Pharyngeal Chemosensitivity in Patients with Obstructive Sleep Apnea and Healthy Subjects

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Abstract

Signs of pharyngeal neurodegeneration have been detected in patients with obstructive sleep apnea (OSA). Along with this neurodegeneration, a decreased pharyngeal sensitivity to mechanical stimulation has been described. The decreased sensitivity may play a role in the pathophysiology of this disease. The aim of the study was to investigate the chemosensitivity of the pharyngeal mucosa in patients with OSA compared with controls. Healthy controls and patients with OSA (age: 30–60 years) were included. Testing of oropharyngeal chemosensitivity was performed with subjective intensity ratings of capsaicin (SIR, visual analogue scale 0–10), air puffs (presented with an olfactometer), and stimulation with CO\textsubscript{2} at the posterior pharyngeal wall. A 2-point discrimination test at the soft palate, an intensity rating of capsaicin at the tongue, and a nasal laterization test were performed. Twenty-six patients with OSA and 18 healthy controls were included. No differences were detected in the SIR of capsaicin at the tongue or in the nasal laterization test. At the pharynx, a decreased sensitivity to capsaicin (OSA: 6.8±2.3; healthy control: 8.6±1.3), air puffs (OSA: 2.8±1.9; healthy control: 4.2±1.6), and stimulation with CO\textsubscript{2} (OSA: 1.5±1.7; healthy control: 2.8±1.8) were demonstrated in patients with OSA (all \(P<0.05\)). Two-point discrimination at the soft palate was reduced with statistical significance in the OSA group (OSA: 11.5±5.4 mm; healthy control: 5.0±2.4 mm). The results suggest reduced pharyngeal chemosensitivity in OSA patients in addition to the reduced mechanical pharyngeal sensitivity shown with 2-point discrimination. This demonstrates peripheral neurodegeneration in the context of this disease.

Key words: neurodegeneration, obstructive sleep apnea, pharyngeal chemosensitivity, trigeminal, vibration damage soft palate

Introduction

The etiology of obstructive sleep apnea (OSA) is complex. Several factors contribute to the instability of the upper airway, leading to repetitive upper airway collapse. The major risk factors for OSA are obesity, age, gender, and craniofacial abnormalities. Furthermore, additional risk factors are known including smoking, alcohol consumption, pregnancy, decreased chemosensitivity of the cerebral breathing center, and preexisting diseases such as rheumatism, acromegaly, and hypothyroidism (Young et al. 2002, 2008).

Five issues should be considered regarding the pathophysiology of OSA (Campana et al. 2010): upper airway anatomy (Malhotra et al. 2002), arousal threshold (Younes 2004), regulation of ventilation by the feedback control system that is moderated by the chemical control system (Younes et al. 2001), functional residual capacity of the lungs (Stanchina et al. 2003), and sensitivity of the pharyngeal dilator muscles to respiratory changes during sleep (Mezzanotte et al. 1992).

In addition, the dysfunction of the pharyngeal upper airway dilator muscles seems to play a major role in the pathophysiology of OSA. In previous studies, an increase of type II fibers of the uvula muscle could be demonstrated...
The hypothesis of our study was that in addition to a reduced mechanical sensitivity, a reduced chemosensitivity is present in patients with OSA. To test this hypothesis, the following tests were performed:

### Mechanical sensitivity:
- Two-point discrimination test at the soft palate
- Air puffs at the posterior pharyngeal wall

### Chemosensitivity at the pharynx:
- Sensitivity to capsaicin at the posterior pharyngeal wall
- Sensitivity to CO₂ at the posterior pharyngeal wall

### Chemosensitivity outside the pharynx:
- Olfactory function (Sniffin’ Sticks test)
- Gustatory function (taste strips)
- Sensitivity to capsaicin at the tongue
- Nasal lateralization test

The mechanical tests (air puffs, 2-point discrimination) were conducted first. These tests were done to confirm the decrease in sensitivity described in previous studies (Larsson et al. 1992; Kimoff et al. 2001), thereby serving as an internal control for the correct setting of the study. To test our hypothesis of a decrease in pharyngeal chemosensitivity, we performed the capsaicin test at the posterior pharyngeal wall, as well as the CO₂ stimulation. The nasal and lingual tests (Sniffin’ Sticks, taste strips, and nasal lateralization test) were conducted to assess if potential differences between both groups are specific to the pharynx (and thereby related to OSA) and therefore do not reflect a general reduction of (chemo)sensitivity in OSA patients.

### Patients and healthy controls
Twenty-six patients with OSA (respiratory disturbance index [RDI] > 15/h; 23 males, 3 females; mean age 45 ± 9) and 18 healthy controls (11 male, 7 female; mean age, 40 ± 8) were included in the study. In the OSA group, 2 patients had already the diagnosis of an OSA 1 year before. Both were already provided with a CPAP (continuous positive airway pressure) since 1 year, but they did not use it cause to the fact that it was too uncomfortable for them. Both patients wanted to have an alternative treatment option. The presence or absence of OSA was assessed by an overnight, fully attended polysomnography (PSG). The PSG was scored according to the American Academy of Sleep Medicine Manual 2007 (Iber et al. 2007). Body mass index (BMI, kg per m²) and the score on the Epworth Sleepiness Scale (ESS) (Johns 1991) were also recorded. Further inclusion criteria were age between 30 and 60 years. Exclusion criteria included a history of smell or taste disorders, the use of medication known to affect chemosensory function, nicotine abuse (>10 cigarettes per day), and previous pharyngeal surgeries such as tonsillectomies. This study complies with the Declaration of Helsinki for Medical Research involving Human Subjects.
as tonsillectomy. At the screening visit, patients with relevant nasal pathologies, such as mucosal inflammation, significant septal deviation, and nasal polyposis, were ruled out through clinical examination that included nasal endoscopy. Subjects were not allowed to eat, drink, or smoke for 1 h before testing to avoid chemosensory desensitization. Participants were asked to perform a subjective intensity ranking (SIR, visual analogue scale 0–10) for the following chemosensitivity and mechanical tests: sensitivity to capsaicin at the posterior pharyngeal wall/tongue, air puffs at the posterior pharyngeal wall, and sensitivity to CO₂ at the posterior pharyngeal wall.

**Mechanical sensitivity**

Two-point discrimination was performed with a pair of compasses elongated with toothpicks (Guilleminault et al. 2002). At the margin of the soft palate just above the uvula, a series of tests with fixed interprobe distances ranging from 2 to 40 mm were performed. First, the patients underwent an orientation procedure that told them if 1 or 2 toothpicks had been applied. The test started with the largest intertoothpick distance of 40 mm. The pressure slightly indented the surface of the mucosa and was applied for 2 s. If the patient correctly reported that the toothpick had 2 points, the next smaller intertoothpick distance was used. The smallest noticeable intertoothpick distance was determined for every subject, and the test was repeated 4 times. Mean values of the 4 tests were used for further analysis for every subject.

Mechanical stimulation of the posterior pharyngeal wall was conducted via endoscopy by placing a tube through one of the nasal cavities directed to the posterior pharyngeal wall at a distance of approximately 1 cm. Air puffs were used for stimulation with a computer-controlled air-dilution olfactometer (OM6; Burghart Instruments). Stimulus duration for every puff was 1 s with an interstimulus interval of 30 s. Stimuli were presented randomly with 3 different intensities (flows): 2L/min, 6L/min and 10L/min 5 times each. The subjects rated the intensity of the stimulus on a visual analogue scale (SIR) from 0 to 10 for every stimulus intensity. Mean values of the 3 intensity tests were used for further analysis for every subject.

**Chemosensitivity at the pharynx**

Chemosensitivity was tested with capsaicin on the tongue and the posterior pharyngeal wall and with CO₂ on the posterior pharyngeal wall.

A single-use dropping glass was used for “capsaicin testing” on the posterior pharyngeal wall (Just et al. 2007). Four different concentrations of capsaicin (0.1%, 0.01%, 0.001%, and 0.0001%) dissolved in a neutral oil emulsion were applied in random order. Each concentration was applied only once, as repeat testing would have caused desensitization to the substance, and significant interstimulus intervals of 10 min were needed between the tests. Again the subjects rated the intensity on a scale from 0 to 10.

Chemical stimulation of the posterior pharyngeal wall was performed via endoscopy by placing a tube through one of the nasal cavities directly opposite to the posterior pharyngeal wall as previously described. Three different concentrations of CO₂, an odorless gas (20% v/v, 40% v/v, and 60% v/v), in a constant flow of odorless humidified air (stimulus duration 1 s, interstimulus interval 30 s, flow 8 L/min, 36 °C) were used for trigeminal stimulation. The concentrations for CO₂ were chosen with regard to the concentrations used for chemosensory testing during wakefulness, as it is known that these concentrations produce distinct but tolerable trigeminal sensations. The different concentrations were presented in a randomized manner 5 times each. The intensity of the stimuli was rated on a visual analogue scale (SIR) from 0 to 10 for every stimulus intensity. Mean values of the 5 courses of testing were used for further analysis for every subject.

**Chemosensitivity outside the pharynx**

All participants underwent olfactory testing using the Sniffin’ Sticks test kit (Hummel et al. 1997). Testing involved assessment of n-butanol odor threshold (T), odor discrimination (D), and odor identification (I). From the sum of these 3 scores, a composite TDI score was used to quantify olfactory function (Wolfensberger et al. 2000). The maximum achievable score was 46 points.

Furthermore, all participants underwent gustatory testing using “taste strips” (Mueller et al. 2003). Spoon-shaped filter paper was impregnated with 4 different taste qualities (sweet, sour, bitter, and salty) in 4 different concentrations for each taste quality and placed in the middle of the tongue. The sum of the correctly identified tastes was scored. The maximum achievable score was 16 points.

Capsaicin testing on the tongue was performed according to the same procedure previously described for capsaicin testing of the posterior pharyngeal wall (Just et al. 2007). The same 4 concentrations were applied to the middle of the tongue in random order. Again the subjects rated the intensity on a visual analogue scale from 0 to 10.

The lateralization test was performed to assess nasal trigeminal sensitivity (Wysocki et al. 2003). The test is based on the hypothesis that an irritating odorant can be lateralized in contrast to a nonirritating odorant (Ackerman and Kasbekar 1997). The participants were given 2 polypropylene squeeze bottles (one was filled with water and the other one with eucalyptol). Each bottle had a nosepiece that fit into the nostril. The bottles were squeezed simultaneously, and the participants were asked to indicate whether the impulse of eucalyptol was on the right or left side. The side of stimulation and the order of the administration of stimuli were randomized. Each subject had to repeat the test 40 times. The sum of correct identifications of the
side stimulated with eucalyptol was scored. The maximum achievable score was 40 points.

Statistical analysis

Statistical analysis was performed using PASW Statistics 18.0 (SPSS). Values were expressed as % and (n) or mean and standard deviation for each study group. For categorical variables, the differences between the OSA patients and the control group were assessed through chi-square tests. For continuous variables, the differences in mean values among the different groups were assessed through analysis of variance covariate by sex and age. Bonferroni correction was applied in the case of multiple comparisons. Variables with no normal distribution were transformed. Multiple repeated measures analyses of variance (rm-ANOVAs) with Bonferroni post hoc tests were performed, using perception of capsaicin at different concentrations (0.0001%–0.1%) and place of stimulus (tongue vs. pharynx) as within-subject factors, and using group (OSA patients vs. controls) as a between-subjects factor, with sex and age as covariate.

Results

A total of 44 subjects were included in the study (26 OSA patients; 18 controls). Upon the 26 OSA patients, 24 were newly diagnosed, 1 was diagnosed a year ago but was intolerant to CPAP, and 1 was recently on CPAP for a short period only. Table 1 gives an overview of the subjects’ characteristics. No statistically significant differences could be detected between OSA and control patients with regard to age. Twice as much female subjects were included in the control group compared with the OSA group. As expected, significant differences could be found in RDI, BMI, and ESS. All OSA patients were nonsmokers at the time of investigating. Only 1 person smoked in the control group but less than 5 cigarettes per day.

The results of the different tests for mechanical and chemosensitivity testing are summarized in Table 2.

Table 1  Overview of the patients’ characteristics between both groups (OSA and control)

<table>
<thead>
<tr>
<th></th>
<th>OSA</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>26</td>
<td>18</td>
</tr>
<tr>
<td>Age (mean and standard deviation) in years</td>
<td>45±9</td>
<td>40±8</td>
</tr>
<tr>
<td>Sex</td>
<td>3 f; 23 m</td>
<td>7 f; 11 m</td>
</tr>
<tr>
<td>RDI (mean and standard deviation) per hour</td>
<td>42±23*</td>
<td>6±2*</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>35±6*</td>
<td>26±4*</td>
</tr>
<tr>
<td>ESS</td>
<td>11±4*</td>
<td>6±2*</td>
</tr>
</tbody>
</table>

N, numbers; f = female; m = male; means and standard deviation are reported. *P < 0.05.

Means and standard deviations are reported. *P < 0.05.

Mechanical sensitivity

In the 2-point discrimination and air puffs at the posterior pharyngeal wall tests, the OSA group showed a decreased perception of mechanical stimuli (see Table 2; Figures 1 and 2). The statistical analysis revealed a statistically significant difference in the 2-point discrimination ($F_{(1, 42)} = 19.349; P < 0.001$) between the patients with OSA and the control group. The differences in mechanical sensitivity between OSA patients and controls as measured with the air puffs were also statistically significant ($F_{(3, 39)} = 4.22; P = 0.011$). Post hoc tests with Bonferroni adjustment revealed a statistically significant difference between the groups for the 2L/min ($P = 0.007$) and the 6L/min ($P = 0.008$) conditions but not in the 10L/min ($P = 0.066$) condition.

<table>
<thead>
<tr>
<th></th>
<th>Control group (n = 18)</th>
<th>OSA patients (n = 26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanical sensitivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two-point discrimination (in mm)</td>
<td>5.0±2.4</td>
<td>11.5±5.4*</td>
</tr>
<tr>
<td>Air puffs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2L/min</td>
<td>1.4±1.1</td>
<td>0.8±1.1*</td>
</tr>
<tr>
<td>6L/min</td>
<td>4.2±1.6</td>
<td>2.8±1.9*</td>
</tr>
<tr>
<td>10L/min</td>
<td>6.2±1.8</td>
<td>5.1±2.2</td>
</tr>
<tr>
<td>Chemosensitivity at pharynx</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capsaicin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0001%</td>
<td>1.1±1.0</td>
<td>0.6±1.7*</td>
</tr>
<tr>
<td>0.0010%</td>
<td>3.2±1.6</td>
<td>2.2±2.2*</td>
</tr>
<tr>
<td>0.0100%</td>
<td>6.6±2.2</td>
<td>4.6±2.2*</td>
</tr>
<tr>
<td>0.1000%</td>
<td>8.6±1.3</td>
<td>6.8±2.3*</td>
</tr>
<tr>
<td>CO₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20%</td>
<td>1.3±0.9</td>
<td>1.2±1.6</td>
</tr>
<tr>
<td>40%</td>
<td>2.8±1.8</td>
<td>1.5±1.7*</td>
</tr>
<tr>
<td>60%</td>
<td>6.5±2.6</td>
<td>3.7±2.9*</td>
</tr>
<tr>
<td>Chemosensitivity outside pharynx</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sniffin’ Sticks [TDI]</td>
<td>33.5±2.1</td>
<td>32.2±4.3</td>
</tr>
<tr>
<td>Taste strips</td>
<td>12.3±1.8</td>
<td>11.3±2.5</td>
</tr>
<tr>
<td>Capsaicin tongue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0001%</td>
<td>0.7±1.0</td>
<td>0.5±0.9</td>
</tr>
<tr>
<td>0.0010%</td>
<td>1.3±1.3</td>
<td>1.0±1.5</td>
</tr>
<tr>
<td>0.0100%</td>
<td>4.7±1.9</td>
<td>3.6±2.1</td>
</tr>
<tr>
<td>0.1000%</td>
<td>7.3±2.1</td>
<td>6.8±1.9</td>
</tr>
<tr>
<td>Nasal lateralization test</td>
<td>34.1±3.4</td>
<td>33.3±3.5</td>
</tr>
</tbody>
</table>
Chemosensitivity at the pharynx

Chemosensitivity testing at the pharynx with capsaicin showed statistically significant differences between the 2 groups ($F_{(4, 38)} = 5.22; P = 0.002$; Figure 3) for every concentration. The rm-ANOVA Mauchly's test indicated that the assumption of sphericity for concentration had been violated ($\chi^2 = 375.3, P < 0.001$); therefore, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\varepsilon = 0.34$). The results showed that there was a significant main effect of place of stimulus ($F_{(1, 41)} = 6.98; P = 0.012$) and concentration ($F_{(1.02, 41.61)} = 8.58; P = 0.005$). There were also significant interactions between place of stimulus and group ($F_{(1, 41)} = 11.22; P = 0.002$), between concentration and group ($F_{(3, 39)} = 4.05; P = 0.013$), and between place of stimulus and concentration and group ($F_{(3, 39)} = 3.57; P = 0.023$). Post hoc tests revealed...
a statistically significant difference between the 2 groups ($P = 0.001$).

For the trigeminal testing with CO$_2$ at the posterior pharyngeal wall, the mean (40%) and highest concentrations (60%) were perceived to be less intense in the OSA group (see Figure 4). The difference in the sensitivity to retronasal stimulation with CO$_2$ between the 2 groups was statistically significant ($F_{(3, 37)} = 4.08; P = 0.013$). Post hoc tests with Bonferroni adjustment revealed that the groups differed significantly in the 40% ($P = 0.006$) and 60% ($P = 0.003$) conditions but not in the 20% ($P = 0.256$) condition (Figure 4).

**Chemosensitivity outside the pharynx**

All subjects were normosmic and normogeusic regarding the normal values for this age group. The OSA group’s TDI and taste strip scores tended to be lower, but the difference was neither clinically nor statistically significant. (TDI OSA: 32±4; TDI control: 33±2); (taste strip score OSA: 11±2; taste strip score control: 12±2)

The subjective intensity of the stimulation of the tongue with capsaicin did not show relevant or statistically significant differences between both groups ($F_{(4, 39)} = 1.07; P = 0.387$; Figure 5).

**Figure 3**  Perception of capsaicin concentration at the pharynx (mean and standard deviation) in OSA patients compared with controls. *, $P < 0.05$.

**Figure 4**  Subjective stimulus intensity of retronasal stimulation with CO$_2$ (mean and standard deviation) in OSA patients compared with controls. *, $P < 0.05$. 
The nasal lateralization test did not show statistical differences between both groups either (Table 2).

Discussion

The results of the present trial demonstrate a reduced pharyngeal chemosensitivity in OSA patients compared with controls in addition to the reduced mechanical pharyngeal sensitivity that has been previously documented. In contrast, a reduction of chemosensitivity at areas outside the pharynx could not be detected. We therefore conclude that a peripheral neurodegeneration in the pharynx of OSA patients plays an important role in the pathogenesis of this disease.

The theory of peripheral neurodegeneration was introduced in the late 1990s. Larsson et al. (1992) and Kimoff et al. (2001) reported impaired sensitivity in OSA patients compared with healthy controls. Larsson et al. (1992) tested the temperature thresholds for warmth and cold at the tonsillar pillar in 15 OSA patients and 15 matched pairs of healthy controls. The authors found that OSA patients were less able to detect temperature changes compared with control subjects. Furthermore, the group exhibited a higher temperature threshold at the tip of the tongue in OSA patients. The authors concluded that a neuropathy in the pharynx is present in OSA patients because of a progressive trauma to the pharyngeal structures from vibration induced by snoring. The final conclusion was that the trauma caused by snoring transforms a snorer into an OSA patient. Although we were able to confirm the results of the 2-point discrimination test, we did not perform long-term follow-up or therapeutic intervention.

Kimoff et al. (2001) showed the presence of a selective impairment of upper airway mucosal sensory function in patients with OSA using 2-point discrimination and a vibration test at the soft palate. Thirty-seven patients with OSA, 12 primary snorers, and 15 control subjects were included in the trial. Sensory thresholds were clearly reduced in the upper airway (soft palate) in the OSA and snoring groups compared with controls, although no statistically significant differences could be detected between the OSA and snoring groups. A follow-up of 6 months later also showed an improvement of sensory function in OSA patients treated with CPAP compared with untreated patients. Although we were able to confirm the results of the 2-point discrimination test, we did not perform long-term follow-up or therapeutic intervention.

In the late 1990s, Friberg et al. (1997) investigated the pathophysiological mechanisms and the progression of the disease in OSA patients. Biopsies of the mucosa at the soft palate were compared between OSA patients, habitual snorers, and nonsnoring control subjects and were then analyzed immunohistochemically. Signs of afferent nerve lesions were demonstrated in the snorers and even more in the OSA patients. Later Friberg et al. (1998) performed Laser Doppler perfusion monitoring combined with electrical stimulation to investigate the afferent nerve regulation of the microcirculation of the palatal mucosa. In OSA patients, the vasodilation was significantly reduced compared with controls.

Inferentially, in 1999 Friberg (1999) postulated that heavy snoring is a progressive, local neuropathic disease. Apart from weight gain, the collapse of the upper airway in OSA patients is due to sensory neuronal damage. The patency of the upper airway depends on the balance between the dilating muscle and the negative intrapharyngeal pressure. Snoring
leads to vibrations at the pharyngeal wall, causing chronic neuronal damage of the afferent nerve endings, which are important for the reflexogenic dilatation (Suratt et al. 1983; Henke and Sullivan 1993). A loss in these fibers increases pharyngeal collapsibility. Already Suratt et al. (1983) could show that OSA patients have a significantly narrower cross-sectional area of the pharyngeal airway by performing a lateral fluoroscopy and computed tomographic scan. They concluded that this phenomenon plays an important role in the pathogenesis of OSA. Woodson et al. (1991) showed atrophied muscle fibers of the soft palate and mucous gland hypertrophy with ductal dilatation and an extensive edema of the tissue in OSA patients and snorers. Using an electron microscope, Woodson et al. (1991) revealed a focal degeneration of myelinated nerve fibers and axons, which was indicated as a local nerve lesion. These lesions cause an increase of pharyngeal collapsibility. These degenerative changes contribute to an airway instability and results in OSA. As well other studies could show that using a topical anesthesia at the pharynx—blocking pharyngeal sensory—leads to an increase in the apnea index (Deegan et al. 1995). The authors conclude that an impairment of the upper airway protective reflexes takes place in OSA patients.

The studies described above demonstrated various aspects of neurodegeneration of the upper airway, both in terms of reduced mechanical and thermal sensitivity, as well as in terms of histological changes to the mucosa. Chemosensitivity at the upper airway, however, has not been investigated to date. With the results of our trial, we were able to demonstrate for the first time that the neurodegeneration of the upper airway does not only lead to a reduced mechanical sensitivity but also to a reduced chemosensitivity in patients with OSA compared with controls.

References


