

Modulation of Respiratory Rhythm in the Goat by α_2 -Adrenoceptors

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The purpose of this study was to determine phrenic and recurrent laryngeal nerve (RLN) activities in response to intravenous administration of α_2 -adrenoceptor agonists in anesthetized goats. Phrenic and RLN activities were used as indices of the inspiratory and postinspiratory (stage I expiratory) phases of the respiratory cycle, respectively. We hypothesized that prolonged phrenic apneas and respiratory arrhythmias that we have observed in awake and in anesthetized goats would be correlated with an augmentation of RLN postinspiratory activity rather than a widespread depression of breathing. Systemic infusions of the selective α_2 agonists clonidine or guanabenz resulted in phrenic apneas of varying lengths, and there was a maintained increase of tonic RLN activity throughout the length of the apnea, consistent with our hypothesis. The RLN exhibited a mixture of responses, including inspiratory, postinspiratory, and expiratory-related activities. The results suggest that α_2 agonists differentially affect spinal (phrenic) and cranial (RLN) motoneurons in a reciprocal fashion. Apneas induced by α_2 agonists are correlated with tonic postinspiratory-related activity of the RLN, which may reflect inhibition of medullary inspiratory neurons. Although the mechanism by which α_2 -adrenoceptors augment

postinspiratory-related RLN is unknown, we suggest that an endogenous α_2 -mediated inhibitory tone, possibly noradrenergic in origin, to medullary postinspiratory neurons may be an important feature of this process. The withdrawal of an α_2 -mediated inhibitory input would allow tonic activation of postinspiratory neurons which, in turn, inhibit inspiratory neurons and cause prolonged apneas. Control of postinspiratory-related activity has been implicated as an essential component in the maintenance of respiratory rhythm. Hence, our results are consistent with the three-phase model for mammalian respiratory rhythm generation.

I. Introduction

A current model for the generation of respiratory rhythm in mammals suggests that the ventilatory cycle comprises three distinct phases: inspiration, post-inspiration (also called stage I expiration), and late (stage II) expiration (1,2). These three phases have been correlated with intracellular recordings from ventral brainstem neurons (1-6) and coincide with neural activities of phrenic (inspiratory), laryngeal (postinspiratory) and internal intercostal (late expiratory) motoneurons innervating muscles that control airflow during breathing (7-13).

A recent computer-generated model (14) of respiratory rhythmogenesis based on the three-phase model suggests that postinspiratory activity, in particular, has an "executive" role in the maintenance of respiratory rhythm; that is, changes in tonic drive to postinspiratory neurons exert a greater influence on respiratory rhythm than other neuronal types within the network (5,14,15). Experimental support for this concept includes studies showing that maneuvers to induce apnea, such as the stimulation of laryngeal afferents, activate medullary postinspiratory neurons and cause disturbances of respiratory rhythm (3,5,6,16).

Previous work from our laboratory indicates that intravenous administration of selective α_2 -adrenergic agonists such as clonidine cause respiratory disturbances, including reductions of T_I and prolongations of T_E (apneas). These effects have been observed in awake (17) and anesthetized, artificially ventilated, vagotomized goats with peripheral chemoreceptor afferents removed, suggesting that the apneas are central in origin (18). However, it was not clear from those studies whether α_2 receptor agonists caused a widespread central depression of ventilatory effort or whether there was possibly a differential recruitment of expiratory-related activity that would be consistent with the three-phase model of respiratory rhythm described above. In the context of the network model proposed by Richter and colleagues (1,2,5), we hypothesized that apneas would be correlated with tonic activation of postinspiratory-related neurons. Efferent activity of the recurrent laryngeal nerve (RLN), which innervates vocal cord abductors and adductors, exhibits both inspiratory and

postinspiratory-related activities in the cat and has been used as an index of medullary postinspiratory activity (7,11-13).

In this study we sought to determine if (a) the RLN of goats also exhibits similar respiratory-related activities compared with other species and, if so, (b) whether phrenic apneas caused by systemic administration of α_2 agonists are correlated with augmented postinspiratory-related activity of RLN. Our results suggest that pharmacological modulation of respiratory rhythm by α_2 receptors may involve changes in tonic activity to postinspiratory brainstem neurons, which is consistent with the three-phase network oscillator model.

II. Materials and Methods

A. Animal Preparation

A total of six goats were used in this study. After induction of anesthesia with sodium thiamylal (15 to 20 mg/kg) for intubation, animals were placed in dorsal recumbency under a heated blanket to maintain normal body temperature (38 to 40°C). Anesthesia was maintained with intravenous α -chloralose by continuous infusion (100 to 150 mg kg⁻¹ h⁻¹). Femoral arterial and venous catheters were implanted for blood sampling and drug administration. The animals were bilaterally carotid body denervated.

The left whole recurrent laryngeal nerve (RLN) was identified immediately lateral to the trachea, cut distally, and desheathed. In three of the six animals, the contralateral vagus was also cut. The C6 phrenic nerve root was dissected free from surrounding tissue low in the neck, cut, and desheathed for neural recording. Each nerve was placed on bipolar platinum hook electrodes and immersed in mineral oil to prevent desiccation. Nerve activity was preamplified 10,000 times, filtered, and further amplified; the output was visualized on an oscilloscope (Tektronix 5113) and chart recorder (Gould TA 2000). The amplified signal was rectified and integrated to obtain a moving average of peak nerve activity.

B. Measurements

Once surgical preparations had been completed, the animals were paralyzed with a mixture of metubine iodide/pancuronium bromide and artificially ventilated. Respiratory frequency was set between 15 and 20 breaths/min and tidal volume between 300 and 500 mL, depending on the size of the animal. $F_I O_2$ was adjusted to maintain $P a O_2$ above 90 mm Hg. It was necessary to add CO_2 to the inspired gases so that breathing was above the apneic threshold for stable phrenic recording. Arterial blood gases were sampled frequently to ensure maintenance of blood gas and acid-base homeostasis during artificial ventilation.

C. Protocol

Once the surgery had been completed and the preparation stabilized, the apneic threshold for ventilation was first determined by lowering the inspired CO_2 level until phrenic activity disappeared. PaCO_2 was determined at this point and F_1CO_2 was then raised until PaCO_2 was approximately 5 to 8 torr above the threshold level and phrenic activity was stable. Drugs were given intravenously after stable neural activities were recorded for at least 5 min.

D. Drugs

Clonidine was dissolved in saline to obtain a stock solution (1 mg/mL), which was diluted 1:10 for IV administration. Stock solutions (1 mg/mL) of guanabenz and SKF-86466 were dissolved in dilute acetic acid and saline (1:100) before use. Clonidine and guanabenz were obtained from Sigma Chemicals (St. Louis, MO); SKF-86466 was obtained from Smith, Kline, Beecham (King of Prussia, PA).

III. Results

Phrenic and RLN activities were recorded simultaneously in all six animals. Because RLN activity was determined for whole nerve or branches contained within the main RLN trunk, we recorded from nerves bound for laryngeal adductors and abductors, and probably other upper airway muscles, known to be active during different phases of the respiratory cycle. Not surprisingly, we observed a variety of responses, which are illustrated in several neurograms (Figs. 1 to 4), that are informative for pointing out some general features of the responses that were obtained.

A. Phrenic and RLN Neural Activities

Representative neurograms showing phrenic and RLN activities from two different animals with intact vagi are shown in Figure 1. In normocapnia, RLN activity is apparent during the inspiratory phase, which clearly parallels phrenic activity. Moreover, activity occurs also in the postinspiratory phase; that is, the onset of activity starts at the end of phrenic activity, is confined to early expiration, and exhibits a decremting profile (Fig. 1, upper panel). A second example (Fig. 1, lower panel) illustrates a different type of RLN activity that we obtained: expiratory-related activity of the RLN begins during the postinspiratory phase but is sustained throughout the entire expiratory phase.

B. Effects of α_2 Agonists on Phrenic and RLN Activities

Owing to the anatomical location of the bifurcation of the RLN and main vagal trunk, we could not perform a vagotomy on the ipsilateral side, from which

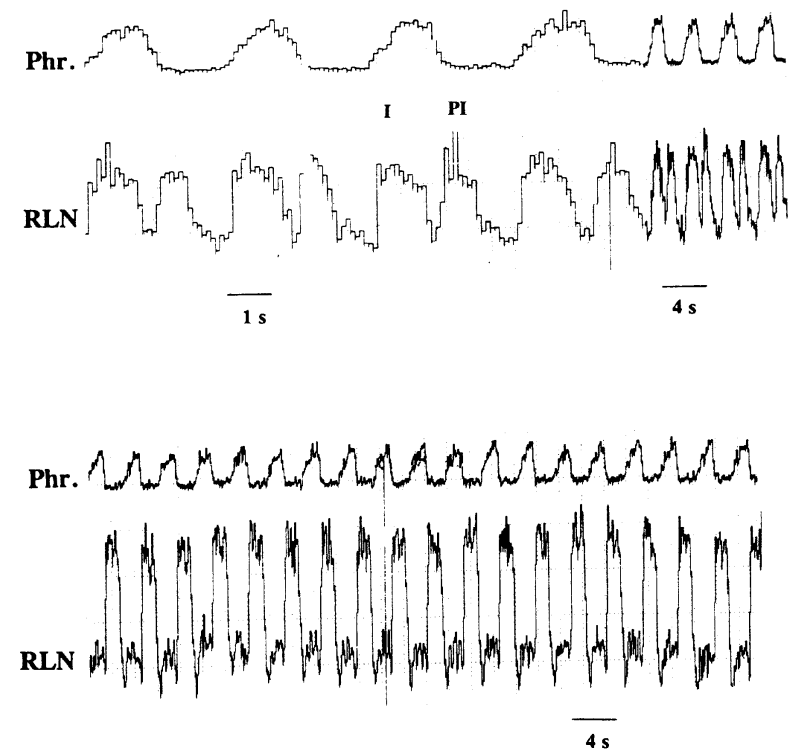


Figure 1 Examples phrenic (Phr.) and recurrent laryngeal nerve (RLN) efferent activities in two chloralose-anesthetized goats with intact vagi. In one animal (upper panel), RLN shows predominantly inspiratory (I) and postinspiratory (PI)-related activity. Inspiratory activity occurs in parallel with Phr. activity, while PI-related activity is confined to early expiration and exhibits a decremting profile. In another animal (lower panel), RLN I-related activity is less pronounced and exhibits mainly expiratory-related activity, which spans the entire expiratory phase, with no clear PI-related component.

RLN activity was recorded. We have examined the effects of α_2 agonists on phrenic activity in animals that were bilaterally vagotomized and glomectomized (18). Figure 2 shows an example of the effects of intravenously administered clonidine ($0.5 \mu\text{g}/\text{kg}$) on phrenic activity from our previous study for comparison with vagi-intact, glomectomized goats in this study. These studies reveal no basic differences in the ventilatory responses to α_2 agonists in goats with or without intact vagi. In both groups, there is a slight yet significant reduction of T_I and a significantly increased T_E . Phrenic amplitude shows variable responses, but generally there is a trend for reductions of phrenic amplitude in both groups.

An example of phrenic and RLN responses to clonidine ($0.5 \mu\text{g}/\text{kg}$) in a vagi-intact goat is shown in Figure 3. Control phrenic and RLN activities are typically rhythmic and RLN activity is primarily confined to the expiratory

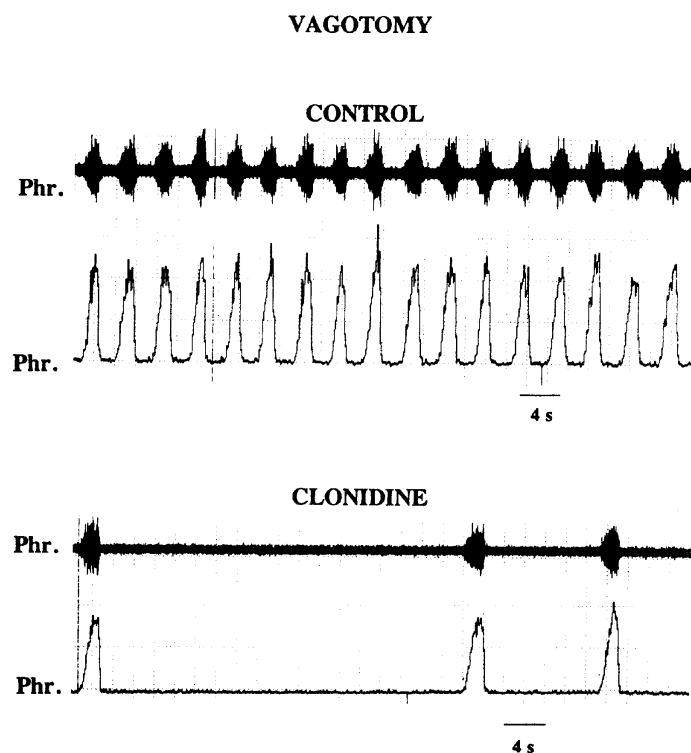


Figure 2 Effects of clonidine ($0.5 \mu\text{g}/\text{kg}$, IV) on Phr. activity in a vagotomized, anesthetized, glomectomized goat. These results were obtained in a previous study (18) and were used to compare with results obtained from vagi-intact goats in this study.

phase in this example (Fig. 3, top). Approximately 1 min after clonidine administration, there was a cessation of respiratory phrenic rhythm but a maintained increase in tonic RLN activity (Fig. 3, middle). Some 5 min after the initial dose of clonidine, phrenic activity resumed, but it was slower and more irregular (Fig. 3, bottom) than during the control period. After resumption of phrenic activity, the RLN shows reciprocal inhibition and excitation with the phrenic activity; that is, during inspiration, the RLN is inhibited and is then facilitated during the expiratory phase.

The effects of guanabenz (Fig. 4, top), another selective α_2 agonist, were similar to those of clonidine but required higher dosages (10 to $20 \mu\text{g}/\text{kg}$). Inspiratory activity was clearly inhibited and associated with a tonic increase of RLN activity during apneas. Respiratory rhythm could be restored with SKF-86466 (19), a highly selective α_2 antagonist (Fig. 4, bottom). The antagonist returned RLN activity to a more phasic pattern with a reduction in expiratory-related activity.

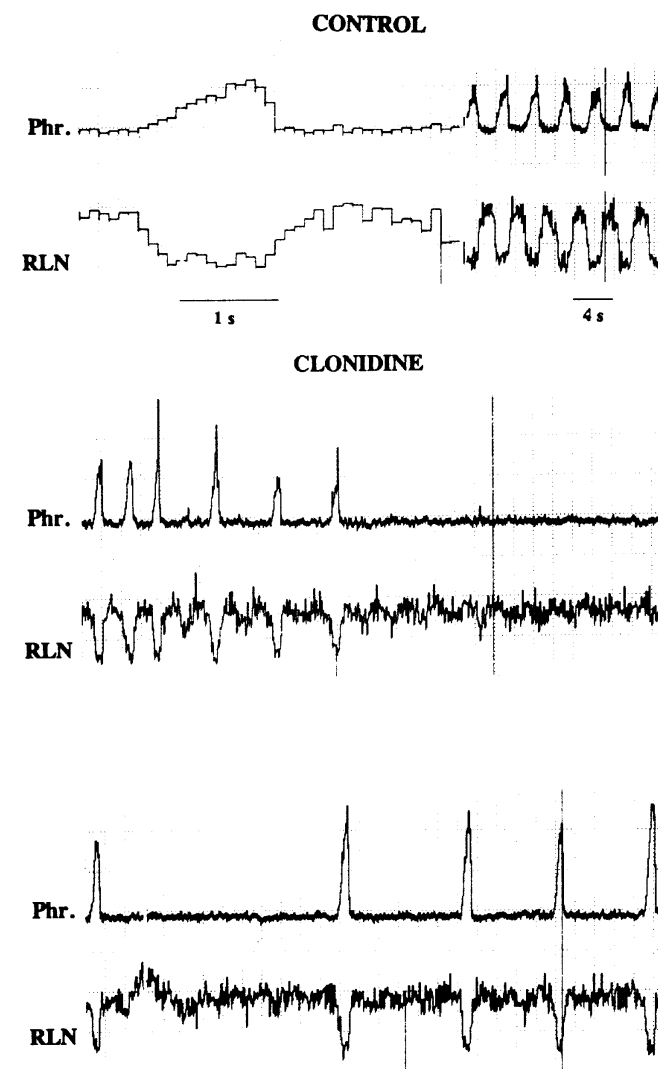


Figure 3 Effects of clonidine ($0.5 \mu\text{g}/\text{kg}$, IV) on Phr. and RLN activities in a vagi-intact goat. Control activities are shown in the top panel, followed by the response approximately 1 min (middle panel) and 5 min (bottom panel) after clonidine injection. Note the cessation of Phr. activity coinciding with tonic activation of RLN activity after 1 min. Arrhythmic breathing with prolonged apneas is apparent after 5 min, which also are correlated with tonic RLN activity.

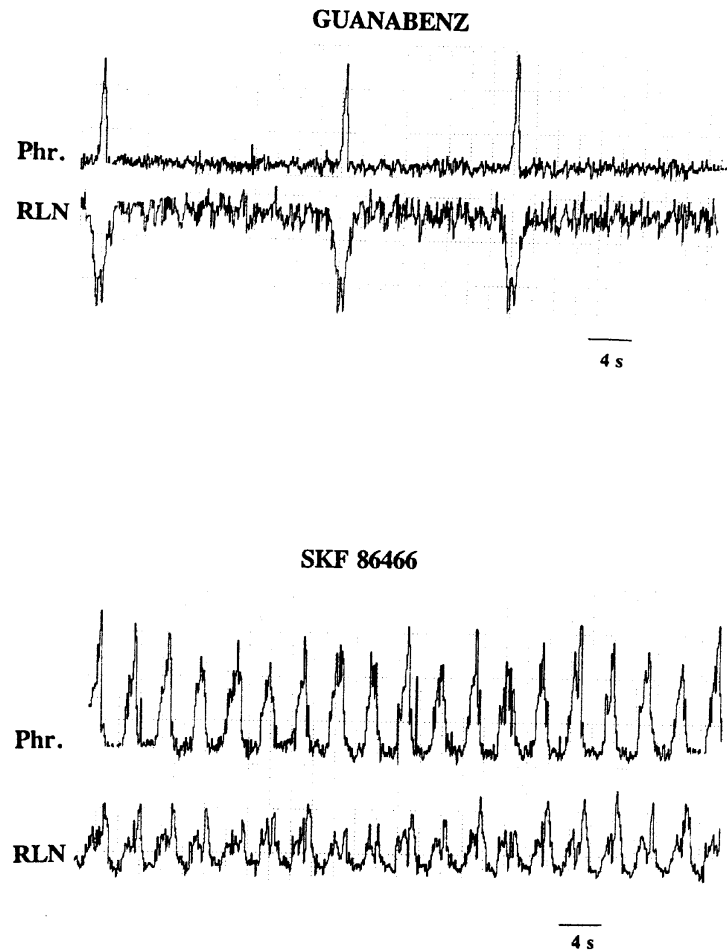


Figure 4 Effects of a different α_2 agonist, guanabenz ($20 \mu\text{g}/\text{kg}$, IV), on Phr. and RLN activities in an anesthetized goat (upper panel). Note similarity with effects of clonidine shown in Fig. 3. A selective α_2 -antagonist, SKF-86466, was given to reverse the effects of guanabenz (lower panel). After the antagonist, there was a significant reduction of postinspiratory-related activity of RLN.

Changes in respiratory timing were consistent in all six goats and are summarized in Figure 5. T_I was significantly reduced after clonidine and there was a profound increase of T_E . This is consistent with our previous results using goats that were bilaterally vagotomized (18).

IV. Discussion

There are two important findings from our study: (a) RLN activity in goats exhibits inspiratory and postinspiratory-related activity—in some cases there

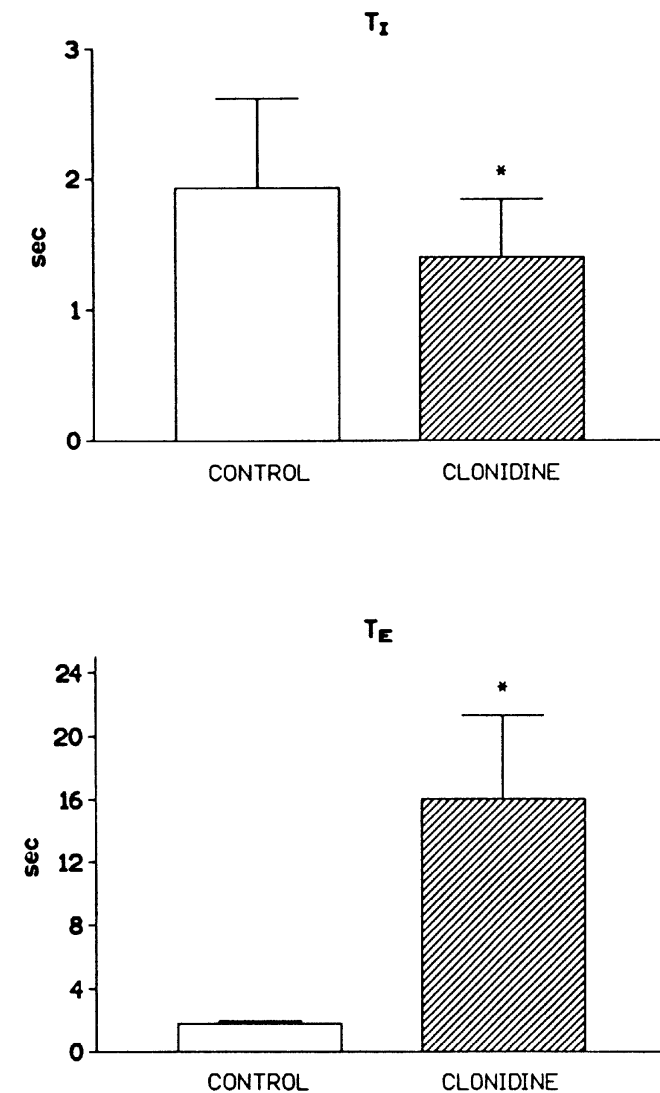


Figure 5 Summary of the effects of clonidine on inspiratory (T_I) and expiratory (T_E) durations in six anesthetized goats. There were significant reductions of T_I and increases of T_E ($P < 0.05$; Wilcoxon paired sample test).

is activity that spans entirely the expiratory phase, and (b) α_2 agonists affect inspiratory and expiratory-related motoneuronal activities in a reciprocal fashion; that is, arrhythmic breathing and phrenic apneas were correlated with tonic increases of RLN activity throughout the apneic period. Our interpretation of these results is that augmented RLN activity is indicative of increased central postinspiratory activity, which may account for apneic breathing and

respiratory disturbances. The data are consistent with the three-phase model for respiratory rhythmogenesis (1,2) in that central postinspiratory neuronal activity is a major determinant of respiratory rhythm.

A. Phrenic and RLN Activities

The RLN in goats exhibited a mixture of activities, which is not surprising considering that we recorded from whole nerve rather than isolating and recording from branches to specific laryngeal muscles. We therefore cannot draw firm conclusions about RLN activity as an index of any particular phase of the respiratory cycle. Zhou et al. (13) have demonstrated the importance of recording efferent activities of branches within the RLN that innervate particular laryngeal muscles. This nerve carries motor fibers innervating both vocal cord abductors (e.g., posterior cricoarytenoid, or PCA) and adductors (e.g., thyroarytenoid, or TA). Vocal cord abduction and adduction are important for regulating airflow during inspiration and early (stage I) expiration, respectively. This has been confirmed with electromyographic (EMG) data and neural recordings from PCA and TA muscles in awake humans and anesthetized animals (13,20–23).

Despite the limitation of recording whole RLN activity in this study, the results are generally consistent with several other studies in decerebrate cats (10–13). We observed both inspiratory- and expiratory-related activity in whole RLN; however, in the majority of preparations, we obtained mainly expiratory-related activity. The onset of this expiratory activity began at or immediately following peak phrenic activity, in most cases was confined to the early (post-inspiratory) portion of the expiratory phase, and exhibited a decrementing profile (Fig. 1, top). Taken together, the data strongly suggest that postinspiratory-related activity is present in the RLN of anesthetized goats. In some preparations, much of this activity spanned the entire expiratory phase (Fig. 1, lower panel), which has also been noted in cats (13).

B. Effects of α_2 Agonists

Few studies have examined the effects of α_2 -adrenoceptor stimulation or blockade on motor nerve activities in anesthetized animals. The general effect noted, however, is one of respiratory depression. For example, McCrimmon and Lalley (24) demonstrated that phrenic nerve activity was completely inhibited by clonidine in artificially ventilated cats. They did not observe any disruption of respiratory rhythm, but this may be related to the higher dosages used in their study (ca. 50 $\mu\text{g}/\text{kg}$) compared with this study. Higher doses of clonidine in anesthetized goats also inhibit phrenic activity (unpublished observations). Clonidine has also been shown to inhibit activity of medullary respiratory-related neurons within the ventral respiratory group (25,26). Our phrenic recordings are therefore consistent with previous studies showing that α_2 agonists cause respiratory (i.e., inspiratory) depression.

The most unique finding from our study is that clonidine and guanabenz are tonically active the RLN during the expiratory phase (Figs. 3 and 4). This is particularly surprising in light of the inhibitory effects mediated by α_2 -receptor agonists in other studies. Stimulation of α_2 receptors has been shown to hyperpolarize CNS neurons (see Ref. 27). Our interpretation of the results is that α_2 agonists cause, by an as yet unidentified mechanism, an increase of tonic postinspiratory-related brainstem neuronal activity. We suggest that RLN activation is likely to be caused by a central α_2 -mediated pathway rather than a peripheral reflex mechanism. The similarity of phrenic responses to α_2 agonists between vagi-intact and vagotomized goats (Figs. 2 and 3) and lack of carotid body chemoreceptor afferents rule out most feedback pathways that could result in augmented postinspiratory activity.

Several studies have shown that electrical stimulation of superior laryngeal nerve (SLN) afferents powerfully inhibits breathing and causes sustained apneas (e.g., Refs. 10,11,28); furthermore, SLN stimulation depolarizes medullary post-inspiratory neurons throughout the apnea (3,5,16). This is similar to our findings of augmented RLN postinspiratory activity after administration of α_2 agonists. Because postinspiratory neurons have been shown to synaptically inhibit inspiratory and late-expiratory neurons (1,15), it is thought that postinspiratory neurons have an overall "executive" role in the brainstem respiratory oscillator network. A recent mathematical model based on this concept suggests that respiratory rhythm is highly dependent upon changes in tonic activity to postinspiratory neurons (14). The results from this study essentially support this concept, because tonic activation of RLN (presumably) postinspiratory-related activity by α_2 receptor agonists caused prolonged T_E and respiratory disturbances, as predicted by this model. Our results further imply that α_2 -adrenoceptor pathways perhaps provide one source of tonic input to postinspiratory neurons (see below).

The differential and antagonistic influence of α_2 agonists on phrenic and RLN activities is similar to the differential recruitment patterns of inspiratory and expiratory motoneurons with changes in central or peripheral chemoreceptor drive. For example, cessation of phrenic activity by superior laryngeal stimulation (28) or hypocapnia (29) augments expiratory-related activity of bulbospinal or intercostal motoneurons in cats. Similarly, augmentation of phrenic activity by hypoxia inhibits abdominal expiratory nerve activity (30). The reasons for these reciprocal changes in inspiratory and expiratory activity are not entirely clear but are thought to stem primarily from differential effects of general anesthesia on central outflow from cranial and bulbospinal motoneurons to upper airway and intercostal muscles. It should be mentioned that the three-phase model of respiratory rhythmogenesis (2) predicts no overlap between inspiratory, postinspiratory, and late-expiratory phases of the respiratory cycle. Evidence from anesthetized animals generally supports this prediction (7,10–12); however, in awake dogs (31,32) and goats (33), there is clear overlap of respiratory EMG activities during the inspiratory-postinspiratory transition with central or peripheral chemoreceptor stimulation.

The neonatal rat brainstem preparation in vitro has provided additional evidence that α_2 -adrenoceptors are involved in respiratory rhythmogenesis. Hilaire et al. (34) showed that the pontine A5 (noradrenergic) group inhibits the medullary rhythm generator, since medullary respiratory frequency increased after removal of the pons or injecting the A5 region with α_2 antagonists norepinephrine (NE) applied to the brainstem preparation both increases and decreases respiratory frequency (35); NE isolated to the pons consistently increases frequency, while NE applied to the medulla decreases frequency. Removing the pons also increases the respiratory frequency of the medullary oscillator (36). Clonidine mimicked the NE-induced increase in frequency when applied to the pons but had no effect when applied to the medulla (35). These results suggest a pontine inhibitory input to the medullary respiratory rhythm generator, possibly from the A5 noradrenergic group, in the neonatal rat brainstem. A significant reduction in the activity of "preinspiratory" neurons was noted in the rostral ventrolateral medulla after clonidine application (37), an effect that was blocked with forskolin, an activator of adenylate cyclase, suggesting that cAMP plays a role in mediating the effects of clonidine on respiratory rhythm. Thus, although there is evidence for α_2 receptor-mediated effects on respiratory rhythm, more studies are needed to clarify the mechanisms and pathways involved.

C. Significance of α_2 -Adrenoceptors in Modulation of Respiratory Rhythm

Alpha₂ receptors in the CNS are generally involved in presynaptic autoregulation of NE, and stimulation of these receptors with agonists such as clonidine causes widespread reductions of NE turnover and release from neurons (38). The central effects of NE release, when mediated by α_2 receptors, have been shown to hyperpolarize neurons at pontine and medullary respiratory-related sites such as the locus ceruleus (LC) (39), the dorsal motor nucleus of the vagus (40), and the ventral respiratory group (26). Given that α_2 -mediated effects cause hyperpolarization, the results from this study showing postinspiratory-related depolarization by α_2 agonists suggests one of two likely possibilities: (a) depolarization of postinspiratory neurons by a direct excitatory effect or (b) a withdrawal of an inhibitory input that unmasks a tonic excitation. We favor the second possibility, since α_2 -receptor agonists are known to reduce NE release and turnover from presynaptic terminals (41). It is unknown whether NE augments or inhibits postinspiratory neurons, but a recent study on medullary inspiratory neurons of the nucleus ambiguus showed opposing effects of NE and serotonin (5-HT): NE caused inhibition while 5-HT excited inspiratory neurons (25). Alpha₂ agonists such as clonidine acting at presynaptic NE terminals could reduce the release of NE and thereby remove an inhibitory noradrenergic input, allowing tonic excitatory inputs to depolarize these neurons. There is evidence for serotonergic (25) and glutamatergic (42) excitation of

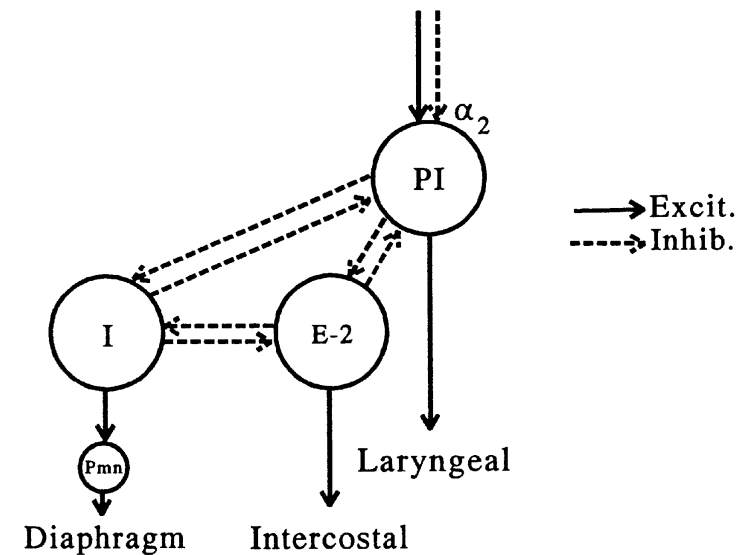


Figure 6 A hypothesis for the possible role for α_2 -adrenergic receptors in the control of respiratory rhythm incorporating the three-phase network model for respiratory rhythmogenesis (1,2). Respiratory rhythm is initiated with inspiratory (I) neurons, which excite the phrenic motor nucleus (Pmn) and the diaphragm. The expiratory phase is illustrated by early (postinspiratory; PI) and late (E-2) expiratory neurons with motoneuronal outflows to laryngeal and internal intercostal muscles, respectively. I, PI, and E-2 neurons are mutually inhibitory (dashed lines) to each other and transmit excitatory (solid lines) outflow to respiratory muscles. We hypothesize that α_2 -adrenergic receptors control a source of tonic inhibitory input to PI neurons (see text for details).

neurons within the nucleus ambiguus that could provide a source of tonic drive to postinspiratory neurons. Because clonidine strongly inhibits pontine A5 and LC neurons (see above) and neurons in these locations exhibit postinspiratory-related activity (43), a possible pontine noradrenergic inhibitory pathway to postinspiratory neurons in the medulla may be present. As mentioned earlier, there is evidence for this hypothesis in the neonatal rat brainstem model (34–36).

Figure 6 illustrates a hypothesis for the incorporation of α_2 -adrenergic control of respiratory rhythm within the context of the three-phase network oscillator model (1,2). In this scheme, control of respiratory rhythm is achieved in part by a balance of excitatory and inhibitory inputs to medullary postinspiratory neurons. We hypothesize that α_2 -adrenoceptors serve to control an inhibitory, possibly noradrenergic, pathway to medullary postinspiratory neurons. Changes in tonic drive to postinspiratory neurons constitute a key feature of the "executive" role for these neurons affecting respiratory rhythm (14). It has also been suggested that inhibitory and excitatory inputs provide the source of "conditional" drive to pacemaker cells for central rhythm generation of breathing in the neonatal rat brainstem in vitro (44,45). Whatever the mechanisms

involved in the initiation and maintenance of central respiratory rhythm, α_2 -receptor-mediated pathways appear to significantly contribute to its modulation.

Acknowledgments

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Multifunctional Ventral Brainstem Respiratory Neurons

Reorganization of Their Activities to Produce Vomiting

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I. Summary

Vomiting is a highly complex behavior that is generated primarily by the coordinated contraction of the major respiratory muscles. These muscles are activated in different patterns during respiration and vomiting. In particular, the diaphragm and abdominal muscles co-contract in a series of bursts of activity during both the retching and expulsion phases of vomiting. This review considers how the activity of brainstem respiratory neurons is reorganized during vomiting. Many of these neurons take an active role in generating the pattern of respiratory muscle discharge during vomiting as well as during breathing and thus can be considered to be multifunctional neurons. Other respiratory neurons are silenced during vomiting, presumably because their discharge would interfere with the unique pattern of respiratory muscle activation that occurs during vomiting. In addition, not all of the neurons in the vomiting pattern generator have yet been identified, including those that provide excitatory drive to phrenic motoneurons.

II. Introduction

Nausea and vomiting (emesis) occur frequently due to a variety of causes, including cancer chemo- and radiation therapy, surgery, pregnancy, motion and space sickness, and various diseases. The input mechanisms by which vomiting is triggered are better understood for some conditions (cancer chemotherapy, radiation) than for others (pregnancy, postsurgery, delayed emesis following chemotherapy) (1-3). Lesion studies have demonstrated that the essential coordinating circuitry for producing vomiting is located within the medulla of the brainstem. The exact organization of this coordinating circuitry is unknown and somewhat controversial. Recent studies of the distribution of cells expressing *c-fos* immunoreactivity (a marker of cell activation) in response to drug-induced emesis indicate that neurons involved in coordinating vomiting radiate from the area postrema and nucleus of the solitary tract to an arc of neurons in the lateral tegmental field implicated in somatoautonomic integration (4).

III. The Motor Act of Vomiting

Vomiting is produced primarily by intrathoracic and intraabdominal pressure changes that are generated by the coordinated action of the major respiratory muscles. These changes are accompanied by the integrated activation of the gastrointestinal tract and upper airway and postural musculature (2,5). The patterns of activity of these muscles during the retching and expulsion phases of vomiting are illustrated schematically in Figure 1. Note in particular that the diaphragm and abdominal muscles, which are activated out of phase with each other during breathing, co-contrast in a series of bursts of activity during vomiting. The expulsion phase can be divided into two parts. During the first, the diaphragm and abdominal muscles co-contrast, as during retching. During the second part of expulsion, phrenic discharge is inhibited while abdominal discharge continues. The periesophageal portion of the crural diaphragm relaxes during expulsion and to a lesser extent during retching, facilitating rostral movement of gastric contents. This relaxation is part of the central motor program for vomiting and does not depend on movement of vomitus within the gastrointestinal tract (6). Intercostal activity is not illustrated in Figure 1 since different patterns of discharge have been reported, presumably reflecting a complex and variable pattern of activity during vomiting. The predominant pattern observed in our laboratory in decerebrate, paralyzed cats is that the nerves to the external intercostal muscles are mainly active in phase with bursts of coactive phrenic and abdominal discharge, while those to the internal intercostals are mainly active between retching bursts and during the expulsion phase (7). The activity of the upper airway muscles during vomiting has been described in detail (8).

Muscles or organs (function)	Innervation	Retch		Retch		Expulsion	S
Digastricus (I) (jaw opener)	Facial & trigeminal nerves	[Solid black bar]					[Small peak]
Genioglossus (I) (tongue protruder)	Hypoglossal nerve	[Peak]		[Peak]		[Solid black bar]	[Small peak]
Geniohyoideus (UES opener)	Hypoglossal nerve			[Peak]		[Solid black bar]	[Small peak]
Pharyngeal dilator muscles (I)	Glossopharyngeal nerve	[Peak]		[Peak]		[Solid black bar]	
Pharyngeal constrictor muscles (I + E)	Pharyngeal vagus nerve		[Peak]		[Peak]		[Small peak]
Larynx (I) (glottis opener)	Recurrent & superior laryngeal nerves						
Larynx (E) (glottis closer)	Recurrent nerve	[Peak]		[Peak]		[Solid black bar]	[Small peak]
Sternal & costal diaphragm (I)	Phrenic nerve	[Peak]		[Peak]		[Peak]	
Crural diaphragm (I)	Phrenic nerve	[Peak]		[Peak]			
Abdominal muscles (E)	Thoracic & lumbar ventral rami	[Peak]		[Peak]		[Solid black bar]	
Trapezius (I) (shoulder muscle)	Spinal accessory n. & cervical ventral rami	[Peak]		[Peak]		[Solid black bar]	
Triceps brachii (Extensor of elbow joint)	Radial nerve	[Solid black bar]					
External anal sphincter	Pudendal nerve	[Peak]		[Peak]		?	
Urethral sphincter	Pudendal nerve	[Peak]		[Peak]		?	

Figure 1 Schematic representation of muscle activity during vomiting. The episode illustrated consists of two retches and an expulsion, followed by swallowing (S). Timing of muscle activation is indicated in black. I and E indicate inspiratory and expiratory activities during breathing. No data indicated by "?". Abbreviations: n, nerve; UES, upper esophageal spincter. (From Ref. 5.)

IV. Activity of Medullary Respiratory Neurons During Vomiting

The contributions of brainstem respiratory neurons to the generation of respiratory muscle discharge during (fictive) vomiting are being investigated in cats that are decerebrated, paralyzed, and artificially ventilated. Fictive vomiting is identified by the same characteristic pattern of respiratory muscle nerve discharge that would generate retching and expulsion in nonparalyzed animals (9). Some types of medullary respiratory neurons are actively involved in generating the muscle discharge pattern during vomiting, while other neurons are silenced. This difference is strikingly illustrated in the firing patterns of expiratory and inspiratory medullary output neurons that convey excitatory drive to spinal respiratory motoneurons (Fig. 2). Augmenting (AUG) expiratory (E) bulbospinal neurons in the caudal ventral respiratory group (VRG) fire in two different patterns appropriate to drive abdominal or internal intercostal motoneurons during vomiting (9). One subgroup of these neurons fires in phase with abdominal and coactive phrenic discharge (E-AUG neurons in Fig. 2). Cutting the axons of these neurons by a midsagittal section between the obex and C1 abolishes abdominal discharge during both respiration and fictive vomiting (10). A second group of these E-AUG neurons fires when internal intercostal motoneurons are active between retching bursts and during expulsion (not illustrated). In contrast, most inspiratory (I) bulbospinal neurons in both the dorsal and ventral respiratory groups are actively inhibited and mainly silent during vomiting (I-AUG neurons in Fig. 2) (11,12). The medullary neurons responsible for exciting phrenic and external intercostal motoneurons during vomiting remain unidentified.

The discharge patterns of excitatory bulbospinal neurons are produced in part by inhibitory respiratory neurons with extensive medullary connections (13,14) that fire during different phases of vomiting (Fig. 2). Many inspiratory neurons with a decrementing (DEC) discharge pattern in the rostral VRG fire during coactive phrenic and abdominal bursts and thus could inhibit inspiratory bulbospinal neurons, as well as other cell types, during this phase of vomiting (15). Another subgroup of I-DEC neurons is silent throughout the vomiting episode (not illustrated). These I-DEC neurons may normally inhibit those caudal VRG E-AUG neurons that drive abdominal motoneurons; thus, their continued firing would interfere with abdominal discharge during vomiting. Another type of inhibitory neuron, augmenting expiratory neurons located in the Bötzing complex of the rostral VRG, fires between coactive phrenic and abdominal nerve bursts and could inhibit phrenic motoneurons, inspiratory bulbospinal neurons, and other medullary neurons during this phase (BOT E-AUG neurons in Fig. 2) (16). In addition to being inhibited between retching bursts, phrenic motoneurons are also inhibited during the second phase of expulsion (17). The firing behavior of Bötzing E-AUG neurons seems insufficient

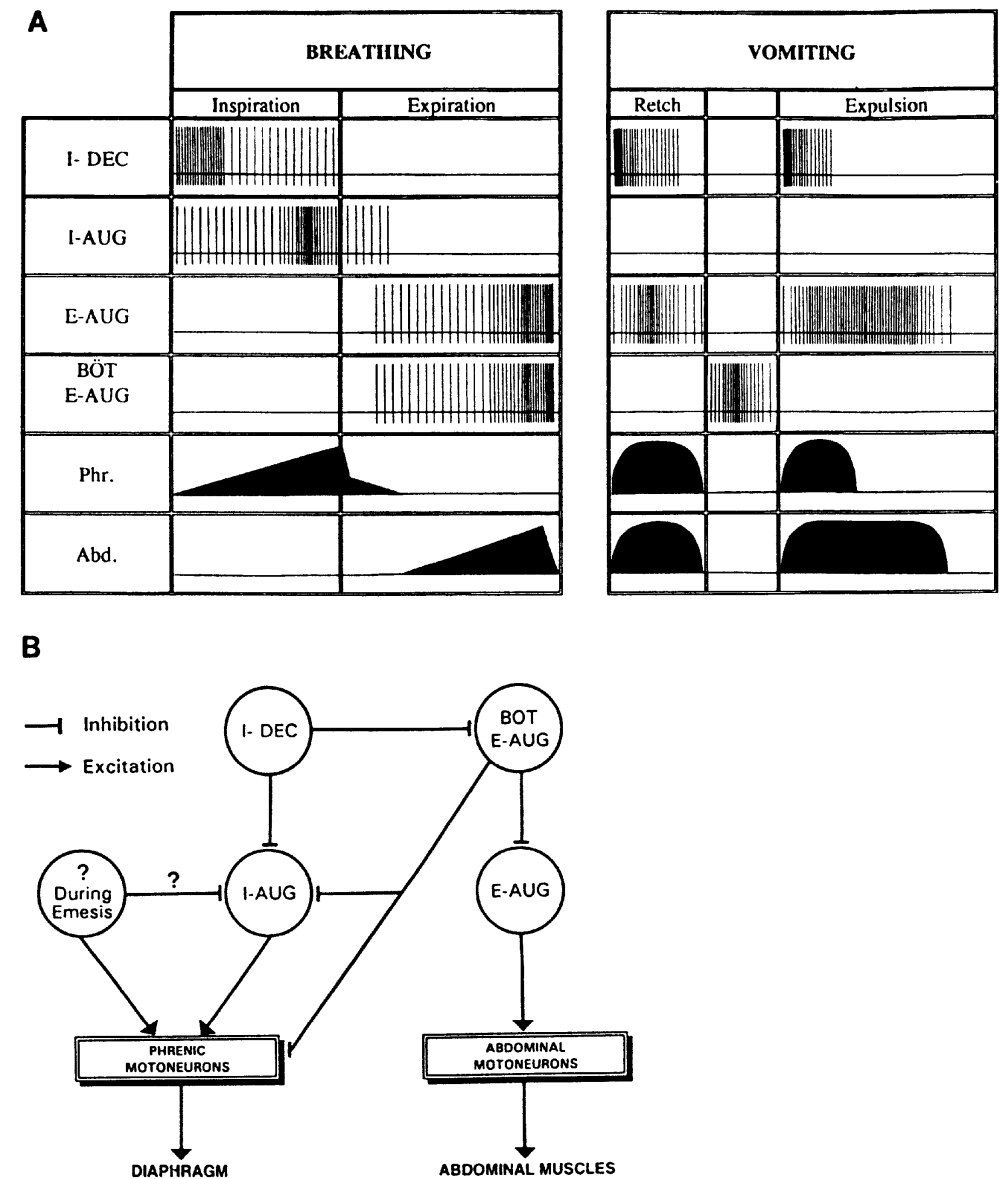


Figure 2 Simplified schematic representation of the activities of four types of medullary respiratory neurons during breathing and vomiting (A) and their connections (B). Note that the behaviors of the two expiratory (E) and two inspiratory (I) cell types are quite different during vomiting. Abbreviations: AUG, augmenting; BOT, Bötzing; DEC, decrementing. Some cell types have been omitted from the figure for simplicity. (From Ref. 5.)

to produce this expulsion-related phrenic inhibition; thus additional yet unknown mechanisms must be involved.

Other propriobulbar cell types are mainly silenced during vomiting, presumably because their discharge would impair vomiting-related activation of spinal or cranial motoneurons (15). Included are decremting expiratory neurons, which are known to inhibit vagal expiratory motoneurons that fire during vomiting, and most inspiratory neurons with a constant discharge pattern, some of which are known to excite inspiratory bulbospinal neurons and vagal motoneurons. The small percentage of inspiratory constant neurons that increase their discharge during vomiting may excite upper airway motoneurons (hypoglossal, glossopharyngeal) that are active during inspiration and vomiting; however, possible connections between these cell types have not been investigated.

At the level of the spinal cord, most inspiratory propriospinal neurons in the upper cervical cord (C1-C3) fire during vomiting (18). However, dysfunction of this cell group caused by the application of kainic acid has shown that these neurons are not essential for apparently normal activation of phrenic, intercostal, or abdominal motoneurons during vomiting (7). The functional significance of upper cervical inspiratory neurons remains unknown. At the level of the phrenic motor nucleus, there are respiratory-related interneurons that exhibit different responses during vomiting and presumably contribute to the output pattern observed on the motoneurons (19).

In sum, the activity of brainstem respiratory neurons during vomiting is complex. Many multifunctional neurons appear to take an active role in generating the pattern of respiratory muscle discharge during vomiting as well as breathing. These include bulbospinal VRG excitatory augmenting expiratory neurons, bulbospinal BOT inhibitory augmenting expiratory neurons, and many propriobulbar VRG inhibitory decremting inspiratory neurons. Other respiratory neurons are mainly silenced during vomiting, presumably because their discharge would interfere with the unique pattern of respiratory muscle activation that occurs during vomiting. Included in this group are most bulbospinal excitatory augmenting inspiratory neurons and VRG propriobulbar neurons, including inhibitory decremting expiratory neurons, most but not all excitatory constant inspiratory neurons, and a subgroup of decremting inspiratory neurons. Still other as yet unidentified neurons are important for some components of the vomiting pattern generator, including excitation of phrenic motoneurons during retching and expulsion and inhibition of these motoneurons during the second phase of expulsion.

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