Intensity and frequency dependence of laryngeal afferent inputs to respiratory hypoglossal motoneurons

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Mifflin, Steven W. Intensity and frequency dependence of laryngeal afferent inputs to respiratory hypoglossal motoneurons. J. Appl. Physiol. 83(6): 1890–1899, 1997.—Inspiratory hypoglossal motoneurons (IHMs) mediate contraction of the genioglossus muscle and contribute to the regulation of upper airway patency. Intracellular recordings were obtained from antidromically identified IHMs in anesthetized, vagotomized cats, and IHM responses to electrical activation of superior laryngeal nerve (SLN) afferent fibers at various frequencies and intensities were examined. SLN stimulus frequencies <2 Hz evoked an excitatory-inhibitory postsynaptic potential (EPSP-IPSP) sequence or only an IPSP in most IHMs that did not change in amplitude as the stimulus was maintained. During sustained stimulus frequencies of 5–10 Hz, there was a reduction in the amplitude of SLN-evoked IPSPs with time with variable changes in the EPSP. At stimulus frequencies >25 Hz, the amplitude of EPSPs and IPSPs was reduced over time. At a given stimulus frequency, increasing stimulus intensity enhanced the decay of the SLN-evoked postsynaptic potentials (PSPs). Frequency-dependent attenuation of SLN inputs to IHMs also occurred in newborn kittens. These results suggest that activation of SLN afferents evokes different PSP responses in IHMs depending on the stimulus frequency. At intermediate frequencies, inhibitory inputs are selectively filtered so that excitatory inputs predominate. At higher frequencies there was no discernible SLN-evoked PSP temporally locked to the SLN stimuli. Alterations in SLN-evoked PSPs could play a role in the coordination of genioglossal contraction during respiration, swallowing, and other complex motor acts where laryngeal afferents are activated.

upper airway patency; control of breathing; neonatal respiration; airway-maintaining muscles and reflexes

THE OROPHARYNX plays a critical role in the coordination of swallowing, coughing, gagging, and respiration (1, 13). For example, compromised oropharyngeal patency has been implicated in the etiology of obstructive sleep apnea (22). The genioglossus muscle, which is innervated by the hypoglossal nerve, is an important determinant of oropharyngeal patency. Therefore, information regarding the integration of peripheral afferent inputs by hypoglossal motoneurons will provide insight into the reflex regulation of upper airway patency and the coordination of complex motor acts such as swallowing and respiration.

The larynx is richly supplied with mechanoreceptors that can have profound effects on respiration. Electrical activation of laryngeal afferent fibers or pressure changes in the upper airway increase the activity recorded in the genioglossus electromyogram (15, 20), the whole hypoglossal nerve (4, 24), and single hypoglossal motoneurons (7, 29). Mathew et al. (15) suggested that laryngeal-hypoglossal reflexes serve a load compensation function so that pressure changes that promote upper airway closure will activate the tongue protruder muscles and decrease the likelihood of collapse of the upper airway. These reflexes also play a role in terminating apneic episodes resulting from relapse of the tongue and upper airway closure (6, 8, 22).

Studies have also reported that activation of laryngeal afferent fibers can influence respiration beyond the actual periods of stimulation (7, 15). Such prolonged effects on respiratory hypoglossal motoneurons serve a protective function and prevent the recurrence of tongue relapse and upper airway closure in the face of the negative intrathoracic pressure generated by inspiratory activity.

Therefore, knowledge of the laryngeal input to hypoglossal motoneurons that discharge during inspiration (IHMs) is important to our understanding of the regulation of upper airway patency at the level of the oropharynx under normal circumstances (e.g., swallowing) and during instances where this regulation is compromised (e.g., obstructive sleep apnea). To this end, intracellular recordings were obtained from antidromically identified IHMs and their responses to electrical stimulation of laryngeal afferent fibers at various frequencies and intensities were examined. The results indicate that inhibitory laryngeal afferent inputs to IHMs undergo a frequency-dependent filtering. It is possible that this filtering could play a role in determining the reflex responses of the IHMs and thereby influence reflex regulation of upper airway patency.

METHODS

Experiments were performed on adult cats of either sex weighing 2.2–4.7 kg. Anesthesia was induced by halothane inhalation (3.5–4% in 100% O2 at 3–5 l/min). After placement of catheters in the femoral artery and vein for measurement of arterial pressure and injection of drugs, respectively, halothane inhalation was discontinued and anesthesia was continued by intravenous injection of pentobarbital sodium (Nembutal, 35 mg/kg). Maintenance doses of pentobarbital sodium (5 mg) were given as needed as determined by stability of arterial pressure, phrenic nerve discharge, and absence of withdrawal reflexes.

The trachea was cannulated well below the larynx, and the animal was mechanically ventilated. The animal was paralyzed (gallamine triethiodide, 20 mg initially supplemented with 4 mg as needed) and given a bilateral pneumothorax to reduce respiratory movements of the brain stem. After paralysis, depth of anesthesia was assessed by stability of arterial pressure and injection of drugs, respectively, and maintained at 4–5% by adjusting lung volume and/or respiratory rate. Arterial blood gases were periodically sampled, and any metabolic acidosis was
corrected by intravenous injection of warmed 1 M sodium bicarbonate. Temperature was measured by a rectal probe and maintained at 37 ± 1°C by a ventral pad circulating warmed water and a dorsal infrared lamp.

The animal was placed in a stereotaxic frame and suspended by cervical and lumbar vertebral clamps. The muscles covering the back of the head were removed, and the brain stem was exposed by occipital craniotomy. The caudal cerebellum was displaced cranially or removed by suction to provide better access to the hypoglossal nucleus. Intracellular recordings were obtained from within a 1-mm pressure foot placed lightly on the surface of the brain to stabilize the area of electrode placement and facilitate intracellular recording.

The common trunk of the hypoglossal nerve and the superior laryngeal nerve (SLN) ipsilateral to the central recording site were placed intact on separate bipolar electrodes for electrical stimulation. In some animals the ipsilateral glossopharyngeal nerve was isolated and placed intact on bipolar stimulating electrodes. The phrenic nerves were cut bilaterally, and the central ends were desheathed and placed on bipolar recording electrodes. The activity in either phrenic nerve was used as an index of central respiratory activity. Because the lungs were inflated independently of phrenic nerve discharge, the cervical vagus nerves were sectioned bilaterally to remove the inhibitory effects of lung stretch afferent inputs on IHM discharge (9, 24, 29). All nerves prepared were covered with a mixture of petroleum jelly and mineral oil. Phrenic nerve discharge frequency was measured by counting the discriminated discharge with an analog-to-digital frequency meter (model 5301A, Hewlett-Packard). Nerves were stimulated by square-wave pulses delivered from constant-current isolated stimulators (model A300, WPI). Stimulus frequency, pulse width, and delay were controlled using a WPI Masterpulse stimulator. The hypoglossal nerve was stimulated with 25- to 100-µA 0.2-ms pulses at a frequency of 1 Hz. The SLN was stimulated with 0.2-ms pulses of variable intensity and frequency. The SLN stimulus intensities are presented as multiples of the threshold intensity necessary for a single shock to produce an inhibition of phrenic nerve activity, e.g., 2 and 10 times threshold (2T and 10T). These threshold intensities ranged from 20 to 45 µA. Intensities of 10T were considered to activate all the afferent fibers in the SLN (19).

Intracellular recordings were obtained from hypoglossal motoneurons identified by their antidromic activation after electrical stimulation of the hypoglossal nerve. Standard criteria for classifying a response as antidromic were used (12): collision of antidromic with orthodromic action potentials and invariant antidromic response onset latency. A voltage follower equipped with capacitance compensation and a bridge circuit (model 767, WPI) was used. Electrodes filled with a mixture of 2 M K-citrate and 0.2 M KCl (direct-current resistance = 20–80 MΩ) were used. A horseshoe-shaped pressure foot (≈1 mm ID) was occasionally used to stabilize the medulla. Recordings were considered satisfactory if membrane potential exceeded −50 mV.

In addition to the above experiments in adult animals, intracellular recordings were obtained from antidromically identified hypoglossal motoneurons in ten 166- to 244-g kittens at 6–13 days after birth. The surgical preparation of these animals was the same as preparation of the adult animals with the following exceptions. In the newborns, anesthesia was induced by an intraperitoneal injection of pentobarbital sodium (35 mg/kg). The newborn animals were not suspended by vertebral clamps, and a pressure foot was not used to stabilize the medulla.

All parameters (arterial pressure, membrane potential, end-tidal CO₂, phrenic nerve activity) were displayed on a chart recorder (model TA2000, Gould), digitized, and recorded on videotape (model 4000, Vetter). Faster time base sweeps of membrane potential responses to nerve stimulation were viewed on a digital oscilloscope (model 320, Niccol). Signal averaging was done off-line using the Cambridge Electronic Design 4100. Values are means ± SE. A one-way analysis of variance with Scheffé’s F procedure for post hoc comparisons was used to determine statistical significance. When responses were normalized to a 100% control value, the analysis was run on the differences between the individual groups and the control group.

RESULTS

To examine the laryngeal input to single IHMs, intracellular recordings were obtained from 72 antidromically identified IHMs. An IHM was defined as a hypoglossal motoneuron that discharged action potentials and/or exhibited a membrane depolarization during periods of central inspiratory activity coincident with phrenic nerve discharge. Membrane potentials averaged −61 ± 1 mV, and antidromic latencies averaged 1.2 ± 0.1 ms.

Effects of changing stimulus intensity during low-frequency stimulation. Figure 1 illustrates averaged responses of an IHM to SLN stimulation at a frequency of 1 Hz and at two SLN stimulus intensities. In this IHM, SLN stimulation at 2T evoked only an excitatory postsynaptic potential (EPSP), whereas increasing the intensity to 10T evoked an EPSP-inhibitory postsynaptic potential (IPSP) sequence. Electrical stimulation of the SLN at 1 Hz and 10T, which should synchronously activate the entire population of SLN afferent fibers (19), evoked only an EPSP in one cell (latency 11.1 ms), an EPSP-IPSP sequence in 54 cells (latency 6.6 ± 0.4 ms, range 2.6–15.0 ms), and only an IPSP in 17 cells (latency 10.1 ± 0.5 ms, range 5.6–13.6 ms). In 12 of the 54 IHMs responding to SLN stimulation with an EPSP-IPSP sequence, a later, secondary EPSP was also observed. In 34 IHMs that responded to SLN stimulation at 10T with an EPSP-IPSP sequence, lowering stimulus intensity to 2T revealed that the EPSP was the lower threshold input in 24, whereas in 10 an EPSP-IPSP sequence was evoked at 2T. All IHMs tested responded to a 2T stimulus with a postsynaptic response (n = 43).

Figure 1 also illustrates how SLN-evoked postsynaptic potentials (PSPs) varied with the respiratory cycle. The latencies, rise times, and peak amplitudes of EPSPs evoked during inspiration were similar to those evoked during expiration (P < 0.05 for all 3 comparisons). The amplitude of SLN-evoked IPSPs, whether generated as the sole response or as a component of an EPSP-IPSP sequence, was greater in inspiration than in expiration (Fig. 1B). IPSP amplitudes were 3.1 ± 0.6 mV larger when evoked during inspiration than during expiration (P < 0.05, n = 11).

Effects of changing stimulus intensity during high-frequency stimulation. The effects of altering SLN stimulus intensity were examined at higher SLN stimulus frequencies. Figure 2 illustrates the responses of
Fig. 1. Averaged responses of an inspiratory hypoglossal motoneuron (IHM) to 2-times-threshold (2T, A) and 10-times-threshold (10T, B) superior laryngeal nerve (SLN) stimulation at 1 Hz during inspiration (top traces) and expiration (bottom traces). Each trace is averaged membrane potential response (25 sweeps) with corresponding average of phrenic nerve activity below. Distance between phrenic nerve and membrane potential sweeps represents a direct-current level of potential to illustrate that membrane potential was hyperpolarized during expiration (−69 mV) compared with inspiration (−63 mV). ○, Application of SLN stimulus.

Fig. 2. Responses of IHM in Fig. 1 to 10-Hz SLN stimulation at 2T (top) and 10T (bottom) during periods indicated by horizontal bars. Traces represent (from bottom to top) arterial pressure, phrenic neurogram, and membrane potential of IHM (action potentials are truncated).
the same cell depicted in Fig. 1 to a 30-s, 10-Hz period of SLN stimulation. During 10-Hz SLN stimulation at 2T, membrane potential depolarized and the cell discharged action potentials (top). The depolarization of membrane potential and action potential discharge declined, despite continued SLN stimulation. During the stimulation period, there was no phrenic nerve activity and the inspiratory depolarization of IHM membrane potential was absent. At the end of the stimulation the duration of the inspiratory depolarization was increased for two respiratory cycles. The response pattern illustrated in Fig. 2 (top) of early excitation/depolarization, which decayed and was followed by a period of no rhythmic, respiratory membrane potential oscillations, was observed in 12 IHMs.

Figure 2, bottom, illustrates the response of the same IHM to 10-Hz SLN stimulation at 10T. During the stimulation period there was an inhibition of phrenic nerve discharge as during the 2T stimulation. However, in contrast to the response to 2T stimulation, at the onset of SLN stimulation there was an immediate hyperpolarization of membrane potential. During the hyperpolarization there were large waves of depolarization that evoked very-high-frequency action potential discharge. At the end of the SLN stimulation the duration of the inspiratory depolarization was increased for two respiratory cycles, and discharge frequency during the inspiratory depolarization was increased for four cycles. The response pattern illustrated in Fig. 2 (bottom) of hyperpolarization and irregularly occurring, high-frequency bursts of action potential discharge was observed in 20 IHMs tested.

The post-SLN stimulation increase in inspiratory drive to IHMs is further illustrated in Fig. 3 for another IHM that was not discharging action potentials before the SLN stimulation. In 21 IHMs, after 30 s of 10-Hz SLN stimulation at 10T, membrane potential was depolarized and the amplitude of the inspiratory depolarization was markedly increased for ~3 min. Thirty seconds of 10-Hz SLN stimulation at 10T increased action potential discharge frequency in 10 of 12 IHMs that were discharging action potentials before the SLN stimulation. The increased discharge frequency persisted for 14–260 s after cessation of SLN stimulation. SLN stimulation evoked action potential discharge in six of nine IHMs that were not discharging before the SLN stimulation. The increased discharge persisted for 23–190 s after cessation of SLN stimulation. In IHMs that did and did not discharge before SLN stimulation (n = 21), the amplitude of the inspiratory depolarization was increased for 32–238 s after high-frequency SLN stimulation.

Frequency-dependent changes in SLN-evoked PSPs. During 10-Hz SLN stimulation, SLN-evoked PSPs underwent dramatic changes. Figure 4 illustrates the SLN-evoked PSPs during 10-Hz stimulation in the cell shown in Figs. 1 and 2. During 10-Hz SLN stimulation at 2T, the SLN-evoked EPSP underwent no discernible change with time (Fig. 4A). However, during 10-Hz stimulation at 10T, the IPSP component of the EPSP-IPSP sequence...
sequence decayed (Fig. 4B). There was no change in the amplitude or rise time of the initial component of the EPSP. At the end of 10 s, when the IPSP component was absent, the SLN evoked an EPSP similar to that evoked by 2T stimulation.

The SLN-evoked EPSP-IPSP sequences during 10-Hz stimulation from two different IHMs are illustrated in Fig. 5. In seven of nine IHMs, SLN stimulation at 10T evoked a later, secondary EPSP that followed the EPSP-IPSP sequence. During 10-Hz SLN stimulation as the IPSP decayed the initial EPSP merged with the secondary EPSP. The 10-Hz SLN stimulation evoked a secondary EPSP in five IHMs that exhibited no such late EPSP before the stimulation.

The frequency-dependent reduction in IPSP amplitude could be due to an increase in a depolarizing input that is facilitated and obscures the IPSP, or it could result from a reduction in the IPSP due to presynaptic and/or postsynaptic factors. As illustrated in Fig. 6, the frequency-dependent reduction was observed in IHMs in which SLN stimulation evoked only an IPSP. Therefore, the frequency-dependent reduction in inhibitory inputs can occur in the absence of any depolarizing input to the IHM, suggesting that it results from a reduction in IPSP, not an increase in EPSP.

Figure 7 illustrates the time course of the reduction in IPSP amplitude during a 10-Hz SLN stimulus at 10T. Within 2 s IPSP amplitude was ~40% of its amplitude during a 1-Hz stimulus. Within 10 s of the onset of stimulation, only three IHMs still responded with an IPSP, and this was reduced to <5% of control amplitude. Only one IHM did not exhibit a reduction in IPSP amplitude during 10-Hz SLN stimulation (the IHM illustrated in Fig. 3). At stimulus frequencies of 10 Hz, variable changes were observed in the initial EPSPs when they were present. In 7 IHMs the initial EPSP decreased in amplitude, in 10 IHMs it increased in amplitude, and in 31 IHMs it did not change.

The absolute magnitude and time course of IPSP decay at 10 Hz were dependent on stimulus intensity (Fig. 6). In 12 IHMs in which a comparison was made, the IPSP evoked by 2T stimulation invariably decayed more rapidly and to a greater extent than the IPSP evoked by 10T stimulation.

The time course of the decay in inhibitory inputs was also found to be dependent on the SLN stimulus frequency, as illustrated for a representative IHM in Fig. 8. The SLN-evoked IPSP did not decrease in amplitude until stimulus frequency reached 5 Hz. As stimulus frequency was increased above 5 Hz, the time necessary for the IPSP to reach its steady-state level decreased. At stimulus frequencies of 10 Hz, the SLN-evoked IPSP was selectively reduced, with EPSPs, when present, exhibiting no consistent change in amplitude. However, at SLN stimulus frequencies of 25 Hz, initial EPSPs were also abolished along with the inhibitory inputs.

The depressed IPSP recovered quite rapidly, and the recovery time was independent of the preceding stimulus frequency. After a 30-s, 10-Hz, 10T SLN stimulation...
period, the IPSP returned to its control amplitude within 1–3 s in the 21 IHMs in which it was possible to maintain the SLN stimulation for this period of time.

Other inputs to IHMs and other hypoglossal motoneurons. The frequency-dependent reductions were not restricted to laryngeal afferent inputs, inasmuch as qualitatively similar reductions were observed in PSPs evoked by glossopharyngeal nerve stimulation (not illustrated). In 24 of the 54 IHMs that responded to SLN stimulation with an EPSP-IPSP sequence, glossopharyngeal nerve stimulation also evoked an EPSP-IPSP sequence (latency 6.4 ± 0.5 ms, range 3.2–14.9 ms). Inhibitory glossopharyngeal inputs were depressed at stimulus frequencies >5 Hz, and excitatory and inhibitory glossopharyngeal nerve inputs were attenuated at stimulus frequencies >25 Hz.

The frequency-dependent depression of SLN-evoked inhibitory inputs was not restricted to IHMs (not illustrated). An identical process was observed in 12 expiratory hypoglossal motoneurons with membrane potential of −60.0 ± 2.4 mV and antidromic latency of 1.2 ± 0.1 ms. Similar frequency-dependent reductions in SLN-evoked inputs were observed in 24 nonrespiratory hypoglossal motoneurons. In all cases, inhibitory inputs began to decline in amplitude at stimulus frequencies of 2 Hz, whereas excitatory inputs did not decline until stimulus frequency reached 25 Hz.

Laryngeal inputs to hypoglossal motoneurons in newborns. The frequency dependence of SLN inputs to 17 hypoglossal motoneurons in newborns was examined. In 6 IHMs and 11 nonrespiratory hypoglossal motoneurons, the effects of increasing SLN stimulus frequency from 1 to 25 Hz was the same as observed in adult animals (Fig. 9). The steady-state level of reduction was reached in the same amount of time (P > 0.3), the steady-state level of the PSPs was reduced to the same amount (P > 0.5), and recovery times were similar in kittens and adults (P > 0.2).

DISCUSSION

IHMs mediate contraction of the genioglossus muscle and protrusion of the tongue (1, 13). This function is vital to the maintenance of upper airway patency and airflow during sleep, and reductions in genioglossal tone contribute to obstructive sleep apnea (22). IHMs must also integrate disparate inputs to coordinate genioglossal movements during complex motor acts such as swallowing and speaking. All these functions involve some degree of laryngeal afferent activation, which implies an important role for laryngeal afferents in the regulation of IHM discharge and genioglossal contraction. Therefore, it is not surprising that electrical activation of the SLN evokes complex waves of PSPs in most IHMs (7, 25, 29; present study).

Missing from many previous analyses of IHMs was an examination of SLN afferent inputs within the physiological range of laryngeal afferent fiber discharge frequency. Studies have shown that, within physiological ranges of transmural pressure, laryngeal mechano-
receptor afferent fibers discharge at frequencies between 10 and 20 Hz (5). This is well within the range of SLN frequencies utilized in the present study. Electrical stimulation of afferent fibers provides a temporal control of the stimulus that permits analysis of individual PSPs evoked by individual stimuli. In addition, when central neuronal responses to sustained afferent activation are examined, electrical activation of afferent fibers bypasses any contribution receptor adaptation (5) might make to the responses. However, the functional interpretation of data obtained using electrical activation of laryngeal afferent fibers should be done with caution, since afferent fibers are synchronously activated regardless of receptive modality. Nevertheless, the excitation and inhibition of IHMs during SLN stimulation (Figs. 2 and 3) have also been observed during “natural” activation of laryngeal receptors in animals (4, 15, 25, 28, 29) and humans (10, 11, 30).

A second factor to consider in the present study is the fact that the animals were vagotomized. Although this has been done in other studies of hypoglossal motoneurons (4, 29), it is possible that removal of pulmonary afferent-mediated inhibition of IHMs (24, 29) could alter the responses observed in the present study. In a recent study, Gauda et al. (3) found a dramatic increase in the genioglossal electromyogram response to a negative step pressure in the larynx after vagotomy. In addition, the study of Jiang et al. (7) was performed in cats, where the vagi were intact “in most experiments,” and found qualitatively and quantitatively similar IHM responses to SLN stimulation. These results indicate that pulmonary afferents do not qualitatively alter the response to laryngeal stimulation. Certainly, studies of

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Fig. 6. Response of an IHM to 10-Hz SLN stimulation at 2T (A) and 10T (B). Traces are arranged as in Fig. 4 with response to 1-Hz SLN stimulation designated as control. This IHM responded to SLN stimulation with only an inspiratory postsynaptic potential.

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Fig. 7. Inspiratory postsynaptic potential (IPSP) amplitude as a function of time after onset of 10-Hz SLN stimulation at 10T. IPSP amplitude, expressed as a percentage of control (Cont) response to 1-Hz SLN stimulation, was averaged for 20 IHMs at 2-s intervals after onset of SLN stimulation. Number of IHMs still exhibiting an IPSP at end of each interval is given in parentheses above bars. At all intervals, IPSP amplitude was significantly decreased (P < 0.05).
interactions between different afferent inputs at the level of single IHMs are needed to further understand the integrated responses of these neurons.

Intensity-dependent alterations in the SLN input. In most IHMs, the response to low-intensity SLN stimulation, regardless of stimulus frequency, was excitation. However, at higher stimulus frequencies the excitation decayed and respiratory rhythmic oscillations in membrane potential were absent. In contrast, the response to high-intensity SLN stimulation, regardless of stimulus intensity, was inhibition. At higher stimulus frequencies the IPSPs temporally locked to individual SLN stimuli decayed and were eventually absent in most IHMs.

The reduction in SLN-evoked IPSP amplitude during expiration is likely due to the hyperpolarization of membrane potential, inasmuch as IHMs do not appear to be postsynaptically inhibited during expiration (29). Unlike inspiratory neurons in the dorsal and ventral respiratory groups, which oscillate between synaptic excitation and synaptic inhibition (23), IHMs appear to receive only a wave of synaptic depolarization during inspiration. This is also suggested by the observation that there was no decrease in the amplitude of SLN-evoked EPSPs during expiration (Fig. 1), which one would expect if the neuron was postsynaptically inhibited during this phase of the respiratory cycle.

With the caveats previously discussed regarding electrical stimulation of afferent fibers in mind, the data suggest that low-threshold laryngeal afferents are recruited or a larger number of afferents are simultaneously activated, IHMs receive predominantly an inhibitory drive. These results suggest a complex regulation of IHM discharge and excitability as a function of the relative number of the laryngeal inputs activated in response to a given stimulus and the relative thresholds of the afferent fibers activated.

Frequency-dependent alterations in the SLN input. During high-frequency (25-Hz) stimulation of the SLN, the excitatory and inhibitory components of the SLN input were attenuated as a function of stimulus frequency, regardless of stimulus intensity. The attenuation of inhibitory laryngeal inputs was evident in a previous intracellular study of IHMs (see Fig. 7 in Ref. 29). Whether this attenuation occurs at the level of the IHM or at another site of the reflex pathway remains to be definitively determined, and attempts to discern a mechanism for the frequency-dependent reductions in PSP amplitude are speculative at this point. Several studies have reported activity-dependent reductions in afferents are recruited or a larger number of afferents are simultaneously activated, IHMs receive predominantly an inhibitory drive. These results suggest a complex regulation of IHM discharge and excitability as a function of the relative number of the laryngeal inputs activated in response to a given stimulus and the relative thresholds of the afferent fibers activated.

Fig. 8. Decay in IPSP amplitude in a representative IHM during 10T SLN stimulation at various frequencies. IPSP amplitude is expressed as a percentage of control response measured during 1-Hz SLN stimulation at various points in time after onset of SLN stimulation. Symbols represent different SLN stimulus frequencies.

Fig. 9. Response of IHM from an 8-day-old kitten to 10-Hz SLN stimulation at 10T. Traces are arranged as in Fig. 4 with response to 1-Hz SLN stimulation designated as control.
IPSP amplitude as a result of changes at the level of the postsynaptic neuron (e.g., changes in driving force and/or conductance due to intracellular chloride accumulation or changes in membrane potential) (16, 26) or the presynaptic nerve terminal (e.g., reduction of inhibitory transmitter release through activation of presynaptic γ-aminobutyric acid type B receptors) (2, 27). The rapid recovery of the IPSP suggests that postsynaptic alterations in driving force and/or conductance are not major factors, inasmuch as changes in these factors typically take minutes to recover.

In contrast to changes at the level of the hypoglossal motoneuron, the reduction in SLN-evoked IPSP might be the result of changes in afferent integration antecedent to the IHM. For example, a previous study reported qualitatively similar frequency-dependent reductions in SLN afferent inputs to neurons within the nucleus tractus solitarius (NTS) (17). In the present study, at intermediate frequencies of SLN stimulation (2–10 Hz), inhibitory components were selectively attenuated while excitatory components were unaltered or enhanced. In studies of the frequency dependence of afferent inputs to NTS neurons, selective frequency-dependent attenuation of excitatory compared with inhibitory inputs was rarely observed (18). However, in the study of SLN inputs a population of monosynaptically activated NTS neurons was identified that did not exhibit frequency-dependent inhibition (17). Therefore, the possibility exists that the excitatory SLN inputs to IHMs are relayed via these NTS neurons.

In addition to the SLN-evoked PSP directly linked to the stimulus, high-frequency SLN stimulation also evoked large waves of depolarization in IHMs, which resulted in high-frequency bursts of action potentials. These bursts are analogous to those recorded in hypoglossal motoneurons during chewing and swallowing (25, 28). Although one might speculate that the frequency-dependent attenuation of IPSPs makes an IHM more responsive to excitatory (swallowing) inputs, Fig. 2 suggests that these waves occur during, and despite, SLN-evoked IPSPs. The frequency-dependent reduction in IPSP might make the discharge evoked by these waves of depolarization more robust and/or prolonged, which would facilitate tongue protrusion.

The convergent excitatory and inhibitory effects of laryngeal afferent activation on IHMs indicate that a great deal of flexibility is possible in the IHM responses to activation of specific laryngeal receptors. This is no doubt a reflection of the variety of functions that the tongue serves. For example, during intense laryngeal stimulation, the early inhibition of IHM discharge could serve a protective function by preventing aspiration. With sustained stimulation, the frequency-dependent reduction in IPSPs coupled with laryngeal mechanoreceptor adaptation (5) could facilitate mechanisms favoring tongue protrusion. The balance between excitatory and inhibitory inputs is dependent on the specific stimulus, i.e., the receptor properties and locations. The selective attenuation of inhibitory inputs at lower stimulus frequencies suggests that under conditions where inhibitory and excitatory inputs are activated the excitatory inputs will provide the major input to the IHMs.

SLN inputs to newborns. Laryngeally evoked apneas are typically short lived in the adult (6, 8, 22), inasmuch as arousal mechanisms terminate the apneic episode. A contributing factor to this phenomenon might be the observation that SLN-evoked IPSPs decline as a function of time at stimulus frequencies >5 Hz. The presence of material in the trachea and/or increases in tracheal pressure could activate excitatory and inhibitory inputs to IHMs. Initially the inhibitory inputs might predominate, and the reduction in IHM discharge could promote upper airway closure. The inhibitory inputs would decline in a frequency- and time-dependent manner, leaving the IHM more responsive to the remaining excitatory laryngeal inputs or excitatory, arousal inputs from other sources.

In contrast to the adult, in the newborn, laryngeally evoked apneas are long lasting and can lead to death (14, 21). One possible explanation is that, in the newborn, SLN-evoked IPSPs are not attenuated as a function of frequency. The presence of material in the larynx and/or increases in laryngeal pressure could activate excitatory and inhibitory afferent inputs to IHMs. The inhibitory inputs might predominate for the duration of the stimulus, leading to a sustained closure of the upper airway. However, no evidence was found to suggest that the frequency-response characteristics of laryngeal inputs to hypoglossal motoneurons in the newborn were different from those in the adult.

Poststimulus facilitation. The poststimulus facilitation of IHM discharge that followed SLN stimulation was originally described by Mathew et al. (15) and subsequently studied at the single cell level by Jiang et al. (7). In agreement with these studies, the present study found that after SLN stimulation the discharge of IHMs was enhanced for a period of minutes. This was due to the fact that after SLN stimulation the inspiratory wave of depolarization was increased so that even previously silent IHMs now discharged action potentials. Therefore, the poststimulus facilitation of the activity recorded as the genioglossus electromyogram (15) would appear to be the result of increased discharge of those IHMs already firing and recruitment of previously silent IHMs.

The protective role of such an enhancement of IHM discharge after a period of laryngeal stimulation has been discussed previously (7, 15). The point of interest in the context of the present study is the observation that this enhancement far outlasts any change in the “directly evoked” SLN inputs, which return to control levels within a few seconds after cessation of high-frequency SLN stimulation.

Perspective

The results indicate that, in addition to receptor adaptation, central mechanisms exist that can also contribute to the adaptation of laryngeal reflex responses during sustained stimulation. During a relapse of the tongue and subsequent apnea, the attenuation of the inhibitory laryngeal components would be function-
ally advantageous, inasmuch as it would make the cell amenable to excitatory inputs that would protrude the tongue and terminate the apneic episode. This potentially protective mechanism appears to be functional in the newborn.

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