

Differential respiratory muscle recruitment induced by clonidine in awake goats

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Hedrick, Michael S., Melinda R. Dwinell, Patrick L. Janssen, Josue Pizarro, and Gerald E. Bisgard. Differential respiratory muscle recruitment induced by clonidine in awake goats. *J. Appl. Physiol.* 84(4): 1198–1207, 1998.—The purpose of this study was to test the hypothesis that dysrhythmic breathing induced by the α_2 -agonist clonidine is accompanied by differential recruitment of respiratory muscles. In adult goats ($n = 14$) electromyographic (EMG) measurements were made from inspiratory muscles (diaphragm and parasternal intercostal) and expiratory muscles [triangularis sterni (TS) and transversus abdominis (Abd)]. EMG of the thyroarytenoid (TA) muscle was used as an index of upper airway (glottal) patency. Peak EMG activities of all spinal inspiratory and expiratory muscles were augmented by central and peripheral chemoreceptor stimuli. Phasic TA was apparent in the postinspiratory phase of the breathing cycle under normoxic conditions. During dysrhythmic breathing episodes induced by clonidine, TS and Abd activities were attenuated or abolished, whereas diaphragm and parasternal intercostal activities were unchanged. There was no tonic activation of TS or Abd EMG during apnea; however, TA activity became tonic throughout the apnea. We conclude that 1) α_2 -adrenoceptor stimulation results in differential recruitment of respiratory muscles during respiratory dysrhythmias and 2) apneas are accompanied by active glottic closure in the awake goat.

control of breathing; electromyograms; apnea; thyroarytenoid muscle

MOTOR OUTPUTS to specific muscles responsible for generating airflow and regulation of upper airway resistance are the effectors of the central rhythm-generating network. The various muscle groups that generate or regulate airflow are subject to control by a variety of factors, including central and peripheral chemoreceptor stimulation (26, 35–37), vagal feedback (1), anesthesia (12), and posture (9). However, there is little information on specific neurochemical regulation of respiratory muscle activity in awake animals.

Previous studies from our laboratory have shown that systemic administration of α_2 -agonists, such as clonidine and guanabenz, causes profound breathing instabilities in awake and anesthetized goats (14–16). In awake, standing goats, α_2 -agonists induce dysrhythmic ventilatory patterns characterized by alternating episodes of tachypnea and apneas [increased expiratory time (TE)] of variable lengths. Phrenic activity measured in vagotomized, chemodenervated goats anesthetized with chloralose is highly irregular, with apneas of variable lengths, but tachypneic breathing is abolished. We have also shown that efferent activity of

the recurrent laryngeal nerve (RLN) becomes tonic throughout the apneic period in anesthetized goats (15). These findings are consistent with several other studies that show that induction of apneas by a variety of maneuvers, principally by mechanically induced hypocapnia, often results in glottic closure in sleeping humans (24) and in awake, nonsedated animals (22) or results in increased RLN activity in anesthetized animals (8). However, a recent study in lambs (31) showed that barbiturate-induced apneas also resulted in tonic thyroarytenoid (TA) electromyogram (EMG) activity, suggesting that a general depression of central respiratory drive causes upper airway (glottic) closure. Because α_2 -agonists are routinely used as sedatives (32), it is possible that α_2 -agonist-induced apneas in awake goats (16) are produced by a similar mechanism. At present, although it is clear that low dosages of α_2 -adrenoceptor agonists induce dysrhythmic breathing, tonic RLN activity, and possibly upper airway obstructions in goats, it is not clear to what extent these agents affect the central motor output to respiratory muscles.

This study examined the effects of α_2 -adrenoceptor stimulation on EMG activity of selected inspiratory and expiratory muscles in the awake, standing goat. In this study, clonidine preferentially attenuated EMG activity of spinal (thoracic and abdominal) expiratory muscles; in addition, apneas were always associated with activation of the TA muscle throughout the length of the apnea, thus suggesting active glottic closure.

METHODS

Animal preparation. Studies were conducted on 14 adult goats of mixed breed (mean wt 52.8 kg). All goats were prepared under general anesthesia (halothane, nitrous oxide, and O₂) with bilateral common carotid artery translocations to a subcutaneous position to facilitate insertion of arterial catheters for experimental procedures. Some goats with carotid artery translocations from a previous study were prepared during a second surgery with EMG wires inserted into specific muscles for this experiment. The remaining goats were implanted with EMG wires and received carotid artery translocations in the same surgical procedure. In general, two sets of bipolar, Teflon-coated, stainless steel EMG wires were implanted per muscle, except in the case of the TA muscle, in which only one set of wires was implanted. The technique for implantation of EMG wires in goats has been described previously in detail by Smith et al. (37). For the TA muscle, a small C-shaped incision was made with a scalpel in the thyroid cartilage to expose the muscle, and the EMG wire was sewn in place with the use of direct visualization (22). The wire was led out of the thyroid cartilage, and the cartilage was closed with a single suture. All EMG wires

were sewn into a subcutaneous position to facilitate access for recording on the day of the experiment. EMG measurements were taken from five different muscles in the 14 animals; at most, EMG measurements were taken from four muscles in any one animal. Successful EMG recordings were obtained from two inspiratory pump muscles [the costal diaphragm (Dia; $n = 14$) and the parasternal intercostal (PS; $n = 4$)] and from two expiratory pump muscles [the triangularis sterni (TS; $n = 7$) and the transversus abdominis (Abd; $n = 12$)]. These muscles were chosen on the basis of previous studies in goats (10, 37). The EMG was recorded from one upper airway (glottal adductor) muscle, the TA ($n = 7$), on the basis of its function during apneas in humans and animals (19, 22, 24) and on the basis of our previous studies on the effects of α_2 -agonists on RLN activity in goats (14).

The animals were allowed at least 1 wk recovery from surgery before any experiments began. During this 1-wk period, we trained the animals to stand quietly in a stanchion while wearing a muzzle mask. One day before an experiment, arterial catheters were inserted into each carotid artery for anaerobic blood sampling for blood-gas measurements and measurement of blood pressure. A catheter (PE 90) was also placed into one jugular vein for drug injections. All catheters were flushed with heparinized saline and closed until the day of the experiment. On the day of the experiment, the incision overlying the EMG wires was infiltrated liberally with local anesthetic (2% lidocaine), and the wound was reopened to expose the wires.

Measurements. Small alligator clips were used to connect the EMG wires to a differential alternating current-coupled amplifier (CWE or A-M Systems). The EMG signal was amplified $\times 10,000$, filtered (band pass 10–5 kHz) and recorded on tape (Vetter or Hewlett-Packard) for off-line analysis. Ventilatory data were recorded with goats wearing a tightly fitting muzzle mask attached to a low-resistance one-way breathing valve (Hans Rudolph). Inspired gases were delivered to the animal via 3-cm ID tubing. Expired gases were collected in a spirometer (120 liters) for measurements of minute ventilation (\dot{V}_E). Inspired ventilatory flow was measured with a pneumotachograph (Fleisch) and was electronically integrated to obtain tidal volume (V_T). An O_2 analyzer (Applied Electrochemistry) was used to monitor inspired O_2 fraction ($F_{I_{O_2}}$), and expired CO_2 was monitored with an infrared CO_2 analyzer (Anarad).

A six-channel polygraph (Gilson) was used to record end-tidal CO_2 , V_T , \dot{V}_E , and systemic arterial blood pressure. Arterial blood samples were analyzed for pH, PCO_2 , and PO_2 with a blood-gas analyzer (model ABL3M, Radiometer). A rectal thermistor probe was used to monitor body temperature throughout the experiment for blood-gas temperature correction.

Protocol. After the face mask was placed on the animal, the goat breathed room air for 20–30 min to allow measurement of baseline control values. Once a stable ventilatory baseline was established, the animal was subjected to a hypercapnic stimulus with inspired CO_2 fraction ($F_{I_{CO_2}}$) raised to 0.03 for 10 min and then to 0.065 for 10 min before returning the animal to control (room air) conditions. The 6.5% CO_2 stimulus was used as a reference stimulus to which all EMG signals for that animal were compared (37). All EMG values were therefore expressed as a percentage of reference in subsequent analyses.

After baseline \dot{V}_E was reestablished, the animal was subjected to isocapnic hypoxia [arterial PO_2 (Pa_{O_2}) ~ 37 Torr] for 10–20 min. Arterial blood samples were collected frequently during the hypoxic stimulus to verify isocapnic hypoxic conditions. After the hypoxic stimulus was com-

pleted, the animal was once again returned to room air until baseline conditions were reestablished. Peripheral chemoreceptor stimulation was also accomplished with an intra-arterial bolus injection of NaCN (1,000 μg in 0.2 ml saline). \dot{V}_E and EMG values were measured during the 5- to 20-s interval after NaCN injection, the time when these values reach peak levels. After this, the ventilatory responses to three doses of dopamine (DA; 0.5, 1.0, and 5.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$) were measured. The order of the infusions was randomized, with sufficient time between infusions to return to baseline \dot{V}_E . Drug infusions were carried out at a rate of 5 ml/min for 1 min into a carotid artery. During the infusion, \dot{V}_E and EMG activity were measured during the 15- to 45-s interval, the period of maximal \dot{V}_E inhibition by DA (33). An arterial blood sample was taken immediately before the infusion started and just before the end of the infusion.

Drugs. Clonidine (Sigma Chemical) was dissolved in saline to obtain a stock solution (1 mg/ml) that was diluted 1:10 in saline for intravenous (iv) administration. Clonidine was given by bolus injection via the jugular catheter at concentrations ranging from 0.5 to 10 $\mu g/kg$ (cumulative final dose) to achieve maximal ventilatory effects without eliciting the excessive sedation that can occur with these drugs (16).

Data analysis and statistics. Ventilatory measurements of \dot{V}_E , frequency (f), and V_T as well as peak EMG values for each muscle were analyzed with a repeated-measures ANOVA. Where significant differences were found, data were further analyzed post hoc with the Student-Newman-Keuls multiple range test. EMGs were quantified on a breath-by-breath basis (10–20 breaths per condition per muscle) and were normalized to the response achieved in each goat while it was breathing an $F_{I_{CO_2}}$ of 0.065 (percentage of reference). All EMG data expressed as percentages were converted to their arc sine values before statistical comparisons were made. Paired t -tests were used to compare preinfusion control EMG values with EMG values obtained during the 15- to 45-s time interval during intra-arterial DA infusion. Statistical significance was accepted at $P < 0.05$.

RESULTS

Preclonidine ventilatory patterns and EMG measurements. Hypercapnia [mean arterial PCO_2 (Pa_{CO_2}) 51.2 Torr, mean $Pa_{O_2} > 110$ Torr], isocapnic hypoxia (mean Pa_{O_2} 37 Torr), and intracarotid NaCN injection resulted in an overall augmentation of peak EMG activities of inspiratory (Dia) and expiratory (Abd and TS) muscles compared with control (room air) responses (Fig. 1; Table 1). The effects of these stimuli were quite uniform among both inspiratory and expiratory muscles (Table 1). Phasic activity was usually present in the TA muscle during eupnea; the onset of TA activity occurred in the early expiratory (i.e., postinspiratory) phase of the respiratory cycle. In contrast to other respiratory muscle EMG activity, TA activity was suppressed by hypercapnia; that is, there was an overall reduction in phasic activity that was normally present in eupneic conditions.

We occasionally observed augmented breaths that were characterized by a large expiratory effort followed by an apnea that was three to four times longer than a normal T_E (Fig. 2). These augmented breaths were usually accompanied by an increase in TA activity that persisted throughout the length of the apnea. We did

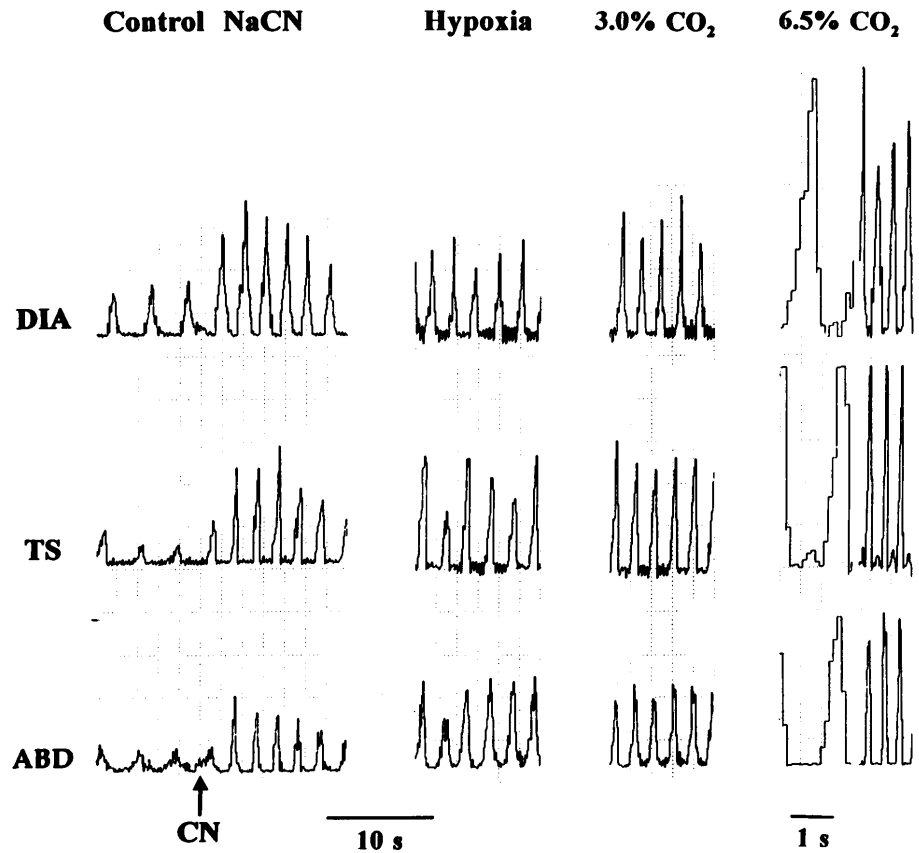


Fig. 1. Integrated electromyographic (EMG) activity from diaphragm (Dia), triangularis sterni (TS), and transversus abdominis (Abd) with NaCN injection (CN; arrow), isocapnic hypoxia (hypoxia) [arterial P_{O_2} (P_{aO_2}) ~ 40 Torr], and hypercapnia [inspired O_2 fraction (F_{ICO_2}) = 0.03 and 0.065] (3.0 and 6.5% CO_2 , respectively).

not, however, note any tonic activation of other expiratory muscles during augmented breaths.

Effects of DA infusion. Intra-arterial infusion of DA ($0.5\text{--}5.0 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), which causes an overall reduction in \dot{V}_E by carotid body (CB) feedback inhibition (5, 33), reduced \dot{V}_E and V_T in this study and, in addition, caused a significant attenuation of peak inspiratory (Dia and PS) and expiratory (TS and Abd) EMG activity. Figure 3 shows the mean difference between peak EMG during control and the mean value during the DA

Table 1. Mean values of peak EMG activity for inspiratory (Dia and PS) and expiratory (TS and Abd) muscles for goats breathing different gas mixtures, after NaCN injection, and after clonidine

Muscle	Condition				
	NaCN	3% CO_2	Hypoxia	Control	Clonidine
Dia	66.5 ± 6.4^a	57.6 ± 3.4^a	43.0 ± 4.1^b	34.9 ± 3.9^b	34.7 ± 3.7^b
<i>n</i>	10	13	14	14	14
PS	62.7 ± 16.5^a	59.0 ± 8.5^a	39.7 ± 3.9^a	32.0 ± 9.9^a	30.5 ± 11.8^a
<i>n</i>	3	4	4	4	4
TS	74.3 ± 7.8^a	$59.8 \pm 6.2^{a,b}$	49.2 ± 7.0^b	36.7 ± 5.3^b	1.2 ± 0.7^c
<i>n</i>	5	6	7	7	7
Abd	79.7 ± 11.0^a	54.8 ± 3.8^b	$43.9 \pm 4.0^{b,c}$	35.4 ± 4.1^c	8.1 ± 2.0^d
<i>n</i>	8	11	12	12	12

Values are means \pm SE in %reference values; *n*, no. of awake goats. Dia, costal diaphragm; PS, parasternal intercostal; TS, triangularis sterni; Abd, transversus abdominis. Within a given muscle type, values with 1 or more of the same superscripts are not significantly different ($P > 0.05$) with the Student-Newman-Keuls test (see METHODS).

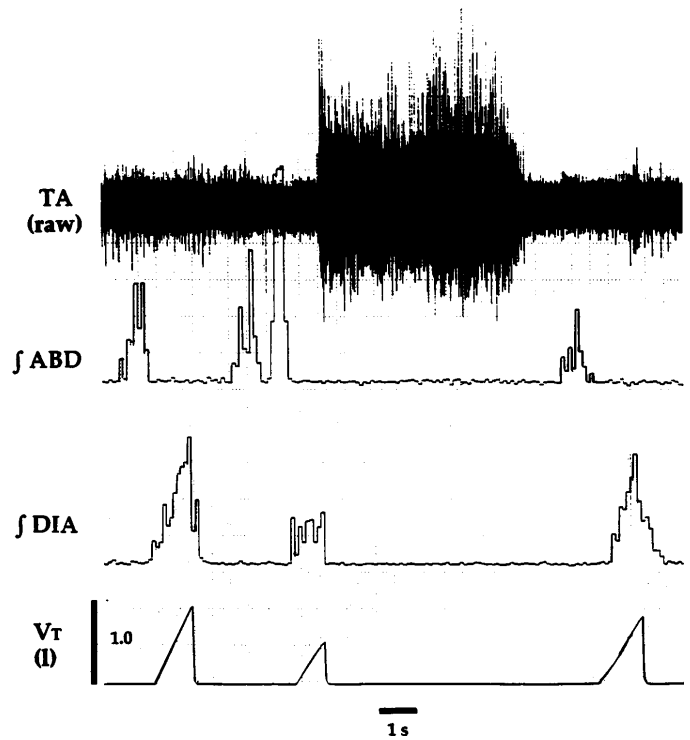


Fig. 2. Example of tonic thyroarytenoid (TA) EMG activity occurring during a brief apnea after an augmented breath in isocapnic hypoxia. Note large expiratory breath in Abd and shortened inspiratory breath in Dia followed by apnea. V_T , tidal volume.

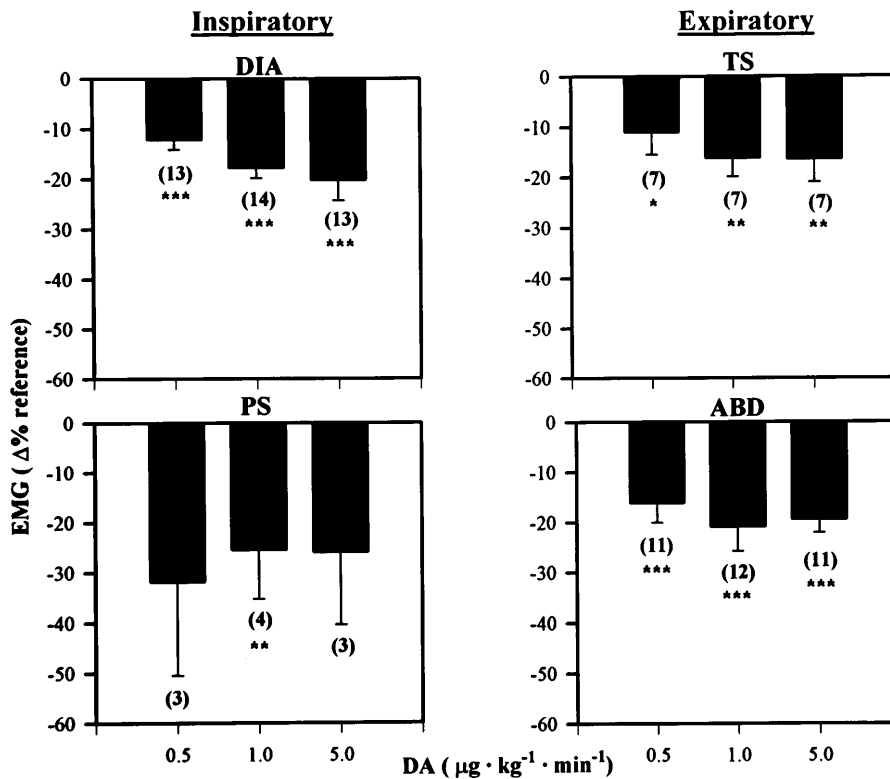


Fig. 3. Inhibitory effects of intra-arterial infusion of dopamine (DA; 0.5, 1.0, 5.0 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) on peak EMG activity of inspiratory [Dia and parasternal intercostal (PS)] and expiratory (TS and Abd) muscles in awake goats. Values are mean change ($\Delta\%$ reference) in peak EMG activity between control (preinfusion) and DA infusion (see METHODS). Negative values indicate reductions in peak EMG activity during infusion. Nos. in parentheses, no. of goats for which EMG activity of that muscle was recorded. Significantly different from control values (paired t-test). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

infusion for each dose. There were significant reductions in peak EMG activity in response to DA infusion for inspiratory and expiratory muscles. TA EMG activity generally increased during DA infusion when ventilation was reduced.

Effects of iv clonidine. In response to iv infusion of clonidine (1.0–10.0 $\mu\text{g}/\text{kg}$), ventilatory patterns in goats became very dysrhythmic with prolonged and variable TE. Pa_{O_2} decreased from a mean control value of 96 ± 2 (SE) to 81 ± 3 Torr, and mean Pa_{CO_2} increased from a control value of 37 ± 1 to 42 ± 1 Torr. During tachypnea alternating with apneas, the mean Pa_{O_2} and Pa_{CO_2} values were 85 ± 3 and 40 ± 1 Torr, respectively. We have previously documented the ventilatory and cardiovascular responses to clonidine in the awake, standing goat model (16), and the results here are qualitatively and quantitatively similar. In this study, peak EMG measurements during clonidine-induced dysrhythmic breathing episodes revealed that there is a significant attenuation of expiratory (TS and Abd) muscle activity compared with control conditions (Fig. 4). The attenuation and/or abolition of TS and Abd EMG activities was pronounced despite large increases in Pa_{CO_2} that occurred in some animals as the result of hypoventilation after clonidine administration (Fig. 5) (16). The attenuation of TS and Abd EMG activity occurred consistently and independently of the prevailing breathing pattern. That is, whether apnea or tachypnea occurred, there was a significant attenuation of expiratory EMG activity, whereas inspiratory (Dia and PS) muscle EMG amplitude was variably affected but, on average, was unchanged relative to control conditions (Fig. 6). As in our previous study (16), clonidine injection decreased

mean arterial blood pressure ~ 20 Torr (control 108 ± 4 to 88 ± 5 Torr with clonidine). This decrease was not likely to influence the ventilatory response to clonidine, because a decreased baroreceptor input would most likely stimulate, rather than inhibit, ventilation (6).

Effects of clonidine on TA activity. The normal response to clonidine in awake goats results in periods of tachypnea interspersed with apneas of variable lengths. In each goat in which TA EMG activity was measured, during apneic periods there was a clear increase of tonic TA activity that was maintained throughout the length of the apnea (Fig. 7). In extreme instances, apneas up to 40 or 50 s occurred and were always accompanied by tonic TA activity that persisted throughout the length of the apnea. Prolonged apneas were potentiated when peripheral chemoreceptor drive was attenuated by DA infusion into the carotid artery (Fig. 8) or by 100% O_2 (Dejours test) given for four to five breaths during DA infusion (data not shown). In some cases, glottal closure, as indicated by tonic TA activity, appeared to be powerful enough to prevent airflow despite inspiratory and expiratory ventilatory efforts (Fig. 9).

DISCUSSION

The important findings from this study are as follows. 1) Clonidine preferentially attenuates or abolishes activity of spinal expiratory muscles (TS and Abd) during dysrhythmic breathing. Moreover, we find no evidence for tonic activation of these muscles during prolonged apneas induced by these drugs. 2) Apneas were accompanied by tonic activation of the TA muscle, a glottal adductor, throughout the length of the apnea.

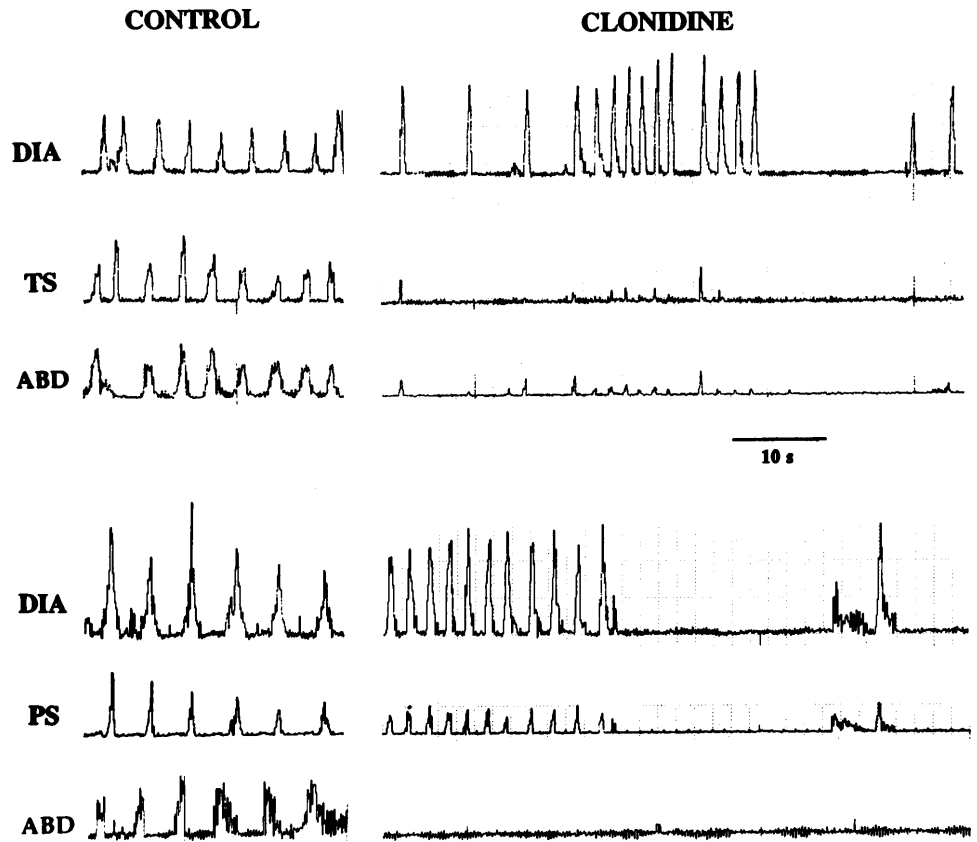


Fig. 4. Effects of iv clonidine on EMG activity in 2 awake goats. *Top*: EMG activity in Dia, TS, and Abd in control condition and after clonidine infusion. *Bottom*: EMG activity in Dia, PS, and Abd in control condition and after clonidine infusion. Note presence of apneas interspersed with tachypnea after clonidine infusion in both animals; also note attenuation/abolition of EMG activity preferentially in expiratory muscles (TS and Abd).

After administration of clonidine, the animals clearly exhibited more prolonged apneas with attenuation of CB feedback by DA infusion. With increased TA activity, it is likely glottic closure would also be increased under these conditions. 3) Dysrhythmic breathing resulting from systemic injection of clonidine is corre-

lated with widespread differential activation and/or attenuation of respiratory pump and upper airway musculature.

Activity of spinal inspiratory and expiratory muscles. Spinal inspiratory and expiratory muscles are clearly influenced by a variety of factors that help to regulate

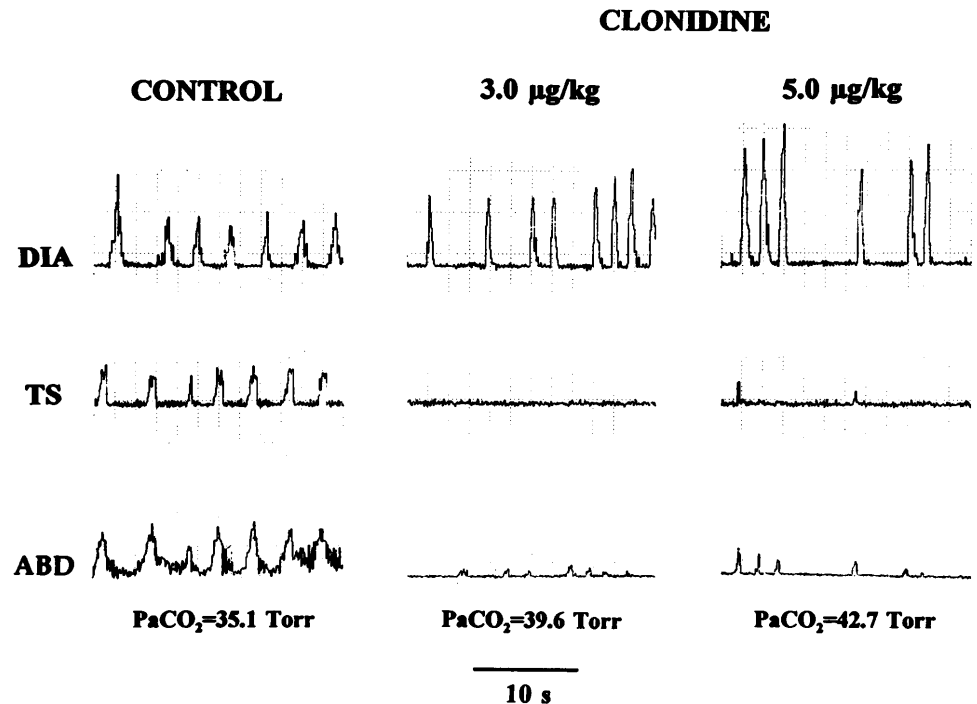


Fig. 5. Attenuation of peak EMG activity in expiratory muscles (TS and Abd) after clonidine administration in awake goat. Two doses of clonidine (3.0 and 5.0 µg/kg) produced significant increases in arterial PCO₂ (PaCO₂) resulting from hypoventilation. Peak EMG of Dia increased with increased hypercapnia, whereas there was little change in attenuation of TS and Abd peak EMG activity despite extreme increase of PaCO₂.

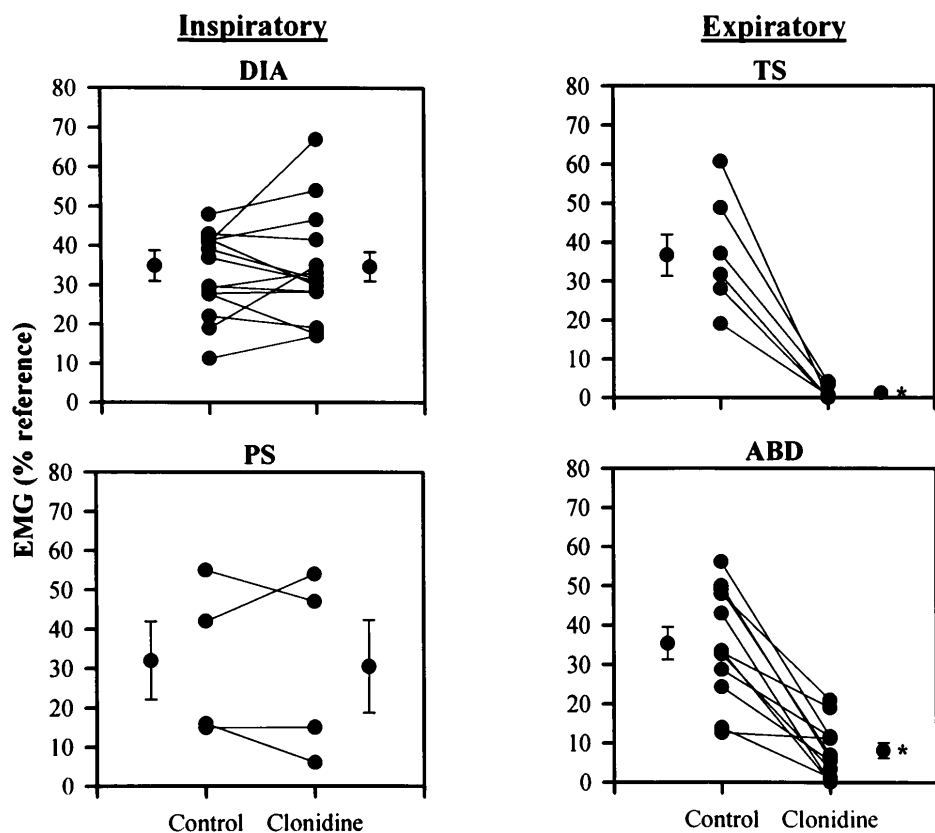


Fig. 6. Summary of peak EMG activities (%reference) of inspiratory (Dia and PS) and expiratory (TS and Abd) muscles in individual awake goats. Each mean control value (preclonidine) is connected to peak mean EMG value after iv clonidine. Means \pm SE for each muscle group before and after clonidine are also indicated. *Significant reductions in peak EMG activity were noted in expiratory but not in inspiratory muscles (ANOVA; see Table 1).

and optimize ventilation to adapt to prevailing demands (2). Recent experiments have revealed a considerable difference in the responses of respiratory muscles to various stimuli in animals under anesthesia com-

pared with awake animals. For example, it is generally accepted that a hypoxic ventilatory stimulus provides little or no augmentation of expiratory muscle activity compared with an equivalent hyperpnea elicited by

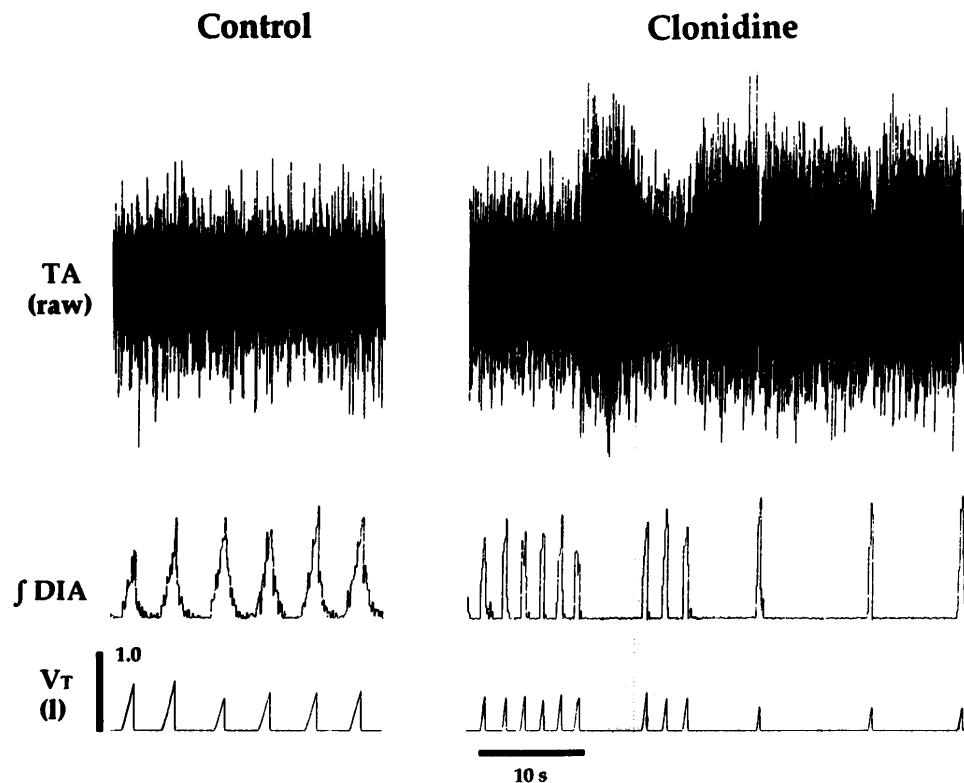


Fig. 7. Raw EMG activity in awake goat after clonidine. Note appearance of apneas as indicated in integrated Dia and tidal volume (V_T). Each apnea is accompanied by increased continuous TA EMG activity throughout each apnea.

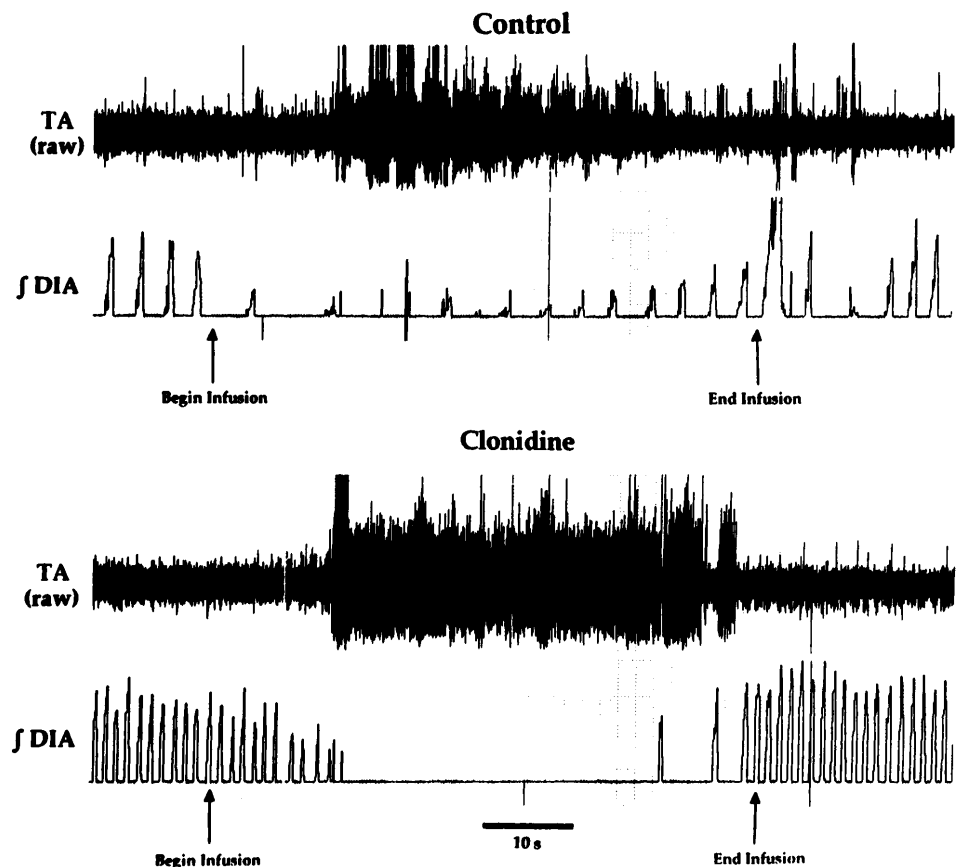


Fig. 8. Synergistic effect of clonidine and DA infusion (arrows). *Top*: 1-min infusion of DA ($5.0 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) during control. Note attenuation of Dia activity and increased tonic and phasic TA activity. *Bottom*: same dose of DA after iv clonidine in same animal. Prolonged apnea during infusion is accompanied by tonic TA activity. Single breath near end of infusion caused break in TA activity during the breath.

hypercapnia (11, 12). This effect has been attributed, in part, to a relatively greater stimulation of inspiratory muscles by CB chemoreceptors or to the depressant effects of central hypoxia and/or hypocapnia. This differential effect on inspiratory and expiratory motor outflow has been termed "the inspiratory shift" (35). However, recent studies in awake dogs (34) and awake goats (10, 37) have shown that equivalent hypoxic or hypercapnic stimuli result in an overall augmentation of both inspiratory and expiratory motor output, with no evidence for an asymmetrical recruitment pattern characteristic of anesthetized animals. Therefore, data collected from awake animals argue against an inspiratory shift and, instead, suggest that both inspiratory and expiratory muscles are recruited to similar degrees with increased central or peripheral chemoreceptor drive.

This study is also consistent with the recent studies of awake dogs (36) that suggest that inhibition of CB feedback by DA infusion results in an overall reduction in ventilatory motor output to both inspiratory and expiratory muscles. It thus appears that, under normal conditions in awake animals, inspiratory and expiratory muscles are tightly coupled with respect to the prevailing respiratory drive. That is, increased central or peripheral chemoreceptor drive causes an overall augmentation of respiratory muscles, whereas inhibition of respiratory drive causes an overall attenuation of respiratory muscles. Although the nature of this coupling is unclear, it is presumably the final integra-

tive output of the brain stem respiratory controller for respiration and serves to maintain optimal levels of ventilation for maintenance of respiratory homeostasis (2).

Systemic infusion of clonidine appears to decouple the relationship between inspiratory and expiratory muscle activity. More specifically, there is a preferential attenuation of phasic expiratory (TS and Abd) muscle activity relative to inspiratory (Dia and PS) muscle activity. Our results do not allow for a complete mechanistic explanation for the disruption of central respiratory rhythm and differential outflow to specific respiratory muscle groups. However, several lines of evidence argue strongly for the importance of α_2 -adrenoceptors in regulating central respiratory output. Anatomical studies have shown a widespread distribution of α_2 -adrenoceptors throughout the brain stem in mammals (38). More recently, the α_{2a} -adrenoceptor subtype has been shown to be predominant in areas associated with central respiratory rhythmogenesis (3, 13). There is considerable evidence that α_2 -adrenoceptors regulate sympathetic outflow from the brain stem and, therefore, provide the hypotensive effects of systemically administered clonidine (28, 32). The actions of α_2 -agonists on membrane function have been attributed to increased K^+ conductance and/or decreased Ca^{2+} conductance (27). Application of clonidine to brain stem respiratory-related neurons causes hyperpolarization of these neurons (7). Bulbospinal expiratory neurons to the thoracic spinal cord project mainly from the caudal

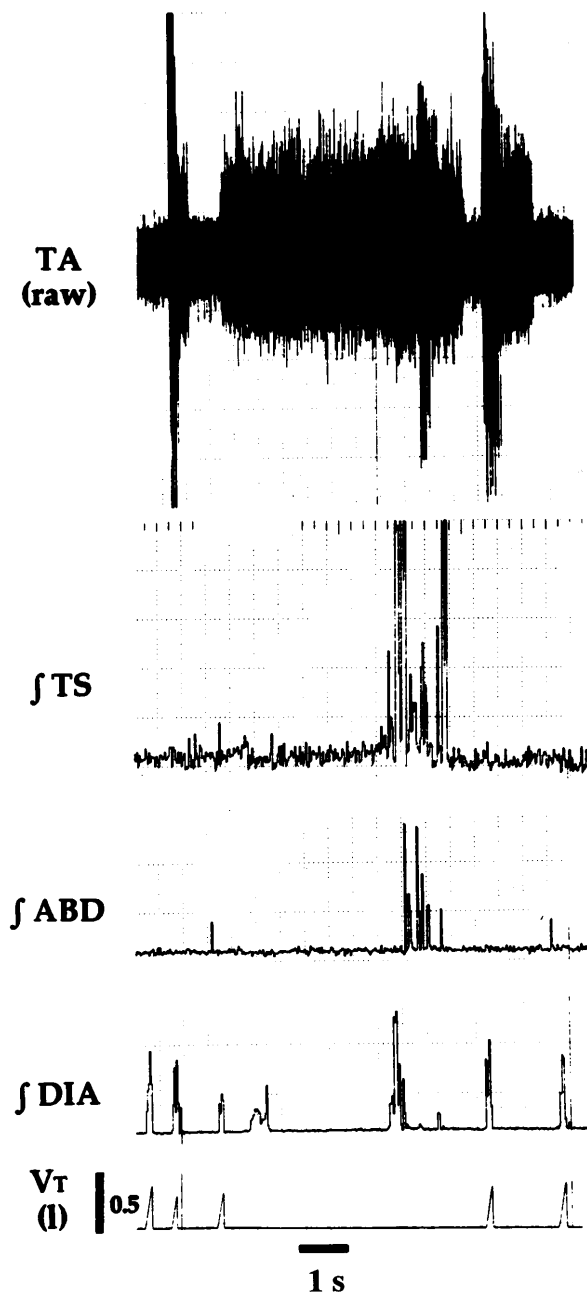


Fig. 9. Tonic TA activity during inspiratory and expiratory efforts in awake goat. Raw TA activity (*top*) persists during brief (5.5 s) apnea. Note the 2 inspiratory efforts indicated in integrated Dia trace and the expiratory effort in integrated TS and Abd traces without any measurable change in air flow indicated in VT trace.

ventral respiratory group and, more specifically, from the nucleus retroambiguus (2, 3). Many of the bulbospinal expiratory neurons projecting to the spinal cord are located in the Bötzinger complex (2), and anatomical studies have shown the presence of α_2 -adrenoceptors in this area of the medulla (38). However, it has recently been shown that presympathetic neurons and expiratory neurons of the Bötzinger complex are anatomically and functionally distinct neuronal populations (21).

Our study does not allow us to distinguish between many different possibilities that would explain the

attenuation of expiratory motor output by systemically administered α_2 -agonists, but a direct hyperpolarizing effect of α_2 -agonists on brain stem expiratory-related neurons is consistent with our observations. Thus, although it is clear that, in awake goats, there is a profound disruption of normal respiratory rhythmogenesis accompanied by attenuation of expiratory motor output, the manner in which α_2 -adrenoceptors decouple inspiratory and expiratory motor output is unclear.

Our study has also revealed an important function of CB feedback drive under normal conditions that maintains phasic spinal respiratory muscle activity. Infusion of DA, which reduces V_E by a CB inhibitory mechanism (5, 33), resulted in an overall attenuation of inspiratory and expiratory muscle groups (Fig. 3). To our knowledge, this is the first evidence for a role of tonic CB afferent feedback providing a background facilitatory drive to respiratory motor activity in an awake animal. Our results also indicate that there is an additive effect of DA inhibitory feedback and α_2 -agonists: DA infusion caused a greater degree of attenuation of respiratory muscle activity in the presence of clonidine than in the control condition (Fig. 8). This synergistic effect may occur peripherally, centrally, or both. In the cat, guanabenz has a direct inhibitory effect on carotid sinus nerve discharge (23) which would enhance the inhibitory effect of DA on CB afferent activity. However, we have also shown that the respiratory responses to α_2 -agonists are not affected by CB denervation in awake goats (16); this suggests that there may be a central interaction between α_2 -agonists and reduced CB afferent feedback by DA infusion.

Previous studies have noted tonic activation of spinal expiratory muscles during hypocapnia-induced apneas. Most notable is the finding that TS activity in awake or sleeping dogs becomes tonic with mechanically induced hypocapnia (17, 18). In this study, we found no evidence for tonic TS activity during apneas in awake, standing goats. Whether these differences can be attributed to species differences, posture (9), or the mechanism to produce the apnea is unclear.

Effects of α_2 -agonists on TA activity. A general observation we have made in these studies is that, when respiratory pump muscles were stimulated by a variety of means, TA activity was suppressed. Conversely, when the pump muscles were depressed by clonidine or DA, TA activity was increased. This study has shown clearly that apneas associated with systemic administration of clonidine are accompanied by tonic activation of TA and, although we realize that EMG activity does not give an accurate estimate of mechanical activity, it appears likely that glottic closure is also induced in awake goats (Fig. 7). The data from awake goats directly corroborate our previous study in anesthetized goats (15) which showed that prolonged apneas induced by clonidine are associated with tonic RLN activation. The RLN controls glottal adductor activity, and our results indicate that apneas induced by α_2 -agonists activate this motor efferent, with the result of glottic closure in goats. On occasion, this mechanism of glottic

closure appeared to be powerful enough to prevent airflow in the presence of ventilatory efforts (Fig. 9).

Our results are also consistent with recent studies that show glottic closure and/or TA EMG activity becomes tonic during sleep apneas (19) and during apneas induced in awake or sleeping animals or humans by hypocapnic hyperventilation (22, 24). What is observed in these studies is that TA activity becomes tonic during the hypocapnia-induced apneic period. One interpretation of these results is that glottic closure helps to preserve lung gas exchange by preventing alveolar gas from flowing from the lung and restoring normocapnia (22). However, a recent study in lambs (31) has shown that barbiturate-induced apneas also result in tonic TA activity, indicating that glottic closure accompanies central respiratory depression and need not require central hypocapnia. Because clonidine and other α_2 -agonists are clinically important for inducing anesthesia, sedation, and analgesia (32), our results appear to be consistent with the results from lambs and may suggest that glottic closure is a general response to depression of central respiratory drive (31). In our previous study with anesthetized goats, which showed tonic RLN activity with systemic clonidine and guanabenz (15), we found that systemic pentobarbital resulted in apneas that were not accompanied by tonic RLN activity (unpublished results). Instead, there was a general depression of both respiratory drive and RLN activity. Thus our results may suggest a specific effect of α_2 -agonists on RLN and, therefore, TA activity. Regardless of the precise mechanism for activation of the TA, it is clear from studies with awake goats and lambs that glottic closure can occur in the absence of hypocapnia-induced apneas.

Our observations of tonic TA activity also have important clinical implications. Recent studies in humans (20, 29) and horses (25) have indicated that α_2 -agonists cause significant upper airway obstructions and apneas which lead to arterial O_2 desaturation. Although the nature of the upper airway obstruction was not identified in those studies, the results of the present study suggest that the larynx is the principal site for obstructive apneas associated with systemically administered clonidine.

It is unclear what mechanism(s) results in tonic activation of the TA during apneas in this study. The RLN motoneurons of the vagus, which control the activity of the TA, originate in the nucleus ambiguus (NA; Ref. 4). This area of the ventral lateral medulla is associated with cardiovascular and respiratory integration and is subject to a wide variety of inputs from areas within the brain stem. Because a preponderance of evidence indicates that α_2 -agonists primarily hyperpolarize neurons, our results showing tonic activation (depolarization) of TA suggest that RLN motoneurons are disinhibited in the presence of α_2 -agonists. At present, there is little direct information on the role of specific neurotransmitters within the NA that may explain this observation. Recently, an electrophysiological study that used preparations of brain stem slices from the NA of guinea pigs (30) revealed that applica-

tion of norepinephrine to brain stem slices blocked a Ca^{2+} -activated K^+ current that resulted in greater depolarization of NA neurons. The effect of norepinephrine on this current was blocked by the β -adrenoceptor antagonist propranolol. Although the authors did not investigate α -adrenergic mechanisms on NA neurons, it is clear that norepinephrine has a direct effect on NA neurons that is consistent with the tonic activation of motoneurons in this region of the medulla. Further studies are needed to determine the precise mechanism(s) by which α_2 -agonists influence central respiratory drive, but it is clear that systemic administration of these agents causes widespread changes in central rhythmic activity and efferent motor output to specific respiratory muscles in awake goats.

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