Serotonin-Induced Cortisol Release in CPAP-Treated Obstructive Sleep Apnea Patients*

David W. Hudgel, MD; and Elizabeth A. Gordon, BS

Previously, we demonstrated elevated cortisol production/release in response to the administration of the serotonin precursor, L-5-hydroxytryptophan (L-5-HTP) in untreated patients with obstructive sleep apnea (OSA). We hypothesized that if this elevated cortisol response to L-5-HTP was related to OSA, this finding would not be present in OSA patients treated with nasal continuous positive airway pressure (nCPAP). Eleven OSA patients treated for at least 1 month with nCPAP were studied. On two different days, we measured blood cortisol level every 15 min for 4 h following the ingestion of L-5-HTP, 0.4 mg/kg, or placebo, both given with carbidopa, a peripheral tryptophan decarboxylase inhibitor, used to prevent peripheral L-5-HTP metabolism before brain absorption. For a given subject, the cortisol response was calculated as the difference between the area under the curve of the L-5-HTP and placebo responses. In the nCPAP-treated OSA patients, this net cortisol response, 577±240 min · µg/dL, was less than the value found in the previously studied untreated OSA group, 1,198±227 min · µg/dL (p<0.05) and not different from the previously studied nonapneic control group, 469±154 min · µg/dL. From these results, we speculate that nCPAP treatment reverses the elevated cortisol response to serotoninergic stimulation seen in untreated OSA patients. (CHEST 1997; 111:632-38)

Key words: cortisol; cortisol stimulation test; 5-hydroxytryptamine; obstructive sleep apnea; serotonin; sleep apnea

Abbreviations: BMI=body mass index; 5-HIAA=5-hydroxyindole acetic acid; 5-HT=serotonin; L-5-HTP= L-5-hydroxytryptophan; nCPAP=nasal continuous positive airway pressure; NS=not significant; OSA=obstructive sleep apnea; SaO₂=arterial oxygen saturation

Previously, we demonstrated that patients with obstructive sleep apnea (OSA) had an elevated cortisol response to the ingestion of the serotonin (5-HT) precursor, L-5-hydroxytryptophan (L-5-HTP). Since patients had a normal corticotropin stimulation test result and since we used a peripheral decarboxylase inhibitor to block the potential direct effect of L-5-HTP on tissues peripheral to the blood-brain barrier, we concluded that this increased cortisol response to L-5-HTP was due to increased brain serotonergic stimulation of corticotropin releasing factor secretion by the hypothalamus. If this effect was associated with the presence of OSA in our subjects, we hypothesized that a group of OSA patients treated with nasal continuous positive airway pressure (nCPAP) would not have an elevated cortisol response to L-5-HTP. We tested this hypothesis by measuring the cortisol response to L-5-HTP relative to placebo administration in 11 nCPAP-treated OSA patients. Comparisons were made with the previously studied untreated OSA patients and the nonapneic control group.

Materials and Methods

Subjects

Both untreated and treated OSA patient volunteers were obtained from the referral patient population of the MetroHealth Medical Center’s Sleep Disorders Program. All patients had a history of heavy snoring and excessive daytime sleepiness. A polysomnogram, performed by standard techniques and scored in the classic fashion, demonstrated OSA in each patient, characterized by episodic upper airway closure and arterial oxygen desaturation. If patients chose nCPAP treatment, a second polysomnogram was performed to determine the amount of nCPAP pressure required to eliminate apneas, arterial oxygen desaturation, and snoring. OSA patients who were treated with nCPAP for at least 1 month were asked to volunteer for this study.

Techniques

The dose of L-5-HTP was 0.4 mg/kg. Twenty-five milligrams of carbidopa was also administered. Carbidopa is a decarboxylase...
inhibitor that prevents the conversion of L-5-HTP to 5-HT.\textsuperscript{3} Since carbidopa does not cross the blood-brain barrier, this decarboxylase inhibition was limited to tissues peripheral to that barrier. By using carbidopa, we limited the production of 5-HT from the L-5-HTP to the brain.

Serum cortisol was measured by radioimmunoassay (Diagnostic Products Corp; Los Angeles). Samples were analyzed in duplicate and the two results were averaged to obtain the final result for a given sample. The interassay and intra-assay coefficients of variation were 8.2\%±3.3\% (±SEM) and 5.6\%±0.9\%, respectively, for the cortisol determinations in the nCPAP-treated patients. Placebo and L-5-HTP test-day plasma samples from the same subject were processed during the same analysis. All determinations were performed by the same technician.

Protocol

The study was conducted in the Case Western Reserve University General Clinical Research Center, MetroHealth Medical Center. The protocol was approved by the institutional review board. Subjects underwent placebo and L-5-HTP challenges on separate days, usually a week apart. The order of testing was performed randomly across subjects.

Subjects reported to the General Clinical Research Center the evening prior to the study at approximately 6 PM. All subjects were given the same low-protein, high-carbohydrate meal on each of the testing days. The purpose of this meal was to standardize the L-tryptophan intake before the study so that the L-5-HTP gut and brain absorptions at the time of the study would not vary because of diet.\textsuperscript{4} At bedtime, a finger-pulse oximeter (model N-100; Nellcor; Pleasanton, Calif) was placed on an index finger, and recorded on chart paper at a speed of 30 cm/h. nCPAP was administered throughout the night, utilizing the patient’s nCPAP machine. On arising in the morning, subjects were allowed to shower, but were kept “nothing by mouth” (NPO) except for small sips of water. An IV catheter was placed in a hand or forearm vein. Catheter patency was maintained with heparin, 100 U/mL dilution. Carbidopa, 25 mg, was given orally at 7 AM.

After three baseline blood samples, 3 mL each, were obtained at 15-min intervals, L-5-HTP, 0.4 mg/kg, or an identical-appearing placebo was orally administered at 8 AM. Three milliliters of blood was sampled for the cortisol assay every 15 min for 3 h and every half hour for 1 h. Blood samples were centrifuged at 500 g for 20 min. Plasma was removed, divided from duplicate measurements, and stored at -70°C.

Data Analysis

Areas under the curve of the L-5-HTP-cortisol and placebo-cortisol responses over the 4 h were calculated using a computer data analysis program (MATLAB; Mathworks Inc; South Natieck, Mass). The cortisol response of each volunteer was calculated as the difference between the areas under the curve for the L-5-HTP and placebo challenges. Cortisol response data across groups, nCPAP-treated OSA patients, untreated OSA patients, and nonapneic control subjects were compared by analysis of covariance, with age and body mass index (BMI, kg/m\textsuperscript{2}) as covariates. Post hoc, Duncan’s multiple range test was used. Statistical significance was taken as \(p\leq0.05\).

RESULTS

Subject Characteristics

Characteristics of the three subject groups—untreated OSA patients, nCPAP-treated patients, and nonapneic control subjects—are given in Table 1. Significant differences among the groups were the following: BMI was higher in the nCPAP-treated OSA groups than in the nonapneic control subject group and in the untreated OSA patient group. The latter two groups were not different. As would be expected, the apnea/hypopnea index was higher, the percent sleep period time spent hypoxic (arterial oxygen saturation [\text{SaO}_2] <90\%) was higher, and the \text{SaO}_2 nadir during sleep was lower in the untreated OSA patients than in the nonapneic control subjects or the nCPAP-treated OSA patients. No differences existed in these variables between the nonapneic control subjects and the nCPAP-treated patients. Age was not different across all these groups. nCPAP-treated patients had an average duration of treatment of 16±3 months.

L-5-HTP-Cortisol Challenge

The baseline cortisol blood level was 11.5±1.2 µg/dL for the nCPAP-treated OSA patients, 10.6±1.2 µg/dL for the untreated patients, and 11.2±0.7 µg/dL for the control subjects (\(F=0.2, p=\text{not significant [NS]}\)). The results of individual L-5-HTP-cortisol and placebo-cortisol challenges for the nCPAP-treated patients are shown in Table 2. Table 3 presents L-5-HTP cortisol challenge group data for the nonapneic control subjects, untreated OSA patients, and nCPAP-treated patients. The cortisol responses of the nCPAP-treated OSA patient group to placebo and to L-5-HTP are shown in Figure 1. The area under the curve for the cortisol response to placebo was different among the groups (\(F=3.0, p<0.05\) (Fig. 2). The area under the curve of the cortisol response to placebo for the nCPAP-treated patients was 3,157±126 min ∙ µg/dL, compared to 2,227±236 min ∙ µg/dL for the untreated patients (\(p<0.2\times10^{-2}\)) and 2,792±694 min ∙ µg/dL for the control subjects (\(p=\text{NS}\)). The area under the curve of the cortisol response to L-5-HTP for the

<table>
<thead>
<tr>
<th>Table 1—Subject Characteristics (Mean±SEM)*</th>
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<tbody>
<tr>
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<tr>
<td>--------------------------------------------</td>
</tr>
<tr>
<td>N 11 (all male)</td>
</tr>
<tr>
<td>Age, yr</td>
</tr>
<tr>
<td>BMI, kg/m\textsuperscript{2}</td>
</tr>
<tr>
<td>AHI, events/h</td>
</tr>
<tr>
<td>Sleep period time</td>
</tr>
<tr>
<td>\text{SaO}_2 &lt;90%, %</td>
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<tr>
<td>\text{SaO}_2 nadir, %</td>
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\*\text{AHI}=apnea/hypopnea index.  
\textsuperscript{1}p<0.05 from other two groups by analysis of covariance.
nCPAP-treated patients was 3,735 ± 239 min · µg/dL, compared to 3,425 ± 225 min · µg/dL for the untreated patients (p = NS) and 3,261 ± 218 min · µg/dL for the control subjects (p = NS). The area under the curve for the L-5-HTP-cortisol net response (L-5-HTP cortisol response minus the placebo response) was different among the groups (F = 3.49, p = 0.05) (Fig. 3). L-5-HTP-cortisol net area under the curve for the nCPAP-treated patients was 577 ± 240 min · µg/dL, which was different from the 1,198 ± 227 min · µg/dL value for the untreated patients (p < 0.04) but not different from the 469 ± 154 min · µg/dL value for the nonapneic control subjects (p = NS from the nCPAP-treated OSA patients, p < 0.02 from the untreated patients). The placebo and L-5-HTP order of testing was the following: for the control group, five had L-5-HTP first; for the untreated patients, six had L-5-HTP first; and for the nCPAP-treated patients, four had L-5-HTP first.

**DISCUSSION**

The results of this study show that the increase in the cortisol response to L-5-HTP ingestion in untreated OSA patients compared to nonapneic control subjects was not present when nCPAP-treated OSA patients were studied. The L-5-HTP-cortisol response of the nCPAP-treated OSA patients was lower than the level of the response in the untreated patients, and was not significantly different from the cortisol response to L-5-HTP found in the control group. Not only does cortisol production/release in response to L-5-HTP appear to be changed with nCPAP treatment, but there is an increased cortisol response to placebo with nCPAP treatment relative to the untreated patients and nonapneic control subjects. Therefore, nCPAP treatment may lead to an increase in the morning unstimulated cortisol production/release or to a change in the diurnal rhythm of cortisol homeostasis, in addition to changing the response of the cortisol axis to serotonergic stimulation.

**Rationale for Technique Used**

We used the serotonergic stimulation of cortisol production/release in response to L-5-HTP compared to the cortisol production/release to placebo over the same time frame as a probe of brain and hypothalamic 5-HT activity. Enhanced brain 5-HT activity in response to L-5-HTP would stimulate hypothalamic production/release of corticotropin-releasing hormone, which in turn would stimulate corticotropin pituitary production/release and subsequent adrenal cortisol production/release. A heightened response, as found in our untreated OSA patients, would be consistent with the presence of an upregulated brain 5-HT system, likely secondary to decreased brain 5-HT production.

**Technical Considerations for This Study**

Technical and design factors could affect our results. We must be concerned that L-5-HTP is directly stimulating the pituitary or the adrenal glands and not working through brain serotonergic receptors. That is why we added carbidopa, a peripheral-acting decarboxylase inhibitor that will stop 5-HT production from L-5-HTP in organs other than the brain. To address this issue, we measured blood 5-HT and the 5-HT metabolite 5-hydroxyindole acetic acid (5-HIAA) blood in our initial study. We showed that blood 5-HT did not increase following L-5-HTP and carbidopa administration, but 5-HIAA increased considerably. We interpret these results to indicate that the orally administered L-5-HTP was not converted to 5-HT in peripheral tissues because of the effect of the carbidopa. The increase in 5-HIAA likely indicates that there was an increase in 5-HT production from the L-5-HTP administered, and that this increase in 5-HT production

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**Table 2—L-5-HTP Stimulation of Cortisol in nCPAP-Treated Patients**

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Placebo Carbidopa*</th>
<th>5-HTP Carbidopa*</th>
<th>Net*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3,078.6</td>
<td>3,694.8</td>
<td>616.2</td>
</tr>
<tr>
<td>2</td>
<td>2,859.7</td>
<td>3,729.9</td>
<td>870.2</td>
</tr>
<tr>
<td>3</td>
<td>3,837.9</td>
<td>4,251.3</td>
<td>413.4</td>
</tr>
<tr>
<td>4</td>
<td>3,126.6</td>
<td>3,655.8</td>
<td>529.2</td>
</tr>
<tr>
<td>5</td>
<td>3,325.7</td>
<td>3,222.5</td>
<td>-103.2</td>
</tr>
<tr>
<td>6</td>
<td>3,106.3</td>
<td>3,913.9</td>
<td>807.6</td>
</tr>
<tr>
<td>7</td>
<td>3,365.1</td>
<td>2,195.7</td>
<td>-1,169.4</td>
</tr>
<tr>
<td>8</td>
<td>3,205.3</td>
<td>3,371.7</td>
<td>166.4</td>
</tr>
<tr>
<td>9</td>
<td>2,144.6</td>
<td>3,340.3</td>
<td>1,195.7</td>
</tr>
<tr>
<td>10</td>
<td>3,242.0</td>
<td>4,320.4</td>
<td>1,078.4</td>
</tr>
<tr>
<td>11</td>
<td>3,438.1</td>
<td>5,384.6</td>
<td>1,946.5</td>
</tr>
</tbody>
</table>

Mean ± SEM 3,157.26 ± 125.89 3,734.63 ± 238.89 577.36 ± 240.22

*Area under curves, min · µg/dL.

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**Table 3—L-5-HTP and Placebo Cortisol Response**

<table>
<thead>
<tr>
<th></th>
<th>Nonapneic Control Subjects</th>
<th>Untreated OSA Patients</th>
<th>nCPAP-Treated OSA Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>2,792 ± 694</td>
<td>2,227 ± 236</td>
<td>3,157 ± 126*</td>
</tr>
<tr>
<td>L-5-HTP</td>
<td>3,261 ± 218</td>
<td>3,425 ± 225</td>
<td>3,735 ± 239</td>
</tr>
<tr>
<td>Net (L-5-HTP minus placebo)</td>
<td>469 ± 154*</td>
<td>1,198 ± 227*</td>
<td>577 ± 240*</td>
</tr>
</tbody>
</table>

*p < 0.05 between marked groups by analysis of covariance.

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Clinical Investigations
FIGURE 1. Plasma cortisol response to administration of L-5-HTP (solid squares) and placebo (open squares) in nCPAP-treated OSA patients. Bars are SEM. Agents were administered at 0 time.

most likely occurred in the brain with transfer of 5-HIAA to the bloodstream, as is known to occur. In addition, there is no reason why there would be a systematic difference in protein-bound and free plasma cortisol among these experimental groups. Thereby, the cortisol assay should not be affected across the groups by a potential difference in free vs bound cortisol.

In addition, there may be a concern about the comparability of cortisol measurements made at different times. If the techniques of analysis varied at all, then the results of the cortisol measurements made in the nCPAP-treated patients could not be compared with the previously measured cortisol levels made in nonapneic control subjects and untreated OSA patients. The same commercial assay was used with the same technique by the same technician in both cases. The interassay and intra-assay coefficients of variation were similar for the two testing times. The interassay mean coefficient of variation was 9.3 in the first study and 8.2 in this study. The intra-assay mean coefficient of variation was 7.4 previously and 5.6 currently. Thus, we conclude that the serum cortisol concentrations can be compared across these groups as we have done.

In the present study, one female OSA patient was included, while in the study of untreated OSA, all subjects were male. It is unlikely that this gender difference would have significantly affected our results since the cortisol response to L-5-HTP and placebo was not different from the remainder of the test population (No. 6 in Table 2). Another potential problem is that the patient groups were not identical for BMI. However, our analysis of covariance compensated for this difference in BMI. This analysis showed that BMI was not a significant variable accounting for the differences in the cortisol response to L-5-HTP among our three experimental groups. Although our analysis did not identify subject group differences responsible for our findings in this cross-sectional study, it will be important to confirm our findings in another group of OSA patients who would be studied longitudinally before and during nCPAP treatment.

It also must be pointed out that both the nCPAP-treated OSA patients and the relatively obese control subjects studied might still have some brain 5-HT deficiency due to their obesity, insulin resistance, hypertension, and mild daytime hypoxemia, if present. We do not know the extent of this deficiency since we did not test a thin control group.

**Ventilatory Effects of Brain 5-HT**

Although the cortisol response to L-5-HTP does not directly examine the ventilatory effect of brain 5-HT of OSA patients, there are implications to ventilatory control that can be made from this
analysis. To a large extent, the ventilatory action of 5-HT occurs via the midline juxtaposed raphe nuclei, an area whose efferent serotonergic neural activity occurs during both wakefulness and sleep. We assume that changes in raphe nuclei serotonergic activity would occur when brain serotonergic activity in general is changed by the L-5-HTP administration, as is evidenced by our test results. It is also possible that heightened hypothalamic serotonergic activity stimulates raphe nuclei serotonergic activity since interaction between changes in raphe nuclei and hypothalamic function occurs. In addition, raphe nuclei serotonergic activity is known to affect upper airway ventilatory function and diaphragm activity via innervation of the hypoglossal nucleus and phrenic motoneurons. Therefore, when changes in brain 5-HT activity occur, we speculate that raphe nuclei activity is changed, and thereby ventilatory control, especially of upper airway muscles, may be changed. The specific ventilatory control response to fluctuations in brain serotonergic activity in humans currently is being evaluated.

**Potential Mechanism(s) of the Difference in the L-5-HTP-Cortisol Response Between the Untreated and nCPAP-Treated OSA Patients**

The elevated cortisol response to L-5-HTP in the untreated OSA patients is evidence of upregulation of receptor(s) in the brain 5-HT production pathway. This receptor upregulation is an indication that a 5-HT deficiency state exists in the brain.

Why would there be such a 5-HT deficiency in patients with OSA? Evidence exists that insulin resistance, obesity, and hypertension, common problems in OSA patients, contribute to decreased brain 5-HT concentration. In addition, mild hypoxemia, which is often present in obese subjects, may contribute to insulin resistance and is also a predisposing factor contributing to low tissue 5-HT concentrations. Recently, it has been reported that insulin resistance is present specifically in OSA patients. Insulin resistance in OSA patients improves with nCPAP treatment. Insulin action is important in the brain uptake of tryptophan, the amino acid 5-HT precursor. Without the insulin-induced transfer of neutral amino acids (other than tryptophan) into cells, competition for the amino acid blood-brain barrier protein transporter increases. Thereby, less tryptophan is transported across this barrier. With less insulin resistance, more tryptophan will cross the blood-brain barrier and brain levels of 5-HT will increase. If our untreated OSA patients had significant insulin resistance, as appears to occur even in nondiabetic OSA patients, then improvement of their insulin resistance with nCPAP treatment might result in an

![Figure 2](https://example.com/figure2.png)
increase in brain tryptophan levels. Consequently, a decrease in 5-HT production might not be so likely to occur and serotonin receptors would not become upregulated.

In addition, altered adrenergic activity, often present in OSA, also might alter brain 5-HT activity. Since the activity of the rate-limiting enzyme in 5-HT production, tryptophan hydroxylase, is reduced in the presence of significant hypoxemia, 5-HT production could be further decreased during nocturnal OSA-induced hypoxemia. Thus, there is good rationale to support the idea that untreated OSA patients have a brain deficiency in 5-HT. Several variables are improved with nCPAP treatment of OSA. Apnea, sleep disruption, intermittent hypoxemia, and heightened adrenergic output at least are partially resolved with this treatment. Resumption of normoxia during sleep with nCPAP treatment could be a major factor in preventing at least a portion of the heightened serotonergic activity in the nCPAP-treated OSA patients.

Either one or all of these mechanisms could play a role in brain 5-HT dynamics observed in the untreated and treated OSA patients we have studied. The obvious question that comes from these data is whether the abnormality in brain 5-HT identified in untreated OSA patients is related to the pathophysiologic condition of sleep-disordered breathing. The findings of this study suggest that the deficiency in brain 5-HT identified in untreated OSA patients likely is related to OSA. The question as to whether this brain deficiency of 5-HT is a predisposing variable contributing to abnormal upper airway muscle activity in sleep is currently under investigation.

FIGURE 3. L-5-HTP plasma cortisol response in nCPAP-treated OSA patients (solid squares), nonapneic control subjects (triangles), and untreated OSA patients (open squares). Agents were administered at 0 time. (Standard error bars were eliminated for clarity.)

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