Effects of pentobarbital on upper airway patency during sleep

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**Question of the study:** We hypothesized that pentobarbital would improve upper airway mechanics based on an increase in: a) latency to arousal, b) amplitude of the phasic genioglossus electromyogram, and decrease in: c) the active upper-airway critical closing pressure (Pcrit).

**Materials and methods:** Twelve healthy subjects received pentobarbital 100 mg or placebo in a double-blind, cross-over protocol. During wakefulness, we measured the genioglossus reflex response to negative-pressure pulses. During sleep, carbon dioxide was insufflated into the inspired air. Airway pressure was then decreased in a stepwise fashion until arousal from sleep.

**Results:** With basal breathing during sleep, flow-rate was lower in volunteers given pentobarbital, end-tidal carbon dioxide concentration and upper airway resistance were greater, and Pcrit was unaffected (pentobarbital: -11.7±4.5 vs. placebo: -10.25±3.6 cm H2O, p=0.11). Pentobarbital increased the time to arousal (297±63s vs. 232±67s p<0.05), at which time phasic genioglossus EMG was higher (6.2±4.8 %max vs. 3.1±3%, respectively, p<0.05) as were carbon dioxide levels. The increase in genioglossus electromyogram after carbon dioxide administration was greater after pentobarbital vs. placebo. Pentobarbital did not affect the genioglossus negative pressure reflex.

**Conclusion:** Pentobarbital increases the time to arousal and stimulates genioglossus muscle activity, but it increases upper airway resistance during sleep.

**Key words:** Arousal Threshold, Airway, Lung, Obstructive Sleep Apnoea Hyopnoea Syndrome, Sleep-Disordered Breathing.
INTRODUCTION

Obstructive sleep apnoea (OSA) is a common disorder[1], characterized by repetitive pharyngeal collapse during sleep[2]. Arousal from sleep is traditionally believed to be an important mechanism for re-establishing airway patency in OSA. However, recent data suggest that an excessively low arousal threshold may predispose an individual to recurrent arousals, and the hyperventilation that occurs following arousal may produce hypocapnia during subsequent sleep[3].

Reduced carbon dioxide values may lead to either central or obstructive apnoea, depending on the prevailing upper-airway mechanics[4, 5]. Thus, a low arousal threshold may contribute to sleep apnoea, at least in some individuals[3, 6, 7]. Premature arousal during an obstructive event may prevent adequate upper-airway muscle recruitment because there is inadequate accumulation of respiratory stimuli (i.e. carbon dioxide and negative intrapharyngeal pressure). Recent data suggest that treatment with certain hypnotics may not be deleterious and may even improve this condition in certain patients[8-11]. A number of medications have been demonstrated to raise arousal threshold, and recent data suggest that treatment with some hypnotics and antidepressants may improve OSA manifestations[8-11]. Triazolam and ethanol have been shown to increase the arousal threshold in response to airway occlusion in normal subjects[12, 13], and the antidepressants mirtazapine and trazodone may improve manifestations of sleep apnoea[8]. Trazodone co-administered with L-tryptophan can treat sleep-disordered breathing in an animal model of obstructive sleep apnoea[14]. In humans, trazodone may improve airway mechanics by raising the arousal threshold[10] and potentially allowing both negative airway pressure and carbon dioxide to increase thereby activating
pharyngeal dilator muscles. The upper-airway dilator muscles (e.g. genioglossus) are known to respond during sleep to combinations of negative pressure and hypercarbia better than to either stimulus alone[15].

Recent data show that pentobarbital can increase genioglossus phasic activity in the rat[11],[16]. However, in the rat, pentobarbital also produces some less desirable effects on airway physiology: it causes a dose-dependant reduction in both diaphragmatic activity and tonic (expiratory) genioglossus activity[16]. Large doses of pentobarbital can also impair the genioglossus negative pressure reflex (i.e. reflex activation in response to a sudden decrease in pressure) [16], which may adversely affect upper-airway patency.

Based on preclinical data, we hypothesized that pentobarbital would delay arousal in human subjects following a standardized negative pressure stimulus and that this delay would augment genioglossus muscle activity and improve upper-airway closing pressure.

To test these hypotheses, we performed a randomized, double-blind, placebo-controlled, cross-over study comparing 100 mg of pentobarbital to placebo.
MATERIALS AND METHODS

The protocol was approved by the Institutional Review Board of Brigham and Women’s Hospital. Twelve healthy adult volunteers (ages 18-48 years; BMI <25 kg/m^2) were recruited to participate in the study. We excluded people with concurrent cardiopulmonary disease including untreated hypertension, kidney disease, liver disease, neuromuscular disease, sleep disorders, and psychiatric disease. We also excluded those taking medications known to affect sleep, upper airway muscles or respiratory function (e.g., oral contraceptives, hormone replacement therapy, theophylline, acetazolamide, stimulants, sedatives, thyroxine, and antidepressants). Finally, we excluded those with a history of lignocaine or barbiturate allergy or acute intermittent porphyria. Subjects were recruited through posted flyers, email and newspaper advertisements.

Protocol:

Subjects were studied twice, once with pentobarbital 100 mg (diluted in cherry syrup) and once with placebo treatment (cherry syrup) in a randomized, double-blind fashion with at least 10 days in between treatments (see figure 1).

On study days, subjects were admitted into our Clinical Research Center at approximately 8pm. Pre-menopausal women underwent urinary pregnancy test prior to medication administration. After the study procedures were explained, adhesive surface electrodes were attached to the scalp (EEG), face (EOG), and chin (EMG). Following this, both nostrils were decongested (oxymetazoline HCl), and one nostril and the back of the throat were anesthetized with ~0.5 ml of topical 4% lignocaine (20 - 40mg). Airway
pressure was monitored at the level of the epiglottis (epiglottic pressure [Pepi]) using a pressure-tipped Millar catheter that was inserted through the anesthetized nostril and secured with tape. Three surface electrocardiogram electrodes were placed on the chest and shoulders.

The area under the tongue (3-4 mm lateral to the frenulum on each side) was topically anesthetized with lignocaine for insertion of genioglossus muscle electrodes. Two needles (25 gauge) containing 30-gauge stainless steel recording electrodes were inserted into the genioglossus muscle. The needles were then quickly removed leaving the recording electrodes in place. Recordings were bipolar with a forehead ground. The subject was instructed to perform several manoeuvres to determine maximal activity of the genioglossus muscle (maximal tongue protrusion, swallowing, negative inspiratory force).

A nasal CPAP mask placed over the subject’s nose and held in place with a head strap permitted measurement of breathing rate, inspired volume (integrated inspiratory flow signal from a pneumotachograph), mask pressure and carbon dioxide levels. An arterial oxygen saturation probe was attached to one of the subject’s fingers or earlobes to monitor oxygenation.

Before and 60 minutes after administration of the study drug, baseline data were collected during a ten minute period of normal breathing while the subject was awake (see figure 1). In addition, approximately 40 brief pulses of negative airway pressure (200 ms) were delivered during early inspiration every 2-8 breaths to measure the genioglossus negative pressure reflex as described previously[17, 18].
Subjects were then allowed to fall asleep while breathing room air at atmospheric pressure. When breathing was stable for a period of 5 minutes, the respiratory response to carbon dioxide was assessed. Sufficient carbon dioxide (10% balanced with nitrogen) was added to the inspired air to produce stable elevations of end-tidal carbon dioxide that were first 5, then 10 mmHg higher than baseline.

Subjects were then awoken and placed on 3 cmH₂O CPAP. Once they had reached stable NREM sleep again, we increased the CPAP level to alleviate any degree of flow limitation. When steady state stage-II sleep without flow limitation was achieved, we reduced airway opening pressure in 2 cm H₂O steps, and subjects were monitored for arousal (i.e., presence of alpha wave activity on the EEG). If the subject did not have an arousal for 1 minute we proceeded to the next step. Continuous negative airway pressure was used if sub-atmospheric pressures were required. This titration procedure (hereafter called a negative pressure “ramp”) was repeated up to a maximum of 15 times throughout the night. Following data collection, all equipment was removed and the subjects were allowed to recover in the General Clinical Research Center for at least 8 hours after drug ingestion and until they felt alert enough to go home. Discharge readiness was confirmed by a licensed physician who was not involved in the study.
Data analysis

A single experienced registered sleep technician, blinded to the experimental manipulations, defined the presence of arousal and sleep stage according to standard criteria[19]. For analysis of arousability, we have only included data in the analysis that occurred more > 30 seconds after onset of a pressure reduction.

The effect of pentobarbital and placebo on the excitation component of the genioglossus negative pressure reflex was compared within subjects pre versus post administration according to methods previously described[17, 18]. Analysis was performed blinded to the study condition. Briefly, the genioglossus electromyogram signal was rectified and averaged for all negative pressure pulses that were free from movement and swallow artifact. The amplitude of the initial genioglossus electromyogram peak was expressed as a percent of the baseline activity. Reflex latency was defined as the time to peak genioglossus electromyogram from time zero (the last point preceding the sudden decrement in the ensemble-averaged pressure signal).

Wakefulness, phasic and tonic genioglossus electromyogram, airflow, upper-airway resistance, and end-tidal carbon dioxide were measured during quiet breathing, before, and 60 minutes after study drug. Maximal genioglossus activation manoeuvres allowed an electromyogram scale to be created for each subject between electrical 0 and the single highest value encountered (100%)[20]. During sleep, genioglossus EMG just prior to arousal was calculated by averaging the value during three breaths immediately before arousal.

A standardized protocol for assessing the active Pcrit was implemented as previously described (figure 2)[21]. When inspiratory flow limitation was stable, nasal pressure and
maximum inspiratory flow were obtained from three breaths at the end of a 2-min period of stable stage 2 sleep. Flow limitation was defined as: unchanged inspiratory flow despite a further decrease in pharyngeal (epiglottic) pressure. Mask pressure was then plotted versus maximum flow for the flow limited breaths and fitted using a linear regression model.

Time to arousal was defined as the time from onset of the negative pressure ramp to an arousal as detected by electroencephalogram (EEG) alpha-wave activity.

Upper airway resistance (epiglottic catheter to mask) was measured at a flow of 0.2 l/s, which is generally on the linear portion of the pressure/flow curve.

**Statistical analysis**

The primary dependent variable was time to arousal. We tested the hypothesis that time to arousal is significantly longer in subjects following pentobarbital 100 mg compared with placebo. We tested the secondary hypothesis that phasic genioglossus EMG just prior to arousal would be significantly higher in volunteers given pentobarbital compared with placebo. We also tested the exploratory hypothesis that active upper-airway closing pressure would be lower (more negative) in volunteers given pentobarbital vs. placebo.

Based on the observations of Berry and coworkers who observed a longer time to arousal from sleep in volunteers given alcohol[13] and triazolam[12] we anticipated a 30 per cent difference and a standard deviation of 10 per cent. Based on the data of Younes and coworkers[11], who observed a higher phasic genioglossus activity at the time of arousal in rats given pentobarbital compared with placebo, we expected a 50% difference between groups in phasic genioglossus activity (SD: 10%). Finally, based on the
association of phasic genioglossus activity and upper-airway closing pressure in humans, we expected a 10 per cent difference (SD: 10%) in Pcrit between groups[22]. We calculated that a total sample size of 10 volunteers would provide sufficient power to detect a significant difference in the primary and secondary hypotheses (power= 0.8, alpha<0.05). Paired t-tests were used for testing the main hypotheses. We used a general linear model (mixed model) to analyse the genioglossus electromyogram response to carbon dioxide. We used genioglossus electromyogram as the dependent variable and drug (pentobarbital versus placebo), respiratory phase (phasic versus tonic), and carbon dioxide level (baseline, +5 mmHg, and +10 mmHg) as independent variables. The results are expressed as the mean ± SD unless indicated otherwise. SPSS Version 11.0 (SPSS Inc, Chicago, IL) as well as Sigma Stat Version 3.0 (SPSS Inc) were used for statistical analysis.
Results

One volunteer was excluded during the first study night due to inability to sleep, leaving data from 11 volunteers (3 men and 8 women) aged 35±10 years (height: 173±8 cm, weight: 67±8 kg) for analysis.

Effects of pentobarbital during wakefulness

During wakefulness, pentobarbital did not affect breathing or genioglossus muscle function. There was no significant difference in minute ventilation, tidal volume, end-tidal carbon dioxide, duty cycle [Ti/Ttot], flow-rate [Vt/Ti], phasic and tonic genioglossus electromyogram (table 1). Negative pressure reflex activation of the genioglossus electromyogram was robust with pentobarbital and placebo (more than two-fold increase, table 2). Amplitude and latency of the genioglossus reflex activation did not differ before and after pentobarbital administration. Similarly, reflex properties did not differ before versus after placebo.

Effects of pentobarbital during sleep

Respiratory function during normal breathing

During normal stage II sleep (atmospheric mask pressure), flow-rate was significantly lower in volunteers given pentobarbital, while end-tidal carbon dioxide concentration, and upper airway resistance were significantly greater compared with both baseline (same study day), and placebo. Duty-cycle was significantly greater after pentobarbital compared with baseline (table 2). Tidal volume and respiratory rate did not differ between treatments.
Responses to pressure drops

Time to respiratory induced arousal from stage II sleep

For each subject, we decreased CPAP an average of 10±3 times during stage II sleep. Onset of flow limitation occurred at -3.6±2.5 cmH2O versus -3.4±3.2 cmH2O in the placebo and pentobarbital night, respectively, without differences between groups (p=0.8).

There was no difference in the number of pressure drops prior to arousal between placebo and pentobarbital trials. However, arousal from stable stage II sleep occurred significantly later with pentobarbital (297±63 versus 232±67 seconds after stimulus, p<0.05), and mask pressure was therefore lower (-2.9±3.2 versus, -0.5±2.3 cmH2O, p<0.05).

Genioglossus function and upper airway pressure flow relationship just prior to arousal from sleep

Phasic genioglossus activity during flow-limited breathing just prior to arousal was significantly higher after pentobarbital compared with placebo (figure 3). End-tidal carbon dioxide concentration (first breath after termination of pressure drop) was modestly, but significantly higher with pentobarbital versus placebo (45.6±4.6 versus 42±1.1 mmHg, p<0.05).

The range of mask pressure values used for assessment of Pcrit was 1 to -17 cm H2O. The change in Pcrit (pentobarbital: -11.7±4.5 vs. placebo: -10.25±3.6 cm H2O, respectively, p=0.11; figure 4) and the increase in tonic genioglossus activity with pentobarbital compared with placebo did not reach statistical
significance (p=0.082). In assessing whether genioglossus activation was mechanically effective, we found that the rise in tonic (but not phasic) genioglossus electromyogram was predictive of the improvement in airway mechanics (i.e active Pcrit) (R=-0.66, p=0.03, figure 5).

Genioglossus function and peak airflow measured at the same time after starting the negative pressure ramp as at the placebo night.

We analysed genioglossus activity and peak airflow at a standardized time (260±100 seconds after onset of negative pressure drop), defined as the lowest level of mask pressure (at -5.45±2.78 cmH2O) that we were able to apply under both placebo and pentobarbital conditions. Both flow-limited and no flow-limited breaths were included in this analysis. Phasic genioglossus activity was significantly higher (3.9±6.6 % versus 1.3±1.96 % of maximum activation, p=0.08), but tonic genioglossus activity (0.68±1.2% versus 0.49±0.85% of maximum, p=0.3) and peak inspiratory airflow (0.33±0.1 versus 0.3±0.13 l/s, p=0.17) did not differ between groups.

Upstream resistance (Pmask/Vmax) taken at the same time tended to be lower under pentobarbital 19±14 H2O·l⁻¹·s compared with placebo 24±19 cm H2O·l⁻¹·s (p=0.066).

Respiratory response to carbon dioxide

In one pentobarbital trial, and in two placebo trials, awakening from sleep was observed before steady state hypercapnic stimulation could be achieved. Measurements of the respiratory response to carbon dioxide are therefore reported from 9 volunteers.
Increased inspired carbon dioxide augmented the genioglossus electromyogram, and the amplitude of this effect was significantly dependent on drug (pentobarbital>placebo), and state (phasic>tonic, table 3). Administration of carbon dioxide significantly increased upper airway resistance by 145±18% (placebo) and 147±17% (pentobarbital). Flow-rate measured at an end-tidal carbon dioxide 10 mg Hg above baseline was not significantly different between groups (198±76% of baseline for placebo vs. 196±68% for pentobarbital).
DISCUSSION

Our study found that in healthy volunteers pentobarbital (100 mg orally) had no effect on respiratory function during wakefulness and did not impair genioglossus muscle function during the awake or sleep states. During stage II sleep pentobarbital had a mild respiratory depressant effect manifested as a decrease in peak inspiratory flow, and a rise in end-tidal carbon dioxide and upper-airway resistance. However, the hypercapnic responsiveness of the genioglossus muscle improved. Active upper-airway closing pressure did not significantly change following pentobarbital. However, lower Pcrit values were associated with increased tonic genioglossus electromyogram, suggesting clinical relevance of the observed muscle recruitment, i.e., that it was mechanically effective. These results confirm and extend those of Younes and colleagues[11] as well as our own preclinical studies in rats[16].

An interesting finding of our study is that in humans pentobarbital increased time to arousal and genioglossus activation preceding arousal. This particular constellation of effects could be useful for OSA patients with low arousal thresholds, ventilatory control instability, or both. Younes as well as Wellman et al. have suggested that ventilatory control instability can contribute to the pathogenesis of OSA[3, 23]. Concomitant increases in phasic genioglossus activity and time to arousal should help stabilize breathing patterns by allowing the necessary physiological responses to obstructive events to stabilize the airway without producing arousals and subsequent ventilatory overshoot that cause ventilatory oscillation in susceptible patients. In support of this idea, Younes observed that even patients with severe sleep apnoea have some periods of stable
breathing[24], and we have recently observed that these stable breathing periods are associated with high levels of upper-airway dilator muscle activity[25], suggesting that these muscles are necessary and sufficient to protect pharyngeal patency when adequate respiratory stimulation is present for sufficient duration. On the other hand delaying arousal is theoretically deleterious for patients with a high arousal threshold in whom substantial hypoxemia and hypercapnia could develop.

In our study, upper-airway closing pressure was stable at pentobarbital 100 mg, a dose that promotes sleep in humans[26, 27], without affecting normal breathing or the ventilatory response to carbon dioxide[28]. However, pentobarbital increased upper-airway resistance during sleep, leading to decreased peak inspiratory flow rate and mild hypercarbia. In theory, an elevated upper airway resistance may actually be beneficial in those with unstable ventilatory control[29], if the accumulation of respiratory stimuli allows important recruitment of upper airway muscle activity[15, 30].

Interestingly respiratory depression may contribute to upper airway stabilizing effects of pentobarbital. We previously observed in rats a dissociation of pentobarbital’s respiratory effects on the genioglossus (activation) and breathing (inhibition of diaphragmatic activity and consequent hypercarbia)[16]. We therefore speculate that hypercapnia plays a role in mediating pentobarbital’s activating effects on genioglossus which we observed at the time of arousal[31, 32]. To the extent that chemoreflex activation of the genioglossus muscle may occur independently of ventilatory drive, it is possible that elevated carbon dioxide may partially account for the increase in genioglossus activity observed in parallel with decreased ventilatory drive[31]. Another possibility is that when
‘negative effort dependence’ is present, flow may actually improve with reduced ventilatory drive. However, pentobarbital’s narrow therapeutic index makes pentobarbital a problematic candidate agent for being used for treatment of OSA. Further work will be required to determine whether a pharmacological approach to sleep apnoea therapy is viable using either different agents or by carefully selecting patients for treatment.

An increase in genioglossus activity, as been observed in our volunteers during the pentobarbital night prior to arousal from sleep, does not necessarily translate to mechanical improvement. Recently we have observed that pharmacologically evoked genioglossus muscle weakness (partial neuromuscular transmission block) explains only 20% of the variance of the evoked increase in upper airway closing pressure[33]. Moreover, obstructive sleep apnea patients may even have significantly greater basal genioglossal activity compared to controls during wakefulness [20]. Other non-muscular factors such as decreases in lung volume [34-36], and fluid displacement into nuchal and peripharyngeal soft tissues[37] could contribute to narrowing and increased airflow resistance of the pharynx, and predispose to pharyngeal collapse in humans.

In the present study, upper-airway resistance increased during sleep, and the magnitude of the effect was higher when pentobarbital was given. The respiratory duty-cycle was significantly increased by pentobarbital, suggesting that higher resistance was partially offset by an increase in inspiratory time. Our data cannot explain why upper-airway resistance during normal breathing was increased while genioglossal mechano- and chemoresponses were normal or even augmented. Upper-airway resistance is influenced by a variety of mechanisms including airflow pattern, mandibular- and body
position, respiratory timing, as well as end-expiratory lung volume. We speculate that 
pentobarbital’s deleterious effects on upper-airway resistance might in part be explained 
by its reduction of lung volume. In pentobarbital-anesthetized dogs, phasic electrical 
activity increases over time in the expiratory muscles, whereas electrical activity of the 
inspiratory muscles is unchanged[38], which might decrease end-expiratory lung volume. 
In our study, the pentobarbital-induced change in tonic, but not phasic genioglossus 
activity correlated with improved Pcrit values. Tonic upper airway muscle activity is 
critical for maintenance of airway patency[39]. The impact of tonic genioglossus activity 
on airway patency in OSA patients has been emphasized in a recent study reported from 
our laboratory[40]. In OSA, reductions in tonic genioglossus activity during REM are 
associated with hypopnea events and therefore have been suggested to contribute to the 
higher severity of OSA in that stage[40]. In addition, our recent research in single motor 
units in the genioglossus has hi-lighted the importance of tonic motor neurons in 
affecting overall genioglossal activity and airway mechanics[41]. Ostensibly, the 
genioglossus phasic activity may be more mechanically effective when influencing a 
stiffened airway.

Limitations:
We made a comparison between pentobarbital and placebo. Accordingly, our data cannot 
address whether the observed effects are specific to pentobarbital or a class effect of 
barbiturates or even GABA-A agonists.
The increases in genioglossus EMG at the end of the negative pressure ramp may be 
secondary to the prolonged latency to arousal (with lower mask and pharyngeal pressures 
and an increase in carbon dioxide) and increased resistance, but may also include a
specific barbiturate stimulatory effect. The greater increase in the genioglossus response to evoked hypercarbia during the pentobarbital compared with placebo may provide some evidence for a primary stimulatory effect of the barbiturate on genioglossus EMG. Moreover, genioglossus activity measured at a standardized time after onset of a pressure ramp, revealed higher values of phasic genioglossus activity during the pentobarbital compared with placebo, suggesting that barbiturates may have some stimulatory effects on genioglossus muscle.

At the end of the pressure ramps, the volunteers showed considerable flow-limited breathing (even at a fixed flow of 0.2 liters/sec), which complicates resistance determinations. As the pharyngeal tissues collapse, the epiglottic pressure is no longer the downstream pressure and thus airflow resistance is not simply a function of the pressure drop from the mask to the epiglottic catheter. While we have included peak-flow during the ramps, we do not know an ideal way of reporting the resistance at the end of the pressure ramps during flow limitation.

We believe more OSA research should address arousability, a variable that is associated with sleepiness and may have therapeutic implications[42]. We assessed arousability by the arousal response time during step reduction of the airway pressures. During OSA, arousal could be caused by various factors such as hypercapnia, hypoxemia, intrathoracic pressure and interaction among these. The arousal response examined in this study is one aspect of the responses in normal subjects and may not reflect arousablity during OSA.
In summary, in healthy volunteers given pentobarbital, time to arousal and phasic
genioglossus activity immediately prior to arousal were increased and genioglossus reflex
activation was maintained. However, pentobarbital increased upper-airway resistance
during sleep leading to decreased peak flow and mild hypercarbia while active upper-
airway closing pressure was stable. These findings make it difficult to predict whether or
not manipulating arousal threshold with pentobarbital may be a viable therapeutic
strategy for subsets of sleep apnoea patients. Its narrow therapeutic index makes
pentobarbital a problematic candidate agent. Further work will be required to determine
whether a pharmacological approach to sleep apnoea therapy is viable using either
different agents or by carefully selecting patients for treatment.
REFERENCES


Legends to figures

Figure 1.
Study protocol. Subjects were studied twice: during placebo and during pentobarbital treatment. Each study day measurements were performed during wakefulness (before and after test-drug application) and sleep. During sleep, subjects were studied at atmospheric pressure first, to measure normal breathing and the respiratory response to inspiratory CO2 insufflation. Subjects were then put on CPAP (3 mmHg) to avoid flow-limitation, and negative pressure ramps were performed until arousal.

Figure 2.
Method of calculation of upper airway critical closing pressure (Pcrit) during stage II sleep by linear regression in one volunteer. Peak air flow during flow-limited breathing is plotted as a function of mask pressure. Throughout the study night, during 12 negative pressure ramps, flow limitation was observed. Values derived from 31 flow-limited breaths were used for analysis and extrapolated to Pcrit (mask pressure at zero flow) by linear regression.

Note that at a given mask pressure, peak-flow during flow-limited breathing varies throughout the overnight study, suggesting that the balance between the collapsing and dilating forces acting at the upper airway varies throughout the night.

Figure 3.
Effect of pentobarbital on genioglossus activity during negative pharyngeal pressure challenges. Shown are average values of genioglossus EMG just prior to arousal. Phasic
genioglossus activity was significantly higher after pentobarbital 100 mg compared with the control night, and tonic genioglossus activity tended to be higher. * p<0.05 versus placebo, + p<0.1 versus placebo.

**Figure 4.**
Effect of pentobarbital on active upper-airway closing pressure (Pcrit) during sleep.
Shown are average Pcrit values in 11 subjects during the pentobarbital night compared with the placebo night. Note that Pcrit tended to be more negative during the pentobarbital night compared with the placebo night.

**Figure 5.**
Difference in active critical upper-airway closing pressure (Pcrit) during the pentobarbital night and control night vs. the difference in tonic genioglossus activity (as % of maximum value). Measurements during negative pharyngeal pressure challenges during sleep. Average values from all pressure drops.
<table>
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<th>Wakefulness, baseline</th>
<th>Wakefulness, After test drug</th>
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<th>Stage II sleep CPAP</th>
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<td>normal breathing, and negative pharyngeal pressure pulses</td>
<td>normal breathing, and negative pharyngeal pressure pulses</td>
<td>Lights off</td>
<td>active Pcrit: pressure ramps from 3 mmHg CPAP until arousal</td>
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<td>- Mask and epiglottic pressures</td>
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<td>- Polysomnography</td>
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**Figure 1**
Mask pressure (cm H₂O)

Peak flow (l/s)

Figure 2
Figure 3

Genioglossus EMG

Tonic EMG
Phasic EMG

Genioglossus EMG [per cent of maximum activation]

placebo  pentobarbital

placebo  pentobarbital

*
Figure 4

Placebo                                          Pentobarbital

p=0.12

p=0.11

vs. placebo

Active

Pcrit [cmH₂O]

0  -2  -4  -6  -8  -10  -12  -14  -16  -18  -20  -22
Change in tonic Genioglossus activity [% of maximum values]

-1.5 -1.0 -0.5 0.0 0.5 1.0

Change in Pcrit [cm H₂O]

-10 -8 -6 -4 -2 0 2 4

r=0.66, p=0.03

Figure 5
<table>
<thead>
<tr>
<th>Respiratory function during normal breathing</th>
<th>Wakefulness</th>
<th>Sleep</th>
<th>During stage 2 sleep</th>
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</thead>
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<td><strong>Before test drug application</strong></td>
<td>Placebo</td>
<td>Pentobarbital</td>
<td>Placebo</td>
</tr>
<tr>
<td><strong>After test drug application</strong></td>
<td>Placebo</td>
<td>Pentobarbital</td>
<td>Placebo</td>
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<td><strong>Upper airway resistance</strong> [H2O·l⁻¹·s⁻¹]</td>
<td>2.9±4.4</td>
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<td>42.4±3.2</td>
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<td><strong>Minute ventilation [l/min]</strong></td>
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<td>6.4±1.1</td>
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<td><strong>Tidal volume [l/min]</strong></td>
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<td>0.46±0.06</td>
<td>0.44±0.09</td>
</tr>
<tr>
<td><strong>Duty-cycle [Ti/Ttot]</strong></td>
<td>0.43±0.03</td>
<td>0.38±0.07</td>
<td>0.47±0.07</td>
</tr>
<tr>
<td><strong>Flow rate [VVTi, ml/s]</strong></td>
<td>0.36±0.29</td>
<td>0.34±0.19</td>
<td>0.29±0.04</td>
</tr>
<tr>
<td><strong>Phasic GG activity [% max]</strong></td>
<td>1.9±2.4</td>
<td>1.9±1.9</td>
<td>2.1±4.9</td>
</tr>
<tr>
<td><strong>Tonic GG activity [% max]</strong></td>
<td>1.2±2.6</td>
<td>1.5±4.0</td>
<td>0.64±2.2</td>
</tr>
<tr>
<td><strong>Peak GG activity [% max]</strong></td>
<td>3.1±4.8</td>
<td>2.7±5.9</td>
<td>2.7±5.9</td>
</tr>
</tbody>
</table>

*p<0.05 versus placebo treatment, same study day, †p<0.1 vs. placebo treatment, sleep values. GG=genioglossus electromyogram.
Table 2: Negative pressure pulse data collected during wakefulness

<table>
<thead>
<tr>
<th>Genioglossus Negative Pressure Reflex and Stimulus Characteristics</th>
<th>Pre-Pentobarbital</th>
<th>Post-Pentobarbital</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excitation Onset Latency (ms)</td>
<td>27 ± 5</td>
<td>28 ± 3</td>
</tr>
<tr>
<td>Excitation Peak Amplitude (% baseline)</td>
<td>224 ± 30</td>
<td>227 ± 34</td>
</tr>
<tr>
<td>Excitation Peak Latency (ms)</td>
<td>36 ± 4</td>
<td>38 ± 3</td>
</tr>
<tr>
<td>Minimum Mask Pressure (cmH₂O)</td>
<td>-16 ± 2</td>
<td>-17 ± 2</td>
</tr>
<tr>
<td>Number of Artifact Free Pulse Presentations</td>
<td>39 ± 3</td>
<td>35 ± 3</td>
</tr>
<tr>
<td><strong>Pre-Placebo</strong></td>
<td><strong>Post-Placebo</strong></td>
<td></td>
</tr>
<tr>
<td>Excitation Onset Latency (ms)</td>
<td>26 ± 5</td>
<td>21 ± 2</td>
</tr>
<tr>
<td>Excitation Peak Amplitude (% baseline)</td>
<td>236 ± 34</td>
<td>242 ± 28</td>
</tr>
<tr>
<td>Excitation Peak Latency (ms)</td>
<td>42 ± 8</td>
<td>34 ± 4</td>
</tr>
<tr>
<td>Minimum Mask Pressure (cmH₂O)</td>
<td>-19 ± 2</td>
<td>-17 ± 1</td>
</tr>
<tr>
<td>Number of Artifact Free Pulse Presentations</td>
<td>41 ± 2</td>
<td>34 ± 3</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. There were no significant differences between conditions for genioglossus reflex characteristics or stimulus magnitudes. N=10.
Table 3: Respiratory effects of evoked hypercarbia during sleep with out CPAP.

<table>
<thead>
<tr>
<th></th>
<th>Room air</th>
<th>PETCO₂ + 5 mmHG above baseline</th>
<th>PETCO₂ + 10 mmHG above baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pentobarbital</td>
<td>Placebo</td>
<td>Pentobarbital</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td></td>
<td>Placebo</td>
</tr>
<tr>
<td>Upper airway resistance</td>
<td>6.4±8.5*</td>
<td>2.3±2.5</td>
<td>7.0±11.1</td>
</tr>
<tr>
<td>[H₂O·l⁻¹·s⁻¹]</td>
<td>7.0±2.3</td>
<td>2.3±1</td>
<td>9.4±16*</td>
</tr>
<tr>
<td></td>
<td>6.4±8.5*</td>
<td>2.3±1</td>
<td>9.4±16*</td>
</tr>
<tr>
<td></td>
<td>6.4±8.5*</td>
<td>2.3±1</td>
<td>9.4±16*</td>
</tr>
<tr>
<td></td>
<td>6.4±8.5*</td>
<td>2.3±1</td>
<td>9.4±16*</td>
</tr>
<tr>
<td>Minute ventilation [l/min]</td>
<td>6.0±2.3</td>
<td>5.3±1</td>
<td>9.6±3.8</td>
</tr>
<tr>
<td></td>
<td>9.6±3.8</td>
<td>7.9±2.5</td>
<td>12.1±6.5*</td>
</tr>
<tr>
<td>Tidal volume [l/min]</td>
<td>0.41±0.15</td>
<td>0.37±0.07</td>
<td>0.61±0.19</td>
</tr>
<tr>
<td></td>
<td>0.61±0.19</td>
<td>0.53±0.17</td>
<td>0.87±0.33*</td>
</tr>
<tr>
<td>Duty-cycle [Ti/Ttot]</td>
<td>0.43±0.13</td>
<td>0.41±0.04</td>
<td>0.44±0.23</td>
</tr>
<tr>
<td></td>
<td>0.44±0.23</td>
<td>0.42±0.05</td>
<td>0.50±0.30</td>
</tr>
<tr>
<td>Flow rate [Vt/Ti, ml/s]</td>
<td>0.27±0.08</td>
<td>0.27±0.05</td>
<td>0.38±0.10</td>
</tr>
<tr>
<td></td>
<td>0.38±0.10</td>
<td>0.36±0.08</td>
<td>0.52±0.22*</td>
</tr>
<tr>
<td>Phasic GG activity [% max]</td>
<td>4.6±7.9</td>
<td>2.1±4.9</td>
<td>5.8±9.9</td>
</tr>
<tr>
<td></td>
<td>5.8±9.9</td>
<td>2.1±1.9</td>
<td>14±24*</td>
</tr>
<tr>
<td>Tonic GG activity [%max]</td>
<td>2.4±6.3</td>
<td>0.64±2.2</td>
<td>3.8±10.4</td>
</tr>
<tr>
<td></td>
<td>3.8±10.4</td>
<td>0.8±3.8</td>
<td>5.2±13.8*</td>
</tr>
</tbody>
</table>

#p<0.05 for drug effect, i.e., higher genioglossus electromyogram during pentobarbital versus placebo (all genioglossus electromyogram data during carbon dioxide insufflation, general linear model [mixed model]), *p<0.05 versus placebo treatment (Wilcoxon); +p<0.05 versus baseline - same study day, “p<0.1 vs. placebo treatment. GG=genioglossus electromyogram, CO₂= carbon dioxide, PETCO₂=end-tidal carbon dioxide.