjunctionally mediated vasodilatation, Epi may promote sympathetic neural vasoconstriction, as demonstrated in recent human studies (14, 15). Neural release of NE at prejunctional sites appears to be facilitated by Epi stimulation of β₂-adrenergic receptors. The affinity of Epi for β₂-receptors is >200-fold greater than that of NE (8). Either exogenous or adrenal Epi can be taken up by postganglionic sympathetic nerves and released as a cotransmitter with NE for 24 h after its uptake (when plasma concentrations have returned to baseline) (2). Epi augments the simultaneous discharge of endogenous NE, facilitating neurogenic neuroadrenergic signal transmission. Thus, while the acute administration of Epi causes increased blood flow and lower BP, chronic administration can actually increase BP. Epi infusions that do not increase plasma concentration can cause sustained elevation of BP in rats (19, 34).

Thus one scenario for elevated diurnal BP in chronic episodic hypoxia-stimulated rats is that neurogenic sympathetic vasoconstriction is induced by chemoreceptor stimulation. At the same time, the adrenal medulla releases Epi, which is taken up by postganglionic nerves and released as a cotransmitter with NE, accentuating the vasoconstrictive signal to renal, mesenteric, or muscle vasculature for up to 24 h after the episodic hypoxia is stopped. Elimination of the adrenal medulla and virtually all plasma Epi thus reduces this cotransmitter potential. Similarly, removal of the renal artery nerves eliminates the potential for the kidney vasculature to respond to sympathetic output. Thus both renal sympathetic nerves and the adrenal medulla may be necessary for diurnal BP elevation in this setting.

In another scenario, increased SNS activity to the kidneys could be translated into elevated diurnal BP through increased activity of the renin angiotensin system. The kidney contains both α- and β-adrenergic receptors, which respond to sympathetic stimulation with increased renin secretion (26). Hypertension in several animal models, including the spontaneously hypertensive rat, is believed to be related to increased adrenergic activity (28, 29). Increased activity of both splanchnic and renal nerves has been reported in spontaneously hypertensive rats vs. normotensive Wistar-Kyoto control rats (29). Bilateral renal denervation in spontaneously hypertensive rats will ameliorate or delay the usual chronic increase in systemic BP (22).

β-Adrenergic receptors located in the juxtaglomerular apparatus of afferent arterioles (7, 26) as well as NE receptors in the renal tubules that modulate sodium delivery to the macula densa (5) both control kidney renin secretion. Secretion of renin persists in the face of renal artery denervation, and the existence of a humoral factor secreted by an extrarenal site is postulated (3, 4). A likely location of an extrarenal β-adrenergic, renin-stimulating site may be the hypothalamic paraventricular nucleus because this area has afferent baroreceptor input, efferent projections to the sympathetic areas of the spinal cord, and high concentrations of β-receptors (30). It is believed that stress-related renin release is mediated more by renal α-adrenergic- and extrarenal β-adrenergic-receptor sites (30). Hypotheti-
cally, increased BP from recurrent episodic hypoxia may result both because of chronic stimulation of α- or β-adrenergic receptors in the kidney via renal artery sympathetic nerves as well as stimulation of the extrarenal β-adrenergic receptors by circulating Epi from the adrenal medulla. Thus in the second senerio, removal of either of these arms could blunt the usual BP rise seen in this setting.

In summary, this study suggests a dual mechanism for the SNS in promoting sustained diurnal BP changes in the face of repetitive episodic hypoxia. Catecholamine levels measured in plasma and tissue confirmsuccessful for the SNS in promoting sustained diurnal BP changes in this experimental setting.

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