

# Differential Effects of Isoflurane and Propofol on Upper Airway Dilator Muscle Activity and Breathing

Matthias Eikermann, M.D., Ph.D.,\* Atul Malhotra, M.D.,† Philipp Fassbender, M.D.,‡ Sebastian Zaremba,‡ Amy S. Jordan, Ph.D.,§ Shiva Gautam, Ph.D.,|| David P. White, M.D.,# Nancy L. Chamberlin, Ph.D.\*\*

**Background:** Anesthesia impairs upper airway integrity, but recent data suggest that low doses of some anesthetics increase upper airway dilator muscle activity, an apparent paradox. The authors sought to understand which anesthetics increase or decrease upper airway dilator muscle activity and to study the mechanisms mediating the effect.

**Methods:** The authors recorded genioglossus electromyogram, breathing, arterial blood pressure, and expiratory carbon dioxide in 58 spontaneously breathing rats at an estimated ED<sub>50</sub> (median effective dose) of isoflurane or propofol. The authors further evaluated the dose-response relations of isoflurane under different study conditions: (1) normalization of mean arterial pressure, or end-expiratory carbon dioxide; (2) bilateral lesion of the Kölliker-Fuse nucleus; and (3) vagotomy. To evaluate whether the markedly lower inspiratory genioglossus activity during propofol could be recovered by increasing flow rate, a measure of respiratory drive, the authors performed an additional set of experiments during hypoxia or hypercapnia.

**Results:** In vagally intact rats, tonic and phasic genioglossus activity were markedly higher with isoflurane compared with propofol. Both anesthetics abolished the genioglossus negative pressure reflex. Inspiratory flow rate and anesthetic agent predicted independently phasic genioglossus activity. Isoflurane dose-dependently decreased tonic and increased phasic genioglossus activity, and increased flow rate, and its increasing

effects were abolished after vagotomy. Impairment of phasic genioglossus activity during propofol anesthesia was reversed during evoked increase in respiratory drive.

**Conclusion:** Isoflurane compared with propofol anesthesia yields higher tonic and phasic genioglossus muscle activity. The level of respiratory depression rather than the level of effective anesthesia correlates closely with the airway dilator muscle function during anesthesia.

UPPER airway patency depends on an appropriate balance between the dilating force of pharyngeal muscles and the collapsing force of negative intraluminal pressure, which is generated by respiratory “pump” muscles. The genioglossus protects pharyngeal patency in humans. This muscle receives various types of neural drive, including a phasic (in phase with inspiration) and tonic (expiratory) drive, distributed differentially across the hypoglossal motoneuron pool.<sup>1</sup> In addition, reflex genioglossus activation in response to negative pharyngeal pressure stabilizes upper airway patency both in humans<sup>2</sup> and in rats.<sup>3</sup> General anesthetic agents,<sup>4-9</sup> including propofol<sup>7,9</sup> and isoflurane,<sup>8</sup> can predispose the upper airway to collapse, partly by decreasing upper airway muscle activity.<sup>4-7</sup> In contrast, recent data suggest that anesthetics can increase genioglossus phasic activity,<sup>10,11</sup> an apparent paradox. It is not clear which of these disparate observations—activation *versus* inhibition of upper airway dilator function—are anesthetic type/agent dependent or dependent on the study conditions used.<sup>4,10,11</sup> Understanding the mechanism by which anesthetics activate or inhibit upper airway dilator muscle activity is fundamental to their safe use in settings where upper airway patency is at risk, *e.g.*, during conscious sedation.

In theory, anesthetics could affect upper airway dilator activity by several mechanisms, including the following. First, in humans, anesthetics induce a dose-dependent decrease in hypercapnic and hypoxic ventilatory drive.<sup>12-14</sup> Because the activity of hypoglossal motoneurons is in part respiratory related,<sup>4</sup> a decrease in ventilatory drive could result in a suppression of hypoglossal motor activity.<sup>4</sup> Second, anesthetics depress hypoglossal motoneurons,<sup>15,16</sup> both directly and possibly indirectly by enhancing the activity of the inhibitory neurotransmitters  $\gamma$ -aminobutyric acid and glycine,<sup>17</sup> receptor mechanisms that are tonically active at the hypoglossal motor nucleus.<sup>18</sup> Third, animal studies have revealed barbiturates and volatile anesthetics can decrease skeletal muscle contractility directly by a variety of cellular mechanisms.<sup>19,20</sup> Fourth, anesthetics decrease arterial blood pressure,<sup>21</sup> a condition that has been shown to activate phasic geno-

Additional material related to this article can be found on the ANESTHESIOLOGY Web site. Go to <http://www.anesthesiology.org>, click on Enhancements Index, and then scroll down to find the appropriate article and link. Supplementary material can also be accessed on the Web by clicking on the “ArticlePlus” link either in the Table of Contents or at the top of the Abstract or HTML version of the article.

\* Assistant Professor, Department of Anesthesia, Massachusetts General Hospital, and Harvard Medical School; Universitätsklinikum Essen, Klinik für Anästhesie und Intensivmedizin, Essen, Germany. † Assistant Professor, § Instructor, # Gerald E. McGinness Professor of Sleep Medicine, Divisions of Sleep Medicine and Pulmonary/Critical Care, Brigham and Women’s Hospital and Harvard Medical School, Boston, Massachusetts. ‡ Research Associate, Divisions of Sleep Medicine and Pulmonary/Critical Care, Brigham and Women’s Hospital and Harvard Medical School, Boston, Massachusetts; Department of Neurology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts. || Associate Professor, \*\* Assistant Professor, Department of Neurology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts.

Received from the Department of Anesthesia, Massachusetts General Hospital, and Harvard Medical School, Boston, Massachusetts. Submitted for publication August 17, 2007. Accepted for publication January 11, 2008. Supported by grant Nos. P50 HL060292, R01-HL73146, and AG024837 from the National Institutes of Health, Bethesda, Maryland, and Organon International Inc., Roseland, New Jersey. Organon did not participate in generation of the protocol, data analysis and interpretation, or writing. Presented as a talk at the Annual Meeting of the American Society of Anesthesiologists, San Francisco, California, October 14, 2007.

Address correspondence to Dr. Eikermann: Department of Anesthesia and Critical Care, Massachusetts General Hospital, Harvard Medical School, 55 Fruit Street, Boston, Massachusetts 02114-2696. [meikermann@partners.org](mailto:meikermann@partners.org). Information on purchasing reprints may be found at [www.anesthesiology.org](http://www.anesthesiology.org) or on the masthead page at the beginning of this issue. ANESTHESIOLOGY’s articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

glossus activity.<sup>22</sup> Fifth, the vagus nerve is important for mediating the interplay between lung volume and upper airway muscle activity,<sup>23,24</sup> and isoflurane may have vagolytic effects.<sup>25,26</sup> Finally, it has recently been suggested that volatile anesthetics can increase phasic hypoglossal nerve discharge by altering neuronal activity in the Kölliker-Fuse region, which contains hypoglossal premotor motoneurons.<sup>10</sup>

Based on the observations of Roda *et al.*,<sup>10</sup> we tested the *a priori* hypothesis that phasic genioglossus muscle activity is higher during isoflurane anesthesia compared with propofol. We tested the secondary hypothesis that flow rate, a measure of respiratory drive, predicts the effects of anesthetics on phasic genioglossus activation. Third, with an exploratory intention, we sorted out the above mentioned variables which could mediate the effects of isoflurane at the genioglossus<sup>4,10,21,22,25-28</sup> by controlling in subsets of experiments for carbon dioxide concentration, mean arterial blood pressure, Kölliker-Fuse neuron influence, or vagus nerve effects.

## Materials and Methods

Every effort was made to minimize the numbers of animals used and their suffering. All procedures involving animals were approved by the Institutional Animal Care and Use Committee at Harvard Medical School, Boston, Massachusetts. Additional information is available on the ANESTHESIOLOGY Web site at <http://www.anesthesiology.org>.

Fifty-eight adult male Sprague-Dawley rats (300–400 g; Harlan Sprague-Dawley, Indianapolis, IN) were used in these studies. After induction of anesthesia with isoflurane, electromyographic recording electrodes were inserted into the genioglossus (one on each side of the midline by open surgery). The trachea was transected and cannulated with PE-240 tubing through which the rat spontaneously breathed and isoflurane was delivered. In a subset of six chronically instrumented rats, we did not perform tracheostomy but delivered isoflurane by facemask.

### Measurements and Data Analysis

Genioglossus electromyography signals were amplified (Grass Instruments, West Warwick, RI), filtered, moving time averaged (100 ms), and digitized by a computer. Signals were analyzed with Clampfit (Molecular Devices, Sunnyvale, CA) and Igor Pro (WaveMetrics, Inc., Lake Oswego, OR). Phasic genioglossus activity was defined as the entire burst that occurred in phase with each inspiration minus the nadir expiratory activity preceding it in the same respiratory cycle. Tonic genioglossus activity was defined as nadir genioglossus activity during expiration minus genioglossus activity measured in the dead rat after euthanasia (reflecting the degree of electrical noise).<sup>29</sup>

Negative pressure was delivered to the rats' isolated upper airway. For this purpose, the rats' nares were occluded by a plastic cap placed over their muzzle and sealed with glue. Negative pressure was then applied *via* this cap by gating a vacuum source with a solenoid valve. The magnitude of the applied pressure was measured by a pressure-sensitive catheter (Millar Instruments, Houston, TX) inserted by the tracheostomy into the rostral trachea, which we sealed up with a suture subsequently. For evaluation of the negative pressure reflex, the change in genioglossus amplitude during negative pressure was measured by taking the average of the second, third, and fourth breaths after the onset of negative pressure application (4-s duration, delivered at a 20-s duty cycle) and comparing it with the average during the three breaths just before negative pressure application. In each rat, we applied a stimulus that produced a consistent increase in genioglossus activity (fig. E1 of the Web Enhancement). The negative pressure stimulus was kept constant throughout the experiment.

End-tidal carbon dioxide was measured by a CAPSTAR-100 Carbon Dioxide Analyzer (CWE Inc., Ardmore, PA), and intratracheal gas sampling was used to measure isoflurane gas concentration, as described by Pajewski *et al.*<sup>30</sup> Samples were obtained consecutively in duplicate to ensure constant alveolar concentration, and averaged values were used for analysis. Gas samples were assayed using a side-stream infrared analyzer (Capnomac II; DATEX Instrumentarium Corp., Helsinki, Finland). Blood gas analyses of arterial blood samples were performed immediately after blood samples were taken (OPTI CCA-TS; Osmetech, Roswell, GA).

Depth of anesthesia was determined by assessing responses to tail clamping. A clamp was applied to the base of the tail and oscillated (1–2 Hz) for up to 1 min or until the rat displayed gross and purposeful movement.<sup>31</sup>

### Protocols

The protocols applied in this study are depicted in figure 1.

**Protocol 1.** After surgery, we compared the effects of isoflurane (Baxter Healthcare, Deerfield, IL;  $n = 12$ ) or propofol (AstraZeneca Pharmaceuticals, Wilmington, DE;  $n = 6$ ) on genioglossus function.

**Isoflurane-anesthetized Rats.** After surgery during isoflurane anesthesia, we determined the ED<sub>50</sub> (median effective dose) by applying a standardized noxious stimulus on the tail as previously described.<sup>32</sup> Briefly, tail clamping was applied and, depending on the response, the isoflurane concentration was increased or decreased by 0.2%. This procedure was repeated every 10 min until two sequential responses just permitted and just prevented movement.<sup>32</sup> This isoflurane level was taken as the ED<sub>50</sub>. Anesthesia was stabilized at this level for 45 min before measurement of genioglossus activity was performed during basal breathing. We then established a

Induction	Surgery	Intervention	Determination of ED50	Measurement 1	Titration of analgesia	Measurement 2	Titration of analgesia	Measurement 3
<b>Protocol 1:</b> - Isoflurane: n=12, 3 different doses. - Propofol: n=6, ED50 only.		None	<b>ED 50:</b> (Propofol: n=6, isoflurane: n=12)	Norm. breath.   NP   CO <sub>2</sub> 50mmHg		<b>Isoflurane:</b> 1 vol. % (n=12) Norm. breath.   NP   CO <sub>2</sub> 50mmHg		<b>Isoflurane 2% (n=6):</b> 2.25 % (n=6) Norm. breath.   NP   CO <sub>2</sub> 50mmHg
<b>Protocol 2</b> Isoflurane, n=35		Blood pressure normalized (vasopressin, n=6) Blood pressure normalized (phenylephrine, n=11) KF lesion (n=6) Vagotomy (n=6) Chronic instrumentation, 5 days recovery (n=6)	<b>ED 50 of isoflurane</b>	Normal Breathing   NP		<b>Isoflurane 1%</b>		<b>Isoflurane 2%</b>
<b>Protocol 3</b> Propofol, n=6		None	<b>Propofol ED50</b>	Normal breathing   hypoxia   hypercapnia		<b>Isoflurane 1%</b>		

**Fig. 1. Protocols.** Protocol 1: After surgery, we determined the ED<sub>50</sub> (median effective dose) of propofol or isoflurane to compare the effects of equianesthetic doses of these anesthetics on genioglossus activity and breathing. In isoflurane-anesthetized animals, we subsequently measured these variables at two additional dose levels, *i.e.*, 1.49 ± 0.01 (ED<sub>50</sub>) (n = 12) and either 2% or 2.25% (n = 6 each). Protocol 2: We evaluated the effects of different interventions on the dose–response relation of isoflurane at the genioglossus muscle and breathing. In a subset of 6 chronically instrumented animals, we compared the effect of light isoflurane anesthesia between wakefulness and light anesthesia (1% isoflurane). Protocol 3: We evaluated during propofol anesthesia the effects on genioglossus muscle electromyogram of conditions that increased respiratory drive (hypoxia and hypercapnia) to flow rate values that were similar to those observed during isoflurane anesthesia. CO<sub>2</sub> = carbon dioxide; KF = Kölliker-Fuse nucleus; NP = negative pharyngeal pressure application.

dose–response curve for isoflurane, beginning with a steady state concentration of 1.0%. Because we found that we could apply up to, but not more than, three different doses of isoflurane and still achieve full recovery of genioglossus activity and minute ventilation to baseline values, we then subdivided the group to apply to two subsets of six rats either 2% isoflurane (n = 6) or 2.25% isoflurane (n = 6) as the highest dose. In addition, at each concentration of isoflurane (1.49 ± 0.01, 1, and 2%) the genioglossus negative pressure reflex was measured, and outcome variables were also assessed at a standardized end-tidal carbon dioxide concentration of 50 mmHg (carbon dioxide insufflation). This level was chosen because it was the highest carbon dioxide level observed (at 2% isoflurane; fig. E2A of the Web Enhancement).

**Propofol-anesthetized Rats.** After induction of anesthesia and surgery with isoflurane, we reduced the level to 1% isoflurane for 30 min, and recorded genioglossus function during 1% isoflurane. We then discontinued isoflurane administration and started an infusion of propofol (500 μg · kg<sup>-1</sup> · min<sup>-1</sup>). After isoflurane had been discontinued for 45 min, we determined the ED<sub>50</sub> of propofol as described by Orth *et al.*<sup>31</sup>

**Protocol 2.** Because we had observed in protocol 1 that isoflurane increases phasic genioglossus activity in the 1- to 2-vol% range, we applied additional interventions in subsets of rats and analyzed their effect on the dose–response curve of isoflurane.

Isoflurane dose-dependently decreases arterial blood pressure, as depicted in figure E3 of the Web Enhancement. Therefore, mean arterial blood pressure was normalized to values observed at 1% isoflurane, *i.e.*, to a mean arterial pressure of 115 mmHg, by giving either phenylephrine (n = 11) or vasopressin (n = 6).

In six animals, we performed bilateral lesions of the Kölliker-Fuse neurons with orexin-saporin<sup>10</sup> and studied the effects of isoflurane on genioglossus function 7–10 days later. Kölliker-Fuse nuclei were assumed at the following coordinates: anterior–posterior plane: 8.8 mm posterior of bregma; dorsoventral plane: 6.6 mm ventral of the brain surface; right–left plane: 2.6 mm left and right of midline as coordinates of Kölliker-Fuse nucleus. Injections were performed during chloral hydrate anesthesia (350 mg/kg), and orexin-saporin (90 nl; Advanced Targeting Systems, San Diego, CA) was injected by using a fine glass pipette. Injection volumes were monitored by measuring the movement of the fluid meniscus with

an operating microscope equipped with an eyepiece reticule. The correct location of the Kölliker-Fuse neuron lesions was verified by *post hoc* histology (fig. E4 of the Web Enhancement).

In six rats, we performed an acute bilateral cervical vagotomy before studying the dose–response relation of isoflurane. After a medial cervical cutaneous incision, the vagus nerves were isolated from adjacent vascular structures and sectioned under a binocular microscope.

To compare the effects of isoflurane on genioglossus activity with unmedicated conditions, we chronically instrumented six rats with genioglossus electromyography electrodes.<sup>33</sup> After recording of genioglossus baseline activity during quiet wakefulness/sleep for 5 min, anesthesia was induced with isoflurane and stabilized over a period of 45 min to achieve an end-tidal isoflurane concentration of 1%.

**Protocol 3.** In another subset of experiments, which aimed to determine whether suppression of phasic genioglossus activity by propofol could be reversed by conditions that restore respiratory drive, we produced hypoxemia (inspiratory oxygen concentration of 0.15) or hypercapnia (by adding nitrogen or carbon dioxide to the inspired air) during propofol anesthesia (ED<sub>50</sub>). Hypoxemia and hypercapnia were applied to each rat in a random order. The degree of hypoxemia and hypercapnia was titrated to normalize genioglossus activity in each rat to the level observed previously during isoflurane (1 vol%) anesthesia. The effects of the intervention were quantified by arterial blood gas analyses taken before the evoked increase in respiratory drive and 180 s after the target level was set.

#### Statistical Analysis

The primary outcome was phasic genioglossus activity. We used the peak moving time average for statistical analysis, unless indicated otherwise. Rats studied in protocol 1 were included for testing the primary hypothesis, that phasic genioglossus activity is higher during isoflurane anesthesia compared with equianesthetic (ED<sub>50</sub>) propofol anesthesia. The independent sample *t* test was used for making the comparisons between groups. Based on the data of Roda *et al.*,<sup>10</sup> we calculated that a sample size of six rats per group would provide an 80% power to detect a difference in genioglossus activity between anesthetics with an  $\alpha$  error of 5%.

We tested the secondary hypothesis that effects of anesthetics on flow rate predict their effects on phasic genioglossus activation by testing three lines of evidence. In the first step, we tested whether flow rate differs between anesthetics given at an ED<sub>50</sub> (*t* tests, protocol 1). Subsequent statistical comparisons were made by using a linear mixed model. We used pooled data from all rats derived during ED<sub>50</sub> of isoflurane and propofol anesthesia (protocols 1, 2, and 3), and tested for an effect of the independent variables of flow rate

(continuous variable) and anesthetic agent (dichotomous variable) for their predictive value on the dependent variable of phasic genioglossus activity. Finally, we tested whether the interventions that increase respiratory drive, *i.e.*, the increase in isoflurane concentration from 1% to 2% (protocol 1); vagotomy in protocol 2; and hypercapnia or hypoxia in protocol 3 increase phasic genioglossus activity. With an exploratory intention, we tested for an interaction effect on isoflurane's dose–response relation of applied interventions, standardization of (1) blood pressure and (2) carbon dioxide, (3) Kölliker-Fuse nucleus lesion, and (4) vagotomy. Phasic genioglossus activity observed during these conditions (protocol 2) was compared with values observed under control conditions (protocol 1). We used SPSS 11.0 (SPSS Inc., Chicago, IL) and SAS 9.0 (SAS Institute, Cary, NC) for making the statistical analysis.

## Results

Fifty-eight rats were included in this study, and experiments were successfully completed in all but 1 rat. Accordingly, data from 57 rats are presented.

#### Primary Hypothesis: Comparison of the Effects of Different Anesthetics on Genioglossus Function

Data from 18 rats anesthetized with an ED<sub>50</sub> of isoflurane (*n* = 12) or propofol (*n* = 6) were analyzed (protocol 1). The ED<sub>50</sub> of isoflurane amounted to  $1.49 \pm 0.01$  vol%.

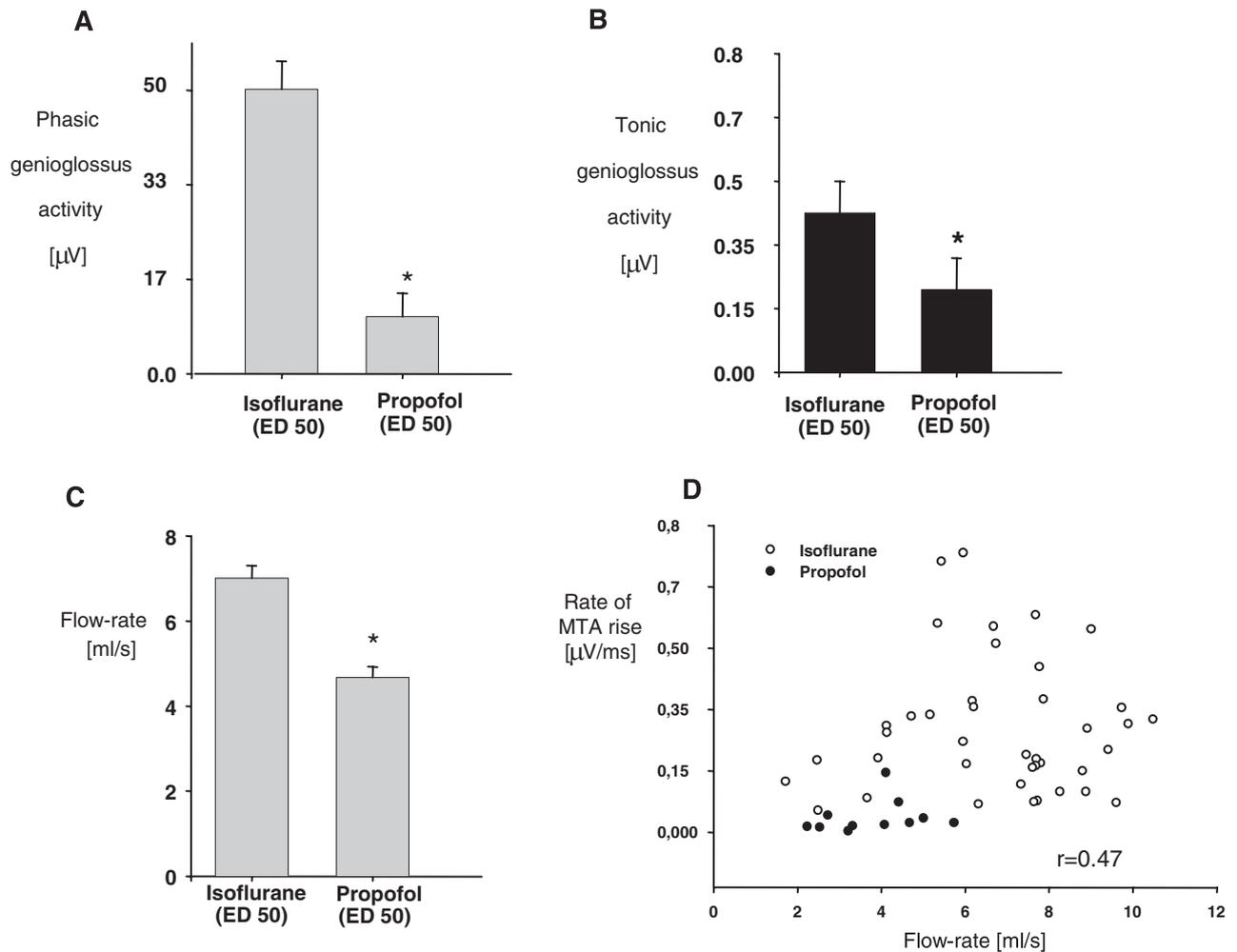
Phasic and tonic genioglossus activities were markedly and significantly higher during isoflurane anesthesia compared with an equivalent dose of propofol (infusion rate:  $880 \pm 41 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ; figs. 2A and B). At ED<sub>50</sub> of isoflurane and propofol, the genioglossus negative pressure reflex was eliminated.

End-tidal carbon dioxide was significantly lower in isoflurane-anesthetized rats compared with propofol, amounting to  $41 \pm 1$  versus  $81.8 \pm 2.6$  mmHg ( $P < 0.05$ ). Respiratory rate was significantly higher in isoflurane-anesthetized rats compared with propofol, amounting to  $80 \pm 2$  versus  $48 \pm 8.3$  breaths/min ( $P < 0.05$ ), and tidal volume tended to be higher ( $P = 0.08$ ), amounting to  $1.84 \pm 0.08$  versus  $1.48 \pm 0.05$  ml, respectively.

During ED<sub>50</sub> of propofol and isoflurane, heart rate ( $317 \pm 10$  vs.  $330 \pm 9$  beats/min) and mean arterial pressure ( $94 \pm 8.6$  vs.  $85 \pm 7.4$  mmHg) did not differ significantly between isoflurane- and propofol-anesthetized rats.

#### Secondary Hypothesis: Association between the Effects of Anesthetics on Flow Rate and Phasic Genioglossus Activity

Inspiratory flow rate (tidal volume/inspiratory time) was positively correlated with phasic genioglossus activ-



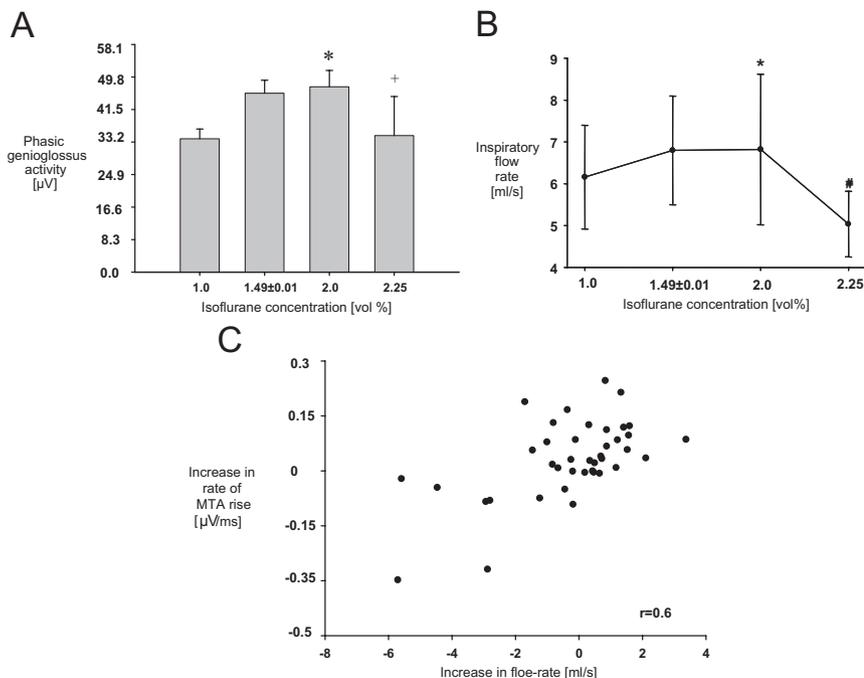
**Fig. 2.** Genioglossus activity and flow rate during isoflurane (ED<sub>50</sub> [median effective dose]) and propofol (ED<sub>50</sub>). Values are given in microvolts. \*  $P < 0.05$  versus isoflurane. **A–C:** Data from protocol 1 ( $n = 18$ ). **D:** Pooled data from protocols 1 and 2 ( $n = 46$ ). **(A)** Phasic genioglossus activity measured at time of assessment of genioglossus activity. Genioglossus activity was significantly lower during propofol anesthesia compared with isoflurane, and carbon dioxide levels were higher ( $82 \pm 3$  vs.  $41 \pm 1$  vs. mmHg;  $P < 0.05$ ). **(B)** Tonic genioglossus activity. Tonic genioglossus activity was significantly lower during propofol anesthesia compared with isoflurane. **(C)** Flow rate. Flow rate was significantly lower ( $P < 0.05$ ) during propofol anesthesia compared with isoflurane. MTA = moving time average. **(D)** Phasic genioglossus activity at ED<sub>50</sub> (given as rate of MTA rise) as a function of flow rate. Pooled data from protocols 1–3 ( $n = 53$ ). Flow rate correlated significantly with phasic genioglossus activity.  $r = 0.47$ ,  $P < 0.05$ . *Open circles* = isoflurane; *closed circles* = propofol.

ity ( $P < 0.0001$ ; fig. 2C, protocols 1–3), and was higher during isoflurane compared with propofol anesthesia (fig. 2D). Phasic genioglossus activity and flow rate increased with increasing isoflurane concentration in the 1- to 2-vol% range, (fig. 3), and the increase in flow rate observed with increasing isoflurane concentrations from 1% to 2% correlated significantly with the paralleled increase in rate of rise of phasic genioglossus activity ( $r = 0.6$ ; fig. 3).

Comparison of genioglossus activity in vagotomized animals (protocol 2) with controls (protocol 1) revealed a significant ( $P = 0.05$ ) interaction between vagotomy and isoflurane dose at the genioglossus electromyography. Phasic genioglossus activity and flow rate were significantly ( $P < 0.05$ ) higher in vagotomized rats compared with controls (fig. 4), and no longer increased with isoflurane concentration.

In propofol-anesthetized rats, comparison of phasic genioglossus activity during evoked hypoxemia and hypercapnia (conditions that increased respiratory drive) with control conditions revealed a significant ( $P < 0.05$ ) interaction of the intervention (evoked hypoxemia and hypercapnia; fig. 5, protocol 3). The decreasing effects of propofol on inspiratory genioglossus muscle function were fully reversed to baseline levels (1% isoflurane level) when flow rate and minute ventilation were restored with carbon dioxide (end-tidal carbon dioxide tension =  $134 \pm 7$  mmHg), and partially reversed with hypoxemia (end-tidal oxygen tension =  $44 \pm 2$  mmHg).

Analysis of all data derived during ED<sub>50</sub> of isoflurane and propofol anesthesia (protocols 1–3) revealed that flow rate ( $F = 67.32$ ,  $P < 0.001$ ) and anesthetic agents ( $F = 35.2$ ,  $P = 0.004$ ) independently predicted phasic genioglossus activity.



**Fig. 3.** Effects of isoflurane on genioglossus activity and respiratory function. Data are mean  $\pm$  SEM. (A) Phasic genioglossus activity at different isoflurane concentrations. Repetitive measurements at 1.0% ( $n = 12$ ),  $1.49 \pm 0.01$  (ED50) ( $n = 12$ ), and 2% ( $n = 6$ ) or 2.25% ( $n = 6$ ) isoflurane. Phasic genioglossus activity increased dose dependently with isoflurane dose but was significantly lower at 2.25% versus  $1.49 \pm 0.01$  (ED50) (paired  $t$  test). \*  $P < 0.05$  for increase of genioglossus activity with isoflurane dose (linear mixed model). +  $P < 0.05$  versus  $1.49 \pm 0.01$  (ED50) isoflurane (paired  $t$  test). (B) Flow rate. Flow rate (tidal volume/inspiration time) increased significantly with isoflurane dose. \*  $P < 0.05$  for dose effect (linear mixed model). #  $P < 0.05$  versus  $1.49 \pm 0.01$  (ED50) isoflurane (paired  $t$  test). (C) Increase in flow rate from 1–2% isoflurane versus increase in genioglossus activity from 1–2% isoflurane. Isoflurane evoked increase in genioglossus activity correlated significantly with evoked increase in flow rate ( $r = 0.6$ ,  $P < 0.05$ ;  $n = 35$  rats). MTA = moving time average.

#### Characterization of Different Interventions on the Dose-Response Relation of Isoflurane at the Genioglossus Muscle

Analysis of data derived from protocol 1 revealed that isoflurane in the 1- to 2-vol% range dose-dependently increased phasic genioglossus activity (protocol 1;  $F = 4.99$ ,  $P < 0.05$ ). In contrast, at the highest dose level of isoflurane studied (2.25 vol%), genioglossus activity was significantly (paired  $t$  test) lower compared with values observed at 1.5% (figs. 3A and fig. E5 of the Web Enhancement, protocol 1). During light isoflurane anesthesia (1.0 vol% end-tidal), we measured a robust genioglossus negative pressure reflex (increased phasic genioglossus activity in response to negative pressure pulse) to  $118 \pm 8\%$  of values observed before negative pressure application (fig. 6A). The genioglossus response to negative laryngeal pressure application decreased significantly ( $P < 0.05$ ) with increasing isoflurane doses. Isoflurane dose-dependently increased end-tidal carbon dioxide concentration, amounting to  $39 \pm 0.8$ ,  $41 \pm 1$ , and  $46.6 \pm 1.8$  mmHg under 1, 1.5, and 2% isoflurane, respectively (fig. E2 of the Web Enhancement, protocol 1). Increasing end-tidal carbon dioxide concentration to 50 mmHg (the highest level observed) increased phasic genioglossus activity at 1% isoflurane to  $40.8 \pm 4.1$   $\mu$ V (end-tidal carbon dioxide = 50 mmHg) versus  $35.8 \pm 2.5$   $\mu$ V (carbon dioxide =  $41 \pm 1$  mmHg;  $P < 0.05$ ), respectively. With carbon dioxide held constant, isoflurane still dose-dependently increased phasic genioglossus activity in the 1–2% range ( $40.8 \pm 4.1$ ,  $48.3 \pm 14.1$ , and  $55.1 \pm 24.16$   $\mu$ V at 1, 1.5, and 2% isoflurane, respectively; fig. E2 of the Web Enhancement). These values were not significantly different from those of control animals.

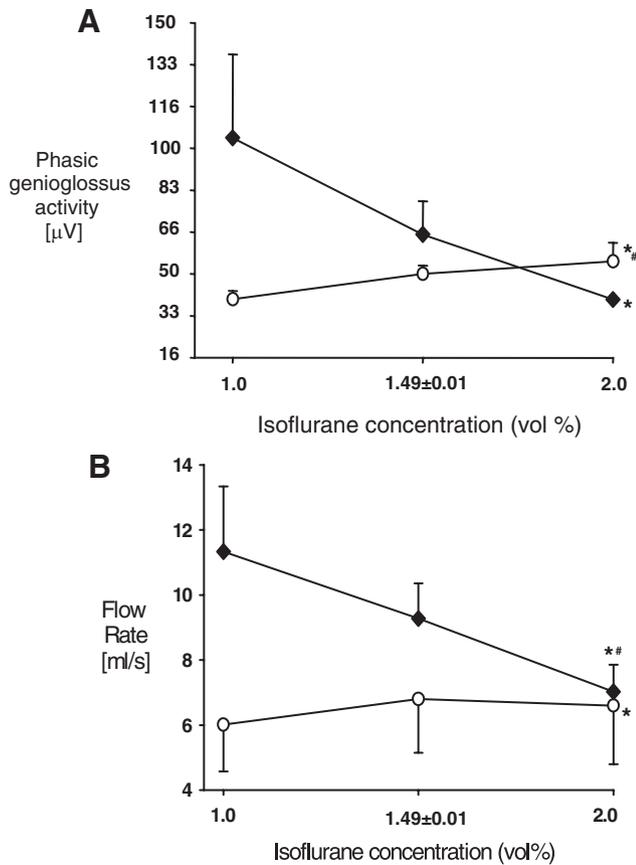
In chronically instrumented, awake animals (protocol 2), respiratory rate amounted to  $90 \pm 4.8$  breaths/min, and decreased significantly (paired  $t$  test) to  $64 \pm 3.9$  breaths/min during isoflurane anesthesia (1 vol%). Tonic genioglossus activity decreased with isoflurane anesthesia compared with the unmedicated condition, and decreased further with increasing isoflurane concentrations ( $P < 0.05$  for dose effect; figs. 6B and C). Phasic genioglossus activation was small and variable in unmedicated awake rats but always appeared in the presence of isoflurane (fig. E5 of the Web Enhancement).

Isoflurane dose-dependently decreased mean arterial blood pressure from  $119 \pm 17$  mmHg at 1 vol% to  $99 \pm 4$  mmHg at 2 vol% (protocol 1). In rats in which mean arterial pressure was normalized to values observed at 1 vol%, either by phenylephrine or by vasopressin (protocol 2), isoflurane dose-dependently increased phasic genioglossus activity as it did in control rats (fig. E3 of the Web Enhancement).

In Kölliker-Fuse-lesioned rats (protocol 2; for histology, see fig. E4 of the Web Enhancement), isoflurane dose-dependently increased phasic genioglossus activity as it did in control animals.

#### Discussion

Genioglossus activity is higher during isoflurane compared with propofol anesthesia at equianesthetic doses, and effects of anesthetics on phasic genioglossus activity resemble those on flow rate. Isoflurane dose-dependently decreases tonic and increases phasic genioglossus activity, effects that persist with normalization of carbon dioxide and arterial blood pressure and Kölliker-Fuse

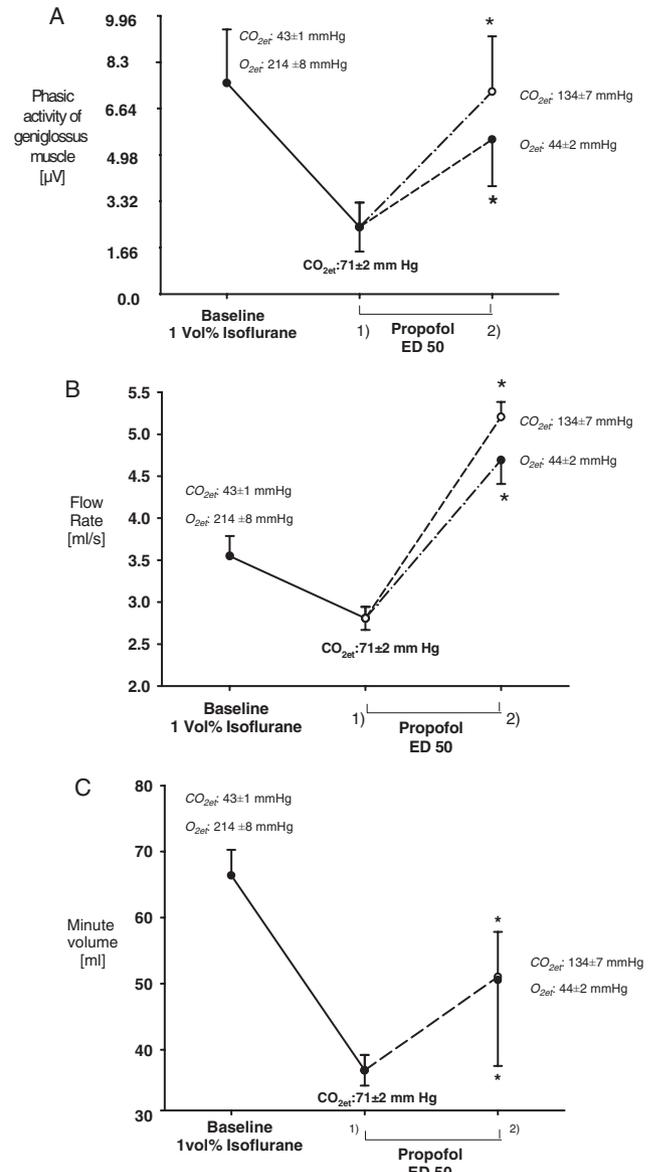


**Fig. 4.** Influence of vagal nerve section on genioglossus activity and breathing. *Open circles* = vagal nerves intact; *diamonds* = vagotomized rats. Data are mean  $\pm$  SEM. \*  $P < 0.05$  for dose effects of isoflurane (within groups). #  $P < 0.05$  versus controls (between-subjects effects). (A) Phasic genioglossus activity. At 1% isoflurane, phasic genioglossus activity was significantly higher in vagotomized rats compared with controls. Isoflurane increased genioglossus activity dose dependently in controls, whereas it decreased it in vagotomized rats. (B) Flow rate. At 1% isoflurane, flow rate was significantly higher in vagotomized rat compared with controls.

lesion. Vagotomy reversed the increasing effect of isoflurane on phasic genioglossus activity.

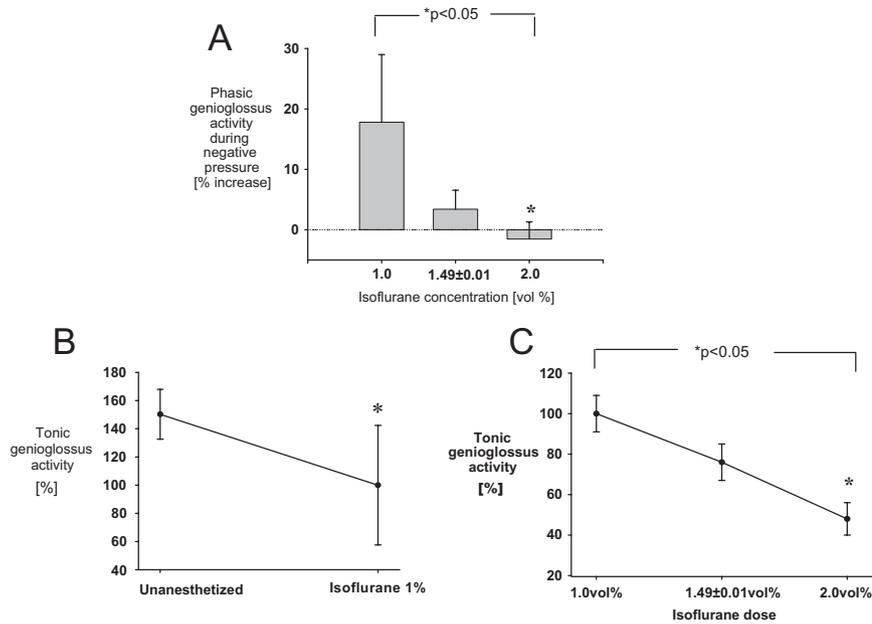
#### Association of Effects of Anesthetics on Flow Rate and Genioglossus Activity

The increasing effect of isoflurane on inspiratory drive seemingly accounts for the relatively preserved genioglossus phasic activity compared with propofol. This effect was abolished in vagotomized rats, where isoflurane dose-dependently decreased both the flow rate and genioglossus phasic activity, consistent with previous reports.<sup>5,34</sup> Therefore, our data contribute to a better understanding of the “paradox” of an increasing<sup>10,11</sup> versus decreasing<sup>5,34</sup> effects of anesthetics on phasic genioglossus activity. The vagus nerve is important for mediating the interplay between lung volume and upper airway muscle activity, and lung inflation decreases genioglossus muscle activity, an effect that is mediated by the vagus nerve as the afferent pathway.<sup>23,24</sup> We speculate that isoflurane increases phasic genioglossus activity



**Fig. 5.** Effects of hypoxemia and hypercapnia on phasic genioglossus activity, and flow rate during propofol anesthesia (ED<sub>50</sub> [median effective dose]). Arterial partial pressures of carbon dioxide and oxygen concentration were measured at time of assessment (mean  $\pm$  SEM). \*  $P < 0.05$  versus condition 1 (paired  $t$  test). CO<sub>2</sub> = carbon dioxide; et = end-tidal; O<sub>2</sub> = oxygen. (A) Phasic genioglossus activity. Genioglossus activity increased significantly during evoked hypoxemia and hypercapnia. (B) Flow rate (tidal volume/inspiratory time). Flow rate increased significantly during evoked hypoxemia and hypercapnia. (C) Minute ventilation. Minute ventilation decreased significantly during propofol anesthesia and increased subsequently during evoked hypoxemia and hypercapnia. \*  $P < 0.05$  versus before evoked hypoxemia/ hypercapnia.

by increasing flow rate, possibly by a vagolytic mechanism. However, differences in flow rate do not account completely for the differences in phasic genioglossus activation observed between anesthetics. Anesthetic agent type also explains some variance of phasic genioglossus activity independently of its effects on flow rate by an unidentified mechanism.



**Fig. 6.** (A) Genioglossus negative pressure reflex. The increase in phasic genioglossus activity in response to negative pharyngeal pressure application was inhibited with increasing isoflurane concentrations. \*  $P < 0.05$  for decreasing genioglossus negative pressure effects with increasing isoflurane dose, and for lower values at 2% isoflurane compared with 1% isoflurane. (B and C) Tonic activity of genioglossus muscle. Data are percent of values measured at 1% isoflurane. (B) Nonanesthetized state versus 1% isoflurane ( $n = 6$ ). Chronically instrumented rats. Tonic activity of genioglossus muscle was significantly higher under room air compared with anesthesia. \*  $P < 0.05$  versus non-anesthetized rats. (C) Dose-response effect of isoflurane. Isoflurane dose-dependently decreased tonic genioglossus activity. Data from 12 rats. \*  $P < 0.05$  for decreasing tonic genioglossus activity with increasing isoflurane dose, and for lower values at 2% isoflurane compared with 1% isoflurane.

Our data show that the increasing effects of isoflurane on inspiratory genioglossus activity are not consequences of its decreasing effects on arterial blood pressure or its increasing effects on carbon dioxide concentration. The genioglossus effects of isoflurane also persisted when Kölliker-Fuse nucleus was lesioned. Therefore, although our data support the findings of Roda *et al.*<sup>10</sup> suggesting that intravenous anesthetics reduce hypoglossal activity much more than volatile anesthetics,<sup>10</sup> our study contradicts their hypothesis that these changes are mediated by premotor neurons in the Kölliker-Fuse; in the study of Roda *et al.*,<sup>10</sup> halothane was strongly associated with a robust phasic inspiratory activity in the hypoglossal nerve. It seems likely that the Kölliker-Fuse activation observed after halothane anesthesia<sup>10</sup> was not causative for the effects of halothane on upper airway dilator muscle activity.

#### Effects of Anesthetics on Genioglossus Reflex Activation

Genioglossus muscle activity in rats increases in response to negative pressure in the upper airway<sup>35–37</sup> as it does in humans.<sup>38,39</sup> In accord, we observed during light isoflurane anesthesia (1 vol%; fig. 6) a strong reflex activation of the genioglossus in response to negative pressure in the upper airway. This reflex was abolished at anesthetic doses of propofol and isoflurane and, in the case of isoflurane, at a dose range that nonetheless increased phasic genioglossus activity. Therefore, we suggest that effects of anesthetics on phasic and reflex activation of the genioglossus are induced by different mechanisms.

#### Limitations

The effects of anesthetics on respiratory muscles differ between species,<sup>40</sup> and it is unclear whether our

finding, preserved upper airway dilator muscle activity during isoflurane, translates to humans. Comparative studies on differential effects of equianesthetic concentrations of anesthetics on upper airway muscle patency are clearly needed.

A major difference between the animal preparation used in the current study and other studies undertaken in humans is that the upper airway has been bypassed in the current model. This means that there will be minimal respiratory-related changes in flow or pressure within the upper airway, and activation of pharyngeal/laryngeal mechanoreceptors by these stimuli. Because the tracheostomized animal preparation used in this study is less physiologic than models in which an animal breathes *via* the intact upper airway, we adopted an additional protocol of chronically instrumented rats breathing by the normal route. Interestingly, the phasic and tonic genioglossus activity observed in these rats during 1% isoflurane was almost identical to the values observed in tracheostomized animals, suggesting that the anesthesia-related changes in electromyographic activity were central in origin.

We did not measure upper airway collapsibility. It is likely, though, that the marked depressive effects of propofol on the muscles of the upper airway (genioglossus) would increase the propensity of the upper airway to collapse compared with isoflurane anesthesia. Phasic genioglossus activation increases the size of the airway<sup>41</sup> and decreases collapsibility,<sup>42</sup> and may be of particular importance under conditions where the negative pressure reflex is absent. We have recently shown by magnetic resonance imaging that a quantitatively similar decrease in respiratory genioglossus activity in rats is associated with marked decreases in inspiratory upper airway volume.<sup>29</sup>

### Possible Clinical Implications

Some studies in humans suggest that subhypnotic concentrations of propofol have stronger decreasing effects on upper esophageal sphincter resting tone than isoflurane,<sup>43</sup> whereas other data suggest the opposite.<sup>7,8</sup> To our knowledge, it is unclear what the effects of equianesthetic concentrations of propofol and isoflurane on upper airway dilator muscle function might be. Our data suggest that respiratory depressant doses, rather than equivalent anesthetic doses, of anesthetics most likely determine the level of vulnerability of the airway dilator muscle function. Propofol in rats is more respiratory depressant than isoflurane given at equianalgesic doses, which seems to be similar in humans, during anesthesia,<sup>44</sup> and during sedation under spontaneous ventilation.<sup>45</sup> If our results obtained in rats translate to the clinical situation of patients scheduled to undergo surgery, then the choice of the anesthetic agent might influence the safety of patients with airways unprotected by an endotracheal tube or a laryngeal mask, during conscious sedation,<sup>43,46</sup> or in extubated patients recovering from general anesthesia.<sup>47</sup>

The strong impairing effects of propofol on upper airway dilator muscle function were reversed by carbon dioxide administration, which increased respiratory drive. It would be interesting to know whether upper airway dilator muscle function can be restored by increasing respiratory drive in humans by administering low doses of carbon dioxide to the inspiration air, *e.g.*, using dead space or rebreathing or with other respiratory stimulants. The depressing effects of isoflurane on human hypoxic and hypercapnic ventilatory response can be reversed by antioxidant administration.<sup>13,48</sup> Volatile anesthetics can increase reactive oxygen species production, and antioxidants reverse the reduction of the human hypoxic ventilatory response by isoflurane.<sup>13</sup> Therefore, it might be interesting to study whether some of the impairing effects of anesthetics on phasic genioglossus activity and respiratory drive observed in our study could be reversed by antioxidant treatment.

In summary, our data show that isoflurane compared with propofol anesthesia allows higher tonic and phasic genioglossus muscle activation at equianesthetic doses, but both anesthetics abolish its reflex activation. The level of respiratory depression rather than the level of effective anesthesia correlates closely with the airway dilator muscle function during anesthesia.

The authors thank Carl Rosow, M.D., Ph.D. (Professor of Anesthesia), Jeevendra Martyn, M.D. (Professor of Anesthesia, Department of Anesthesia, Massachusetts General Hospital, Boston, Massachusetts), and Julian Soboisky, Ph.D. (Research Fellow in Medicine, Division of Sleep Medicine, Brigham and Women's Hospital, Boston, Massachusetts), for their useful comments and criticism. The authors thank Quan Ha (Research Assistant, Beth Israel Deaconess Medical Center, Boston, Massachusetts) for excellent technical support.

### References

- Butler JE: Drive to the human respiratory muscles. *Respir Physiol Neurobiol* 2007; 159:115-26
- Pierce R, White D, Malhotra A, Edwards JK, Kleverlaan D, Palmer L, Trinder J: Upper airway collapsibility, dilator muscle activation and resistance in sleep apnoea. *Eur Respir J* 2007; 30:345-53
- Fuller DD, Williams JS, Janssen PL, Fregosi RF: Effect of co-activation of tongue protruder and retractor muscles on tongue movements and pharyngeal airflow mechanics in the rat. *J Physiol* 1999; 519(pt 2):601-13
- Hwang JC, St John WM, Bartlett D Jr: Respiratory-related hypoglossal nerve activity: Influence of anesthetics. *J Appl Physiol* 1983; 55:785-92
- Ochiai R, Guthrie RD, Motoyama EK: Differential sensitivity to halothane anesthesia of the genioglossus, intercostals, and diaphragm in kittens. *Anesth Analg* 1992; 74:338-44
- Drummond GB: Influence of thiopentone on upper airway muscles. *Br J Anaesth* 1989; 63:12-21
- Eastwood PR, Platt PR, Shepherd K, Maddison K, Hillman DR: Collapsibility of the upper airway at different concentrations of propofol anesthesia. *ANESTHESIOLOGY* 2005; 103:470-7
- Eastwood PR, Szollosi I, Platt PR, Hillman DR: Collapsibility of the upper airway during anesthesia with isoflurane. *ANESTHESIOLOGY* 2002; 97:786-93
- Norton JR, Ward DS, Karan S, Voter WA, Palmer L, Varlese A, Rackovsky O, Bailey P: Differences between midazolam and propofol sedation on upper airway collapsibility using dynamic negative airway pressure. *ANESTHESIOLOGY* 2006; 104:1155-64
- Roda F, Pio J, Bianchi AL, Gestreau C: Effects of anesthetics on hypoglossal nerve discharge and c-Fos expression in brainstem hypoglossal premotor neurons. *J Comp Neurol* 2004; 468:571-86
- Younes M, Park E, Horner RL: Pentobarbital sedation increases genioglossus respiratory activity in sleeping rats. *Sleep* 2007; 30:478-88
- Davies RO, Edwards MW Jr, Lahiri S: Halothane depresses the response of carotid body chemoreceptors to hypoxia and hypercapnia in the cat. *ANESTHESIOLOGY* 1982; 57:153-9
- Teppema LJ, Romberg RR, Dahan A: Antioxidants reverse reduction of the human hypoxic ventilatory response by subanesthetic isoflurane. *ANESTHESIOLOGY* 2005; 102:747-53
- van den Elsen M, Sarton E, Teppema L, Berkenbosch A, Dahan A: Influence of 0.1 minimum alveolar concentration of sevoflurane, desflurane and isoflurane on dynamic ventilatory response to hypercapnia in humans. *Br J Anaesth* 1998; 80:174-82
- Brandes IF, Zuperku EJ, Stucke AG, Hopp FA, Jakovcovic D, Stuth EA: Isoflurane depresses the response of inspiratory hypoglossal motoneurons to serotonin *in vivo*. *ANESTHESIOLOGY* 2007; 106:736-45
- Sirois JE, Pancrazio JJ, Lynch C III, Bayliss DA: Multiple ionic mechanisms mediate inhibition of rat motoneurons by inhalation anaesthetics. *J Physiol* 1998; 512(pt 3):851-62
- Solt K, Forman SA: Correlating the clinical actions and molecular mechanisms of general anesthetics. *Curr Opin Anaesthesiol* 2007; 20:300-6
- Morrison JL, Sood S, Liu H, Park E, Liu X, Nolan P, Horner RL: Role of inhibitory amino acids in control of hypoglossal motor outflow to genioglossus muscle in naturally sleeping rats. *J Physiol* 2003; 552:975-91
- Ingalls CP, Warren GL, Lowe DA, Boorstein DB, Armstrong RB: Differential effects of anesthetics on *in vivo* skeletal muscle contractile function in the mouse. *J Appl Physiol* 1996; 80:332-40
- Haeseler G, Stormer M, Bufler J, Dengler R, Hecker H, Piepenbrock S, Leuwer M: Propofol blocks human skeletal muscle sodium channels in a voltage-dependent manner. *Anesth Analg* 2001; 92:1192-8
- Sessler DI, McGuire J, Moayeri A, Hynson J: Isoflurane-induced vasodilation minimally increases cutaneous heat loss. *ANESTHESIOLOGY* 1991; 74:226-32
- Garpestad E, Basner RC, Ringler J, Lilly J, Schwartzstein R, Weinberger SE, Weiss JW: Phenylephrine-induced hypertension acutely decreases genioglossus EMG activity in awake humans. *J Appl Physiol* 1992; 72:110-5
- Bailey EF, Jones CL, Reeder JC, Fuller DD, Fregosi RF: Effect of pulmonary stretch receptor feedback and CO(2) on upper airway and respiratory pump muscle activity in the rat. *J Physiol* 2001; 532:525-34
- van Lunteren E, Strohl KP, Parker DM, Bruce EN, Van de Graaff WB, Cherniack NS: Phasic volume-related feedback on upper airway muscle activity. *J Appl Physiol* 1984; 56:730-6
- Picker O, Scheeren TW, Arndt JO: Inhalation anaesthetics increase heart rate by decreasing cardiac vagal activity in dogs. *Br J Anaesth* 2001; 87:748-54
- Kato M, Komatsu T, Kimura T, Sugiyama F, Nakashima K, Shimada Y: Spectral analysis of heart rate variability during isoflurane anesthesia. *ANESTHESIOLOGY* 1992; 77:669-74
- Kohli JD, Tuttle RR, Dresel PE, Innes IR: Influence of anesthetics and of arterial blood pressure on the functional refractory period of atrioventricular conduction. *J Pharmacol Exp Ther* 1966; 153:505-10
- Onal E, Lopata M, O'Connor TD: Diaphragmatic and genioglossal electromyogram responses to CO2 rebreathing in humans. *J Appl Physiol* 1981; 50:1052-5
- Eikermann M, Fassbender P, Malhotra A, Takahashi M, Kubo S, Jordan AS, Gautam S, White DP, Chamberlin NL: Unwarranted administration of acetylcholinesterase inhibitors can impair genioglossus and diaphragm muscle function. *ANESTHESIOLOGY* 2007; 107:621-9

30. Pajewski TN, DiFazio CA, Moscicki JC, Johns RA: Nitric oxide synthase inhibitors, 7-nitro indazole and nitro<sup>G</sup>-L-arginine methyl ester, dose dependently reduce the threshold for isoflurane anesthesia. *ANESTHESIOLOGY* 1996; 85:1111-9
31. Orth M, Barter L, Dominguez C, Atherley R, Carstens E, Antognini JF: Halothane and propofol differentially affect electroencephalographic responses to noxious stimulation. *Br J Anaesth* 2005; 95:477-84
32. Jinks SL, Martin JT, Carstens E, Jung SW, Antognini JF: Peri-MAC depression of a nociceptive withdrawal reflex is accompanied by reduced dorsal horn activity with halothane but not isoflurane. *ANESTHESIOLOGY* 2003; 98:1128-38
33. Lu J, Sherman D, Devor M, Saper CB: A putative flip-flop switch for control of REM sleep. *Nature* 2006; 441:589-94
34. Nishino T, Kohchi T, Yonezawa T, Honda Y: Responses of recurrent laryngeal, hypoglossal, and phrenic nerves to increasing depths of anesthesia with halothane or enflurane in vagotomized cats. *ANESTHESIOLOGY* 1985; 63:404-9
35. Chamberlin NL, Eikermann M, Fassbender P, White DP, Malhotra A: Genioglossus premotoneurons and the negative pressure reflex in rats. *J Physiol* 2007; 579:515-26
36. Hwang JC, St John WM, Bartlett D Jr: Afferent pathways for hypoglossal and phrenic responses to changes in upper airway pressure. *Respir Physiol* 1984; 55:341-54
37. Ryan S, McNicholas WT, O'Regan RG, Nolan P: Reflex respiratory response to changes in upper airway pressure in the anaesthetized rat. *J Physiol* 2001; 537:251-65
38. Horner RL, Innes JA, Murphy K, Guz A: Evidence for reflex upper airway dilator muscle activation by sudden negative airway pressure in man. *J Physiol (Lond)* 1991; 436:15-29
39. Malhotra A, Fogel RB, Edwards JK, Shea SA, White DP: Local mechanisms drive genioglossus activation in obstructive sleep apnea. *Am J Respir Crit Care Med* 2000; 161:1746-9
40. Warner DO, Joyner MJ, Ritman EL: Anesthesia and chest wall function in dogs. *J Appl Physiol* 1994; 76:2802-13
41. Kobayashi I, Perry A, Rhymer J, Wuyam B, Hughes P, Murphy K, Innes JA, McIvor J, Cheesman AD, Guz A: Inspiratory coactivation of the genioglossus enlarges retroglossal space in laryngectomized humans. *J Appl Physiol* 1996; 80:1595-604
42. Oliven A, O'Hearn DJ, Boudewyns A, Odeh M, De Backer W, van de Heyning P, Smith PL, Eisele DW, Allan L, Schneider H, Testerman R, Schwartz AR: Upper airway response to electrical stimulation of the genioglossus in obstructive sleep apnea. *J Appl Physiol* 2003; 95:2023-9
43. Sundman E, Witt H, Sandin R, Kuylenstierna R, Boden K, Ekberg O, Eriksson LI: Pharyngeal function and airway protection during subhypnotic concentrations of propofol, isoflurane, and sevoflurane: Volunteers examined by pharyngeal videoradiography and simultaneous manometry. *ANESTHESIOLOGY* 2001; 95:1125-32
44. Ashworth J, Smith I: Comparison of desflurane with isoflurane or propofol in spontaneously breathing ambulatory patients. *Anesth Analg* 1998; 87:312-8
45. Bonnin M, Therre P, Albuissou E, Beaujard H, Barthelemy I, Mondie JM, Bazin JE: Comparison of a propofol target-controlled infusion and inhalational sevoflurane for fiberoptic intubation under spontaneous ventilation. *Acta Anaesthesiol Scand* 2007; 51:54-9
46. Rodrigo MR, Rosenquist JB: Isoflurane for conscious sedation. *Anaesthesia* 1988; 43:369-75
47. D'Honneur G, Rimaniol JM, el Sayed A, Lambert Y, Duvaldestin P: Midazolam/propofol but not propofol alone reversibly depress the swallowing reflex. *Acta Anaesthesiol Scand* 1994; 38:244-7
48. Zakyntinos S, Katsaounou P, Karatza MH, Roussos C, Vassilakopoulos T: Antioxidants increase the ventilatory response to hyperoxic hypercapnia. *Am J Respir Crit Care Med* 2007; 175:62-8