Activation of central adenosine A$_{2A}$ receptors enhances superior laryngeal nerve stimulation-induced apnea in piglets via a GABAergic pathway

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Abu-Shaweesh JM. Activation of central adenosine A$_{2A}$ receptors enhances superior laryngeal nerve stimulation-induced apnea in piglets via a GABAergic pathway. J Appl Physiol 103: 1205–1211, 2007. First published July 26, 2007; doi:10.1152/japplphysiol.01420.2006.—Activation of the laryngeal mucosa results in apnea that is mediated through, and can be elicited via electrical stimulation of, the superior laryngeal nerve (SLN). This potent inhibitory reflex has been suggested to play a role in the pathogenesis of apnea of prematurity and sudden infant death syndrome, and is attenuated by theophylline and blockade of GABA$_A$ receptors. However, the interaction between GABA and adenosine in the production of SLN stimulation-induced apnea has not been previously examined. We hypothesized that activation of adenosine A$_{2A}$ receptors will enhance apnea induced by SLN stimulation while subsequent blockade of GABA$_A$ receptors will reverse the effect of A$_{2A}$ receptor activation. The phrenic nerve responses to increasing levels of SLN stimulation were measured before and after sequential intracisternal administration of the adenosine A$_{2A}$ receptor agonist CGS (n = 10) and GABA$_A$ receptor blocker bicuculline (n = 7) in ventilated, vagotomized, decerebrate, and paralyzed newborn piglets. Increasing levels of SLN stimulation caused progressive inhibition of phrenic activity and lead to apnea during higher levels of stimulation. CGS caused inhibition of baseline phrenic activity, hypotension, and enhancement of apnea induced by SLN stimulation. Subsequent bicuculline administration reversed the effects of CGS and prevented the production of apnea compared with control at higher SLN stimulation levels. We conclude that activation of adenosine A$_{2A}$ receptors enhances SLN stimulation-induced apnea probably via a GABAergic pathway. We speculate that SLN stimulation causes endogenous release of adenosine that activates A$_{2A}$ receptors on GABAergic neurons, resulting in the release of GABA at inspiratory neurons and subsequent respiratory inhibition.

control of breathing; sudden infant death syndrome hypoponosis

ACTIVATION OF THE LARYNGEAL mucosa either chemically (through instillation of water or acid) or mechanically results in apnea that is usually associated with adduction of upper airways and swallowing movements. This potent inhibitory reflex apnea, traditionally termed the laryngeal chemoreflex, has been well characterized in newborn infants (24, 25) and animals of different species and is mediated through the superior laryngeal nerve (SLN), a branch of the vagus nerve. Electrical stimulation of the SLN can induce a similar apneic response. The laryngeal chemoreflex is developmentally regulated because it is strongest in newborns (21) and has been proposed to play a role in the pathogenesis of apnea of prematurity (25), gastro-esophageal reflux-induced apnea (17) and sudden infant death syndrome (6, 33). However, the exact mechanisms, neuronal connections, and neurotransmitters involved in this response have not been well characterized.

The maturation of reflex apnea in piglets is very well described, and newborn animals are known to exhibit an exaggerated response to laryngeal stimulation (18). We have established in our previous studies the involvement of γ-aminobutyric acid (GABA) in SLN stimulation-induced apnea. Blockade of GABA$_A$ receptors through intracisternal or intravenous injections of bicuculline resulted in attenuation of SLN stimulation-induced apnea in newborn piglets (1). Theophylline has also been shown to prevent the laryngeal chemoreflex in newborn piglets (18). The methylxanthines caffeine and theophylline are widely used in the treatment of apnea of prematurity and have been traditionally thought to exert their action centrally by blocking receptors of the inhibitory neurotransmitter adenosine (7, 12). Recent reports have documented an interaction between adenosine and GABA during regulation of breathing and sleep. We have reported that blockade of GABA$_A$ receptors abolishes the inhibitory effect of the adenosine A$_{2A}$ agonist CGS-1680 (CGS) on phrenic activity in piglets (34). Additionally, A$_{2A}$ receptors were found to colocalize with GABAergic neurons in the medulla oblongata of both piglets (34) and rats (35). Furthermore, activation of adenosine A$_{2A}$ receptors caused increased release of GABA in the tuberomammillary nucleus of rats (13). These data together with the ability of both bicuculline and theophylline to block SLN stimulation-induced apnea raise the possibility of an interaction between GABA and adenosine in the pathogenesis of this inhibitory reflex response, although such interaction has not yet been described.

We hypothesized that 1) activation of central adenosine A$_{2A}$ receptors will enhance the inhibition of phrenic activity induced by SLN stimulation and allow apnea to occur at lower levels of electrical stimulation and 2) subsequent blockade of GABA$_A$ receptors will reverse the effects of activation of adenosine A$_{2A}$ receptors. In this study, we aimed to test this hypothesis by 1) examining the effect of intracisternal administration of the adenosine A$_{2A}$ receptor agonist CGS on SLN stimulation-induced apnea and 2) evaluating the effect of subsequently administering the GABA$_A$ receptor antagonist bicuculline on this response in the newborn piglet.

MATERIALS AND METHODS

All experimental procedures were approved by the Institutional Animal Care and Use Committee of Case Western Reserve University School of Medicine. The experiments were performed in 3- to 7-day-old piglets (n = 10), a well-identified model for studying laryngeal reflex apnea. The average weight of the animals was 2.1 ± 0.1 kg. The animals were initially sedated with an intramuscular mixture of xylazine (2.8 mg/kg) and ketamine (14.4 mg/kg), followed by intra-

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venous thiopental sodium at a dose of 25–30 mg/kg. The trachea was intubated through a cervical tracheostomy and the animals were placed on a volume-controlled ventilator (Harvard Apparatus). The volume and frequency of tidal breaths were adjusted to keep a stable eupneic arterial PCO₂ (PaCO₂, 35–45 Torr), using an end-tidal CO₂ analyzer, while using an inspired O₂ concentration of 100%. Femoral venous and arterial lines were inserted for administration of fluid and medication, and for blood pressure monitoring and blood gas analysis, respectively.

To avoid the known confounding effects of anesthesia on breathing and laryngeal reflex apnea (8, 18, 21), midcollicular decerebration was performed as previously described in newborn piglets (1, 5). Anesthesia was discontinued after decerebration was completed, and the animals were allowed to recover for at least 1 h following the surgical preparation before any experimental recordings were performed. Gallamine triethiodide (10 mg·kg⁻¹·h⁻¹) was used for paralysis.

Both vagal nerves were identified and cut in the caudal cervical area, below the origin of the SLNs to avoid the effect of lung inflation and other afferent pulmonary inputs on respiratory timing. The phrenic nerve was dissected, sectioned, desheathed, and placed on a bipolar recording electrode. The phrenic nerve electroneurogram was amplified (model P511, Grass Instruments) and rectified, and the amplified (model P511, Grass Instruments) and rectified, and the bipolar recording electrode. The phrenic nerve electroneurogram was connected to an analog computer. The moving average signal was connected to an analog computer. The SLN on one side was identified, dissected, sectioned, desheathed, and placed on a bipolar electrode connected to a stimulator (model S11, Grass Instruments). We defined SLN threshold, as the minimal amount of electrical current needed to decrease phrenic nerve amplitude or frequency. The electrical current needed to induce threshold stimulation averaged 6.6 ± 0.6 with a range of 4–11 μA. Fifteen seconds of SLN stimulation was performed at threshold, 1.5, 2, and 4× threshold using impulses with pulse duration of 4.5 ms and pulse interval of 50 ms applied through a photoelectric isolation unit (model PSIU6, Grass Instruments). The animals were placed prone, the back of the neck was dissected, and muscles were separated in the midline. The occipitoatlantal membrane was visualized, and the tip of a 10-to 15-cm, 25-gauge soft venous catheter was inserted through the membrane. Proper placement was confirmed by cerebrospinal fluid leak. The phrenic nerve response to multiple levels of SLN stimulation was measured at baseline and after sequential intracisternal administration of J) 0.1–0.3 ml of DMSO, the vehicle for CGS (n = 6); 2) 0.1–0.3 ml of a 10 μM solution of adenosine A₂A receptor agonist CGS (n = 10); and 3) 0.1–0.2 ml of 1 mg/ml solution of the GABA_A receptor blocker bicuculline (n = 7). These concentrations were chosen based on the minimal dose needed to cause a consistent effect from our previous studies in piglets (1, 34). The average duration between CGS and bicuculline administration was 118 ± 18 min. This represented the duration needed for adjustment of ventilator rate to allow for recovery of phrenic activity following CGS administration plus the duration of the SLN stimulation.

**Table 1. Effect of increasing levels of SLN stimulation on phrenic activity**

<table>
<thead>
<tr>
<th>Stimulation/Phrenic Activity</th>
<th>Baseline</th>
<th>Threshold</th>
<th>1.5× Threshold</th>
<th>2× Threshold</th>
<th>4× Threshold</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area, %</td>
<td>100</td>
<td>90±11</td>
<td>51±10</td>
<td>13±5</td>
<td>11±8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Frequency, %</td>
<td>100</td>
<td>83±4</td>
<td>69±12</td>
<td>27±12</td>
<td>7±5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Minute activity, %</td>
<td>100</td>
<td>82±5</td>
<td>105±5</td>
<td>13±8</td>
<td>7±5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T₈, s</td>
<td>0.68±0.05</td>
<td>0.74±0.11</td>
<td>0.63±0.12</td>
<td>0.39±0.14</td>
<td>0.15±0.11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T₉₅, s</td>
<td>0.8±0.2</td>
<td>1.0±0.5</td>
<td>4.2±2.0</td>
<td>12.8±3.5</td>
<td>38.5±16.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Duration of apnea, s</td>
<td>0</td>
<td>0</td>
<td>3.2±2.2</td>
<td>14.6±3.1</td>
<td>37±16.4</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means±SE. Superior laryngeal nerve (SLN) stimulation caused significant inhibition of phrenic area, frequency, and minute activity. The decrease in frequency was secondary to significant prolongation in expiratory time (TE) associated with significant shortening of inspiratory time (Ti) secondary to apnea. *P value refers to the effect of increasing levels of stimulation on phrenic activity via 1-way ANOVA. Post hoc multiple comparison analysis using Student-Neuman-Keuls test. **P < 0.05 for all paired comparisons except baseline and threshold and between 2× and 4× threshold. ***P < 0.05 for all paired comparisons except between baseline and threshold and between threshold and 1.5× threshold. $P < 0.05 only between 4× threshold and all other values.
Activation of Central A2A Receptors

The effect on baseline phrenic activity. Intracisternal CGS administration caused a significant decrease in phrenic nerve activity and resulted in apnea in four animals, (Fig. 1). In seven animals, recovery of phrenic activity required an increase in PaCO2, that was achieved by decreasing the ventilator rate. This resulted in an increase in average PaCO2 in all the animals from 39.7 ± 1.4 to 46.4 ± 3.3 Torr before and after CGS, respectively (*P* = 0.017, 1-way ANOVA) and in recovery of phrenic activity such that phrenic area and frequency were 66 ± 12% and 121 ± 10% of pre-CGS levels, respectively [not significant (NS)].

The effect on blood pressure and heart rate. Intracisternal CGS administration caused a significant decrease in mean arterial blood pressure (MAP) secondary to a decrease in both systolic and diastolic pressures (Fig. 1). The MAP was 54.7 ± 2.1 and 40.3 ± 4 mmHg before and after CGS administration (*P* < 0.01, 1-way ANOVA). Furthermore, CGS caused a significant increase in heart rate from 193 ± 11 to 273 ± 8 beats/min (*P* < 0.001, 1-way ANOVA).

The effect of CGS on the laryngeal reflex. As during control stimulation, increasing levels of SLN stimulation caused a significant decrease in phrenic area, frequency, minute phrenic activity, and Ti, as well as a significant prolongation of Te and duration of apnea (all *P* < 0.01). Additionally, compared with control, CGS administration caused significantly more inhibition of phrenic activity in response to increasing levels of SLN stimulation (Fig. 2). CGS administration exaggerated both the decrease in phrenic area, frequency, minute phrenic activity, and Ti and the prolongation of Te and duration of apnea responses to increasing levels of SLN stimulation with a significant interaction between the effect of CGS administration and SLN stimulation (*P* < 0.01 for all, 2-way ANOVA; Figs. 3 and 4). The effect of CGS administration was more obvious at lower levels of stimulation threshold (1.5× threshold than at 2× and 4× threshold), probably secondary to the higher stimulations causing apnea in the majority of animals during the control period (Figs. 3 and 4). However, the duration of apnea following CGS administration was significantly longer compared with control in response to all levels of SLN stimulation beyond threshold (*P* < 0.001 for all paired comparisons; Fig. 5). The number of animals exhibiting apnea at each level of stimulation was also significantly higher than during control (*P* < 0.05; Fig. 5).

Subsequent Blockade of GABAA Receptors

The effect on baseline phrenic activity. Intracisternal administration of bicuculline following CGS caused recovery of phrenic activity that allowed for a decrease of PaCO2 from the high levels needed after CGS administration toward control values. PaCO2 following bicuculline was 40 ± 2 Torr (*P* < 0.05 and 0.66 vs. CGS and control, respectively). Even after PaCO2 was lowered to control levels, bicuculline still caused a significant increase in phrenic area to 192 ± 50% (*P* < 0.05), compared with prebicuculline, post-CGS levels, whereas phrenic frequency and minute phrenic activity did not change.

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**Fig. 1.** Effect of CGS on baseline phrenic activity and blood pressure (BP). Under similar end-tidal CO2 (ETCO2 in Torr) levels, CGS decreased both systolic and diastolic BP and phrenic activity. After ETCO2 was increased (not shown) by decreasing the ventilator rate, only the phrenic activity recovered, whereas the decrease in BP persisted.

**Fig. 2.** Effect of CGS on the phrenic nerve responses to superior laryngeal nerve (SLN) stimulation. CGS administration enhanced the inhibitory effects of SLN stimulation on phrenic activity. A: at 1.5× threshold, 15-s stimulation of the SLN caused a mild decrease in phrenic activity under control conditions and apnea after CGS. B: at 2× threshold, 15-s stimulation of the SLN caused apnea under control conditions that lasted longer and extended beyond the duration of stimulation after CGS.
Although SLN stimulation continued to cause significant decrease in phrenic area and minute ventilation after bicuculline, these responses were significantly less inhibited than after CGS administration ($P < 0.001$ for the interaction between the effects of bicuculline administration and SLN stimulation vs. CGS, 2-way ANOVA). Additionally, after bicuculline administration SLN stimulation failed to produce a significant effect on phrenic frequency, $T_i$, or $T_e$ (Figs. 3 and 4). Furthermore, bicuculline prevented the inhibitory responses of the phrenic area and frequency to 2× and 4× threshold stimulation of the SLN, respectively, compared with both control and CGS responses (Figs. 3 and 4). Phrenic frequency and minute ventilation were 59 ± 20% and 32 ± 15% of baseline, respectively, following 4× threshold stimulation, whereas phrenic area was 58 ± 16% of baseline following 2× threshold stimulation ($P < 0.05$ for all vs. both control and CGS at the same stimulation level). The preservation of the frequency response to SLN stimulation was secondary to the ability of bicuculline to prevent prolongation of $T_e$ (Fig. 4). Bicuculline also decreased the duration of apnea in response to SLN stimulation compared with control, pre-CGS levels (NS).

(79 ± 10% and 135 ± 21%, respectively; NS). However, phrenic area, frequency, and minute activity following bicuculline were not different from control, pre-CGS levels; i.e., bicuculline blocked the effects of CGS and returned phrenic activity toward control values. Following bicuculline administration, the phrenic area, frequency, and minute activity were 83 ± 14, 89 ± 8, and 77 ± 16% respectively compared with control pre-CGS levels (NS).

The effect on blood pressure and heart rate. Intracisternal bicuculline caused recovery of MAP toward control, pre-CGS levels. The MAP was 50 ± 5.9 mmHg following bicuculline ($P < 0.01$ and 0.71 vs. CGS and control, respectively). However, the tachycardia seen after CGS continued following bicuculline administration despite recovery of blood pressure. The heart rate was 287 ± 6 beats/min following bicuculline, significantly higher than control ($P < 0.001$) but not different from CGS ($P = 0.13$).

The effect on the laryngeal reflex. The overall effect of bicuculline was to reverse the enhanced inhibition induced by SLN stimulation after CGS toward control pre-CGS values.

Fig. 3. The effect of CGS and bicuculline on the phrenic area and frequency responses to SLN stimulation. Increasing levels of SLN stimulation caused progressive inhibition of phrenic area and frequency that was significantly more pronounced after CGS administration. Bicuculline reversed the enhanced decrease in phrenic area and frequency in response to SLN stimulation seen after CGS. Furthermore, after bicuculline administration, 2× and 4× threshold stimulation caused a smaller decrease in the phrenic area and frequency, respectively compared with control ($P < 0.05$). The effect of different levels of stimulation depended on CGS and bicuculline administration with a statistically significant interaction between SLN stimulation and the administration of CGS vs. both control and bicuculline ($P < 0.001$) but not between SLN stimulation and bicuculline administration vs. control (2-way ANOVA). Additionally, after bicuculline administration SLN stimulation vs. both control and CGS ($P < 0.001$) but not between SLN stimulation and bicuculline administration vs. control (2-way ANOVA). Post hoc analysis of the effect at different levels of stimulation: *$P < 0.05$ vs. CGS at the same stimulation level; ‡$P < 0.05$ vs. control at same stimulation level.

Fig. 4. The effect of CGS and bicuculline on phrenic respiratory timing responses to SLN stimulation. There was no effect of either CGS or bicuculline on baseline respiratory times. CGS administration enhanced the prolongation of expiratory time and shortening of inspiratory time in response to SLN stimulation while bicuculline reversed the effects of CGS on respiratory timing. Furthermore, bicuculline prevented the prolongation of expiratory time and shortening of inspiratory time in response to 4× threshold stimulation compared with control. The effect of different levels of stimulation depended on CGS and bicuculline administration with a statistically significant interaction between SLN stimulation and CGS administration vs. control and bicuculline ($P < 0.01$) but not between SLN stimulation and bicuculline administration vs. control (2-way ANOVA). Post hoc analysis of the effect at different levels of stimulation: *$P < 0.05$ vs. CGS the at same stimulation level; ‡$P < 0.05$ vs. control at same stimulation level.
ADENOSINE A2A AND GABAA RECEPTORS IN LARYNGEAL REFLEX APNEA

with both control and CGS \( (P < 0.05 \text{ for the interaction between SLN stimulation and bicuculline administration vs. both control and CGS}; \text{ Fig. 5}) \). The duration of apnea at 4× threshold was \( 8.3 \pm 6.2 \text{ s} \ (P < 0.01 \text{ vs. CGS and control}; \text{ Fig. 5}) \). Furthermore, bicuculline caused apnea in fewer animals in response to all levels of SLN stimulation compared with CGS \( (P < 0.05 \text{ for all paired comparisons}; \text{ Fig. 5}) \).

**DISCUSSION**

The main findings of our study in the newborn piglet are that activation of central adenosine A2A receptors through intracerebral administration of CGS caused 1) inhibition of phrenic activity that recovered after \( \text{PaCO}_2 \) was increased, 2) facilitation of the inhibitory effects of SLN stimulation on phrenic activity and apnea duration, and 3) hypotension and tachycardia. Subsequent blockade of GABAergic receptors reversed the inhibitory effects of CGS on baseline phrenic activity, SLN stimulation induced depression of phrenic activity, and hypotension but not tachycardia. We have thus shown that activation of A2A receptors enhances SLN stimulation-induced apnea probably via a GABAergic pathway.

Activation of adenosine A2A receptors with CGS exaggerated the inhibitory effects of SLN stimulation on phrenic responses despite the hypercapnia that was needed to recover phrenic activity following CGS administration. This is of particular interest because hypercapnia increases the threshold for the production of the laryngeal chemoreflex \( (16, 19) \); i.e., the same level of SLN stimulation causes less inhibition of phrenic activity in the presence of hypercapnia. Another confounding variable that might have affected our results is the decrease in blood pressure following CGS administration. Although hypotension is part of the laryngeal chemoreflex in the intact animal, we are not familiar with any reports that link hypotension with an exaggeration of the laryngeal chemoreflex in the absence of metabolic derangements or respiratory inhibition. In our studies the decrease in blood pressure was not associated with metabolic acidosis, suggesting that there was no change in tissue perfusion. Additionally, because hypercapnia reversed the inhibition of the phrenic nerve and restored its activity to baseline levels, the exaggeration of SLN stimulation-induced apnea following CGS administration cannot be attributed to a nonspecific inhibition caused by CGS but rather to its ability to activate A2A receptors.

Bicuculline reversed the effects of CGS on phrenic nerve response to SLN stimulation and produced a reflex response that was less inhibited compared with control, pre-CGS level. The ability of bicuculline to attenuate SLN stimulation-induced apnea in the absence of a significant increase in phrenic activity and/or \( \text{CO}_2 \) levels suggests that the bicuculline response was not secondary to a nonspecific excitation but rather to its ability to block GABAergic receptors. Although bicuculline was administered almost 2 h after CGS, there was continuing evidence of CGS effect in the form of persistent phrenic inhibition, hypotension, and exaggerated phrenic depression in response to SLN stimulation just before administration of bicuculline. Therefore, the recovery of the phrenic activity and blood pressure following bicuculline administration was probably secondary to its ability to block the downstream effects of activation of A2A receptors by CGS. Furthermore, although bicuculline blocked the phrenic inhibition produced by higher levels of SLN stimulation compared with control, most of its effect was to reverse the exaggerated responses seen after CGS, signifying that bicuculline blocked the added effects of activation of A2A receptors by CGS. The residual inhibition of the phrenic activity in response to higher levels of SLN stimulation might suggest the presence of GABA-independent mechanisms or the need for higher dose of bicuculline in the presence of CGS as a similar dose of bicuculline was able to block this response in the absence of CGS \( (1) \).

Adenosine has been implicated in the regulation of breathing and is known to depress neural function \( (7) \). It is ubiquitously released (by \( \text{Na}^+ \)-dependent transporters) and is formed in the extracellular space by breakdown of released ATP (e.g., as a cotransmitter) and sequentially cleaved by ecto-ATPases/apyrases and ectonucleotidases. Four adenosine receptor subtypes \( (\text{A}_1, \text{A}_2A, \text{A}_2B, \text{and A}_3) \) have been cloned and pharma-
Activation of A1 and A3 receptors decreases while activation of A2A and A2B receptors increases adenylyl cyclase activity, respectively (14). Analogs of adenosine have been shown to decrease respiration in neonatal rabbits and pigs (4, 10, 26). Furthermore, both A1 and A2A receptor agonists were found to mediate inhibition of fetal respiratory drive (12, 15, 32). Our data in newborn piglets support these observations and further implicate activation of adenosine A2A receptors in the production of apnea induced by SLN stimulation.

The methylxanthines theophylline and caffeine are widely used in neonatology practice to prevent and treat apnea of prematurity. Xanthines are thought to exert their action by blocking adenosine receptors, especially A1 and A2A, although the exact mechanism and location of action has not been identified. The involvement of adenosine in the laryngeal chemoreflex was initially suggested when theophylline was shown to block the reflex in newborn piglets (18). Our data suggest that methylxanthines are acting centrally by blocking adenosine A2A receptors on GABAergic neurons, thereby preventing the release of GABA and diminishing the respiratory inhibition induced by SLN stimulation; however, a role for adenosine A1 receptors cannot be excluded. We speculate that blockade of this pathway may contribute to the beneficial effects of xanthines in the treatment of apnea of prematurity.

GABA is the major inhibitory neurotransmitter in the central nervous system. It was found to inhibit breathing mainly via activation of GABAA receptors (9, 11). We have shown previously in newborn piglets that blocking GABAA receptors almost abolished apnea induced by SLN stimulation (1). There is an increasing body of evidence of a central interaction between adenosine and GABA. We have previously reported that blockade of GABAA receptors abolishes the inhibitory effect of the adenosine A2A agonist CGS on phrenic activity (34). The adenosine A2A receptor system appears to be one of the presynaptic neuromodulatory systems able to enhance the evoked release of GABA from hippocampal nerve terminals (3) and tuberomammillary nucleus in rats (13). Furthermore, anatomic studies have shown that A2A receptors colocalize with GABAergic neurons in regions of the medulla oblongata in piglets (34) and developing rats (35). These data as well as the ability of both theophylline and bicuculline to prevent the laryngeal reflex apnea point to the possible interaction between GABA and adenosine in the production of apnea during SLN stimulation. Our findings further expand these observations and allow for the speculation that SLN stimulation causes endogenous release of adenosine that activates A2A receptors on GABAergic neurons, resulting in the release of GABA at inspiratory neurons and subsequent respiratory inhibition.

Similar interaction between A2A and GABAA receptors can be proposed to explain the effects of CGS and bicuculline on the blood pressure. Activation of adenosine A2A receptors in the nucleus tractus solitarii as well as GABAA receptors in the rostral ventrolateral medulla (RVLM) have both been shown to cause hypotension and bradycardia through inhibition of sympathetic activity (20, 27–29). Furthermore, blockade of GABAA receptors in the sympathoexcitatory region of the RVLM resulted in an increase in sympathetic activity and blood pressure (2, 27, 31). These studies suggest a common pathway for hypotension induced by activation of GABAA and A2A receptors through inhibition of sympathetic activity, which is in agreement with our findings in vagotomized piglets. The ability of both CGS and bicuculline to regulate blood pressure despite vagotomy excludes a role for the parasympathetic, and it implicates the sympathetic system in this regulation. Unlike previously mentioned studies in which CGS caused bradycardia in anesthetized animals, intracisternal CGS caused tachycardia in our animals. This could be related to vagotomy or to lack of the confounding effect of anesthesia on the heart rate response to CGS, as was previously suggested. We speculate that SLN afferents activated during the laryngeal chemoreflex stimulate second-order neurons in the NTS that release adenosine, which in turn activates A2A receptors on GABA containing neurons resulting in the release of GABA at inspiratory and sympathetic regions causing apnea and hypotension. Our results do not exclude a role for other neurotransmitters and neuromodulators or other adenosine receptors in the laryngeal chemoreflex. Multiple other agents have been implicated in the genesis of apnea during laryngeal stimulation including glycine, β-endorphins, and catecholamines (21, 22, 30). Furthermore, our findings do not directly indicate the involvement of endogenously released adenosine in the regulation of the laryngeal chemoreflex. However, the involvement of endogenous adenosine is suggested by the previously reported ability of theophylline to block the laryngeal chemoreflex.

Our study is further limited by its inability to identify the site of action of either CGS or bicuculline. However, the midcorticallar decerebration performed in our studies together with the intracisternal administration of the chemicals through a catheter placed close to the surface of the medulla oblongata isolates the effect of CGS and bicuculline to the brain stem and especially the medulla. The variable responses obtained in different studies might be secondary to variable diffusion of the chemicals or to a dilution effect from the CSF. Further studies are clearly needed to identify the proposed network and document the interaction between adenosine and GABA in the production of SLN stimulation induced apnea.

In summary, we report in nonanesthetized newborn piglets that 1) central activation of A2A receptors caused an inhibition of phrenic activity, a decrease in blood pressure and tachycardia, and exaggeration of apnea induced by SLN stimulation despite hypercapnia and 2) subsequent central blockade of GABA receptors reversed the effects of activation of A2A receptors on phrenic activity, SLN stimulation-induced apnea, and blood pressure. We conclude that both adenosine and GABA play a role in the respiratory and cardiovascular responses during laryngeal stimulation. We propose a pathway whereby this interaction takes place in the medulla.

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