Genioglossus muscle activity and serotonergic modulation of hypoglossal motor output in obese Zucker rats

Sandeep Sood, Xia Liu, Hattie Liu, and Richard L. Horner

Departments of Medicine and Physiology, University of Toronto, Toronto, Canada

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Obesity is associated with upper airway narrowing in humans (17, 40, 42) and is a known risk factor for obstructive sleep apnea (OSA) (37). The Zucker rat is an established model of obesity (8), and these animals exhibit a narrower upper airway compared with lean littersmates (5, 24). The upper airway of obese Zucker rats is also more collapsible compared with lean controls as judged by more positive critical closing pressures (32, 35), similar to observations made in OSA patients (41, 45). Although measures of upper airway size and mechanics in obese Zucker rats have similarities to OSA patients, studies of the neural control of pharyngeal muscle activity in obese and lean Zucker rats have not yet been performed. Such studies are important if this obese model is to be valuable to evaluate neural as well as anatomical factors in upper airway collapsibility relevant to OSA.

Pharyngeal muscle tone is augmented in awake patients with OSA, a compensatory response thought to be relevant to maintaining airway patency in these individuals with narrow airways (26, 50). However, suppression of this compensatory response in sleep predisposes to OSA (25). Similar increases in pharyngeal dilator muscle activity occur in English bulldogs (15), a breed also with a narrow airway and sleep-disordered breathing (14). Only one study has reported genioglossus (GG) muscle activity in obese Zucker rats and compared this activity with that of lean controls (32). Those recordings, however, were obtained in only two rats that were studied under anesthesia and in the supine position, and those conditions may have contributed to the increased GG activity observed (32). Magnetic resonance imaging showed smaller changes in oropharyngeal size during inspiration in obese, compared with lean, Zucker rats, but there were no differences in pharyngeal wall tissue strain as indicated by noninvasive tissue tagging (5). The results from this study may indicate normal, rather than enhanced, pharyngeal muscle tone in obese Zucker rats, but this has not yet been determined. Accordingly, the first aim of the present study was to record GG muscle activity across sleep-wake states in freely behaving obese Zucker rats to determine if GG activity is augmented compared with lean littersmates.

The increased GG activity observed in two anesthetized, supine, and obese Zucker rats was diminished by intravenous ritanserin, a serotonin (5-hydroxytryptamine; 5-HT) receptor antagonist (32). Similar effects in awake bulldogs (53) implicates a 5-HT neural component to the augmentation of pharyngeal motor tone in these animals with compromised upper airways. Nevertheless, endogenous 5-HT receptor mechanisms at the hypoglossal motor nucleus (HMN), the source of motor outflow to the GG muscle of the tongue, play a minimal role in the normal modulation of GG activity in intact lean rats in wakefulness and natural sleep (47, 48). Taken together, these results suggest that a normally low endogenous 5-HT tone operates at the HMN in intact lean animals but that this role of 5-HT may be increased in obese animals, for example, by reflex compensation for a narrow airway.

In obese Zucker rats, however, the notion that an enhanced 5-HT-mediated excitatory drive augments pharyngeal motor tone and upper airway patency has not been established. For example, the more positive closing pressures after intravenous ritanserin in anesthetized rats (32) is often cited as evidence of a significant role for 5-HT in maintaining upper airway patency in these animals. However, the increases in closing pressures after 5-HT receptor antagonism are small in magnitude in both

Address for reprint requests and other correspondence: R. L. Horner, Rm. 6368, Medical Sciences Bldg., 1 Kings College Circle, Toronto, ON, Canada, M5S 1A8 (e-mail: richard.horner@utoronto.ca).
lean and obese anesthetized Zucker rats, with increases of only 0.8 and 0.7 cmH2O, respectively (32). Similarly, in conscious Zucker rats, systemic administration of ritanserin decreased ventilation (as measured by noninvasive plethysmography) and increased work of breathing (as reflected by oxygen consumption) but only in older (8 mo) obese animals and not in younger lean or obese rats (32). No measurements of the potential effects on upper airway muscle activity were performed (32). Our laboratory has developed an animal model for manipulation of neurotransmission at the HMN in freely behaving rats awake and asleep (1, 6, 47). Given the potential importance of obese Zucker rats as a clinically relevant model of both anatomic and neural control of the upper airway, with the latter not yet determined (5, 24, 32, 35), the second aim of the present study is to determine whether 5-HT receptor antagonism at the HMN reduces GG activity in obese and lean Zucker rats consistent with a role of a significant 5-HT drive contributing to the maintenance of GG activity in this animal model of obesity.

METHODS

Experiments were performed on seven obese [mean 645.1 ± 35.0 (SE) g] and seven age-matched lean (393.1 ± 20.0 g) male Zucker rats. The obese phenotype (fa/fa) is due to a recessive trait, and lean littermates (fa/?) were used as the controls (32, 35). Importantly, the source (Vassar College, Poughkeepsie, NY), weights (see above) and ages (9.2 ± 0.7 and 8.9 ± 0.3 mo, respectively) of rats were the same as a previous study demonstrating increased upper airway collapsibility in anesthetized obese compared with lean Zucker rats (35), and the study implicating a role for 5-HT modulation of ventilation and upper airway stability in obese conscious rats via indirect measurements of work of breathing and O2 consumption (32). All procedures conformed to the recommendations of the Canadian Council on Animal Care, and the University of Toronto Animal Care Committee approved the experimental protocols.

Anesthesia and Surgical Procedures

Sterile surgery was performed under general anesthesia induced with intraperitoneal ketamine (85 mg/kg) and xylazine (15 mg/kg) as described previously (1, 47, 48). In addition, rats were given saline (3 ml, 0.9% ip) for fluid loading, atropine sulfate (1 mg/kg ip) to reduce airway secretions, and buprenorphine (0.03 mg/kg ip) to reduce potential postoperative pain. Following the onset of surgical levels of anesthesia, as judged by abolition of the pedal withdrawal and corneal blink reflexes, the abdomen, neck, and head regions were shaved and cleaned with 70% alcohol and the antiseptic-germide solution Triadine (10% povidone-iodine, Triad Disposables, Brookfield, WI). Sterile 1% chlorhexidine ointment (Vetcom, Upton, PQ, Canada) was applied to the cornea to prevent drying. An anesthesia mask (12) was placed over the snout, and the rats spontaneously breathed a 50:50 mixture of air and oxygen for the remainder of the surgery. Any additional anesthesia was given by inhalation (isoflurane, typically 0.2–2%).

With the rats supine the ventral surface of the GG muscle was exposed via a submental incision and dissection of the overlying geniohyoid and mylohyoid muscles. Two insulated, multistranded stainless steel wires (AS631, Cooner Wire, Chatsworth, CA) were implanted into GG muscle and secured with sutures and tissue glue. Section of the medial branches of the hypoglossal nerves has shown that GG activity is recorded with such electrode placements (30). To record diaphragm activity, two insulated, multistranded stainless steel wires (AS636, Cooner Wire) were sutured onto the costal diaphragm via an abdominal approach. The size, configuration, and placement of the GG and diaphragm electrodes were consistent across experiments.

To assist in electrode placements during surgery, the GG and diaphragm signals were monitored for respiratory-related activity (AM8 Audio Amplifier, Grass). To ensure that the GG electrodes were positioned correctly, incrementing test voltages were applied (Isolated Pulse Stimulator, model 2100, A-M Systems) to confirm tongue movements that were identified visually. The GG and diaphragm wires were tunneled subcutaneously to an incision in the neck, and the submental and abdominal incisions were closed with absorbable sutures.

The rats were then placed in a stereotaxic frame (model 962, Kopf, Tujunga, CA) with blunt ear bars. To ensure consistent positioning between animals the flat skull position was achieved with an alignment tool (model 944, Kopf). Two multistranded stainless steel wires were sutured onto the dorsal neck muscles to record the neck electromyogram (EMG). To record the cortical electroencephalogram (EEG), two stainless steel screws attached to insulated wire (30 gauge) were implanted in the skull. One of the screws was implanted 2 mm anterior and 2 mm to the right of bregma, whereas the other was implanted 3 mm posterior and 2 mm left of bregma (1, 47, 48). A similar screw that served as a ground electrode was implanted adjacent to the sagittal suture ~8 mm anterior to bregma on the nasal bone. Finally, a small hole was drilled at the junction of interparietal and occipital bones for placement of the microdialysis guides (CMA/11, Acton, MA). The guides were lowered 13.96 ± 0.04 mm posterior to bregma, 7.37 ± 0.19 mm ventral to bregma, and 0.30 mm lateral to the midline for the obese rats, and they were implanted 14.00 ± 0.00 mm, 7.14 ± 0.32 mm, and 0.30 mm, respectively, for the lean rats. At these coordinates the guides were targeted 3 mm above the HMM (1, 47). A dummy cannula was placed inside the microdialysis guide to keep it patent until the day of the experiment. At the end of surgery, all the electrodes were connected to pins and inserted into a miniature plug (STC-89PI-220ABS, Carleton University, Ottawa, ON, Canada). The plug and microdialysis guides are affixed to the skull with dental acrylic and anchor screws.

After surgery, the rats were transferred to a clean cage and kept warm under a heating lamp until full recovery as judged by normal locomotor activity, grooming, drinking, and eating. The rats were given soft food for the first day after surgery. The rats recovered for an average of 9.3 ± 0.7 days before the experiments.

Recording Procedures

For purposes of habituation the rats were placed in the recording chamber (MD-1500, BAS, West Lafayette, IN) with fresh bedding, food, and water on the evening before the experiments. The recording chamber was placed inside an electrically insulated, sound-attenuated cubicule (EPC-010, BR/S/EVE, Laurel, MD). For recordings, a light-weight electrically shielded cable was connected to the plug on the rat’s head and was attached to a counterbalanced swivel that permitted free movement. A video camera allowed continuous visual monitoring without disrupting the rat. The chamber was covered with a customized lid with a center hole that allowed passage of the cable carrying the electrical signals and microdialysis tubing. The volume inside the rat chamber was ~20.5 liters.

Air or CO2 mixtures (see below) were delivered to the chamber at a flow rate of 5 l/min after humidification over a water reservoir (16, 47, 48). To disperse air as it flowed into the chamber, the lid contained three inlet ports equipped with small computer fans (model FP-108GD; 12 V, Commonwealth). Measurements of sleep-wake states and respiratory muscle activities were made during both room air and CO2-stimulated breathing; 7.5% inspired CO2 was chosen because this normally provides robust GG stimulation in awake and sleeping rats (16, 47, 48) and is sufficient to overcome the strong vagal inhibition of GG muscle in the intact rat (2, 16). Some evidence also suggests that 5-HT raphe neurons are activated by CO2 (52, 54) and may also contribute to GG activation in hypercapnia, at least in reduced preparations (7, 46), although this notion has recently been
questioned from studies in intact animals in vivo (48). The CO₂ concentration inside the rat chamber was measured continuously (Beckman LB-2) at a sample flow rate of 500 ml/min with the sampled air returned to the chamber.

The electrical signals were amplified and filtered (Super-Z head-stage amplifiers and BMA-400 amplifiers/filters, CWE, Ardmore, PA). The GG and diaphragm signals were recorded at the same amplification across all experiments. The EEG signal was filtered between 1 and 100 Hz while the GG, neck, and diaphragm EMGs were filtered between 100 and 1,000 Hz. The electrocardiogram was removed from the diaphragm EMG using an oscilloscope and electronic blanker (model SB-1, CWE). The moving-time averages (time constant = 200 ms) of the EMGs were also obtained (model MA-821, CWE). All signals were recorded on computer (Spike 2 software, 1401 interface, CED, Cambridge, UK).

**Microdialysis**

The rats were gently restrained while the internal cannula was removed from the guide and the microdialysis probe (CMA/11 14/01) was inserted. The probes were 240 µm in diameter with a 1-mm cuprophane membrane and a 6,000-Da cutoff. The probes projected 3 mm from the tip of the guide and were targeted to the HMN. In each rat a transient burst of GG activity was recorded when the probe was initially inserted and penetrated the HMN, and this was useful as a preliminary confirmation of probe placement, and did not occur in the neck or diaphragm signals (1, 47).

The probes were connected to Teflon tubing (inside diameter = 0.12 mm) and 1.0 ml syringes via a zero-dead-space switch (Uniswitch, BAS, West Lafayette, IN). During the experiments the probes were continually flushed with artificial cerebrospinal fluid (ACSF) at 2.1 µl/min using a syringe pump and controller (MD-1001 and MD-1020, BAS). The composition of ACSF (mM) was 125 NaCl, 3 KCl, 1 K₂HPO₄, 2 CaCl₂, 1 MgSO₄, 25, NaHCO₃, and 30 d-glucose (30), with the ACSF made fresh on the day of each experiment. The ACSF was bubbled with CO₂ to a pH of 7.39 ± 0.01 for both the lean and obese rats. The experiments began the morning after insertion of the microdialysis probes and were performed during the day when the rats normally sleep.

**Protocol**

During microdialysis perfusion of ACSF into the HMN, recordings were made across sleep-wake states during both room air breathing and hypercapnia (see above). These measurements were made during a 24-h period before the perfusion medium was switched to mianserin dissolved in ACSF (mianserin HCl formula weight = 300.8, Sigma). In the presence of mianserin, recordings were also performed during room air breathing and hypercapnia, again for a 24-h period. Mianserin is a broad-spectrum 5-HT receptor antagonist with affinity for 5-HT₂ receptors (56), i.e., those implicated in mediating the excitatory effects at the HMN (9, 10, 23, 36). Mianserin was applied at a dose of 100 µM. Importantly, our laboratory has shown using the exact same methodology that 100 µM mianserin at the HMN in anesthetized rats produces robust decreases in GG activity during both room air and hypercapnia (46), i.e., showing the efficacy of the interventions. Our laboratory has also shown that similar effects to mianserin are observed with the more specific 5-HT₂ receptor antagonist MDL-100907 at the HMN in intact conscious rats (47). However, MDL-100907 is less cost effective than mianserin for studies requiring continual perfusion in intact rats. Mianserin was also chosen because we had difficulty in dissolving other more specific 5-HT receptor antagonists such as ritanserin in ACSF (53). The mianserin solution was applied at a pH of 7.41 ± 0.01 and 7.40 ± 0.01 for the obese and lean rats, respectively, i.e., similar to that of the ACSF controls (see above).

**Data Analysis**

The data were analyzed in consecutive 5-s time bins. The GG, diaphragm, and neck EMG signals were analyzed from the respective moving-time average signals (above electrical zero) and were quantified both in arbitrary units and the percentage of maximum activity recorded during the experiments for each individual rat. Electrical zero was measured as the voltage recorded with the amplifier inputs grounded. The GG and diaphragm signals were analyzed by breath, which corresponded to ~7–12 breaths for each 5-s epoch. For each breath, the analysis of the GG EMG was time locked to breathing as defined by the peak and trough of the diaphragm signal. For each breath, GG activity was quantified as mean tonic activity (i.e., basal activity in expiration), peak activity and phasic respiratory activity (i.e., peak inspiratory activity — tonic activity), and average values for these measures of GG activity were calculated. Average diaphragm amplitudes (i.e., phasic respiratory diaphragm activity) and respiratory rates were also calculated for the breaths in each 5-s time bin. Neck muscle activity was quantified as the mean signal over each 5-s period. These average values of GG, diaphragm, and neck EMGs for each 5-s period were then taken to a spreadsheet for later correspondence with the prevailing sleep-wake state, level of respiratory stimulation, and drug at the HMN. Each rat served as its own control with all interventions performed in one experiment, therefore allowing for consistent effects of experimental condition (e.g., ACSF and mianserin) to be observed across sleep-wake states within and between rats. The EMG signals were recorded at the same amplifications across all experiments. Data were analyzed at least 60 min after a switch between drugs.

Sleep-wake states were identified by visual inspection and were classified into wakefulness, non-rapid eye movement (REM) and REM sleep using standard criteria (16, 28, 29, 47, 48). Periods of quiet wakefulness were characterized by the lack of overt body movements, identified from the EMG signals and video recordings, in contrast to active wakefulness that included overt behaviors such as grooming and exploring. Such periods of wakefulness were differentiated because 5-HT caudal raphe neurons are thought to be activated more during motor tasks associated with such behaviors (18, 39), and hence may be more responsive to the effects of 5-HT receptor antagonism at those times, whereas the 5-HT raphe inputs may have a lesser role in modulating the respiratory component of GG activity during basal breathing (44). Only those epochs comprising at least 30 s of uninterrupted sleep without arousal, or wakefulness without drowsiness, were included in the analysis. The data are presented as means of all 5-s epochs of a particular sleep or awake state. To minimize bias in selecting periods, the EEG and neck EMG were scored for sleep-wake states without reference to the GG or diaphragm signals.

**Function of GG Electrodes and Histology**

At the end of the experiments the rats were reanesthetized with ketamine and xylazine as described above, and the GG electrodes were again stimulated to confirm tongue protrusion. In all rats, the electrodes were in place from the beginning to the end of the study. To ensure that the lesions were confined to the intended areas, the rats were again stimulated to confirm tongue protrusion. In all rats, the GG electrodes were included in the analysis. The data are presented as means of all 5-s epochs of a particular sleep or awake state. To minimize bias in selecting periods, the EEG and neck EMG were scored for sleep-wake states without reference to the GG or diaphragm signals.

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### Statistical Analysis

For all comparisons, differences were considered significant if the null hypothesis was rejected at $P < 0.05$ using a two-tailed test. Statistics were performed using analysis of variance with repeated-measures (ANOVA-RM) and post hoc $t$-tests performed using Bonferroni-corrected $P$ values. All data are expressed as means ± SE. Analyses were performed using Sigmasstat (Jandel Scientific, San Rafael, CA).

### RESULTS

In the group of obese rats, a total of 31,854 5-s epochs (i.e., a total of 44.2 h of data) were included in the analysis, of which 25,939 epochs (81.4%) were from periods of wakefulness, 5,402 epochs (17.0%) were from non-REM sleep, but only 513 epochs (1.6%) were from REM sleep. Similarly, in the group of lean rats, a total of 24,697 5-s epochs (i.e., 34.3 h of data) were included in the analysis, of which 19,154 epochs (77.6%) were from wakefulness, 5,039 (20.4%) were from non-REM sleep, and only 504 (2.0%) were from REM sleep. Because of this low amount of data obtained in REM sleep in both the lean and obese rats, data analysis was restricted to wakefulness and non-REM sleep.

### Function of GG Electrodes and Histology

The GG electrodes were in place and functional throughout the study. Stimulation of the GG electrodes at the end of the experiment showed that the voltages required to cause tongue movements were not different from at the time of surgery ($0.92 ± 0.14$ vs. $0.93 ± 0.13$ V for the lean rats, and $0.90 ± 0.14$ vs. $0.87 ± 0.13$ V for the obese rats; both $P > 0.610$,

![Fig. 1. Example and group data showing location of the microdialysis probes. A: histological section showing an example of a lesion site made by the microdialysis probe immediately adjacent to both hypoglossal motor nuclei (HMN). Also shown are the distribution of individual microdialysis sites from all lean (B) and obese rats (C). The sizes of the bars represent the apparent size of the lesions from the histological sections. AP, area postrema; Cer, cerebellum; Sol, nucleus of the tractus solitarius; 4V, fourth ventricle.](image-url)
paired t-tests). Similarly, the voltages required to cause tongue movements were similar for lean compared with obese rats ($P > 0.740$, unpaired t-test).

Figure 1A shows an example of a lesion site made by the microdialysis probe in the HMN. The locations of all the microdialysis sites from each of the lean and obese rats are shown in Fig. 1, B and C, respectively. In all the experiments, the microdialysis sites were within the HMN or in the midline immediately adjacent to both the nuclei.

Breathing Pattern in Obese and Lean Rats

There was no evidence for any sleep-disordered breathing in the obese rats (e.g., no recurring diaphragm recruitment or periodic disturbances in sleep associated with abnormal breathing episodes). Figure 2 shows examples of normal breathing during sleep in the lean and obese Zucker rats.

Figure 3 shows values of respiratory variables recorded in the lean and obese Zucker rats in active wakefulness, quiet wakefulness, and non-REM sleep. There was a statistically significant difference in respiratory rate between the lean and obese Zucker rats ($P = 0.040$, 2-way ANOVA-RM) that did not depend on sleep-wake state ($P = 0.417$), i.e., occurred across all states. Post hoc analysis confirmed that respiratory rate was increased in the obese vs. lean Zucker rats in both wakefulness and sleep ($P = 0.048$, post hoc unpaired t-test; Fig. 3A). As shown in Fig. 3, B and C, this increased respiratory rate was due to reduced expiratory time in the obese compared with the lean rats ($P = 0.007$, post hoc unpaired t-test after 2-way ANOVA-RM showed differences between groups, $P = 0.005$). Inspiratory time was not different between the lean and obese rats ($P = 0.588$, 2-way ANOVA-RM).

The amplitude of the diaphragm EMG signal was not statistically different between the lean and obese Zucker rats whether analyzed as arbitrary units ($P = 0.632$, 2-way ANOVA-RM, Fig. 3D) or the percentage of maximum of diaphragm activity (Fig. 3F; $P = 0.782$, 2-way ANOVA-RM). This lack of effect persisted even if diaphragm amplitude was normalized for body weight ($P = 0.111$, 2-way ANOVA-RM), a normalization procedure routinely applied to the tidal volume signal in studies with noninvasive plethysmography (32). The maximum level of diaphragm activity recorded during the experiments was also similar between the lean and obese Zucker rats ($P = 0.696$, unpaired t-test). Diaphragm minute activity (i.e., diaphragm amplitude/respiratory rate, the surrogate for neural ventilation) was not significantly different between groups when analyzed as arbitrary units or the percent of maximum diaphragm activity ($P = 0.168$ and 0.661, respectively, 2-way ANOVA-RM). Similarly, this lack of effect persisted even if the diaphragm minute activity was also normalized for body weight ($P > 0.128$, 2-way ANOVA-RM).

GG Muscle Activity During Wakefulness and Non-REM Sleep in Obese and Lean Zucker Rats

Figure 4 shows examples of GG muscle activities recorded in an obese and lean Zucker rat during room air and CO$_2$-stimulated breathing in quiet wakefulness, in the presence of
ACSF and mianserin at the HMN. Note the presence of prominent tonic GG activity during room air breathing in wakefulness as well as modest respiratory-related GG activity, which is increased by hypercapnia, i.e., patterns typical of previous studies (16, 28, 29, 48). Also note that GG activity is not decreased by 5-HT receptor antagonism at the HMN.

Figure 5 shows group mean GG muscle activity recorded in the lean and obese Zucker rats in active wakefulness, quiet wakefulness and non-REM sleep. The data in Fig. 5 confirm that GG muscle activity across sleep-wake states was not increased in the obese rats compared with lean rats, and it even had a tendency to be decreased; i.e., there was no evidence for increased GG activity as stated in the hypothesis. Statistical analysis showed that respiratory-related, tonic and peak GG activities, whether expressed as arbitrary units (Fig. 5, A–C) or the percent of maximum (Fig. 5, D–F), showed robust changes across sleep-wake states (all \( P \leq 0.001 \), 2-way ANOVA-RM). However, although there was a tendency for GG activity to be decreased in the obese compared with the lean rats this difference was not statistically significant whether analyzed as arbitrary units or the percent of maximum (all \( P > 0.227 \), 2-way ANOVA-RM). The maximum level of GG activity recorded during the experiments was also similar between the lean and obese Zucker rats (\( P = 0.641 \), unpaired \( t \)-test).

5-HT Receptor Antagonism at the HMN on GG Activity in Obese and Lean Zucker Rats

Figure 6 shows GG muscle activity recorded in the lean and obese Zucker rats with 5-HT receptor antagonism at the HMN during both room air and CO\(_2\)-stimulated breathing. Data are shown for respiratory-related, tonic, and peak GG activities, both from the raw signal (Fig. 6, A–C, respectively), as well as the percent of the maximum (Fig. 6, D–F, respectively). These data illustrate that there was no evidence for suppression of GG activity with 5-HT receptor antagonism at the HMN in either the lean or obese rats. Statistical analysis confirmed that there was no significant difference in GG activity between ACSF and mianserin at the HMN for any comparison (i.e., when analyzed as arbitrary units or the percent of maximum) except for a significant effect on respiratory-related GG activity in non-REM sleep (asterisk in Fig. 6, A and D), and
tonic and peak GG activities in quiet wakefulness in the obese rats (asterisk in Fig. 6, E and F) (all \( P < 0.05 \) from 2-way ANOVA-RM). For these latter specific comparisons, however, GG activity was increased in the presence of 5-HT receptor antagonism (all \( P < 0.05 \), post hoc paired \( t \)-test), i.e., in the opposite direction predicted by the hypothesis, and a direction of change also observed in a previous study (47).

**DISCUSSION**

The results of the present study show that GG activity across sleep-wake states was similar between the lean and obese Zucker rats, and there was no evidence for an augmented 5-HT drive to the HMN. Similarly, there was no evidence of sleep-disordered breathing in these obese rats. Overall, these results suggest that despite the upper airway narrowing in obese Zucker rats (5, 24, 32, 35) these animals have a sufficiently stable upper airway such that pharyngeal muscle activity is normal across sleep-wake states. Accordingly, the obese Zucker rat appears to be a model most suited to investigations of the impact of obesity on upper airway mechanics (5, 32, 35) and ventilation (8), rather than OSA per se or neuromuscular compensation for a narrow airway as occurs in humans (26, 50).

**Methodological Considerations**

Data across sleep-wake states. Data analysis was restricted to periods of wakefulness and non-REM sleep because of the practical constraint of low amounts of data obtained in REM sleep. This relative lack of REM sleep, unlike our laboratory’s previous studies (1, 6, 16, 47, 48), was probably because the protocol was limited to measurements in a 2.5-h period in each rat to accommodate each drug with both room air and hypercapnia. We did not wait for multiple REM episodes to occur in a longer protocol because REM sleep is also limited by hypercapnia (16). The percentage of data obtained in wakefulness was also higher than in our laboratory’s previous studies, again likely because of the use of CO2 (16) or because these animals were older, to be in the same age range as a relevant previous study in obese Zucker rats (32). Nevertheless, sufficient data were obtained in wakefulness and non-REM sleep for the purposes of this study, especially to address the potential role of 5-HT receptor mechanisms in modulating GG activity.

Quantification of EMG activity. Because comparisons of EMG activity between groups of lean and obese animals using the raw signal may theoretically be complicated by the possibility of fat acting as an electrical insulator, potentially reducing EMG activity, the GG and diaphragm signals were ana-
analyzed both in arbitrary units and normalized to the percentage of maximum activity recorded during the experiments. We did not rely solely on the normalized signal for the analyses, however, because unlike humans in whom behavioral tongue activity can be standardized for comparisons between groups, e.g., by maximum voluntary protrusion (26), maximum activity in such a freely behaving animal model cannot be controlled and so data were analyzed also in arbitrary units. Analysis showed that regardless of the method used to quantify muscle activity, GG and diaphragm activity was similar between the obese and lean Zucker rats across sleep-wake states, and there was no evidence for a decrease in GG activity with 5-HT receptor antagonism at the HMN. This lack of difference in GG activity between lean and obese rats, with or without 5-HT receptor antagonism, was not an artifact due to a lesser ability to generate muscle tone in either group because maximum GG activity was also similar between lean and obese rats, as was maximum diaphragm activity. In addition, although tongue movements in response to electrical stimulation of the GG wires were only identified visually, it was noted that the voltages required to cause such movements were similar for the lean and obese rats.

Respiratory Motor Activity in Lean Obese Rats

The obese Zucker rats showed increased respiratory rates, compared with lean animals, because of decreased expiratory times (Fig. 3). The increased respiratory rate is in agreement with a previous study using the same age and weights of rats purchased from the same source (32). Interestingly, both tidal volume and overall lung ventilation were reduced in the obese Zucker rats when quantified by noninvasive plethysmography (32), whereas measures of diaphragm muscle activity and diaphragm minute activity (a surrogate for neural ventilation) were similar to lean controls when measured by EMG, either quantified as the raw activity or the percent of maximum. This result suggests that obesity imposes limitations to the mechanical consequences of respiratory muscle activation in these animals that leads to reduced tidal volumes for a given diaphragm activation (8, 32), an effect that is offset (at least in part) by increased respiratory rates (32). Similar changes in the pattern of breathing pattern are also observed in genetically obese mice (34, 51).

Although the accuracy of tidal volume measurements by whole body plethysmography in rats has been questioned, measurements of respiratory rate are accurate with this technique (31). Of note, therefore, the average respiratory rates of 140–160 breaths/min reported for lean and obese Zucker rats in the previous study investigating the role of systemically administered 5-HT receptor antagonists in modulating breathing (32) are high compared with the average respiratory rates of 101–120 breaths/min observed in quiet or active wakefulness in the present study (Fig. 3). In the former study, the rats were monitored in a small (4 liters) plethysmography chamber and within a restrainer that did not permit backward rotation (32). This restricted environment may have contributed to the elevated respiratory rates in that study. In comparison, the rats in the present study were placed in a larger (20.5 liters) chamber in which they were able to move around and sleep freely, habituated overnight and were free from interruption. The potential for a more aroused animal in the former study is relevant because it is under those conditions that systemic administration of ritanserin increased the work of breathing (as indicated by oxygen consumption) and decreased ventilation measured by noninvasive plethysmography (32). Although muscle activity was not measured in those animals (32), the data were taken to suggest that the increase in work of breathing was due to an effect at the upper airway. However, the principal effect of ritanserin in reducing ventilation in those...
awake rats was via reduced respiratory rate (32), suggesting an effect independent of the upper airway. In addition, the increases in upper airway closing pressures in anesthetized rats after 5-HT receptor antagonism occurred in both lean and obese rats and were small in magnitude (<1 cmH2O), even in the supine position (32). A reinterpretation of the previous observations in conscious rats (32), especially in the context of the present experiments, would suggest that the reduced respiratory rate in those animals by systemically administered ritanserin could have been due to a sedating or anxiety-reducing effect (27, 43) rather than an effect at upper airway dilator muscles.

**GG Activity and 5-HT Receptor Antagonism**

GG activity was similar between the lean and obese Zucker rats regardless of whether GG activity was quantified as the raw signal or percentage of maximum activity. Importantly, there was no evidence of any augmented GG activity in the obese rats (Fig. 5) to indicate a neuromuscular compensation for a narrow airway as occurs in humans (26). Magnetic resonance imaging has shown that upper airway size increases during inspiration in obese and lean anesthetized Zucker rats, but it was less so in the obese animals (5). Simultaneous measurements of pharyngeal wall tissue strain with noninvasive tissue tagging led to the suggestion that obesity imposes a mechanical load that prevents a normal airway response to a given change in pharyngeal wall tissue strain (5). The observation of similar levels of GG activity in obese and lean animals in the present study is consistent with this suggestion.

From studies in reduced preparations, withdrawal of 5-HT has been hypothesized to be a principal mechanism underlying decreased GG activity in sleep (21, 23). However, in vivo studies in conscious rats show that despite robust GG activation with delivery of 5-HT to the HMN (19), endogenous 5-HT plays a minimal role in the normal modulation of GG activity (47). Inhibition of serotonergic medullary raphe neurons, the source of 5-HT inputs to the HMN, confirmed this result (48). The results of the present study support this concept of a minimal role for endogenous 5-HT receptor mechanisms at the HMN in the normal modulation of GG activity across sleep.

![Fig. 6. Group mean data showing levels of GG activity in lean and obese Zucker rats with ACSF or mianserin at the HMN (open and gray bars, respectively). Data are shown for AW, quiet QW, and NREM sleep during both room air and CO2-stimulated breathing. GG is quantified as respiratory-related, tonic, and peak GG activity, and shown both for the raw signal (A, B, and C, respectively) and the percent of activity maximum (%max; D, E, and F, respectively). See text for further details. Values are means ± SE. Data were lacking for NREM sleep in the presence of hypercapnia in lean rats, so these data are not included. *P < 0.05 for mianserin compared with ACSF controls.](http://jap.physiology.org/)
wake states (47, 48), even in obese rats with an anatomically narrow airway (5, 24). At first glance, these recent results may seem to contradict the several previous studies in anesthetized or decerebrate animals in which an endogenous 5-HT drive to the HMN was first demonstrated (7, 10, 22, 23, 55), including our laboratory’s studies in anesthetized rats (46, 48) that used the exact same methodology as the studies in conscious rats (47, 48). However, those previous studies demonstrating an endogenous 5-HT drive to the HMN were all performed in the presence of vagotomy (7, 10, 22, 23, 46, 55), which was subsequently shown to augment the role of 5-HT at the HMN (47, 48). In contrast to the lack of influence of endogenous 5-HT mechanisms modulating GG activity, recent studies in reduced (11) and conscious (6) rats suggest an important role for noradrenergic mechanisms operating at the HMN across sleep-wake states.

It was also noted, however, that GG activity could be increased after mianserin (i., in contrast to the hypothesized decrease), and this was statistically significant for some of the comparisons shown in Fig. 6. Although studies in a larger number of animals may have strengthened the validity of this observation, this direction of change is also consistent with previous experiments using both mianserin and the more specific 5-HT2 receptor antagonist MDL-100907 at the HMN (47, 48). These observations strengthen the case that there is a minimal endogenous excitatory 5-HT drive operating at the HMN to increase GG activity, and that the control by 5-HT of hypoglossal motoneurons may not be as straightforward as first presumed. For example, in vitro evidence suggests that a major component of the raphe inputs to hypoglossal motoneurons are glutamatergic with 5-HT inhibiting the release of this excitatory neurotransmitter via presynaptic effects (4).

If relevant to humans, the lack of a significant role of endogenous 5-HT in modulating pharyngeal muscle activity across sleep-wake states may also help explain the lack of clinically relevant responses to selective 5-HT reuptake inhibitors in OSA (3, 13, 20). Nevertheless, there is one animal model where 5-HT appears relevant to augmenting pharyngeal muscle tone and increasing upper airway stability. Pharyngeal dilator muscle activity is diminished by systemic administration of the 5-HT receptor antagonist ritanserin in awake bulldogs, and this effect causes significant upper airway narrowing (53). It remains to be determined whether this latter effect is mediated by antagonism of an augmented 5-HT drive to pharyngeal motoneurons that these dogs require to compensate for a narrow air space (53). However, the airway narrowing may also be due to antagonism of the afferent relay mechanisms that mediate the neural compensatory reflex response for pharyngeal muscle activation, e.g., via an influence of ritanserin on the 5-HT inputs to the nucleus of the solitary tract, inputs which are also state dependent (55).

GRANTS

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REFERENCES


