Effect of Continuous Positive Airway Pressure and Placebo Treatment on Sympathetic Nervous Activity in Patients With Obstructive Sleep Apnea*

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Study objectives: We studied the effect of continuous positive airway pressure (CPAP) treatment on sympathetic nervous activity in 38 patients with obstructive sleep apnea.

Design: Randomized, placebo-controlled trial.

Setting: Patients underwent polysomnography on three occasions in a clinical research center, and had BP monitored over 24 h at home. All of the patients had sleep apnea with a respiratory disturbance index (RDI) > 15.

Interventions: The patients were randomized blindly to CPAP or placebo (CPAP at ineffective pressure) treatment.

Measurements and results: Prior to therapy, the number of apneas and the severity of nocturnal hypoxia correlated significantly with daytime urinary norepinephrine (NE) levels, but not nighttime urinary NE levels. CPAP treatment lowered daytime BP from 99 ± 2 mm Hg to 95 ± 3 mm Hg (mean ± SEM) and nighttime BP from 93 ± 3 mm Hg to 88 ± 3 mm Hg. Placebo CPAP treatment decreased both day and night mean BP only 2 mm Hg. CPAP, but not placebo, treatment lowered daytime plasma NE levels by 23%, daytime urine NE levels by 36%, daytime heart rate by 2.6 beats/min, and increased lymphocyte β2-adrenergic receptor sensitivity (all p < 0.05). The effect of CPAP treatment on nighttime urine NE levels and heart rate did not differ from placebo treatment. There was a suggestion of an effect of placebo CPAP treatment on nighttime measures, but not on daytime measures.

Conclusion: We conclude that daytime sympathetic nervous activation is greater with more severe sleep apnea. CPAP treatment diminished the daytime sympathetic activation; the potential nighttime effect of CPAP treatment was obscured by a small placebo effect.

(CHEST 2001; 120:887–893)

Key words: β-adrenergic receptors; continuous positive airway pressure; heart rate; norepinephrine; pulse

Abbreviations: AMP = adenosine monophosphate; CPAP = continuous positive airway pressure; MIBG = metaiodobenzylguanidine; NE = norepinephrine; RDI = respiratory disturbance index

Obstructive sleep apnea repeatedly impairs breathing due to relaxation and collapse of the upper airway. Apneas lead to episodic hypoxia and arousal. Epidemiologic studies1 have found associations with hypertension, coronary disease, heart failure, arrhythmia, and death. However, male sex, age, and obesity are risk factors for both cardiovascular disease and sleep apnea, so it is not certain if sleep apnea causes cardiovascular disease or if the two are merely associated with common risk factors.2

Sleep apnea has been associated with increased sympathetic nervous activity during apneas3 and in the daytime.4 However, hypertension, obesity, and advancing age are associated with apnea and with an increase in plasma norepinephrine (NE) levels. Since enhanced sympathetic nervous activity might predispose to cardiovascular disease, establishing a link between apnea and sympathetic activity would be important. Several studies5–8 have noted a decrease in indexes of sympathetic neuronal activity following continuous positive airway pressure (CPAP) treatment of sleep apnea. While these studies have not been placebo controlled, they have been...
fairly consistent. Unfortunately, activities controlled by the sympathetic nerves, such as heart rate and BP, are notoriously responsive to placebo treatment, and a prescription for a mask attached to a CPAP machine may elicit a placebo response.

We conducted a blinded, placebo-controlled trial of CPAP and measured several indexes of sympathetic nervous activity in patients with sleep apnea. If CPAP treatment genuinely decreases sympathetic nervous activity, this might help explain the association between sleep apnea and cardiovascular disease and help guide decisions about the appropriate use of CPAP. A full description of the BP responses of these patients is given elsewhere.9

Materials and Methods

We recruited patients for this study by advertisement and referral. All patients gave oral and written consent to this University of California at San Diego Human Subjects Committee-approved protocol. Patients were eligible if they had never received CPAP, if they were between 35 years and 65 years old, and if they were from 100 to 170% of ideal body weight. Patients underwent a blood count, chemistry panel, ECG, history, and physical examination. Patients with any major medical disorder other than hypertension and sleep apnea were excluded.

Patients receiving antihypertensive medications underwent frequent BP measurements while medicines were slowly tapered and then withheld for 3 weeks prior to study in the clinical research center. All patients underwent home monitoring (Nightwatch system; Respironics; Pittsburgh, PA) to screen for sleep apnea. Patients with a respiratory disturbance index (RDI) revealing $>20$ apneic and hypopneic events per hour of sleep were eligible to continue in the study.

The patients had their pulse rate measured over 24 h using an ambulatory BP monitor (model 90207; Spacelabs Medical; Redmond, WA) prior to treatment and after 1 day and 7 days of treatment. The monitor activated every 15 min from 6 AM to 10 PM and every half hour from 10 PM to 6 AM. We used the standard Spacelabs algorithm to account for artifacts.

Patients stayed at the University of California at San Diego General Clinical Research Center for blood and urine collection, overnight polysomnography recording central and occipital EEG, bilateral electro-oculogram, submental and tibial anterior electromyogram, ECG, nasal/oral airflow using thermistor, and respiratory effort using chest and abdominal inductance belts. Oxyhemoglobin saturation was recorded using a pulse oximeter (Biox 3740; Ohmeda; Louisville, CO). Patients with an RDI $>15$ on the inpatient polysomnogram continued in the study. On the next night, patients were randomized to receive either treatment with CPAP or placebo CPAP. Patients receiving CPAP underwent standard CPAP titration (Devilbiss Horizon model 7353D; Devilbiss; Somerset, PA) and a comfortably fitting mask. Pressure in the mask started at 2 cm H$_2$O and was increased over the night by increments of 2 cm H$_2$O until apneic episodes were obliterated or until a pressure of 8 to 10 cm H$_2$O was reached. Further pressure titration was then done in increments of 1 cm H$_2$O based on the presence of apnea, hypopnea, or snoring associated with arousals. The titration ended when respiratory events were controlled with CPAP.

Individuals randomized to receive placebo CPAP underwent a mock titration night; they wore a special mask that had three 1/4-inch drill holes to create a large leak through the mask. Their CPAP pressure was set at 2 cm H$_2$O and was not advanced. All CPAP units had a hidden compliance clock that allowed measurement of the amount of time the machine was switched on. The patient and all investigators not involved in CPAP titration remained blinded as to treatment assignment. Compliance with CPAP and placebo CPAP treatment was similar. No patients familiar with CPAP were entered into the study. Patients receiving active and placebo treatments reported similar improvements in subjective well-being, suggesting that those receiving inactive CPAP were not aware of their placebo status.

On study day 1, patients arrived at the clinical research center in the afternoon and had urine collected from 5 PM to 10 PM, 10 PM to 6 AM, and 6 AM to 5 PM. Polysomnography was carried out on the evening of day 1. We collected a blood sample in the morning through an indwelling catheter after the patient rested for 20 min in a seated posture. On study day 1, patients began CPAP or placebo CPAP treatment; on study day 2 and study day 10, they continued CPAP or placebo CPAP treatment and provided repeat blood and urine samples. The 24-h urine collections were carried out on the clinical research center. We evaluated the adequacy of urine collections by measures of volume and creatinine excretion. We measured plasma and urine NE levels with a catechol-O-methyltransferase-based radioenzymatic procedure with a purification step prior to assay.10 Urine NE was normalized to body surface area by expressing it as ng/m$^2$/h.

β$_2$-Adrenergic and α$_2$-adrenergic receptor sensitivity and density were determined at day 0 and day 11 according to previously published methods.11–13 β-Receptor sensitivity was determined in whole lymphocytes by quantifying cyclic adenosine monophosphate (AMP) accumulation following a 2-min incubation with 10 μmol/L isoproterenol. α-Receptor sensitivity was determined in whole platelets by quantifying 10 μmol/L epinephrine inhibition of 20 μmol/L prostaglandin E$_2$-stimulated cyclic AMP accumulation following a 2-min incubation. Cyclic AMP levels were determined by radioimmunoassay. β-Receptor density was determined in lymphocyte membranes by saturation radioligand binding using [125I]-iodopindolol. Incubations were for 60 min at 37°C. Specific binding was determined using 10$^{-6}$ mol/L propranolol. α-Receptor density was determined in platelet membranes by saturation radioligand binding using [3H]-rauwolscine. Incubations were for 30 min at 25°C. Specific binding was determined by using 10$^{-5}$ mol/L phentolamine. We analyzed binding by a nonlinear regression receptor-binding software program (GraphPad Software; San Diego, CA).

Statistics

We compared groups by a two-tailed t test or Wilcoxon’s rank sum test for nonnormally distributed data. We evaluated treatment effects using the interaction term for analysis of variance for repeated measures. The β-receptor data were log normalized prior to repeated-measures analysis of variance.

Results

The 38 patients entered into this study had sleep apnea with an RDI $>15$. The 11 hypertensive patients were studied when they were not receiving antihypertensive drugs. The randomized groups were of similar age, race, height, and BP status. However, those randomized to CPAP treatment were heavier and had higher urinary NE levels (Table 1).
**Table 1—Subject Characteristics**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>CPAP Treatment</th>
<th>Placebo Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, No.</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>Age, yr</td>
<td>48.3 ± 1.4</td>
<td>49.7 ± 2.2</td>
</tr>
<tr>
<td>Male/female, No.</td>
<td>14/6</td>
<td>16/2</td>
</tr>
<tr>
<td>White/black/Hispanic, No.</td>
<td>10/4/6</td>
<td>12/2/4</td>
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<tr>
<td>Hypertensive, No.</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Height, cm</td>
<td>175 ± 2</td>
<td>175 ± 2</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>32.5 ± 1.1</td>
<td>28.7 ± 1.3†</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>132 ± 4</td>
<td>131 ± 3</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>83 ± 2</td>
<td>83 ± 2</td>
</tr>
<tr>
<td>Plasma NE, pg/mL</td>
<td>432 ± 31</td>
<td>355 ± 18</td>
</tr>
<tr>
<td>Plasma E</td>
<td>39 ± 9</td>
<td>34 ± 6</td>
</tr>
<tr>
<td>Urine NE, ng/m²/h</td>
<td>1,263 ± 114</td>
<td>861 ± 75†</td>
</tr>
<tr>
<td>RDI</td>
<td>54 ± 5</td>
<td>39 ± 5</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SEM unless otherwise indicated.
†p < 0.05. E = epinephrine

Although all subjects had sleep apnea, the severity of their sleep disturbance and hypoxia correlated with their daytime urinary NE excretion (Table 2). Daytime plasma NE levels correlated with RDI. The correlations of nighttime indexes of sympathetic nervous activity with sleep disturbance and hypoxia were in the predicted direction, but failed to attain statistical significance. The group receiving placebo CPAP spent 5.5 ± 2.7% of their sleep time (mean ± SEM) with an oxygen saturation < 90%, while those in the CPAP-treated group were similarly hypoxic for only 0.6 ± 0.4% of their sleep time (p = 0.006). CPAP treatment lowered mean daytime BP from 99 ± 2 to 95 ± 3 mm Hg and nighttime pressure from 93 ± 3 to 85 ± 3 mm Hg. Placebo CPAP treatment decreased mean BP both day and night only 2 mm Hg.

Treatment with CPAP effectively diminished apneic episodes, abolishing 94% of apneas and hypopneas on the final night of monitoring. By comparison, placebo CPAP treatment was associated with only a 23% drop in RDI. The 94% drop in RDI of the CPAP-treated group was associated with a 23% decrease in daytime plasma NE levels. In contrast, placebo CPAP treatment did not change plasma NE levels (Fig 1), so CPAP treatment was significantly more effective at lowering plasma NE levels than placebo treatment (p = 0.032).

Treatment with CPAP had no immediate effect on daytime urinary NE excretion, but after 10 days of therapy, NE excretion fell by 36%, a response very different than the response to placebo CPAP treatment (p < 0.001; Fig 2). Nighttime urinary NE excretion fell in both CPAP-treated and placebo-treated groups (p = 0.004), but the difference between CPAP and placebo treatment was only marginally significant (p = 0.08; Fig 3). Epinephrine responses to CPAP treatment did not differ from placebo-treatment responses.

Ten days of CPAP treatment lowered daytime heart rate by 2.6 beats/min, and this effect of CPAP differed from placebo (p < 0.05). CPAP treatment lowered nighttime heart rate by 2.1 beats/min, an effect not significantly different from placebo treatment.

CPAP treatment altered lymphocyte β-receptors by increasing β-receptor sensitivity from 4.8 to 5.2, while the placebo group changed from 5.1 to 4.9 (p = 0.01). β-Receptor density followed a similar trend but was not significant (CPAP-treated group, 70 to 82 fmol/mg protein; placebo-treated group, 79 to 76 fmol/mg protein). α-Receptor sensitivity and density were unchanged in either group (CPAP-treated group, 0.96 to 1.2, and 32 to 29 fmol/mg protein; placebo-treated group, 1.1 to 1.06, and 29 to 33 fmol/mg protein, respectively).

**DISCUSSION**

When compared with placebo, CPAP treatment lowered RDI, plasma NE levels, daytime urine NE levels, and daytime heart rate, and increased β₂-adrenergic receptor sensitivity. Diseases exacerbated by sympathetic nervous activity, such as hypertension and angina pectoris, respond to placebo treatment. Wearing a mask attached to an operating machine might elicit a powerful placebo response. A trial of CPAP for sleep apnea used a pill as placebo and found no significant effect on BP. We recently reported that CPAP treatment lowered nighttime BP more than placebo CPAP treatment; the daytime BP.
effect was not significant. We have confirmed the findings of prior uncontrolled studies that CPAP treatment decreases sympathetic nervous activity as manifested by plasma NE levels, urine NE levels, heart rate, and β2-receptor changes. Comparison of placebo- and CPAP-treatment responses revealed that CPAP treatment lowered daytime sympathetic nervous activity as much or more than nighttime sympathetic activity. CPAP treatment decreased daytime urinary NE levels by 36% (p < 0.001 compared to placebo). Nighttime urine NE levels fell by only 16%, and this did not differ from the small fall in nighttime urine NE levels in the placebo-treated group. Similarly, CPAP treatment significantly lowered daytime heart rate, while the effect of CPAP treatment on nighttime heart rate did not differ from placebo.

**Figure 1.** The effect of CPAP and placebo CPAP treatment on plasma NE levels. CPAP decreased NE levels by 23%, differing from the effect of placebo treatment (p = 0.032).

**Figure 2.** The effect of CPAP and placebo CPAP treatment on daytime urinary NE levels. Urine NE excretion fell by 36% in the CPAP group, differing from the effect of placebo (p < 0.001).
placebo treatment, which slightly lowered the nighttime heart rate as well. The effect of CPAP treatment on daytime sympathetic activity is consonant with clinical reports15 and studies of the whole group prior to treatment. Daytime urine NE levels correlated significantly with measures of sleep disturbance and hypoxia, while correlations with nighttime urine NE levels were weaker and not significant (Table 2). We cannot tell from this study if the effects of CPAP treatment were due to fewer arousals from sleep or less hypoxia, as most of the arousals were the result of respiratory disturbance.

The concept that CPAP applied at night has its most apparent effect on sympathetic nervous activity in the daytime seems counterintuitive, but there is experimental support for a long-lasting effect of episodic hypoxia on the sympathetic nervous system. Rats subjected to episodic hypoxia for 7 h/d had elevated renal sympathetic nerve activity while not hypoxic.16,17 Patients with sleep apnea had increased muscle sympathetic neuronal activity in the daytime4 that decreased with CPAP treatment.7 Sleep apnea may increase daytime sympathetic nervous activity by changing cardiovascular reflexes. Sleep apnea changes baroreflex sensitivity to BP18 and chemoreflex sensitivity to oxygen19,20 in ways that enhance and prolong sympathetic neuronal responses. Although normal subjects did have BP increases in response to breathing a hypoxic gas mixture in the daytime, patients with sleep apnea had a pressor response to the same stimulus21 and a prolonged increase in sympathetic nerve activity following hypoxia.22 We have found that daytime hypoxia increased the rate of NE release into the circulation more in apneic subjects than in nonapneic subjects.23 In this study, nighttime sleep apnea elicited daytime sympathetic neuronal activation that was more prominent in our subjects than its nighttime effect. This would not have been apparent without nighttime placebo CPAP treatment. The placebo-treated group had a small decrease in nighttime urine NE level and heart rate, while there was no hint of placebo effect on daytime measures, as might be expected from a treatment applied at night. Other studies without a placebo comparison group have found that CPAP treatment decreased urinary NE levels5 and NE metabolites6 as much in the daytime as at night. Daytime studies24,25 of sympathetic nerve electrical discharge have found increased activity in sleep apnea patients compared to healthy control subjects. In addition, CPAP treatment lowers daytime sympathetic nerve activity,26 and better compliance with CPAP predicts a greater lowering of sympathetic nerve activity.27 We conclude that nocturnal CPAP treatment has at least as large an effect on daytime measures of sympathetic nervous activity as on nighttime measures.

CPAP treatment increased lymphocyte β-receptor sensitivity, as might be expected from a treatment that lowered plasma NE levels. This finding is especially interesting since we have previously shown, both in vitro28 and in vivo,29 that patients
with sleep apnea have desensitized β-adrenergic receptors as compared to nonapneic patients. CPAP treatment normalized both NE levels and β-receptor sensitivity. This suggests that the β-receptor desensitization of apneic patients is a response to increased sympathetic nervous activity. Enhanced β sensitivity may help explain the relatively small (2.6 beats/min) fall in daytime heart rate in the face of a 23% fall in plasma NE levels and a 36% fall in urine NE levels. Other studies have generally not remarked on a change in daytime heart rate with CPAP therapy, leading one to suspect that changes are generally small. This should not be taken to mean that the effects of sleep apnea and CPAP treatment on cardiac sympathetic nerves are small. Cardiac sympathetic nerves handle metaiodobenzylguanidine (MIBG) like NE. Sleep apnea patients released MIBG from the heart too fast; CPAP treatment slowed daytime cardiac MIBG turnover almost to normal. A small decrease in heart rate might be expected from a treatment that lowers sympathetic neuronal activity and permits β-receptors to become more sensitive.

This study has limitations. We studied patients with sleep apnea and hypertension but without other serious illness, so the results might not apply to patients with other illnesses that commonly occur in apneic patients. The sample size was modest. However, the mock CPAP gave insight into potential placebo effects of CPAP treatment and clearly showed an effect of CPAP treatment on daytime sympathetic nervous activity. Patients with sleep apnea have an excess incidence of left and right ventricular hypertrophy, myocardial infarction, hypertension, and arrhythmia. The sympathetic nervous system plays a role in all of these. This study points out the need for controlled studies to see if treatment of sleep apnea can prevent cardiovascular disease.

REFERENCES