The Effects of Ondansetron on Sleep-Disordered Breathing in the English Bulldog

Sigrid C. Veasey,1,2 Jennifer Chachkes,1 Polina Fenik,1 and Joan C. Hendricks1,3

Center for Sleep & Respiratory Neurobiology, 1Division of Pulmonary & Critical Care, Department of Medicine, School of Medicine, 2Department of Clinical Studies, School of Veterinary Medicine, 3University of Pennsylvania

Abstract: Serotonin and serotoninergic drugs have significant effects on respiration, at many sites throughout the nervous system, and serotonin has been implicated in the pathogenesis of obstructive sleep apnea. Thus, understanding the serotoninergic mechanisms underlying respiratory control may help discover novel pharmacotherapies for sleep-disordered breathing. Ondansetron, a serotonin (5-HT) antagonist selective for the 5-HT3 receptor subtype has recently been shown to suppress sleep-related central apneas in rats, particularly in rapid-eye-movement (REM) sleep. To evaluate the potential of ondansetron in the treatment of obstructive sleep-disordered breathing, we have performed randomized trials of two doses of ondansetron (20 and 40 mg orally) and placebo (4 studies for each of the 3 conditions) in our animal model of obstructive sleep apnea, the English Bulldog. Ondansetron significantly reduced the respiratory disturbance index (RDI) in REM sleep from 24.15±4.85 events/hour at placebo to 11.01±1.56 events/hour with high dose treatment, n=4, p<0.05. In contrast, the effects of drug on the RDI in non-rapid-eye-movement (NREM) sleep (5.23±1.30 events/hour, placebo; 4.31±1.36, with 20 mg ondansetron and 2.89±1.30 with 40 mg ondansetron, n=4) were not significant. Ondansetron, however, had no effect on either sleep efficiency or sleep architecture, and there were no effects on either oxyhemoglobin saturation nadirs or on the sleep time with saturations <90%. Although a trend towards reduction in the latter measure of oxygenation was seen at the higher dose of ondansetron. These data suggest a therapeutic potential for ondansetron in obstructive sleep-disordered breathing, particularly REM sleep apnea.

INTRODUCTION

RESEARCH OVER THE PAST THREE DECADES HAS PROVIDED TREMENDOUS INSIGHT INTO THE PATHOGENESIS of obstructive sleep-disordered breathing. It is evident that sleep-state dependent reductions in the activity of upper airway dilator muscles may cause collapse of the upper airway and obstructive sleep-disordered breathing events.1-10 These reductions in upper airway dilator activity are, in turn, the consequence of complex changes in neurochemical input to dilator motoneurons. That is, both reductions in excitatory inputs and increases in inhibitory inputs likely contribute to sleep-related reductions in upper airway dilator activity.11-18

Importantly, sleep state-dependent changes in respiratory drive occur not only at upper airway dilator muscles, but may involve other groups of respiratory muscles, as well.3,19 Indeed, persons with obstructive sleep-disordered breathing may have, in addition to obstructive apneas, central apneas and mixed (central and obstructive) apneas.20 The neurochemical mechanisms underlying central and mixed apneic events are not fully understood.

One potential mechanism for central sleep-disordered breathing events is cardiopulmonary vagal afferent C fiber stimulation. It is known that stimulation of C fibers can produce central apneas in anesthetized cats,21 and this effect can be replicated with serotonin excitation at the nodose ganglion.22 Further, systemic administration of a highly-selective 5-HT3 receptor antagonist can prevent the 5-HT mediated apneas in this preparation.23

Recently, the importance of this vagal 5-HT3 mechanism in central sleep apnea was tested in the rat,24,25 a model of central sleep-disordered breathing.26 Ondansetron, a 5-HT3 antagonist, administered systemically largely prevented REM sleep-related central apneas in the rat,24 and blocked the peripheral 5-HT3-induced central sleep apnea events in REM sleep.25 Therefore, it is likely that a peripheral 5-HT3-mediated effect contributes significantly to central sleep apnea events in the rat.

Whether this peripheral vagal effect contributes to sleep-disordered breathing events in obstructive sleep apnea is unknown. If this 5-HT3-dependent peripheral mechanism is important in obstructive sleep-disordered breathing, then a 5-HT3 antagonist should reduce the frequency and severity of sleep-disordered breathing events in obstructive sleep apnea. We have tested this postulate in a natural animal model of obstructive sleep disordered breathing, the English Bulldog.27

Day-to-day variability in sleep-disordered breathing indices occurs in the English Bulldogs,28 as it does in humans.29 This high baseline variance increases the probability of Type II statistical error in sleep apnea clinical trials. One advantage of an animal model of sleep apnea is the ability to perform multiple studies per animal, and a within-subject design is a powerful statistical tool. Thus, we performed in four bulldogs double-blinded, randomized trials of two doses of ondansetron with placebo controls (four studies for each condition) to determine the effect of drug on sleep and respiration in sleep.
METHODS

Animals. Adult English Bulldogs (two females, two males) were housed individually in a University Lab Animal Research colony at the University of Pennsylvania. The Bulldogs, ranging in age from 7 to 11 years, weighed 19-22 kg. The animals were kept on a 12:12 light/dark cycle with food and water ad libitum. The surgical procedures and experimental protocol were approved by the Institutional Animal Care and Use Committee at the University of Pennsylvania. All dogs had been trained to sleep in the recording cage with the encumbered recording equipment.

Animal preparation. A radio telemetry device was implanted in each Bulldog to obtain long-term electroencephalographic and electromyographic recording signals, (TL 10M3 Bioimplant, Data Sciences, St. Paul, MN), as previously described. Briefly, the dogs were premedicated with 0.005 mg/kg atropine sulfate intramuscularly and 0.2 mg/kg dexamethasone intravenously (IV) to reduce upper airway secretions and edema. General anesthesia was induced with 25—35 mg/kg thiopental IV. The dogs were endotracheally intubated, and general anesthesia was maintained with inhaled isoflurane (1-2.5%). Following implantation of the telemetry units, dogs were allowed to recover from anesthesia, and after 12 hours of close observation, they were returned to their home cages. Studies were conducted after a three-week recovery period.

Electrophysiological recording procedures. Sleep studies were performed with the dogs unrestrained within a 2 cubic meter electrically-shielded Plexiglas recording chamber. EEG and nuchal EMG signals were transmitted to a recorder (RLA 2000, Data Sciences) within the recording chamber. From the receiver, EEG and EMG signals were passed to a receiver multiplexor (RMX 10, Data Sciences), and then amplified and band-passed filtered (BMI-830, CWE, Inc., Ardmore, PA) using settings of 1-100 HZ for the EEG and 50-1000 HZ for the EMG signals. All of these signals were sent to a polygraph recorder (TA11, Gould). The other measured parameters were hemoglobin oxygen saturation, abdominal and rib cage movements, snoring and nasal airflow. Oximetry was measured continuously on the dog’s pinna or upper lip (Biox IIA, Ohmeda). Oximetry readings were recorded manually on the chart recorder paper. Rib cage and abdominal movements were measured with inductive plethysmography bands (Ambulatory Monitor, Respirtrace) with signals sent to the polygraph. Snoring was measured with a snore sensor attached to the ventral neck of the dog (Snore Mike 1200; EPM Systems, Sylmar, CA). At rest, Bulldogs are exclusively nasal breathers. Thus only nasal airflow was measured in this study. Airflow was measured indirectly and qualitatively with a thermistor (Easy Flow, EPM Systems, Midlothian, VA) suspended just over the nares user a wire halter, developed specifically for this purpose. Signals were sent directly to the chart recorder.

Study design and protocol. Each of the four English Bulldogs studied was randomized to the order of 12 separate sleep studies (four placebo studies and four studies at each of two drug dose conditions). The order for each of the 12 trials was randomized. The three experimental conditions varied by drug condition: placebo, 20 mg ondansetron (approximately 1 mg/kg) orally and 40 mg ondansetron (approximately 2 mg/kg) orally. The placebo and drugs were administered 30 minutes prior to the recording session, which lasted six hours from 10:00 A.M. to 4:00 p.m. A washout period of two days occurred between each of the 12 studies. Polysomnography recorders and scorers, were blinded to the conditions. The dogs were allowed to settle in the cage for 15 minutes prior to the start of the study while electrical signals were checked.

Sleep state scoring and sleep architecture. Behavioral state data were analyzed as described previously. Briefly, we studied drug effect on sleep efficiency, and the percentage of test time spent in each behavioral state, waking, light NREM sleep, and slow-wave predominant sleep, using electrophysiological measures described in the previous paper to define each of the behavioral states. The arousal index was measured for both NREM and REM sleep.

Scoring sleep-disordered breathing events. Sleep-disordered breathing events included in the respiratory disturbance index were respiratory effort-related arousals (RERAs), hypopneas and apneas. The RERAs were identified as arousals associated with either increased snoring immediately preceding an arousal or with paradoxical movements of the rib cage and abdomen preceding the arousal but without desaturations of at least 2%. Apneas, hypopneas, and RERAs were grouped together as respiratory disturbances, and the respiratory disturbance index (RDI) was calculated as the average number of number of apneas, hypopneas, and RERAs's per hour of sleep. The total time (in minutes) during SDB events spent with oxyhemoglobin saturations <90% was calculated for each study and expressed as the RDI desaturation time.

Data analysis. Mean±standard error (SE) values for each drug condition were obtained for the four trials/condition for each of the three conditions for: sleep architecture (percentage of time spent in each sleep stage and arousal indices); respiratory disturbance indices for NREM sleep and for REM sleep; oxyhemoglobin saturation nadir per study; and time spent with oxyhemoglobin saturations <90%. The mean values per animal per condition were analyzed to determine group effect of drug condition by repeated measures ANOVA with Scheffe's F test for individual differences; for these determinations the degrees of freedom were always = 3. Statistical significance was reported for p<0.05. Drug effect on RDI’s was also assessed by performing Pearson’s correlation coefficients for drug dose (0, 20 and 40 mg) vs. either REM RDI or NREM RDI, with n=4.

RESULTS

All four English Bulldogs successfully completed the 12 randomized studies to determine the effects of the two doses of ondansetron on sleep-wake behaviors and sleep-disordered breathing. Each dog, then, received four studies with placebo, four with 20 mg ondansetron and four studies with 40 mg of ondansetron. Scorers were blinded to the conditions. Inter-rater agreement (two scorers) for respiratory events scored for 10 complete sleep studies was 92.2±5.3%.

Drug effects on behavioral state and sleep-wake architecture. There were no apparent differences in waking behavior with drug at either dose, and overall, there were no significant differences in sleep architecture. Specifically, the average sleep efficiency (n=4) for the six-hour day study was 34.3±8.6% with placebo, 24.1±8.8% with 20 mg ondansetron, and 35.5±10.8% with 40 mg ondansetron, with repeated-measures ANOVA show-
The effects of ondansetron on sleep-disordered breathing indices in NREMS. As observed in previous studies, most of the NREM sleep-disordered breathing events were either respiratory effort-related arousals or hypopneas with 2% desaturations and arousals. Further, as in previous studies, events in NREM sleep in the bulldogs were infrequent, as described below.

Overall for the four dogs, the NREM sleep RDI was 5.23±1.30 with placebo, 4.31±1.36 with 20 mg of ondansetron, and 2.89±0.70 with 40 mg of ondansetron. While this did not achieve statistical significance, three of the four dogs demonstrated an overall improvement in the RDI with individual repeated-measure ANOVA, averaging all studies for each condition. The individual data for all four dogs are presented in Figures 1A, and the group data are shown in Figure 1B.

The effects of ondansetron on sleep-disordered breathing indices in REMS. In contrast to the types of events seen during NREM sleep in the Bulldogs, many of the REM sleep events were mixed central and obstructive apneas or hypopneas. Typically, at the start of a REM sleep period these events were apneas, while later events in REM sleep were more frequently hypopneas. Overall, the group RDI in REM sleep (n=4) with placebo was 24.15±4.85 events/hour. The REM sleep RDI with 20 mg of ondansetron was 12.83±3.72 events/hour, N.S., and the RDI in REM sleep with 40 mg ondansetron was 11.01±1.56 events/hour. Statistical significance was achieved for placebo vs. 40 mg ondansetron, p<0.05. Individual data are presented in Figure 1C and the group average ±SE are shown in Figure 1D.

The effects of ondansetron on oxygenation in sleep. Despite reductions in the RDI in REM sleep, ondansetron did not significantly change the time spent with oxyhemoglobin saturations <90% in total sleep. With placebo, the time spent with SaO2 values <90% was on average for the four dogs, 6.62±3.96 minutes; with 20 mg ondansetron, the time spent with SaO2 values <90% was 11.07±10.81 minutes and with 40 mg ondansetron, 3.68±3.11 minutes. There were no statistically significant differences in the time spent with SaO2 values <90% for the three conditions. Further, none of the dogs demonstrated an individual oxygenation response to ondansetron. The number of desaturations to SaO2 values <90% also did not change with drug. The SaO2 nadirs for each of the three conditions did not vary. At baseline, the mean nadir for the group was 88.0±3.7%, with 20 mg ondansetron the average SaO2 nadir was 84.8±4.5% and with 40 mg ondansetron the average nadir was 86.8±3.1%. There were no main effects of drug on SaO2 nadirs. In summary, then, while ondansetron reduced the frequency of REM sleep-disordered breathing events, this drug at 20 and 40 mg orally did not affect either the severity or the time spent with oxyhemoglobin desaturation in sleep.

DISCUSSION

We have shown, in our natural animal model of obstructive sleep-disordered breathing, that ondansetron, a 5-HT3 antagonist, reduces sleep-disordered breathing, in REM sleep. While ondansetron does not fully alleviate sleep-disordered breathing in the English Bulldog, we believe that the findings are significant for several reasons. First, these data suggest that a 5-HT3-mediated mechanism may be important in the pathogenesis of obstructive sleep-disordered breathing. Second, this ondansetron effect was observed primarily in REM sleep, in contrast to other studies showing drug effects on NREM SDB. Finally, the significant effect of ondansetron on REM sleep-disordered breathing in the English Bulldogs raises the possibility that ondansetron may be clinically useful in humans with predominantly REM sleep-related obstructive sleep apnea.

Limitations of the model and study design. While the English Bulldog is one of the few natural animal models of obstructive sleep apnea, there are several limitations with this model that deserve discussion. First, this model has very mild obstructive sleep-disordered breathing in NREM sleep, with most events qualifying as respiratory effort-related arousal events and thus for NREM sleep, the Bulldog may be more accurately considered as a model of upper airways resistance syndrome. In light NREM sleep (the equivalent of stages 1 and 2 in humans) respiratory activity fluctuates significantly enough that there are frequent >50% reductions in airflow or respiratory movement. Only rarely, despite prolonged reductions in airflow (typically 10—30 seconds), are these events associated with desaturations. Therefore, in the Bulldogs, these events are not scored as sleep-disordered breathing events, unless such events cause arousals. In contrast to the minimal sleep-disordered breathing the Bulldogs show in NREM sleep, during REM sleep, the events appear strikingly similar to events seen in humans with obstructive sleep-disordered breathing. Desaturations occur with

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Table 1—Effects of ondansetron on sleep architecture in the English bulldog. Mean±SE for the four bulldogs for all studies under each condition. No significant F values with α<0.05.

<table>
<thead>
<tr>
<th>% of Study in Light NREM Sleep</th>
<th>Placebo</th>
<th>Ondansetron 20 mg</th>
<th>Ondansetron 40 mg</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of Study in Slow-Wave Sleep</td>
<td>12.6±0.8%</td>
<td>11.3±1.0%</td>
<td>15.1±4.5%</td>
<td>N.S.</td>
</tr>
<tr>
<td>% of Study in REM sleep</td>
<td>17.6±5.7%</td>
<td>18.7±6.1%</td>
<td>18.3±7.8%</td>
<td>N.S.</td>
</tr>
<tr>
<td>% of Study in REM Sleep</td>
<td>4.6±2.2%</td>
<td>6.0±2.3%</td>
<td>4.2±2.4%</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

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most REM sleep events, and desaturations are lower and apneas are longer in REM sleep than in NREM sleep. In addition, as in humans with obstructive sleep apnea, REM sleep frequently begins with a pronounced mixed central and obstructive event. This animal model of sleep-disordered breathing, the English Bulldog, may thus be best characterized as a model of upper airways resistance syndrome and REM sleep-related obstructive sleep-disordered breathing. Because the pathophysiology of upper airways resistance syndrome (sleep-related reductions in upper airway dilator activity) is very similar to the pathophysiology of obstructive sleep apnea, we believe that drugs affecting upper airways resistance syndrome will likely impact upon obstructive sleep apnea, although higher doses may be required to reduce apneic events.

This study design is limited by a small sample size, necessary to the expense and difficulties in maintaining a larger Bulldog colony and training time required for dogs to sleep with recording equipment. Statistical power analysis based on within and between subject variability shows that for four dogs, we have achieved 80% power to detect a 50% decline in the REM sleep RDI, but would need seven dogs to achieve the same power for NREM sleep. With our study design then, we cannot rule out that an effect of drug would have been seen with a larger sample size. We believe that given the large effect in REM sleep in the Bulldogs, the next step would be to adequately power and then perform human trials to determine if this drug would be of benefit in treating human obstructive sleep-disordered breathing.

A final point about study design concerns the pharmacokinetics of ondansetron. This drug has a clinically effective half-life of approximately four to six hours in humans, with an onset of action 30 minutes after ingestion. The smaller dose used in this study is comparable to the dose showing effectiveness in the rat, given the excellent bioavailability. In addition, we used a dose two times in size to test dose-responsiveness. The intent of this study was not to quantify the reduction in sleep-disordered breathing under steady state drug levels in Bulldogs, but rather to provide support for the hypothesis that one mechanism contributing to central sleep apnea events in rats (afferent vagal stimulation) might also play a role in obstructive sleep apnea.

The effects of ondansetron on breathing and sleep in the bulldog. In this paper, we show that a 5-HT₃ antagonist improves REM sleep-disordered breathing in the English Bulldog. These results at first glance would seem to contradict earlier work showing that serotonin antagonists in the Bulldog produce upper airway obstruction, and several types of serotoninergic drugs reduce sleep-disordered breathing in Bulldogs, as well as in humans. The effects of serotonin agonists and antagonists on SDB depend upon the 5-HT receptor subtypes for which the drugs are active. The mechanism through which 5-HT₃ receptor antagonism reduces REM SDB cannot be determined in the present studies. Possible sites of action include: 1)

![Figure 1](https://example.com/figure1.png)

**Figure 1**—The effects of ondansetron on sleep-disordered breathing in the English bulldog. Figure 1A shows the individual data for the average NREM sleep disordered breathing index (number of events/hour) for each dog under each condition. Sleep-disordered breathing events are rare in this species in NREM sleep, and in the two dogs with lower indices, there was no apparent effect. Figure 1B shows the group mean ± SE. Figure 1C plots the individual average responses for each condition for each of the four dogs in REM sleep. In contrast to NREM sleep, in REM sleep, all dogs demonstrated a reduction in the number of events/hour of sleep-disordered breathing. Figure 1D shows the groups average data for each condition. The asterisk highlights a significant reduction from baseline.
upper airway dilator motoneurons; 2) upper respiratory neurons; 3) respiratory afferents, including vagal; and 4) sites involved in behavioral state control.

Ondansetron in animals and humans has not been shown to significantly alter sleep efficiency or sleep architecture. A detailed analysis of ondansetron effects on phasic vs. tonic REM sleep has not been described in the literature. It is possible that the reduction in REM SDB may have occurred secondary to reductions in phasic REM sleep, rather than changes in respiratory drive.

Whether 5-HT3 receptors are functional at upper airway dilator motor nuclei is at present unknown. Messenger RNA for the 5-HT3 receptor has shown message levels to be present in motor nuclei, including the hypoglossal nucleus. 5-HT3 receptor ligand binding, however, is not detectable within the confines of the motor nuclei for upper airway dilator motoneurons in mammalian species, while binding is abundant within the nucleus tractus solitarius, the hippocampus, the area postrema and the spinal trigeminal nucleus. Future studies are planned to determine if the 5-HT3 receptor is functional in upper airway dilator motor nuclei.

Immunoreactivity for 5-HT3 receptors has also been identified within the nodose ganglion and the carotid body, where in both locations, respiration has been shown modified through 5-HT receptor activation. Systemic 5-HT3 antagonists reduce 5-HT-induced apneas in anesthetized cats. Carley and Radulovacki have shown that ondansetron administered prior to systemic 5-HT largely suppresses central sleep apneas in rats. A likely mechanism, through which ondansetron has an effect in the bulldog, therefore, is through blocking 5-HT3-dependent vagal afferents within the nodose ganglion. Our data support the hypothesis that 5-HT3 mechanisms can contribute to the pathogenesis of obstructive sleep apnea.

Ondansetron reduces the REM SDBI without parallel reduction in either the arousal or desaturation frequency. The time spent in REM sleep with SaO2 <90% was decreased by 40% at the highest dose of ondansetron, and the number of desaturations to <90% decreased by 30%. Neither of these comparisons achieved statistical significance, and whether this was a Type II error (variance is high for these parameters) or a true negative result is unclear in the present study. For most dogs, the magnitude of the decrease in REM sleep RDI was greater than the magnitude of change in the frequency of SaO2 <90% events. Therefore, ondansetron may have a greater impact on the REM sleep events with SaO2 nadirs ≥90%. Similarly, the arousal index in REM sleep was not changed by ondansetron, but again this parameter showed high variance. Typically, REM SDB events in the Bulldog do not terminate in an arousal, so that a change in the arousal index would not be expected. There was a trend towards a reduction in the NREM SDBI without reductions in the number of desaturations <90% or in the NREM sleep arousal index. In contrast to REM SDB, a reduction in the arousal index would be expected with a significant reduction in upper airway resistance syndrome in NREM sleep. Arousal in NREM sleep may occur with SDB events, but may also occur spontaneously within NREM sleep or upon termination of NREM sleep bouts. The Bulldogs in the present study have relatively rare NREM SDB events (two to eight per hour), and the small reduction in these infrequent events may, at least in part, explain the lack of decline in arousal indices with drug. Alternatively, the drug may slightly increase NREM sleep fragmentation.

Ondansetron: its safety and adverse effects in humans.

High doses of ondansetron were required for a statistically significant improvement in the REM sleep RDI. While most of the long-term use ondansetron trials in either healthy persons or in chemotherapy or peri-anesthesia trials have used smaller doses, comparable high doses have been used in one pediatric peri-anesthesia trial without adverse effect, and in healthy humans one-time doses as high as 1.5 mg/kg were used without adverse effect. We believe that the above findings in bulldogs support a trial of single dose ondansetron in persons with REM sleep-disordered breathing.

CONCLUSIONS

In an animal model of obstructive sleep-disordered breathing, high-dose ondansetron significantly reduced the frequency of sleep-disordered breathing events in REM sleep. Although there was a trend towards improvement in the NREM sleep RDI, the effect of ondansetron on NREM SDB cannot be determined in the present study. Nevertheless, the effects of ondansetron in the bulldogs suggest that 5-HT3 mechanisms may contribute to the pathogenesis of obstructive sleep-disordered breathing, and that adequately powered trials of ondansetron should be tested in humans with obstructive sleep apnea, particularly in persons with a predominance of REM sleep events.

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