Low-Dose Mirtazapine Increases Genioglossus Activity in the Anesthetized Rat

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INTRODUCTION

THE ACTIVITY OF THE GENIOGLOSSUS, A TONGUE PROTRUDER, ALONG WITH OTHER UPPER AIRWAY MUSCLES IS IMPORTANT FOR MAINTAINING UPPER AIRWAY PATENCY DURING SLEEP.1 The genioglossus is innervated by the medial branch of the hypoglossal nerve (CN XII). Studies in decerebrate animal preparations2-5 and freely moving animals during natural sleep6 have shown that direct application of serotonin to the hypoglossal motor nuclei can augment hypoglossal nerve activity and genioglossus electromyographic (EMG) activity. Serotonin receptors of the 5-HT2A type on the hypoglossal motor nuclei are thought to be the predominant receptor mediating this effect.5 The effects of systemic increases in serotonin on hypoglossal/genioglossus activity appear to be relatively more complex. Stimulation of different types of serotonin receptors in the central nervous system or periphery6 could have multiple effects on global genioglossus activity. Serotonin does not cross the blood-brain barrier. However, serotonin precursors (tryptophan, 5-hydroxytryptophan)7, selective serotonin reuptake inhibitors [SSRIs], and serotonergic agonists do cross into the central nervous system and can potentially increase genioglossus activity by increasing local serotonin levels or stimulating serotonin receptors. Recent evidence suggests that blockade of the serotonin receptor type 3 can also increase hypoglossal activity.8 This is believed to occur by action at the nodose ganglion. In one study, ondansetron, a 5-HT3 receptor blocker, decreased the apnea-hypopnea index in the English bulldog.9 Hypoglossal activity is also augmented by norepinephrine,10 although this effect has not been studied as extensively as it has been with serotonin.

Mirtazapine is an antidepressant with a number of actions on the serotonergic and noradrenergic systems. The drug increases both serotonin and norepinephrine by blockade of central α2 autoreceptors and heteroreceptors.11,12 In addition, mirtazapine is a blocker of type 2 and type 3 serotonin receptors. Unlike SSRIs, mirtazapine does not impair sleep quality.11,13 The sedating properties are believed to be secondary to the histamine-receptor blockade. The 5-HT2 receptor-blocking activity may also increase slow-wave sleep.13 The combination of beneficial actions of mirtazapine on sleep as well as the ability to increase serotonin and norepinephrine makes it a candidate for use in the treatment of obstructive sleep apnea.

The specific effects of mirtazapine administration on genioglossus activity, however, have not been previously studied. A study in rats during natural sleep found that mirtazapine decreased the amount of central apnea and stimulated ventilation without impairing sleep quality.14 Yet this observation does not directly imply that mirtazapine would decrease obstructive apnea or increase genioglossus activity. Respiratory stimulants (eg, salicylate, acetazolamide, carbon dioxide) tend to decrease obstructive as well as central apnea.15-17 Stimulation of ventilation may reduce obstructive apnea by stabilization of neural input to the upper airway muscles18 rather than by augmenting upper airway

Study Objectives: To examine the effects of mirtazapine on genioglossus and diaphragmatic electromyogram activity in the anesthetized rat.

Design: Parallel-group study.

Subjects: Sprague-Dawley adult male rats, 10 in each of 3 groups were studied.

Interventions: After anesthesia with 1.2 g/kg of urethane, a tracheostomy and bilateral vagotomy were performed. Femoral arterial and venous lines were placed, and fine wire hook electrodes were implanted into the genioglossus and diaphragm muscles.

Measurements: After a baseline period of measurement, either saline, 0.5 mg/kg of mirtazapine, or 5.0 mg/kg of mirtazapine was injected via the intraperitoneal route, and measurements were made for the next 3 hours. The average peak and tonic values of the moving time average of the genioglossus and diaphragm electromyogram for hours 1, 2, and 3 were determined and expressed as a percentage of the corresponding average value during the baseline (preinjection) monitoring period.

Results: At 0.5 mg/kg of mirtazapine, the peak genioglossus electromyogram was significantly higher than in control conditions over hours 2 and 3. At 5.0 mg/kg of mirtazapine, the genioglossus electromyogram was significantly lower than in control conditions for the first 2 hours of monitoring. The peak diaphragmatic electromyogram was slightly but significantly lower in the mirtazapine 5.0-mg/kg group than in controls.

Conclusions: Mirtazapine, at a dose similar to one used clinically, increased genioglossus activity. We hypothesize that, at this dose, the ability of mirtazapine to increase serotonin and norepinephrine or block type-3 serotonin receptors predominated. At the higher dose of mirtazapine, the type-2 blockade effect predominated and genioglossus activity decreased.

Key words: Mirtazapine, genioglossus, serotonin.

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Disclosure Statement

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blood-pressure tracing by the elapsed time. Respiratory rate was determined by dividing the number of respiratory cycles in the first minute of each measurement period by time. Systolic and diastolic blood pressure values were determined for the first 10 blood-pressure pulsations in each measurement time period.

The values of genioglossal EMG, diaphragmatic EMG, heart rate, respiratory rate, systolic blood pressure, and diastolic blood pressure were expressed as a percentage of the baseline period. Significant changes in these variables were determined by performing a repeated-measures analysis using time period (first, second, third) as the repeated variable and drug (saline control, 0.5 mg/kg mirtazapine, 5.0 mg/kg mirtazapine) as the between-group variable using a statistical analysis program (Sigma Stat, SysStat Software, Point Richmond, CA, USA). Where appropriate, posthoc comparisons were made with the Student-Neuman-Keuls test. Changes were considered significant when $P < .05$. All data are reported as mean ± SEM.

RESULTS

Administration of mirtazapine at $0.5\text{mg/kg}$ significantly increased peak genioglossal EMG activity compared to control over hours 2 and 3 of the monitoring period (Figure 1). In contrast, mirtazapine at $5.0\text{mg/kg}$ significantly decreased peak genioglossal EMG activity compared to control over hours 1 and 2 of the monitoring period. The effects of mirtazapine on diaphragmatic activity are shown in Figure 2. In both mirtazapine-injected groups, peak diaphragmatic EMG was reduced slightly relative to vehicle-treated animals. This reduction in peak diaphragmatic EMG, however, was only significantly different from controls following treatment with the higher-dose mirtazapine ($5.0\text{mg/kg}$, $P < .05$). Of note, in the saline-injected animals, peak genioglossal EMG activity tended to decrease over time relative to preinjection levels (Figure 1), but peak diaphragmatic EMG remained steady (Figure 2). The tonic genioglossus and diaphragmatic EMG values did not differ between the 3 treatment groups (Table 1).

The baseline values of systolic and diastolic blood pressure, respiratory rate, and heart rate for each group are shown in Table 2. The corresponding values were not statistically different. The higher-dose mirtazapine significantly ($P < .05$) reduced systolic blood pressure as a percentage of baseline within the first hour following administration and pressure remained significantly reduced relative to both control and low-dose mirtazapine for the remaining 2 hours. The systolic blood pressures in the control and low-dose mirtazapine groups did not differ. The diastolic blood pressure as a percentage of baseline was also lower in the high-dose mirtazapine group than the other 2 groups, although this difference did not reach significance ($P < .06$) (Figure 3).

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

**Figure 1**—Peak genioglossus electromyogram (EMG) activity as a percentage of the baseline period in rats treated with saline (control), mirtazapine $0.5\text{mg/kg}$ (Mirt 0.5 mg), and mirtazapine $5.0\text{mg/kg}$ (Mirt 5 mg) ($n = 10$ in each group). The EMG activity was significantly higher on $0.5\text{mg/kg}$ of mirtazapine compared to control during hours 2 and 3 of monitoring. The EMG activity was significantly lower with mirtazapine $5.0\text{mg/kg}$ compared with that of controls during the first 2 hours of monitoring. The values are expressed as mean ± SEM.
The respiratory rate as a percentage of baseline was higher in the 5.0-mg/kg mirtazapine group than the other 2 groups over the first hour, but the heart rate as a percentage of baseline did not differ between the 3 groups at any point in time (Figure 4). The arterial blood-gas data from the end of the experiment is shown in Table 3. At the end of the experiment, there was a mild respiratory acidosis in all groups. However, the pH, PCO2 and PO2 did not differ between the groups.

DISCUSSION

The interest in the effects of serotonin on upper-airway muscle activity has been generated by the desire to find a pharmacologic treatment for obstructive sleep apnea. Augmentation of upper-airway muscle activity by a medication could theoretically prevent upper-airway closure during sleep. This study found that a dose of 0.5 mg/kg of mirtazapine augmented peak genioglossus activity compared to control in the anesthetized, vagotomized, spontaneously breathing rat. In contrast, at the higher dose of 5.0 mg/kg, peak genioglossal EMG activity was lower than control. Peak diaphragmatic EMG activity was significantly reduced by high-dose mirtazapine compared to control, although the percentage decrease was smaller than the decrease in genioglossus activity. These observations suggest that the multifaceted actions of mirtazapine increase peak genioglossus activity only when administered at lower doses. At the higher dose, mirtazapine actually decreased genioglossus activity.

Direct application of serotonin on the hypoglossal nucleus in animal preparations or via dialysis in freely moving animals has been documented to increase hypoglossal or genioglossus activity. Systemic administration of 5-hydroxytryptophan, the immediate precursor of serotonin, also increases hypoglossal or genioglossal activity in the anesthetized rat. However, to date, studies of serotonergic agents in humans have been disappointing. Most studies have focused on SSRIs. Studies have found evidence of modest augmentation in genioglossus activity during wakefulness or non-rapid eye movement sleep with the SSRI paroxetine. However, this medication decreased the apnea-hypopnea index only during non-rapid eye movement sleep in patients with mild to moderate sleep apnea. Moreover, SSRIs disturb sleep, and no study has shown an improvement in sleep quality.

As norepinephrine also augments genioglossus activity, one might hypothesize that agents increasing both serotonin and norepinephrine might be more effective than SSRIs. Mirtazapine is a novel antidepressant that increases both serotonin and norepinephrine without disturbing sleep quality. The medication is also a blocker of serotonin type-2 and type-3 receptors. The latter effect might be expected to increase genioglossus activity.

![Figure 2](image-url) 

**Figure 2**—Peak diaphragmatic electromyogram (EMG) activity as a percentage of the baseline period in rats treated with saline (control), mirtazapine 0.5 mg/kg (Mirt 0.5 mg), and mirtazapine 5.0 mg/kg (Mirt 5 mg) (n = 10 in each group). The EMG activity was significantly higher in the control group compared with the mirtazapine 5.0 mg/kg group. The values are expressed as a mean ± SEM.
Figure 3—Systolic and diastolic blood pressure as a percentage of baseline in the rats treated with saline (control), mirtazapine 0.5 mg/kg (Mirt 0.5 mg), and mirtazapine 5.0 mg/kg (Mirt 5 mg) (n = 10 in each group). The systolic blood pressure was lower in the higher-dose mirtazapine group than in the other 2 groups. The systolic blood pressures in the other 2 groups did not differ. The diastolic blood pressure was not statistically different across treatments or time. The values are expressed as mean ± SEM.

Figure 4—The respiratory rate and heart rate as a percentage of baseline are shown for rats treated with saline (control), mirtazapine 0.5 mg/kg (grey bar), and mirtazapine 5.0 mg/kg (black bar). The respiratory rate for mirtazapine 5.0 mg was significantly greater than that of the other 2 groups during the first hour of monitoring (P < .05). The heart-rate values did not differ between the groups and changed little from baseline. The values are expressed as mean ± SEM, with 10 animals in each group.
Ondansetron, a 5-HT3 receptor blocker, has been shown to increase hypoglossal activity in high doses when given systemically but not when administered directly on the hypoglossal nucleus. Ondansetron also decreased sleep-disordered breathing in the English bulldog. However, a trial of ondansetron on moderate obstructive sleep apnea found no benefit. On the other hand, 5-HT2 receptor blockers might be expected to decrease genioglossal activity. The receptor on the hypoglossal motor nuclei mediating augmentation of serotonin appears to be of the 5-HT2A type. One might expect the net effect of mirtazapine on genioglossus to depend on the relative importance of its different actions, and these might vary with the level of medication present in the brainstem (dose).

The results of our study demonstrate that, at the lower dose (0.5 mg/kg), mirtazapine increased peak genioglossal activity. Carley et al14 have shown that mirtazapine at all doses ranging from 0.1 mg/kg to 5.0 mg/kg suppressed central apnea in both non-rapid eye movement and rapid eye movement sleep and increased minute ventilation in freely moving rats. Their study, however, did not report respiratory rate separately from measurement of minute ventilation. There was no effect of dose on the changes in minute ventilation. We did not measure minute ventilation but did find the PCO2 to be slightly lower, although not significantly, at the end of the experiment in the mirtazapine groups compared to controls. In the higher-dose mirtazapine group, the respiratory rate was also increased compared to control during the first hour of monitoring.

Our experiment does not allow us to determine if the increase in serotonin-norepinephrine or 5-HT3 blockade (or both) increased genioglossal activity. Veasey and coworkers found that approximately 1 and 2 mg/kg of ondansetron reduced the apnea-hypopnea index during rapid eye movement sleep in the English bulldog. Because systemic administration of ondansetron has been shown to increase hypoglossal activity, this effect on the apnea-hypopnea index may have been secondary to an increase in upper-airway muscle activity. We are not aware of a study comparing the potency of ondansetron and mirtazapine with respect to 5-HT3 blockade.

In the control group, over the 3 hours of monitoring, the peak diaphragmatic activity tended to increase slightly, to approximately 110% of baseline (Figure 2), while peak genioglossal activity in the control group decreased to around 60% of the baseline activity (Figure 1). We can offer 2 possible explanations for this finding. First, it is possible that the fall in genioglossal activity represents a subacute adaptation to the effects of vagotomy. All rats were noted to have an immediate and large increase in peak genioglossal activity following vagotomy. We waited a minimum of 10 minutes after vagotomy until acute changes in the breathing pattern and genioglossal activity had stabilized before starting recording and determining the baseline values. However, there could have been a slower component of adaptation to vagotomy over the next several hours. Second, the fall in genioglossal activity from baseline could represent a progressive effect of anesthesia. Anesthesia has been shown to preferentially decrease upper-airway muscle activity.29 We do not believe that the fall in genioglossal activity in the control group represents a deterioration of the brainstem because blood pressure and heart rate were relatively stable. Nor is there evidence of deterioration of the fine wire electrode recording because, in the lower-dose mirtazapine group, the peak genioglossal EMG activity actually increased over the monitoring period. In any case, we used the same procedure for all groups and, therefore, controlled for the fall in genioglossal activity following vagotomy.

The peak diaphragmatic EMG activity was less than that of the control group in the higher-dose mirtazapine group. As with the effects of mirtazapine on the genioglossus, changes in diaphragm activity could represent competing effects of different actions of the medication. Veasey et al30 found that ritanserin, a 5-HT2 blocker, decreased diaphragmatic activity in the English bulldog. In contrast, Richmonds and Hudge12 found that ritanserin actually increased both hypoglossal and phrenic activity in the anesthetized rat. Mirtazapine, by increasing serotonin and blocking 5-HT2 and 5-HT3 receptors, tends to result in preferential stimulation of 5-HT1 receptors. Application of 5-HT1 agonists to the brainstem increases respiratory frequency in decerebrate cats.11 At the higher dose of mirtazapine, we found evidence of an increase in respiratory frequency over the first hour of monitoring (Figure 4).

In the study of mirtazapine in naturally sleeping rats, the medication increased ventilation and reduced central apnea.14 Tidal volume and respiratory rate were not analyzed separately. We did not find a consistent increase in respiratory rate, and peak diaphragmatic EMG was not increased (actually decreased at the higher dose). However, our rats were vagotomized, tracheostomized, anesthetized, and breathing oxygen-enriched air. Thus, our results could be different because of any or all of these differences in experimental technique. The arterial blood gases at the end of the experiment also did not show a higher PCO2 (actually slightly lower) than control in either mirtazapine group. Hence, it is unlikely that mirtazapine decreased ventilation.

Finally, we acknowledge that there are several limitations of our methodology. First, we studied the anesthetized rat instead of a decerebrate or freely moving animal. As mentioned above, there is a preferential suppression of hypoglossal activity by use of anesthesia.29 Despite, the use of anesthesia we were able to show an increase in genioglossal activity compared to control with the lower dose of mirtazapine. Second, we studied the effects of mirtazapine in a vagotomized animal. Vagotomy increases phasic genioglossal activity and facilitates measurements. However, it is possible that our results may not generalize to animals (or humans) with an intact vagus nerve. Third, we only studied a 1-log difference in dosage (0.5 and 5.0 mg/kg). It is possible that lower or higher doses have different effects. There may be a narrow therapeutic window and a given dose could have different effects in different patients. Certainly further study is needed to better define the dose effect. Finally, the design of our study allowed us to study only the acute effects of mirtazapine. There certainly could be differences between acute and chronic effects.

### Table 3—Arterial Blood Gases at the End of the Study*

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Mirtazapine 0.5 mg/kg</th>
<th>Mirtazapine 5.0 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.24 ± 0.03</td>
<td>7.31 ± 0.01</td>
<td>7.29 ± 0.02</td>
</tr>
<tr>
<td>PCO2, mm Hg</td>
<td>51.3 ± 6.9</td>
<td>48.4 ± 1.9</td>
<td>48.4 ± 4.4</td>
</tr>
<tr>
<td>PO2, mm Hg</td>
<td>131 ± 17.2</td>
<td>143 ± 10.5</td>
<td>151 ± 14.4</td>
</tr>
</tbody>
</table>

*Values are expressed as mean ± SEM, with 10 animals in each group.
of mirtazapine on the genioglossus. Studies of chronic drug administration in freely moving rats would be needed to determine if the effects we demonstrated have clinical relevance. However, our findings do suggest that mirtazapine at a dose used clinically in humans (0.5 mg/kg) may augment genioglossus activity, and, thus, the medication could have clinical utility.

In summary, this investigation demonstrated that, at a dose of mirtazapine similar to that used clinically for depression, genioglossus activity was significantly increased compared to control animals in the anesthetized rat within 2 hours of administration. In contrast, at a higher dose of mirtazapine, genioglossus activity was significantly reduced below control for 2 hours after drug administration. We hypothesize that the differential dose effects were secondary to the predominance of different actions of mirtazapine—some enhancing and others diminishing genioglossus activity.

REFERENCES

17. Sakamoto T, Nakazawa Y, Hashizume Y, et al. Effects of acetazo-