Role of Endothelin in Intermittent Hypoxia-Induced Hypertension

Nancy L. Kanagy, Benjimen R. Walker, Leif D. Nelin

Abstract—Clinical studies suggest that sleep apnea causes systemic hypertension. In addition, patients with sleep apnea have elevated plasma levels of endothelin-I (ET-1). We hypothesized that the intermittent hypoxia/hypercapnia (IH) associated with sleep apnea causes hypertension by increasing ET-1 production. To test this hypothesis, rats with arterial and venous catheters were placed in Plexiglas chambers. IH rat chambers were flushed with an N2–CO2 mixture for 90 seconds to achieve hypoxia/hypercapnia (5% O2–5% CO2) followed by 90 seconds of compressed air to achieve normoxia (21% O2–0% CO2). Control rat chambers were flushed with 90 seconds of air-air cycles. Cycles for both groups were repeated 8 hours per day for 11 days. Resting mean arterial pressure (MAP) and heart rate were recorded daily before the start of exposure. After 11 days, MAP was significantly elevated in IH rats compared with initial MAP (109±5 mm Hg initial, 139±11 mm Hg day 11) and compared with air-air rats (110±4 mm Hg). On day 11, cumulative doses of PD145065 (a nonselective ET-receptor antagonist) were administered intravenously to the rats breathing room air. PD145065 caused a dose-dependent decrease in MAP in IH rats but did not alter MAP in air-air rats. Plasma ET-1 measured by radioimmunoassay was significantly increased on days 5 and 11 in the IH rats compared with day 1 and compared with air-air rats. There was no significant change in plasma ET-1 over time in air-air rats. We conclude that IH exposure increases both MAP and plasma ET-1 and that the increased ET-1 may contribute to the hypertension. (Hypertension. 2001;37[part 2]:511-515.)

Key Words: apnea ■ endothelin ■ rats ■ hypoxia

Sleep apnea is a common problem in the United States, affecting 2% to 4% of adults.1 Systemic hypertension is found in 50% to 90% of patients with sleep apnea,1,2 but the cause of the hypertension remains unknown. Perhaps because of the unknown cause of the condition, the hypertension is difficult to control and standard pharmacological interventions are often ineffective.3 These refractory cases are only corrected by treatment of sleep apnea through invasive techniques such as breathing support or surgery.4 Therefore, understanding the physiological basis for sleep apnea-induced hypertension could provide an effective noninvasive means to pharmacologically treat the increased blood pressure.

Several mediators have been evaluated as contributors to sleep apnea associated hypertension. These include increased sympathetic nervous system activity,5 decreased responses to nitric oxide,6 and elevated endothelin production.7 Clinical studies have shown that plasma endothelin levels are elevated in patients awakened after apneic episodes compared with patients awakened from normal sleep. Furthermore, this same study found that correction of sleep apnea reduced plasma endothelin to control levels.8,9 However, Grimpen and coworkers10 compared patients with obstructive sleep apnea with age- and weight-matched control subjects and found no differences in plasma levels of endothelin and no change in plasma endothelin after correction of the sleep apnea. Therefore, clinical studies do not provide a clear indication of the role of endothelin in sleep apnea-induced hypertension.

Several animal models have been developed to address the mechanism of sleep apnea–induced hypertension. These studies have suggested that plasma catecholamines are elevated in rats exposed to intermittent hypoxia during their sleep period.11 Furthermore, sympathodenervation with 6-hydroxydopamine prevents an increase in blood pressure in rats exposed to intermittent hypoxia.12 However, animal studies have not yet investigated the role of endothelin in sleep apnea–induced hypertension. This is especially intriguing because endothelin can augment activity in the sympathetic nervous system,12 and the sympathetic nervous system appears to be upregulated in dog and rat models of sleep apnea.11 In addition, hypoxia increases expression of endothelin in both cultured cells and in animals,13 and elevated plasma endothelin causes hypertension.14,15 Therefore, increased endothelin production during sleep apnea could cause the hypertension and elevated sympathetic nervous system activity previously observed. We hypothesized that intermittent hypoxia induced by sleep...
apnea stimulates endothelin production; endothelin (ET)-1 production remains elevated during wakeful periods, and ET-1 contributes to hypertension. We tested this hypothesis by using chronically instrumented rats to investigate the time course of hypertension development and the involvement of endothelin in the response.

Methods

Animal Model
Male Sprague-Dawley rats were instrumented with arterial catheters to directly record arterial blood pressure and heart rate and with venous catheters to infuse test substances, as previously described. Briefly, rats were anesthetized with ketamine (91 mg/kg) and acepromazine (0.9 mg/kg); catheters were inserted into the abdominal aorta and inferior vena cava through the femoral artery and vein, respectively. The ends of the catheters were tunneled subcutaneously to the head, where they exited through a stainless steel spring secured to the skull with jeweler’s screws and dental acrylic. The catheters were filled with saline solution containing heparin when not in use and were flushed daily. All animal protocols were approved by the University of New Mexico Animal Use Committee and are in accordance with University of New Mexico and National Institutes of Health guidelines.

Intermittent Hypoxia Protocol
Rats were allowed 3 to 5 days of recovery from surgery before being exposed to either intermittent hypoxia/hypercapnia (IH) or control. Rats were housed in Plexiglas chambers and exposed to either IH or air-air for 7 to 8 hours each day for 11 days. During exposure, the atmosphere in the boxes was controlled by a constant flow of gas through the boxes with a dampening device placed over the gas inlet to dissipate the gas stream. For the IH exposure, the atmosphere alternated every 90 seconds between compressed air (21% O2/79% N2) and hypoxic/hypercapnic air (5% O2/5% CO2/90% N2). The resultant changes in the percent O2 and CO2 in the chamber are shown in Figure 1. For control exposure, the atmosphere alternated every 90 seconds between two room air mixtures, simulating the noise and airflow disturbance associated with the protocol. Resting mean arterial pressure (MAP) and heart rate (HR) were recorded daily before the start of exposure while the animals were in room air by connecting the exposed arterial line to a pressure transducer. The signal from the pressure transducer was fed through an amplifier and recorded on both a chart recorder and on a computer data acquisition system (Codas, Datag). O2 and CO2 content of the chambers was also recorded throughout the exposure period, and the inflow of gas was adjusted to achieve 5% O2/5% CO2 during the IH period and 21% O2/1% CO2 during the air period. Gas flow in air-air exposures was equivalent to that used in the IH exposures.

Endothelin Antagonist Infusions
On day 11, after the daily recording, the nonselective endothelin receptor antagonist PD145065 (dissolved in PBS) was administered through the venous line to evaluate the contribution of endothelin to the maintenance of blood pressure. Five bolus doses were administered sequentially in a cumulative manner (0.3, 3.0, 30.0, and 1000 mmol/kg), 1 every 10 minutes. Blood pressure and HR were recorded continuously and for 30 minutes after the final dose. These doses were chosen on the basis of the published observation that 1- to 10-μmol/kg boluses of PD145065 effectively blocked pressor responses to ET-1 infusion.

Plasma Endothelin Measurement
Plasma was collected from rats on days 1, 5, and 11 before the start of the exposure period by withdrawing 1 milliliter of arterial blood into a heparinized syringe. Blood was centrifuged and the plasma withdrawn and stored at −80°C until analyzed. Red blood cells were resuspended in heparinized saline and returned to the rat through the arterial catheter. At the time of the assay, ET-1 was extracted from thawed plasma samples with Amprep 500-mg C2 columns (Amersham Pharmacia). Plasma was loaded onto the columns, eluted with methanol, and the eluate dried in a centrifugal evaporator. The pellet was reconstituted with 250 mL of 20 mmol/L borate buffer (pH 7.4) and assayed for ET-1. Radioimmunoassay for ET-1 was performed on extracted plasma with a commercially available kit (Amersham Pharmacia).

Statistical Analysis
Data are expressed as mean±SEM. MAP, HR, and plasma ET-1 levels from IH rats and air-air rats over time was compared by means of 2-way ANOVA. Differences were identified by a Student-Newman-Keuls post hoc test, and a significance level of P≤0.05 was used.

Results

Hemodynamic Measurements
Resting MAP and HR measured daily before beginning the exposure period were not significantly different between air-air and IH rats on day 1 of the treatment period (MAP: air-air=109±2 mm Hg, IH=109±5 mm Hg; HR: air-air=340±14 bpm, IH=352±12 bpm). In contrast, resting MAP in IH rats was significantly elevated above that in the air-air rats starting on day 7, whereas MAP in air-air rats did not change over the 11-day exposure period (Figure 2). Heart rate was not different between groups at any time point and did not change over time within groups (Figure 3). Therefore, 8 hours of IH daily during sleep appears to induce systemic hypertension when sustained for >7 days.

Plasma Endothelin Levels
Plasma ET-1 levels, measured on days 1, 5, and 11 before IH or air-air cycling, showed a time-dependent increase in the IH rats but did not change throughout the 11-day protocol in the control rats (Figure 4). These studies demonstrate that IH is associated with increased ET-1 production at a time when the rats are hypertensive.

Response to Endothelin Receptor Antagonist PD145065
To determine the contribution of circulating endothelin to maintenance of arterial pressure, we administered the nonse-
lective endothelin receptor antagonist PD145065. We observed a concentration-dependent decrease in MAP that was significant only in the IH group (Figure 5). The fall in MAP was rapid and sustained in the IH-exposed rats, persisting for $30\text{ minutes after antagonist administration.}$ In the air-air group, there was no significant change in blood pressure in response to PD145065.

**Discussion**

Rats exposed to 20 cycles per hour of IH to simulate the hypoxemia and hypercapnia of apnea during each daily 8-hour sleep period had a progressive increase in blood pressure and plasma ET-1 levels, whereas rats exposed to 20 cycles per hour of air-air had no significant change in either variable. In addition, an ET-1 receptor antagonist dose-dependently reduced arterial pressure in the hypertensive IH rats. This same antagonist did not affect blood pressure in control rats. These data support the hypothesis that elevated circulating ET-1 contributes to the development of sleep apnea–induced hypertension.

Patients with sleep apnea have as many as 100 episodes of apnea per hour of sleep. Apneic periods are caused by complete or partial airway closure. Thus, patients with sleep apnea also have disturbed sleep and daytime sleepiness. In most animal models of sleep apnea, the animals continue to breathe throughout the hypoxic period, and the regulated atmosphere reduces inspired oxygen to simulate apneic conditions. This type of animal model also mimics the disturbed sleep seen in patients with sleep apnea. It is possible that either the IH per se or the disturbed sleep could lead to hypertension. However, it has been demonstrated that periodic waking in dogs who had intermittent tracheal occlusions during sleep had significantly higher daytime arterial pressure compared with dogs that had their sleep disturbed without tracheal occlusion. In addition, exposing rats to 6 seconds of noise stimulation every 30 seconds, 7 hours a day, for 35 days did not cause an increase in systemic blood pressure. Similarly, the air-air rats in the current study were subjected to the same noise and airflow alterations as the IH rats without the change in atmospheric content, and they did not develop hypertension. Together, these studies support that it is IH and not disturbed sleep that causes hypertension.

Previous animal models of sleep apnea with similar exposure protocols with varying degrees of intermittent hypoxia have not all found that IH leads to hypertension. In agreement with our study, Fletcher et al have consistently shown an increase in arterial pressure after 35 days of 8 hours per day IH (3% to 5% O$_2$). In contrast, a study of rats exposed to intermittent hypoxia (6% O$_2$) for 70 days found no change in resting MAP in either normotensive or spontaneously hypertensive rats. Therefore, the exposure conditions and durations.
tion of exposure may affect the magnitude of the IH-induced hypertension. However, in our study, it is apparent that 20 cycles per hour of IH for 8 hours a day elevated blood pressure significantly within 1 week.

Our study provides evidence for the first time that rats exposed to IH have increased circulating ET-1 that could mediate at least part of the developed hypertension. This is in agreement with a clinical study by Phillips et al., who found that after 4 hours of sleep with spontaneous apneic episodes, MAP and ET-1 both increased significantly compared with presleep levels. However, preventing the apneic episodes for an additional 5 hours of sleep with constant positive airway pressure returned MAP and ET-1 levels to presleep levels. This study therefore suggests that intermittent hypoxia can directly stimulate ET-1 production and that the persistent elevation in plasma ET-1 observed in our study was stimulated by the daily IH exposures.

It has been previously shown that elevated plasma ET-1 directly contributes to the development of the systemic hypertension by increasing vascular resistance. In addition, endothelin receptor antagonists lower blood pressure in certain forms of hypertension including spontaneously hypertensive rats and post–myocardial infarction hypertension. Clinical studies indicate that ET-A receptor antagonists are effective antihypertensive agents and improve the prognosis after congestive heart failure. Our data from the nonselective endothelin receptor antagonist PD145065 suggest that elevated circulating ET-1 directly contributes to the hypertension in IH-exposed rats.

In an attempt to more closely mimic sleep apnea, we used hypercapnia in addition to hypoxia. It has been suggested that the combination of hypoxia and hypercapnia may cause a more profound stimulation of the sympathetic nervous system than hypoxia alone. Furthermore, ET-1 release has been shown to enhance sympathetic vasoconstriction. Thus, the mechanism of IH-induced hypertension in the present study could also involve ET-1 stimulation of sympathetic nervous system activity, although the lack of increase in HR does not necessarily support this contention.

Our results suggest that elevated circulating ET-1 is the mechanism of the increased blood pressure after exposure to IH, whereas previous studies indicate that hypoxia is the likely stimulus for increased ET-1 in this setting. For example, hypoxia has been shown to be a potent stimulus for increased ET-1 production in cultured cells. In addition, exposure to chronic hypoxia is associated with increased plasma ET-1 in both animal and human studies. Interestingly, plasma ET-1 levels in IH rats remain elevated after 16 hours of normoxia on days 5 and 11 and increase progressively with repeated IH exposure. This is similar to increases in plasma endothelin observed with chronic hypoxia. However, animals and patients exposed to chronic hypoxia, for example, high altitude, do not develop significant systemic hypertension. Taken together, these data suggest that factors other than hypoxia induction of ET-1 are involved in IH-induced hypertension. It has been shown that chronic hypoxia decreases vascular sensitivity to vasoconstrictors, so that elevated ET-1 levels under that setting may not stimulate increased systemic blood pressure. In contrast, sleep apnea has been associated with increased vasoconstrictor responses and decreased vasodilator responses. Therefore, increased vascular reactivity coupled with elevated circulating ET-1 may contribute to the pathogenesis of IH-induced hypertension.

Conclusions

We found that intermittent hypoxia leads to systemic hypertension during normoxic breathing in the rat, analogous to daytime hypertension in patients with sleep apnea. The hypertension was associated with increased circulating ET-1 plasma levels and was reversed by the infusion of the nonselective endothelin receptor antagonist PD 145,065. Therefore, sleep apnea–induced hypertension may be another form of human cardiovascular disease dependent on elevated ET-1 production and may be responsive to treatment with endothelin receptor antagonists.

Acknowledgments

This research was supported by National Heart, Lung, and Blood Institute grants 03852 (N.L.K.) and 58121 (B.R.W.) and a Grant-in-Aid from the Desert Mountain Affiliate of the American Heart Association (L.D.N.). The authors gratefully acknowledge Heather Nash for conducting the endothelin assays and Kelly Billings and Pam Allgood for technical assistance.

References

3. Kraiczi H, Hedner J, Peker Y, Grote L. Comparison of atenolol, amlo-