Episodic hypoxia induces long-term facilitation of neural drive to tongue protrudor and retractor muscles

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Fuller, D. D. Episodic hypoxia induces long-term facilitation of neural drive to tongue protrudor and retractor muscles. J Appl Physiol 98: 1761–1767, 2005. First published January 7, 2005; doi:10.1152/japplphysiol.01142.2004.—Hypoxic episodes can evoke a prolonged augmentation of inspiratory motor output called long-term facilitation (LTF). Hypoglossal (XII) LTF has been assumed to represent increased tongue protruder motor neuron activity and pharyngeal airway dilation. However, recent studies indicate that tongue protruder and retractor muscles are coactivated during inspiration, a behavior that promotes upper airway patency by reducing airway compliance. These experiments tested the hypothesis that XII LTF is manifest as increased inspiratory drive to both tongue protruder and retractor muscles. Neurograms were recorded in the medial XII nerve branch (XIIMED; contains tongue protruder motor axons), the lateral XII nerve branch (XII LAT; contains tongue retractor motor axons), and the phrenic nerve in anesthetized, vagotomized, paralyzed, ventilated male rats. Strict isocapnia was maintained for 60 min after five 3-min hypoxic episodes (arterial Po2 = 35 ± 2 Torr) or sham treatment. Peak inspiratory burst amplitude showed a persistent increase in XIIMED, XILLAT, and phrenic nerves during the hour after episodic hypoxia (P < 0.05 vs. sham). This effect was present regardless of the quantification method (e.g., % baseline vs. percent maximum); however, comparisons of the relative magnitude of LTF between neurograms (e.g., XIIMED vs. XILLAT) varied with the normalization procedure. There was no persistent effect of episodic hypoxia on inspiratory burst frequency (P > 0.05 vs. sham). These data demonstrate that episodic hypoxia induces LTF of inspiratory drive to both tongue protruder and retractor muscles and underscore the potential contribution of tongue muscle coactivation to regulation of upper airway patency.

LONG-TERM FACILITATION (LTF) is a prolonged augmentation (e.g., minutes to hour) of inspiratory motor output observed most commonly after episodic hypoxia (15), although other stimuli can produce a similar response (34, 46). LTF of inspiratory drive to upper airway muscles has been demonstrated using genioglossus (GG) electromyography (27, 33) and neurograms recorded from the main trunk of the hypoglossal (XII) nerve (3, 7) and inferred from measures of upper airway resistance during sleep (41). Based on the mechanical actions of the GG (i.e., tongue protrusion), it has been suggested that LTF of XII motor output may be unique to this muscle (3, 41).

Recently, Bocchiarro and Feldman (9) presented strong evidence that XII LTF occurs via an activity-independent, postsynaptic mechanism triggered by activation of serotonin (5-HT) receptors within the XII motor nucleus. Thus the segregated distribution of tongue protruder and retractor motoneuron cell bodies within the XII motor nucleus (21, 30, 31) could provide a neuroanatomic substrate for selective expression of LTF in tongue protruder motoneurons. For example, 5-HT release during episodic hypoxia [the proposed in vivo “trigger” of LTF (35)] could be localized to the ventral portions of the XII nucleus that contain predominantly GG motoneuron cell bodies (30). However, dendritic arborizations of protruder and retractor motoneurons are extensively intermingled within the XII nucleus (1), and protruder and retractor motoneurons receive significant common innervation from serotonergic neurons in the medullary raphe nuclei (12). Furthermore, the traditional notion that XII inspiratory activity is unique to GG motoneurons is inconsistent with recent data. Indeed, tongue protruder and retractor muscles are coactivated during inspiration in humans (28) and animals (6, 20, 37, 39). This coactivation pattern confers a mechanical advantage vs. isolated GG contraction by more effectively reducing upper airway compliance [i.e., reduced collapsibility (5, 17)]. Accordingly, these experiments tested the hypothesis that episodic hypoxia-induced LTF is reflected as an increase in inspiratory neural drive to both tongue protruder and retractor muscle groups.

METHODS

Experiments were conducted using 19 male Lewis rats (355 ± 6 g) obtained from Harlan (Indianapolis, IN; rat colony 202B). The Animal Care and Use Committee at the University of Florida approved all experimental procedures.

Experimental preparation. Isoflurane anesthesia was initially induced in a closed chamber and then maintained via a nose cone. A catheter was inserted into the femoral vein, and rats were converted from isoflurane to urethane anesthesia (1.6 g/kg in 5 ml of distilled water). The adequacy of anesthesia was monitored during this period by observing limb withdrawal and palpebral reflexes. A femoral arterial catheter was inserted to enable blood pressure measurements (Statham P-10EZ pressure transducer, CP122 alternating current/direct current strain gauge amplifier, Grass Instruments, West Warwick, RI) and withdrawal of blood samples. The trachea was then cannulated and rats were mechanically ventilated [inspired O2 fraction (FIO2) = 0.50–0.60] for the remainder of the experiment. The vagus nerves were bilaterally sectioned in the medullar region, and the rats were paralyzed with pancuronium bromide (2.5 mg/kg). The latter procedure was necessary to prevent spontaneous breathing movements and entrainment of the neurograms with the ventilator. After paralysis, the adequacy of anesthesia was monitored by observing blood pressure and respiratory responses during application of deep pressure to the paws. A 4:1 solution of lactated Ringer and sodium bicarbonate was continually infused via the venous catheter (2.5 ml/h).

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to help maintain arterial blood pressure and acid-base regulation. Arterial $P_{O_2}$ ($P_{O_2}$) and $P_{CO_2}$ ($P_{CO_2}$) as well as pH were periodically determined (see protocol) from 0.2-ml arterial blood samples (i-Stat, Heska, Fort Collins, CO). The end-tidal $P_{CO_2}$ ($PET_{CO_2}$) was measured throughout the protocol using a rapidly responding mainstream $CO_2$ analyzer placed a few centimeters from the tracheostomy tube on the expired line of the ventilator circuit (Capnogard neonatal $CO_2$ monitor, Novametrix Medical Systems, Wallingford, CT). Rectal temperature was successfully maintained at 37.5 ± 1°C using a rectal thermistor connected to a servo-controlled heating pad (model TC-1000, CWE, Ardmore, PA). At the conclusion of all experiments, rats were euthanized via a rapid bolus injection of urethane.

A ventral approach was used to isolate one phrenic nerve within the caudal neck region, proximal to the communication with the accessory phrenic nerve. The XII nerve was then exposed bilaterally, also with a ventral approach. As the XII nerve reaches the tongue, it bifurcates into distinct medial and lateral branches. The medial XII nerve (XIMED), in turn, has a distinct branch innervating the geniohyoid, and several branches that supply the GG. XIMED also projects to the body of the tongue, innervating the verticalis and transversus linguae muscles (29). The small XIMED branch innervating the geniohyoid muscle was severed ~1 mm from the communication with XIMED. XIMED was then placed over bipolar silver recording electrodes proximal to the projection of fibers to the GG and distal to the geniohyoid branch. Thus XIMED neurograms represented neural drive to the intrinsic verticalis and transversus linguae muscles and the extrinsic GG.

The lateral division of the XII nerve (XII-LAT) projects to two extrinsic tongue muscles [styloglossus (SG) and hyoglossus (HG)] as well as the intrinsic superior longitudinalis and inferior longitudinalis muscles (29). The HG acts to retract and elevate the tongue, whereas the SG retracts and depress the tongue. The XII branch innervating the SG projects from the XII nerve ~0–1 mm distal to the primary bifurcation into medial and lateral branches (29). The HG branch projects from XII-LAT ~2 mm distal to the XII bifurcation (29). XII-LAT was placed over bipolar silver recording electrodes ~1 mm distal to the bifurcation of the main XII nerve trunk into medial and lateral branches. Thus XII-LAT neurograms represented neural drive to the HG as well as the superior and inferior longitudinalis muscles. Based on the anatomy published by McClung and Goldberg (29), it is unlikely that the XII-LAT neurograms reflected neural drive to the SG.

Nerve signals were amplified ($\times 10,000$) and band-pass filtered (phrenic signal, 10–100,000 Hz; XII signals, 100–10,000 Hz) using a differential alternating current amplifier (model 1700, A-M Systems, Carlsborg, WA). The amplified signal was full-wave rectified and moving averaged (time constant, 50 ms) using a model MA-1000 moving averager (CWE, Ardmore, PA) or Spike2 software (CED Limited, Cambridge, UK). All data were digitized and recorded on a computer using a CED Spike2 data acquisition system.

**Experimental protocol.** The protocol used to evoke LTF was similar to that developed by Bach and Mitchell (3). After an adequate plane of anesthesia was established, the $PET_{CO_2}$ was maintained at 40 ± 2 Torr for 60 min. During this period, all rats displayed inspiratory phrenic and XII bursting. The end-tidal $CO_2$ apneic threshold for inspiratory activity was then determined as follows. Rats were first hyperventilated by gradually increasing the ventilator pump rate until inspiratory bursting ceased in the phrenic and XII nerves. The ensuing apnea was maintained for at least 2 min, and then the ventilator rate was gradually decreased, allowing $PET_{CO_2}$ to rise, until inspiratory activity reappeared. $PET_{CO_2}$ was then “clamped” 2 Torr above the value at which inspiratory bursting began by adjusting the ventilator rate as necessary. $PET_{CO_2}$ measurements were used as a guide to help maintain isocapnia. However, $PET_{CO_2}$ values tended to be a few Torr greater than corresponding arterial $CO_2$ values. Therefore, isocapnia was determined exclusively by analyses of arterial blood samples (see below). This method permits baseline and post-hypoxia neural activity to be standardized relative to the individual $CO_2$ apneic threshold of each animal (3).

A few minutes before episodic hypoxia or the sham hypoxia period, an arterial blood sample was drawn. Treated rats were then exposed to five 3-min episodes of hypoxia ($F_{IO_2} = 0.12–0.14$), separated by 3 min of hyperoxia ($F_{IO_2} = 0.50$). An arterial blood sample was drawn during the second hypoxic episode. The particular pattern of hypoxia exposure was selected to maximize the probability of LTF expression. Preliminary experiments ($n = 4$) indicated that three 5-min hypoxic bouts (3) did not produce consistent LTF in the phrenic or XII nerves. Phrenic and XII nerve activities were monitored for 60 min ($F_{IO_2} = 0.50$) after episodic hypoxia. For sham treatment, $F_{IO_2}$ was maintained at 0.50 throughout the protocol. Arterial blood samples were drawn at 30 and 60 min in both episodic hypoxia and sham rats. At the conclusion of the experiment, all rats were exposed to a 3-min hypoxic episode ($F_{IO_2} = 0.12–0.14$) followed 3 min later by a 5-min hypercapnic exposure ($PET_{CO_2} = 70–80$ Torr).

**Data analyses.** Phrenic and XII neurograms were analyzed using Spike2 software (CED Limited). The following parameters were measured in phrenic and XII moving averaged neurograms: end-expiratory amplitude, peak inspiratory amplitude, and burst frequency. The instantaneous burst frequency was calculated by taking the inverse of the period between each inspiratory burst (e.g., frequency = 1/period). In this manner, burst frequency was calculated for the entire experimental protocol. Burst frequencies were then grouped in 30-s bins. Measures of amplitude were averaged over 30-s periods before the first hypoxic episode (baseline), during the peak of each hypoxic response (short-term hypoxic response), 1–2 min before the 30- and 60-min arterial blood samples, and during the final hypoxic and hypercapnic exposures. Baseline neural activity was expressed relative to the hypercapnic response (i.e., percent maximum). Changes in neurogram amplitude and area during and after hypoxia were expressed relative to the value observed during both baseline (i.e., change in percent baseline) and hypercapnia (i.e., change in percent maximum) (3).

Statistical comparisons between episodic hypoxia and time control experiments were done using a two-way ANOVA followed by the Student-Newman-Keuls post hoc test (Sigma-Stat version 1.0; Jandel Scientific, St. Louis, MO). Comparisons between nerves within the episodic hypoxia group (e.g., phrenic vs. XIMED vs. XII-LAT) were made using a two-way repeated-measures ANOVA and the Student-Newman-Keuls post hoc test. Responses within a nerve across time (e.g., peak inspiratory burst amplitude during episodic hypoxic episodes) or changes in blood pressure or gases, were compared with a one-way ANOVA and the Student-Newman-Keuls post hoc test. In cases where the data were not normally distributed, a one-way ANOVA on ranks was used. Differences were considered statistically significant when the $P$ value was ≤0.05. All data are presented as means ± SE.

**RESULTS**

Arterial isocapnia was successfully maintained throughout the LTF and sham hypoxia protocols (Table 1). Mean arterial pressure decreased transiently during hypoxia and then tended to decline slightly as the experimental protocol progressed (Table 1). However, the overall drop in mean arterial pressure was not different between episodic hypoxia and sham rats ($P > 0.05$; Table 1).

Baseline inspiratory burst amplitude was greater in phrenic (44 ± 5% maximum) vs. XIMED (20 ± 2% maximum), and both were greater than XII-LAT (9 ± 2% maximum; $P < 0.05$); these values were not different between episodic hypoxia and sham rats. Baseline inspiratory burst frequency was not different between episodic hypoxia (48 ± 1 bursts/min) and sham rats (48 ± 1 bursts/min). Relative to baseline amplitude, hypoxia induced a greater increase in XII-LAT vs. XIMED.
burst amplitude, and both were greater than phrenic amplitude \((P < 0.05; \text{Fig. 1})\). However, changes in XIILAT and XIIMED amplitude during hypoxia were not different when expressed relative to the maximum response \((P > 0.05; \text{data not shown})\). It should be noted that the relatively large increases in XIILAT and XIIMED activity during hypoxia are similar to a prior report of XII nerve branch activity during chemoreceptor stimulation \((20)\). A significant interaction between the hypoxic episode number and peak inspiratory amplitude was present in XIIMED \((P = 0.005)\), XIILAT \((P = 0.001)\), and phrenic neurograms \((P < 0.001)\). Thus a progressive augmentation of inspiratory burst amplitude \((38)\) was observed across hypoxic episodes in all three neurograms (Fig. 1). The end-expiratory amplitude of the moving averaged XIIMED and XIILAT neurograms transiently increased during hypoxia (Figs. 1 and 3). However, in contrast to the progressive augmentation of burst amplitude, XII end-expiratory amplitude was attenuated over successive hypoxic episodes \((P < 0.05; \text{Figs. 1 and 3})\). There was a significant interaction between hypoxic episode number and end-expiratory amplitude in both XIIMED \((P = 0.001)\) and XIILAT nerves \((P = 0.007)\). End-expiratory phrenic amplitude did not change during hypoxia (Figs. 1 and 3). Inspiratory burst frequency increased at the onset of each hypoxic episode and declined below baseline values on cessation of hypoxia \([\text{posthypoxic frequency decline (PHFD); Fig. 2}]\). However, the peak frequency during hypoxic episodes 2–5 was not significantly different than the preepisodic hypoxia baseline (Fig. 2). The magnitude of PHFD tended to decline with successive bouts of hypoxia, but this effect did not achieve statistical significance \((P = 0.08; \text{Fig. 2})\).

Representative tracings depicting XII and phrenic activity during an episodic hypoxia protocol are presented in Fig. 3. Peak inspiratory burst amplitude was persistently augmented after episodic hypoxia in phrenic, XIIMED, and XIILAT neurograms, an effect that was independent of the procedure used to normalize burst amplitude \((all P < 0.05 \text{vs. sham rats}; \text{Fig. 4})\). There was not a statistically significant difference in burst amplitude between 30- and 60-min postepisodic hypoxia.

### Table 1. Arterial blood gases, pH and MAP during episodic hypoxia and sham protocols

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Hypoxia 30 min</th>
<th>Hypoxia 60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{PaCO}_2) Torr</td>
<td>42±2</td>
<td>38±2</td>
<td>42±2</td>
</tr>
<tr>
<td>Episodic hypoxia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>150±12</td>
<td>35±2</td>
<td>144±12</td>
</tr>
<tr>
<td>pH</td>
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<td>7.37±0.02</td>
<td>7.34±0.02</td>
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<tr>
<td>MAP, mmHg</td>
<td>80±4</td>
<td>44±4</td>
<td>70±7</td>
</tr>
<tr>
<td>Sham</td>
<td>168±15</td>
<td>157±17</td>
<td>155±16</td>
</tr>
<tr>
<td>(\text{PaO}_2), Torr</td>
<td>150±12</td>
<td>35±2</td>
<td>144±12</td>
</tr>
<tr>
<td>Sham</td>
<td>168±15</td>
<td>157±17</td>
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### Fig. 1. Changes in average peak inspiratory activity (left) and average end-expiratory activity (“baseline”; right) in medial hypoglossal (XII) nerve branch activity (XIIMED; A), lateral XII nerve branch (XIILAT; B), and phrenic nerves (C) during hypoxic episodes. All hypoxic episodes were 3 min in duration. The first 5 hypoxic episodes were separated by 3 min; episode 6 occurred 1 h after episode 5. Note that the scale of the y-axes are different on each panel. \(*\)Significantly different from hypoxic episode 1 \((P < 0.05)\).
in any of the neurograms. Interestingly, when the postepisodic hypoxia inspiratory burst amplitude was expressed relative to baseline, LTF was greater in XIILAT compared with both XIIMED and phrenic nerves ($P_{/H11021}<0.05$; Fig. 4). However, when the change in burst amplitude was expressed relative to the maximum response, LTF was not different between XIILAT and XIIMED nerves, and both neurograms had diminished LTF compared with the phrenic response (Fig. 4). Accordingly, the present data establish the presence of LTF in all three neurograms, but no conclusions can be drawn regarding the relative magnitude of LTF.

Relative to baseline, inspiratory burst frequency tended to be elevated at 60 min postepisodic hypoxia ($4 \pm 2$ bursts/min), but this was not different from the sham response ($2 \pm 2$ bursts/min; $P = 0.85$).

**DISCUSSION**

**Summary.** Episodic hypoxia resulted in prolonged increases in inspiratory burst amplitude recorded in both XIIMED and XIILAT nerves. These data indicate that the mechanisms underlying LTF are not unique to subpopulations of motoneurons within the XII motor pool (i.e., GG motoneurons). Rather, XII LTF is manifest as an increase in neural drive to both tongue protruder and retractor motoneurons (i.e., a coactivation pattern). If LTF of upper airway muscle activity serves to defend the pharyngeal airway against narrowing or collapse (41), the present data suggest that tongue muscle coactivation is an important part of this defense mechanism.

**Critique of methods.** In addition to innervating the extrinsic tongue muscles, the XIIMED and XIILAT nerve branches also contain motor axons supplying the muscles that comprise the body of the tongue (i.e., the intrinsic muscles; see METHODS for detailed description). Although the intrinsic tongue muscles have not traditionally been viewed as serving a respiratory-related function, a recent study by Bailey and Fregosi (4) indicates that this view is suspect. These investigators recorded phasic, inspiratory electromyogram (EMG) activity in the intrinsic superior longitudinal muscle of spontaneously breathing, anesthetized rats. During normoxic normocapnia, the area of the integrated inspiratory EMG burst (expressed as a percent of maximum) in the superior longitudinal muscle was approximately one-half of that recorded in the extrinsic HG muscle. Vagotomy increased the EMG activity in both muscles, but ratio of superior longitudinal EMG activity to HG EMG activity remained similar (4). These results indicate that a portion of the XIIMED and XIILAT neurograms in the present study may reflect inspiratory neural drive to the intrinsic tongue muscles. However, the interpretation of the present data is focused on the extrinsic (vs. intrinsic) tongue muscles, primarily because their mechanical actions and functional contribution to breathing are better defined (16). On the other hand, Kier and Smith (24) propose that all tongue movements reflect an interaction between the intrinsic and extrinsic tongue muscles. Thus intrinsic tongue muscle contraction may make a significant contribution to the regulation of pharyngeal airway...
patency during breathing. Further studies are needed to fully appreciate the significance of respiratory-related activity in the intrinsic tongue muscles.

**Respiratory-related control of extrinsic tongue muscles.** During quiet breathing in humans, inspiratory GG activity is either absent or minimal (23, 28, 44) but appears during conditions of increased respiratory drive including exercise and hypoxia (23, 44). There has been only one detailed study of respiratory-related control of human tongue retractor muscles (28). This investigation revealed that inspiratory activity in both tongue protrudor and retractor muscles was absent during eupnea, but these muscles were recruited in parallel during hypoxia and hypercapnia (28). Inspiratory coactivation of tongue protrudor and retractor muscles has also been shown in animal experiments during quiet breathing (6, 20, 37, 39) and chemoreceptor stimulation (20, 45). Inspiratory activity in both tongue protrudor and retractor muscles is similarly inhibited by phasic lung volume feedback (6) and augmented by upper airway negative pressure (39).

**LTF of tongue muscle activity.** LTF of XII motor output has been demonstrated in adult rats (3) and cats (27), neonatal rats (33) and in vitro brain stem slices from neonatal rats (9). These prior investigations, however, have utilized either GG EMG recordings or neurograms recorded from the main trunk of the XII nerve. The present data are the first to document LTF of neural drive to both tongue protruder and retractor muscles is similarly inhibited by phasic lung volume feedback (6) and augmented by upper airway negative pressure (39).

**Frequency response.** A short-term reduction in respiratory burst frequency (i.e., PHFD) is often observed in anesthetized rats after hypoxia (2, 11). Although the mechanisms underlying PHFD are not precisely defined, this behavior appears to depend on neurons located in the ventrolateral pons and ultimately represents a complicated interaction between serotonergic-
pharyngeal airway stability during sleep, the present data reducing upper airway compliance (i.e., reduced collapsibility; rate (13) and dilate both the naso- and oropharyngeal airway dilator muscles during LTF. However, coactivation of tongue upper airway airflow resistance (41). This reduction in airflow Consistent with this suggestion, hypoxic episodes in sleeping during sleep could minimize or prevent apneic events (3, 41). Accordingly, LTF of neural drive to upper airway muscles agreement that a sleep-related decline in upper airway muscle obstructive sleep apnea; Ref. 41). The mechanisms underlying plasticity (7, 9) but also may provide insight into the patho-
be a valuable tool for exploring basic mechanisms of synaptic frequency after episodic hypoxia was found in the present study.

Physiological significance. Respiratory LTF has proven to be a valuable tool for exploring basic mechanisms of synaptic plasticity (7, 9) but also may provide insight into the pathogenesis of upper airway obstruction in sleeping humans (i.e., obstructive sleep apnea; Ref. 41). The mechanisms underlying obstructive sleep apnea are complex, but there is general agreement that a sleep-related decline in upper airway muscle activity contributes to airway narrowing and/or collapse (40). Accordingly, LTF of neural drive to upper airway muscles during sleep could minimize or prevent apneic events (3, 41). Consistent with this suggestion, hypoxic episodes in sleeping humans induce ventilatory LTF that is associated with reduced upper airway airflow resistance (41). This reduction in airflow resistance may indicate selective activation of upper airway dilator muscles during LTF. However, coactivation of tongue protruder and retractor muscles can increase inspiratory airflow rate (13) and dilate both the naso- and oropharyngeal airway (10). Tongue muscle coactivation also confers a mechanical advantage vs. isolated GG contraction by more effectively reducing upper airway compliance (i.e., reduced collapsibility; Refs. 5, 17). Therefore, if upper airway muscle LTF promotes pharyngeal airway stability during sleep, the present data suggest that tongue muscle coactivation is an important part of this defense mechanism.

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GRANTS

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