Central GABAergic mechanisms are involved in apnea induced by SLN stimulation in piglets

JALAL M. ABU-SHAWEESH, ISMAIL A. DRESHAJ, MUSA A. HAXHIU, AND RICHARD J. MARTIN
Department of Pediatrics, Case Western Reserve University, Cleveland, Ohio 44106

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Abu-Shaweesh, Jalal M., Ismail A. Dreshaj, Musa A. Haxhiu, and Richard J. Martin. Central GABAergic mechanisms are involved in apnea induced by SLN stimulation in piglets. J Appl Physiol 90: 1570–1576, 2001.—Stimulation of the superior laryngeal nerve (SLN) results in apnea in animals of different species, the mechanism of which is not known. We studied the effect of the GABA_A receptor blocker bicuculline, given intravenously and intracisternally, on apnea induced by SLN stimulation. Eighteen 5- to 10-day-old piglets were studied; bicuculline was administered intravenously to nine animals and intracisternally to nine animals. The animals were anesthetized and then decerebrated, vagotomized, ventilated, and paralyzed. The phrenic nerve responses to four levels of electrical SLN stimulation were measured before and after bicuculline. SLN stimulation caused a significant decrease in phrenic nerve amplitude, phrenic nerve frequency, minute phrenic activity, and inspiratory time (P < 0.01) that was proportional to the level of electrical stimulation. Increased levels of stimulation were more likely to induce apnea during stimulation that often persisted beyond cessation of the stimulus. Bicuculline, administered intravenously or intracisternally, decreased the SLN stimulation-induced decrease in phrenic nerve amplitude, minute phrenic activity, and phrenic nerve frequency (P < 0.05). Bicuculline also reduced SLN-induced apnea and duration of poststimulation apnea (P < 0.05). We conclude that centrally mediated GABAergic pathways are involved in laryngeal stimulation-induced apnea.

Superior laryngeal nerve; GABA_A receptors; reflex-induced apnea; control of breathing; apnea of prematurity

STIMULATION OF THE LARYNGEAL mucosa results in apnea in humans as well as in animals of different species (10, 19, 27, 37). This reflex apnea is mediated via stimulation of the superior laryngeal nerve (SLN) (10, 21). It has been suggested that an exaggerated reflex response in newborns may be implicated in multiple pathologial processes, including gastroesophageal reflex-induced apnea (19), sudden infant death syndrome (5, 38), and apnea of prematurity (27).

The laryngeal inhibitory reflex has been shown to be influenced by maturation (9, 21, 24, 34). Lee et al. (21) demonstrated respiratory arrest in newborn piglets by maintaining chemical stimulation of the larynx; older piglets did not express a similar response. This maturational change in the laryngeal inhibitory reflex was also found in dogs, monkeys, sheep, and cats (24, 31, 34, 37), as well as in preterm infants (27). However, the mechanisms underlying the maturational changes in the laryngeal reflex are not known. The fact that respiratory arrest may persist beyond the period of laryngeal stimulation suggests stimulation of a central neural mechanism that inhibits respiratory rhythm after cessation of the stimulus (18).

GABA is the major inhibitory neurotransmitter in the central nervous system. GABA expresses its effect through stimulation of ionotropic fast chloride receptors (GABA_A) or metabotropic receptors (GABA_B) to produce slow prolonged inhibitory signals (28). GABA has been implicated in control of breathing (12) and was found to inhibit breathing mainly via activation of GABA_A receptors (12, 13). GABA_A receptors have been shown to undergo maturational changes in rats (39). Xia and Haddad (39) demonstrated a much higher GABA_A receptor density in the newborn than in the adult rat brain stem. It seems, therefore, that GABA is a good candidate to represent the centrally mediated inhibitory mechanism that controls reflex-induced apnea.

We hypothesized that upregulation of inhibitory neurotransmitter-mediated mechanisms in the brain stem during the neonatal period enhances the susceptibility of newborns to reflex-induced apnea. The aim of this study was to characterize the role of GABA in this phenomenon by blocking the effects of the inhibitory neurotransmitter GABA on SLN stimulation-induced apnea.

METHODS

Newborn piglets, 5–10 days old, were purchased from a local vendor (n = 18). The animals were divided into two groups according to the route of administration of the GABA_A receptor blocker bicuculline: intravenously (n = 9) and intracisternally (n = 9). A warming pad was used to maintain body temperature at 37–38°C. In our initial studies (intravenous bicuculline), animals (n = 9) were anesthetized using inhaled methoxyflurane (Metofane, Pitman Moore). However, when methoxyflurane became unavailable, later animals (intracisternal bicuculline, n = 9) were initially sedated with a mixture of intramuscular xylazine (1.4 mg/kg) and...
ketamine (7.2 mg/kg) followed by intravenous thiopental sodium (25–30 mg/kg). The trachea was intubated, and the animals were placed on a volume ventilator (Harvard Apparatus). The volume and frequency of tidal breaths were adjusted to maintain a stable eucapneic arterial PCO2 of 35–40 Torr using an end-tidal CO2 analyzer while using an inspired justed to maintain a stable eucapneic arterial PCO2 of 35–40

RESULTS

SLN stimulation. Stimulation of the SLN before bicuculline administration caused a significant decrease in phrenic nerve amplitude, frequency, T1, and minute phrenic activity (all F < 0.01) that was proportional to the level of stimulation (Fig. 1). Maximal electrical stimulation (at 4 times threshold) caused a decrease in phrenic amplitude to 20 ± 8% of baseline (P < 0.01) and a decrease in minute phrenic activity to 9 ± 6% of baseline (P < 0.01). Maximal SLN stimulation also caused a significant decrease in phrenic nerve frequency from 42 ± 4 to 4 ± 2 breaths/min (P < 0.01). The decrease in frequency was secondary to significant prolongation of T6 (from 0.9 ± 0.1 to 21.1 ± 5.6 s, P < 0.01). SLN stimulation caused apnea (total cessation of phrenic activity throughout stimulation) in a greater number of piglets with increased level of stimulation: 8 of 18 piglets at 1.5 times threshold, 10 of 18 piglets at 2 times threshold, and 14 of 18 piglets at 4 times threshold. The duration of poststimulation apnea was also directly related to level of electrical stimulation (Fig. 1): from 3.3 ± 1.7 s at 1.5 times threshold stimulation to 15.8 ± 7.1 s at 4 times threshold.

Effect of bicuculline on baseline phrenic activity. There was no significant effect of bicuculline administration, intravenous and intracisternal, on the baseline phrenic activity. In nine animals, phrenic nerve amplitude was increased after bicuculline administration; in the other nine animals, phrenic nerve amplitude was decreased. Furthermore, there was no correlation between the effect of bicuculline on the baseline phrenic activity and its ability to block the SLN stimulation-induced decrease in phrenic nerve activity.

Intravenous bicuculline. There was a significant effect of bicuculline on the phrenic nerve amplitude, frequency, and minute activity responses to SLN stimulation (P < 0.05, 2-way ANOVA). Intravenous bicuculline blocked the SLN stimulation-induced decrease in phrenic nerve amplitude, minute phrenic activity, and phrenic nerve frequency. At maximal SLN stimulation, the phrenic nerve amplitude was 70 ± 24% of baseline and the frequency changed from 36 ± 6 to 27 ± 6 breaths/min (not significant). The resultant minute phrenic activity was 71 ± 27% of baseline with maximal SLN stimulation (not significant; Figs. 2 and 3). Bicuculline blocked the decrease in phrenic nerve frequency with SLN stimulation through inhibition of the prolongation in T6 (Fig. 4). There was no difference in the T1 response to SLN stimulation before and after bicuculline administration (Fig. 4).

Intravenous bicuculline almost abolished the episodes of apnea in response to SLN stimulation. In the
nine piglets studied, apnea occurred in one animal at 1.5 times threshold, one animal at 2 times threshold, and two animals at 4 times threshold. Bicuculline also decreased the duration of poststimulation apnea: 15.8 ± 7.1 and 3.1 ± 3.1 s at four times threshold before and after bicuculline, respectively.

Intracisternal bicuculline. There was a significant effect of intracisternal bicuculline on the phrenic nerve amplitude, frequency, and minute activity responses to SLN stimulation ($P < 0.01$, 2-way ANOVA). Intracisternal bicuculline attenuated, but did not completely block, the decrease in phrenic amplitude, frequency, and minute phrenic activity in response to SLN stimulation (Fig. 5). The phrenic nerve amplitude at maximal SLN stimulation decreased to 24 ± 12% of baseline after bicuculline ($P < 0.01$) vs. 7 ± 7% before bicuculline. The phrenic nerve frequency decreased with maximal stimulation from 37 ± 4 to 16 ± 6 breaths/min after bicuculline ($P < 0.05$) vs. 41 ± 3 to 2 ± 2 breaths/min before bicuculline. The resultant decrease in minute phrenic activity with SLN stimulation was 34 ± 20% of baseline ($P < 0.01$) vs. 4 ± 4% before bicuculline. The attenuation in phrenic nerve frequency response to SLN stimulation with bicuculline was achieved by a significant decrease in the prolongation of T$e$ ($P < 0.01$).
Intracisternal bicuculline administered to nine piglets also reduced SLN-induced apnea and duration of poststimulation apnea. No apneic episodes occurred at 1.5 and 2 times threshold stimulation, while apnea occurred in six piglets at 4 times threshold stimulation. The duration of poststimulation apnea at four times threshold was significantly reduced after intracisternal bicuculline: from 11.6 ± 4.1 to 5.7 ± 2.8 s before and after bicuculline, respectively (P < 0.05).

DISCUSSION

In this study, we demonstrated the role of centrally mediated GABAergic mechanisms in mediating SLN stimulation-induced apnea. Inhibition of the effect of endogenously released GABA through blocking GABA\(_\text{A}\) receptors almost abolished reflex-induced apnea. Bicuculline given intravenously or intracisternally was able to block SLN stimulation-induced apnea, indicating that the effect of bicuculline is related to blockade of central GABA\(_\text{A}\) receptors and not peripheral actions of bicuculline. This is further supported by the ability of bicuculline to reduce the duration of poststimulation apnea. To our knowledge, this is the first report linking GABAergic mechanisms with reflex laryngeal-induced apnea.

Reflex-induced apnea elicited through stimulation of the laryngeal mucosa has been well characterized in humans and animals of different species. This reflex apnea is mediated through the SLN, inasmuch as bilateral sectioning of the SLN abolishes laryngeal stimulation-induced apnea (8, 10, 21). This reflex apnea is associated with contraction of the thyroarytenoid muscle, causing closure of the glottis and swallowing movements, signifying active stimulation of brain stem centers. The purpose of such inhibition of breathing combined with closure of upper airways through stimulation of the larynx is not clear. However, Kianicka et al. (15) and Renolleau et al. (29) described spontaneous apneas associated with contraction of the thyroarytenoid muscle and swallowing movements in fetal and preterm lambs. These investigators suggested that glottic closure during apnea might enhance prenatal lung development in fetal lambs by preventing pulmonary fluid efflux from the trachea (15) and might support higher lung volumes and alveolar gas exchange in preterm animals (29). On the other hand, persistence...
of an exaggerated reflex-induced apnea in preterm and full-term infants might predispose to multiple pathological processes such as gastroesophageal reflux-induced apnea (19), apnea of prematurity (27), and sudden infant death syndrome (5, 31, 38).

Newborn animals of different species have been shown to exhibit an exaggerated response to laryngeal stimulation compared with adult animals (9, 21, 24, 34). Chemical stimulation of the larynx in newborn piglets caused respiratory arrest, whereas older piglets did not express a similar response (8, 21). Maturation of the laryngeal inhibitory reflex is also common to a wide variety of other animals, including dogs, monkeys, sheep, and cats (24, 31, 34, 37). Preterm infants also appear to express such an exaggerated inhibitory reflex, inasmuch as they elicit prolonged apnea in response to instillation of saline in the oropharynx (27). The mechanisms underlying such maturational change in reflex-induced apnea are not known; however, they do not seem to be related to changes in laryngeal receptors (11), changes in central synaptic connections, or maturation of the carotid body (7).

GABA is the main inhibitory neurotransmitter in the central nervous system and has been implicated in control of breathing, although the mechanism whereby it induces respiratory inhibition is still under study. Systemic administration of GABA was found to induce apnea in rats (14), thought to occur via activation of GABA_\textsubscript{A} receptors (12, 13). Czyzyk-Krzeska and Lawson (3) reported that hyperpolarization of medullary neurons during SLN stimulation was reversed by chloride current, indicating a chloride-mediated postsynaptic inhibition. This is similar to the hyperpolarization induced by stimulation of GABA_\textsubscript{A} receptors, which causes direct increases in membrane permeability to chloride ions with subsequent hyperpolarization of the membrane potential (28). These data suggest a potential role for endogenously released GABA in mediating reflex-induced apnea. Structural and functional differences in GABA_\textsubscript{A} receptors have been observed during development (1, 16, 23). GABA_\textsubscript{A} receptors are heterooligomers assembled from four different subunits (33). During embryonic and early postnatal development, the “mix” of GABA_\textsubscript{A} receptor subunits differs from that in adults (16, 23). Furthermore, Xia and Haddad (39) demonstrated a much higher GABA_\textsubscript{A} receptor density in the newborn than in the adult rat brain stem. Therefore, maturation of reflex-induced apnea might be related to maturational changes in the GABAergic system.

Multiple investigators have suggested a role for centrally acting inhibitory neurotransmitters that might mediate reflex-induced apnea (3, 17, 18, 20). The strongest evidence for the presence of such an inhibitory neurotransmitter may be the persistence of poststimulation apnea (18). Inhibitory mechanisms and neural factors that have been found to modulate laryngeal stimulation-induced apnea include cholinergic pathways and endorphins (30, 35, 36). Richardson et al. (30) found that blockade of M_3 muscarinic receptors resulted in an age-related decrease in apnea duration induced by laryngeal stimulation. Storm et al. (36) described an increase in levels of β-endorphins in cerebrospinal fluid of newborn piglets that was related to duration of laryngeal stimulation, and pretreatment of these animals with naloxone decreased the duration of apnea in response to laryngeal stimulation. Intracerebral injection of β-endorphins has also been shown to potentiate laryngeal stimulation-induced apnea (35). These data suggest an interactive role for several neurotransmitters and neuromodulators in eliciting laryngeal stimulation-induced apnea. Oertel et al. (25, 26) demonstrated a coexistence of opioid peptides and GABA in the same neurons. Furthermore, several investigators demonstrated reciprocal mediation between GABA and endogenous opioids (2, 4, 32, 40). Systemic administration of morphine caused a significant increase in GABA levels in the substantia nigra (40). Endogenous opioids have also been found to mediate the effect of GABA on the vasopressin system in humans (2), whereas GABA has been found to mediate opioid release in rats (32) and its analgesic effect (4). These data suggest a complex interaction between several central inhibitory neurotransmitters in the regulation of neural output, including respiratory control, as suggested by the present data.

An explanation for our results, other than direct involvement of GABA in the laryngeal stimulation-induced apnea reflex, might be that bicuculline changed the balance between excitatory and inhibitory pathways toward higher chemosensitivity, increasing the threshold for SLN stimulation-induced apnea (22). We and others previously showed that hypercapnia increases, whereas hypocapnia decreases, the threshold for SLN stimulation-induced apnea (17, 22). Cooling of the ventromedullary surface, a technique used to decrease central chemosensitivity by inhibiting synaptic transmission at this site, decreased the threshold for laryngeal stimulation-induced apnea (22). Theophylline, which stimulates respiratory neural output, has been shown to block SLN stimulation-induced apnea (21). Therefore, the overriding level of respiratory neural output or central chemosensitivity might change the threshold for laryngeal stimulation-induced apnea. However, this does not readily explain the ability of GABA receptor blockade to eliminate poststimulation apnea.

Intravenous bicuculline appeared more effective than intracisternal bicuculline in blocking the respiratory inhibition induced by SLN stimulation. The quantitative difference in the responses to intravenous vs. intracisternal bicuculline might be related to several factors. In the intracisternal group, the anesthesia used before decerebration included thiopental, which is known to augment SLN stimulation-induced apnea (24). Although the study was not started until ≥1 h after the last thiopental dose, a residual effect of thiopental might have contributed to the difference between the two responses. In addition, the intravenous...
bicuculline might have reached deeper groups of brain stem neurons that are involved in the reflex apnea.

This study does not identify the site of release of GABA or the respiratory groups involved in such a response. However, we propose that stimulation of the SLN results in excitation of second-order neurons that contain GABA and project to medullary inspiratory neurons. Release of GABA may result in hyperpolarization and inhibition of these inspiratory-related neurons and subsequent apnea. On the other hand, the depressing effect of SLN stimulation on the phrenic neurogram might represent lack of inspiratory premotor neuron activity in response to a subthreshold level activity of chemosensory neurons. Further studies are clearly needed to clarify these GABA-mediated pathways.

We conclude that centrally mediated GABAergic pathways are involved in laryngeal stimulation-induced apnea. We further speculate that upregulation of GABAergic mechanisms in newborns and preterm infants may enhance their vulnerability to reflex-induced apnea and contribute to important neonatal pathologies, such as gastroesophageal reflux-induced apnea, sudden infant death syndrome, or apnea of prematurity.

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REFERENCES


