Effect of almitrine on ventilation and on diaphragm and geniohyoid muscle activity in the rat

Ken D. O’HALLORAN, Aidan K. CURRAN and Aidan BRADFORD
Department of Physiology, Royal College of Surgeons in Ireland, St. Stephen’s Green, Dublin 2, Ireland

(Received 11 December 1995/9 May 1996; accepted 9 May 1996)

INTRODUCTION

Almitrine bismesylate is used in the treatment of chronic obstructive pulmonary disease [1] and hypoventilation syndromes [2]. The stimulant action of almitrine on respiration is well established for rats [3], rabbits [4], cats [5], dogs [6] and man [7], but its effects on the ventilatory responses to hypoxia and hypercapnia are controversial. In man, the ventilatory effects of almitrine were reported to be abolished after bilateral surgical excision of the carotid body as a treatment for breathlessness [8]. This is consistent with the drug’s action as a peripheral arterial chemoreceptor stimulant. However, in cats and dogs, some ventilatory effect remains after carotid sinus nerve section [9], suggesting that almitrine may also act on glomus tissue other than the carotid bodies. The superior laryngeal nerves (SLNs) have been shown to contain paraganglia that morphologically and biochemically resemble the carotid body [10]. Although the functions of the laryngeal paraganglia are unknown, it is speculated that they possess chemoreceptor properties [11].

In order to test the hypothesis that almitrine may exert part of its ventilatory effect through a direct action on the SLNs and to study almitrine’s interaction with hypoxia and hypercapnia, we have examined the ventilatory effects of almitrine before and after SLN section in conscious, tracheostomized rats breathing air and hypoxic and hypercapnic gas mixtures. The rationale for the tracheostomy was that the upper airway was now bypassed and thus effects on SLN afferent activity secondary to the almitrine-induced increase in ventilation would be minimized. An almitrine-induced hyperpnoea would in itself alter the activity of upper airway receptors in the SLNs so that changes in the ventilatory response to almitrine as a result of SLN section could not be ascribed to a direct effect of almitrine on SLN paraganglia unless the upper airway was bypassed with a tracheostomy.

It has been suggested that almitrine may also have a useful role to play in the treatment of diseases involving pharyngeal airway obstruction [12]. Pharyngeal airway obstruction occurs when the dilating and stabilizing force of the upper airway muscles is insufficient to counterbalance the negative pharyngeal pressure produced by the inspiratory pump muscles during inspiration [13]. There is evidence that peripheral arterial chemoreceptor stimulation preferentially increases upper airway compared with diaphragm muscle activity [14] and we hypothesize that almitrine should do likewise. Little
attention, however, has been focused on the effects of almitrine on upper airway dilator muscles, although one report has shown that almitrine causes a reduction in the duration of apnoeic events in adults with obstructive sleep apnoea [15]. The present study, therefore, also examines the effects of almitrine on geniohyoid and diaphragm electromyo- 

graphic (EMG) activity in anaesthetized rats. We chose to study the geniohyoid muscle because it is readily accessible in the rat and because of its importance as an upper airway dilator. Contraction of the geniohyoid causes anterior displacement of the hyoid bone and dilation and stiffening of the hypopharynx [13]. Although the site of upper airway collapse is confined to the oropharynx in approximately 50% of patients with obstructive sleep apnoea, the region of collapse also includes the hypopharynx in the remaining 50% [16].

METHODS

Recording of ventilation in conscious animals

In order to examine the effects of almitrine on ventilation and on the ventilatory response to hypoxia and hypercapnia before and after SLN section, 27 male Wistar rats (body weight 400–500 g) were anaesthetized with diethyl ether and a thin-walled cannula was inserted into a low-cervical tracheostomy. Animals were randomly divided into two groups, one of 18 animals in which the SLNs were exposed bilaterally and cleared from surrounding connective tissue, towards the larynx and the hypopharynx [13]. Although the site of upper airway collapse is confined to the oropharynx in approximately 50% of patients with obstructive sleep apnoea, the region of collapse also includes the hypopharynx in the remaining 50% [16].

Ventilation and ventilatory responses to hypoxia and hypercapnia were measured before and 30 min after the administration of an intraperitoneal injection of almitrine bismesylate (Servier Laboratories Ltd) at a dose of 5 mg/kg or an equal volume of the drug's vehicle (0.6% malic acid). Almitrine was given to nine SLN-intact and nine SLN-sectioned animals and vehicle was given to a separate group of nine SLN-intact animals.

Conscious, unrestrained animals were placed in a 2 l sealed, air-tight, perspex chamber and allowed an exploratory period of at least 30 min to accustom themselves to the chamber before any ventilatory measurements were carried out. The animal chamber was continuously flushed with warmed (24°C), saturated (100% relative humidity) air containing 0, 3, 6 and 9% CO₂ and 10% O₂ in N₂ at a rate of 6 l/min. CO₂ and 10% O₂ gas mixtures were generated by mixing air with 100% CO₂ and with 100% N₂ respectively.

Five minutes were allowed for equilibration with each gas mixture. At the end of this period, the inlet and outlet tubes of the animal chamber were clamped and pressure fluctuations within the chamber (due to the animals breathing) were measured relative to an identical reference chamber using a differential pressure transducer (Type M3; Mercury Electronics Ltd, Glasgow, U.K.) connected in series between the two chambers. The signal was digitized and recorded using a data acquisition system (MacLab Mk3; AD Instruments, NSW, Australia) and stored for later analysis on a microcomputer (Apple Macintosh LC III; Apple Computer Inc., CA, U.S.A.). The O₂ and CO₂ concentrations of the gases flushing the plethysmograph were analysed using fuel cell O₂ and IR CO₂ analysers respectively (Eliza Duo; Gambro Engström AB, Bromma, Sweden). Calibrations were performed by repeatedly injecting 0.1–0.2 ml of air into the chamber using a spring-loaded Hamilton syringe (Becton Dickinson and Co., U.S.A.) and measuring the consequent pressure deflection of the wave-form pattern. The entire system was immersed in a water bath (Model 20E; Gallenkamp and Co. Ltd., London, U.K.) and a constant comfortable temperature of 24°C was maintained within the animal chamber.

Data analysis

Respiratory frequency and inspiratory and expiratory duration were obtained from direct measurement of the recordings obtained. Tidal volume was calculated according to the equations derived by Drorbaugh and Fenn [17]. Minute ventilation was calculated as the product of respiratory frequency and the calculated tidal volume. Values were obtained from 10 to 20 consecutive breaths during a steady-state period for each trial where breathing was regular and movement artefact was absent. Ten to twenty breaths were chosen for analysis because a longer period of recording (and therefore more breaths) would have increased the possibility of rebreathing in the closed system of the plethysmograph. All challenges were preceded and followed by air trials. All data are expressed as mean absolute values or as mean percentage change from control±SD. Values for tidal volume, minute ventilation and mean inspiratory flow are expressed per 100 g of body weight. Statistical analysis was performed using one-way, two-way and three-way analysis of variance where appropriate using almitrine or vehicle, SLN section and gas mixture as independent variables followed by Fisher's least significance test. Differences were considered significant at P<0.05.

EMG recording

Experiments were performed on 11 male Wistar rats (body weight 500–600 g) anaesthetized with 100 mg/kg α-chloralose and 1 g/kg urethane injected intraperitoneally. Animals were placed supine and
rectal temperature was monitored continuously and maintained at 37°C with a heating pad and radiant heat. A jugular vein was cannulated to administer almitrine (0.5 mg/kg) or supplemental anaesthetic as required. A common carotid artery was cannulated to record arterial blood pressure. Since head and jaw position affect upper airway muscle activity [18], the head was placed with the mouth closed so that there was no lateral flexion or rotation of the neck and the amount of neck extension was the natural position assumed which did not vary during the course of the experiments. Animals breathed room air spontaneously through a cannula inserted into a low-cervical tracheostomy with the aid of a binocular microscope. The vagus nerves were identified and sectioned bilaterally. The digastic muscles were identified and carefully separated to reveal the underlying omohyoid muscle which was cut in order to expose the geniohyoid muscles. The geniohyoid muscles consist of two parallel strips of muscle running from the midpoint of the mandible to the hyoid bone and bonded medially by dense connective tissue. Care was taken to preserve the vascular and neural integrity of the muscles. Bipolar, fine copper wire electrodes (outside diameter 0.02 mm, insulated except for 1–2 mm at the tips) were placed in the central portion of the muscle on one side and spaced approximately 5 mm apart. Each wire was passed through a hypodermic needle (23-gauge) which was used to insert the wire into the muscle, and the wire was bent at its tip into a hook to allow it to remain embedded in the muscle after the needle was withdrawn. Similar electrodes were inserted under direct vision into the costal diaphragm through an abdominal incision which was then closed. Electrode locations were verified at the end of each experiment. EMG signals were band-pass filtered between 10 and 6000 Hz, rectified and amplified (Neurolog NL 104; Digitimer Ltd, Hertfordshire, U.K.) and the resulting signal was integrated (Neurolog NL 703; Digitimer Ltd). Raw and integrated EMG activity together with arterial blood pressure signals were digitized and recorded on a microcomputer as described above. The effects of a single intravenous injection of almitrine on EMG activity were examined. In all animals, the effects of vehicle were also examined.

**Data analysis**

Phasic inspiratory diaphragm and geniohyoid integrated EMG activity was quantified as the height of the peak integrated signal from end-expiratory level in arbitrary units. Mean peak integrated EMG activity, respiratory frequency and inspiratory and expiratory duration were calculated for the last 20–30 consecutive breaths during the control period, and for 20–30 consecutive breaths 30 min after almitrine administration when effects were maximal. In order to enhance statistical reliability, it was possible to analyse more breaths compared with the plethysmography measurements since we were not constrained by rebreathing or movement artefact. All values are expressed as mean percentage change from control ± SD. Responses to almitrine were compared with pre-trial control values using Student’s t-test with P<0.05 considered significant.

**RESULTS**

**Effect of almitrine on geniohyoid and diaphragm activity in anaesthetized animals**

In all animals, the geniohyoid muscle had phasic inspiratory activity which slightly preceded that of the diaphragm. Vagotomy caused typical decreases in respiratory frequency and increases in diaphragm and geniohyoid EMG activity. An intravenous injection of almitrine had no significant effect on respiratory frequency or inspiratory and expiratory duration (Fig. 1a) but caused significant increases in peak integrated diaphragm and geniohyoid EMG activity (Fig. 1b). The increase in peak integrated geniohyoid EMG activity (132.0 ± 21.3%) was much greater than that for diaphragm activity (29.3 ± 13.6%). The amount of preactivation of the geniohyoid compared with the diaphragm was unaffected by almitrine. Thus, the time between activation of the geniohyoid and the diaphragm was 76 ± 81 ms and 80 ± 75 ms before and during almitrine respectively. Vehicle administration had no significant effect on any measured variable. Figure 2 shows an example of the effect of almitrine on diaphragm and geniohyoid EMG activity.

**Ventilatory responses to almitrine in conscious animals with SLNs intact**

Data for ventilatory variables in normoxia, hypoxia and hypercapnia before and after an intraperitoneal injection of almitrine in nine tracheostomized animals with intact SLNs are given in Table 1. It shows that while breathing air, almitrine caused significant increases in respiratory frequency, minute ventilation and mean inspiratory flow and significant decreases in inspiratory and expiratory duration, but had no significant effect on respiratory duty cycle or tidal volume. The ventilatory response to 10% O₂ and to 3, 6 and 9% CO₂ before and after injection of almitrine is shown in Fig. 3. When expressed as a percentage change from air, there was no significant difference in the ventilatory response to hypoxia for any of the ventilatory variables measured, before or after almitrine treatment. Ventilatory responses to 6 and 9% CO₂ were increased by almitrine treatment, although statistical significance was achieved for 6% CO₂ only. The ventilatory responses for both tidal volume and minute ventilation were significantly higher for 6% CO₂ after almitrine. After an equal-volume intraperitoneal injection of the drug’s vehicle in nine addi-
Role of the SLNs in the ventilatory response to almitrine

In SLN-intact animals breathing air, almitrine significantly increased minute ventilation and mean inspiratory flow due to an increase in respiratory frequency, with no change in tidal volume. In SLN-denervated animals breathing air, the ventilatory response to almitrine was significantly reduced owing to a reduction in the tidal component of the ventilatory response with no change in the frequency component of the response. In addition, mean inspiratory flow was unaffected in SLN-denervated animals. Thus, in SLN-denervated animals breathing air, there was a small but significant reduction in the ventilatory response to almitrine. In addition, the ventilatory response to hypercapnia after almitrine administration was significantly different in both groups (Fig. 4). In SLN-intact animals, the ventilatory response to CO₂ was increased compared with control, a response significantly different to that in SLN-denervated animals in which hypercapnic sensitivity was unaltered after almitrine administration. Almitrine had no effect on the ventilatory response to hypoxia either in SLN-intact or SLN-sectioned animals (Fig. 4).

DISCUSSION

In the present study, the changes in ventilation caused by almitrine in tracheostomized, conscious rats compare well with those reported in the literature. Dhillon and Barer [3] reported a 47±6% increase in minute ventilation after an intravenous infusion of almitrine (3.5 mg/kg) in anaesthetized rats. Pequignot et al. [19] reported a 30% increase in ventilation after an intraperitoneal injection of almitrine (5 mg/kg) in anaesthetized rats. The effects of tracheostomy itself on ventilation are complex and controversial. In conscious rats, tracheostomy has been reported to either increase [20] or decrease [21] minute ventilation compared with airway-intact animals. Under identical conditions to the present experiments, we have shown previously that tracheostomy reduces minute ventilation [22] in agreement with Nattie [21]. Such effects are probably due to differences in dead space, airway resistance and loss of upper airway sensory function compared with airway-intact animals. Certainly, loss of sensory function is a factor since the present experiments show that SLN section in tracheostomized animals does not affect ventilatory variables while breathing air, whereas our previous work has shown that SLN section does alter ventilation in airway-intact animals [23].

In the present work, the ventilatory response to hypoxia was unaffected by almitrine treatment. This
Almitrine and airway function

(a)

(b)

GH

DIA

Fig. 2. Effect of almitrine on geniohyoid and diaphragm muscle EMG activity. (a) Raw and integrated (J) geniohyoid (GH) and diaphragm (DIA) EMG activity before almitrine. (b) Raw and integrated (J) geniohyoid (GH) and diaphragm (DIA) EMG activity 30 min after an intravenous injection of 0.5 mg/kg almitrine.

Table 1. Effect of almitrine on ventilation in normoxia, hypoxia and hypercapnia in animals with intact SLN. Values are mean ± SD before (control) and after (almitrine) an intraperitoneal injection of almitrine (5 mg/kg). fR, respiratory frequency; V̇E, tidal volume; V̇E, minute ventilation; Tᵢ, inspiratory duration; Tₑ, expiratory duration; Tₑ/Tᵢ = mean inspiratory flow. * indicates significant difference, air control versus air almitrine (one-way analysis of variance). † and ‡ indicate significant difference from air and from control respectively, and $ indicates significant difference in response to inspired gas due to almitrine (two-way analysis of variance).

<table>
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<tr>
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<th>fR (breaths/min)</th>
<th>V̇E (ml/100 g)</th>
<th>V̇E (ml min⁻¹ 100 g⁻¹)</th>
<th>Tᵢ (s)</th>
<th>Tₑ (s)</th>
<th>V̇E/Tᵢ (ml s⁻¹ 100 g⁻¹)</th>
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<tbody>
<tr>
<td>Control (n = 9)</td>
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<tr>
<td>Air</td>
<td>87.97 ± 27.9</td>
<td>0.45 ± 0.09</td>
<td>37.9 ± 11.3</td>
<td>0.34 ± 0.10</td>
<td>0.40 ± 0.14</td>
<td>0.46 ± 0.05 1.29 ± 0.38</td>
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<tr>
<td>10% CO₂</td>
<td>107.8 ± 29.8†</td>
<td>0.54 ± 0.22†</td>
<td>56.8 ± 9.7†</td>
<td>0.25 ± 0.05†</td>
<td>0.36 ± 0.16</td>
<td>0.42 ± 0.07 21.6 ± 0.7†</td>
</tr>
<tr>
<td>3% CO₂</td>
<td>94.43 ± 25.6</td>
<td>0.57 ± 0.16†</td>
<td>53.7 ± 19.9†</td>
<td>0.32 ± 0.09</td>
<td>0.36 ± 0.11</td>
<td>0.48 ± 0.04 1.78 ± 0.58†</td>
</tr>
<tr>
<td>6% CO₂</td>
<td>97.28 ± 29.8</td>
<td>0.64 ± 0.13†</td>
<td>61.7 ± 20.8†</td>
<td>0.30 ± 0.10</td>
<td>0.39 ± 0.17</td>
<td>0.45 ± 0.04 2.13 ± 0.95†</td>
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<td>9% CO₂</td>
<td>92.83 ± 35.0</td>
<td>0.72 ± 0.11†</td>
<td>66.4 ± 19.4†</td>
<td>0.31 ± 0.08</td>
<td>0.39 ± 0.16</td>
<td>0.44 ± 0.05 2.32 ± 1.05†</td>
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<td>Almitrine (n = 9)</td>
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<tr>
<td>Air</td>
<td>106.7 ± 27.6*</td>
<td>0.45 ± 0.12</td>
<td>46.7 ± 13.6*</td>
<td>0.28 ± 0.06*</td>
<td>0.31 ± 0.07*</td>
<td>0.47 ± 0.06 1.63 ± 0.47*</td>
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<tr>
<td>10% CO₂</td>
<td>129.5 ± 22.8</td>
<td>0.54 ± 0.23</td>
<td>68.5 ± 28.6*</td>
<td>0.21 ± 0.05</td>
<td>0.27 ± 0.05</td>
<td>0.43 ± 0.06 2.57 ± 0.74*</td>
</tr>
<tr>
<td>3% CO₂</td>
<td>110.2 ± 32.8</td>
<td>0.55 ± 0.14</td>
<td>64.3 ± 20.3*</td>
<td>0.29 ± 0.10</td>
<td>0.32 ± 0.14</td>
<td>0.48 ± 0.06 1.90 ± 0.66</td>
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<tr>
<td>6% CO₂</td>
<td>101.2 ± 29.9</td>
<td>0.68 ± 0.26‡</td>
<td>85.8 ± 26.13‡</td>
<td>0.28 ± 0.07</td>
<td>0.38 ± 0.21</td>
<td>0.44 ± 0.08 2.86 ± 0.73</td>
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<tr>
<td>9% CO₂</td>
<td>97.18 ± 27.3</td>
<td>0.76 ± 0.22</td>
<td>79.2 ± 23.5‡</td>
<td>0.29 ± 0.06</td>
<td>0.41 ± 0.19</td>
<td>0.44 ± 0.08 2.69 ± 0.83</td>
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Agrees with the finding that almitrine has no effect on the ventilatory response to hypoxia in anaesthetized rats [24], and is also consistent with the finding that there is no difference in the carotid chemosensory response to hypoxia after almitrine administration in anaesthetized cats [25]. On the other hand, several studies have reported increases in hypoxic sensitivity after almitrine administration in the cat [26], dog [9] and rabbit [4]. It has been suggested that the increase in hypoxic sensitivity is due to an almitrine-induced increase in carotid body CO₂ responsiveness [27]. Since an increased CO₂ stimulus augments carotid chemoreceptor responses to hypoxia [28], a similar augmentation of the hypoxic response may be obtained by raising the sensitivity of the chemoreceptors to CO₂ with almitrine. It is possible that almitrine excited peripheral chemoreceptor activity to the extent that the hypoxic stimulus was not capable of exerting its full effects. It cannot, however, be attributed to the mechanical limits of the ventilatory system, since hypercapnia caused further increases in ventilation. Since hypercapnia acts predominantly on central mechanisms and since hypoxia acts like almitrine via the peripheral chemoreceptors, the hypoxic stimulus may have been masked by almitrine.

The effects of almitrine on the ventilatory response to hypercapnia are controversial. Some investigators have reported increases in hypercapnic sensitivity after almitrine in anaesthetized animals.
[26] and man [29]. Others, however, have observed no change in the ventilatory response to hypercapnia after almitrine treatment [7]. In the present experiments, the ventilatory responses to 6 and 9% CO₂ were increased after almitrine treatment. Almitrine, by virtue of its specific effects, has been shown to be hypoxic-mimetic [9, 26]. In light of this, an increased ventilatory response to hypercapnia after almitrine might be expected, since a combined hypoxic–hypercapnic stimulus is more potent than a hypercapnic stimulus alone. Thus, the present results indicate that in the conscious, unrestrained, tracheostomized rat, almitrine administration has no effect on the hypoxic ventilatory response but enhances the ventilatory response to hypercapnia.

In SLN-sectioned animals, almitrine caused a significant increase in respiratory frequency (due to decreases in inspiratory and expiratory duration), minute ventilation and mean inspiratory flow, while breathing air. This pattern of change closely resembled that of SLN-intact animals. However, the tidal component of the ventilatory response to almitrine
was significantly different in SLN-denervated animals, resulting in a reduced ventilatory response to almitrine in the latter. This result is entirely consistent with a brief report of the effects of SLN denervation in tracheostomized, anaesthetized cats [30]. In that report, SLN section reduced the ventilatory response to almitrine owing to a reduction in the tidal component of the ventilatory response, with no change in the frequency component. The results of these studies indicate that the sensory pathway from the larynx affects the tidal component of the respiratory response to almitrine, either from responses in SLN paraganglia or from responses in laryngeal afferents secondary to the hyperpnoea. Since the larynx was bypassed, the latter possibility is unlikely, although it is possible that the activity of receptors sensitive to contraction of laryngeal muscles was increased. The timing component of the ventilatory response to almitrine depends entirely on almitrine's well described peripheral chemoreceptor action. In contrast, however, it has been reported that the ventilatory response to almitrine is not dependent on laryngeal afferents in anaesthetized, tracheostomized cats [31]. Interestingly, however, in this latter study, responses to almitrine were lower in SLN-denervated animals 1 min and 10 min after administration, but no significant difference was observed after 20 min.

There were no significant differences in the ventilatory responses to hypoxia and hypercapnia after almitrine administration in SLN-denervated animals. Thus, the increased ventilatory response to CO₂ in SLN-intact animals was abolished after SLN section. This result suggests that the SLNs also play a role in the enhanced ventilatory response to CO₂ after almitrine administration. There was no significant difference in the ventilatory response to hypoxia after almitrine administration in SLN-denervated animals. Similar results were observed in intact animals.

In the present experiments, a single intravenous dose of almitrine caused a preferential increase in geniohyoid compared with diaphragm muscle activity in anaesthetized animals. Preferential excitation of upper airway compared with diaphragm muscle activity, in response to a variety of sensory stimuli, has been demonstrated in anaesthetized rabbits [14] and dogs [32] and in conscious cats [33]. Almitrine has been used successfully in the treatment of chronic obstructive lung disease [1], a number of hypventilation syndromes [2] and in preventing respiratory depression due to morphinomimetic analgesics [34]. It has been suggested that almitrine may be useful in the treatment of sleep apnoea syndromes with both central and/or obstructive components [12].

The calibre of the upper airway is largely dependent on the balance between the stabilizing and diluting force of the upper airway dilator muscles and the collapsing force produced by the inspiratory pump muscles [13]. Conditions such as obstructive apnoea would be favoured by an imbalance between diaphragm and upper airway dilator muscle activity. Few studies, however, have investigated the effects of almitrine on the upper airway musculature. In agreement with the present study, a dose-dependent increase in phrenic and hypoglossal nerve activity has been reported in response to almitrine in anaesthetized, paralyzed, vagotomized cats [35]. In that study, however, the magnitude of the response was similar for both nerves. In contrast, almitrine was shown to have no effect on phasic inspiratory geniohyoid activity in sleeping cats [36].

A drug that would preferentially increase the inspiratory activity of upper airway muscles compared with the inspiratory pressure-generating muscles could be beneficial in diseases involving pharyngeal airway collapse. It has been shown that in eight patients with the sleep apnoea syndrome given oral almitrine, there was no reduction in the number of

<table>
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<tr>
<th>Table 2. Effect of almitrine on ventilation in normoxia, hypoxia and hypercapnia in animals with cut SLN. Values are mean ± SD before (control) and after (almitrine) an intraperitoneal injection of almitrine (5mg/kg).</th>
<th>( T_\text{Ti} )</th>
<th>( T_\text{Ti/} )</th>
<th>( V_{E} )</th>
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<tr>
<td>Control (n=9)</td>
<td>( T_\text{Ti} )</td>
<td>( T_\text{Ti/} )</td>
<td>( V_{E} )</td>
<td>( V_{E} )</td>
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<tr>
<td>Air</td>
<td>( 91.70 ± 23.2 )</td>
<td>( 3.36 ± 7.01 )</td>
<td>( 0.32 ± 0.11 )</td>
<td>( 0.41 ± 0.11 )</td>
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<td>( 10% \text{ O}_2 )</td>
<td>( 106.7 ± 22.3 )</td>
<td>( 34.0 ± 10.2 )</td>
<td>( 0.24 ± 0.07 )</td>
<td>( 0.35 ± 0.09 )</td>
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<td>( 3% \text{ CO}_2 )</td>
<td>( 92.4 ± 35.8 )</td>
<td>( 38.6 ± 6.1 )</td>
<td>( 0.31 ± 0.08 )</td>
<td>( 0.37 ± 0.12 )</td>
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<tr>
<td>( 6% \text{ CO}_2 )</td>
<td>( 78.18 ± 27.3 )</td>
<td>( 46.4 ± 10.8 )</td>
<td>( 0.34 ± 0.07 )</td>
<td>( 0.51 ± 0.24 )</td>
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<td>( 9% \text{ CO}_2 )</td>
<td>( 85.62 ± 25.9 )</td>
<td>( 50.3 ± 17.5 )</td>
<td>( 0.33 ± 0.07 )</td>
<td>( 0.46 ± 0.16 )</td>
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<tr>
<td>Almitrine (n=9)</td>
<td>( 117.6 ± 34.0 )</td>
<td>( 39.4 ± 13.5 )</td>
<td>( 0.22 ± 0.06 )</td>
<td>( 0.31 ± 0.13 )</td>
</tr>
<tr>
<td>Air</td>
<td>( 133.4 ± 27.7 )</td>
<td>( 41.9 ± 13.7 )</td>
<td>( 0.19 ± 0.05 )</td>
<td>( 0.27 ± 0.05 )</td>
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<tr>
<td>( 3% \text{ CO}_2 )</td>
<td>( 126.3 ± 44.8 )</td>
<td>( 46.5 ± 19.3 )</td>
<td>( 0.25 ± 0.07 )</td>
<td>( 0.28 ± 0.10 )</td>
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<tr>
<td>( 6% \text{ CO}_2 )</td>
<td>( 103.1 ± 26.9 )</td>
<td>( 51.1 ± 13.2 )</td>
<td>( 0.28 ± 0.07 )</td>
<td>( 0.35 ± 0.11 )</td>
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<tr>
<td>( 9% \text{ CO}_2 )</td>
<td>( 90.51 ± 26.9 )</td>
<td>( 56.0 ± 12.6 )</td>
<td>( 0.29 ± 0.06 )</td>
<td>( 0.42 ± 0.16 )</td>
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apnoeic events per hour of sleep, but a diminution of mean duration of apnoea was observed for obstructive and mixed apnoeas [15].

The large preferential increase in geniohyoid (a representative upper airway dilator muscle) compared with diaphragm EMG activity in the present study suggests that almitrine could be effective in the treatment of clinical apnoeas with both central and obstructive components. The increase in geniohyoid activity might be expected to stabilize and dilate the upper airway and reduce airway resistance. It is well known that activation of upper airway muscles precedes that of the diaphragm and that this is also important for maintaining airway stability [37]. However, almitrine did not affect the amount of geniohyoid preactivation.

An important point to consider is that in the present study, animals were anaesthetized, tracheostomized and vagotomized. Each of these factors has profound effects on upper airway muscle activity and thus caution must be exercised in extrapolating this effect of almitrine to the sleeping, airway- and
vagus-intact state. Anaesthesia is known to depress upper airway muscle activity more than diaphragm activity [13]. As pointed out earlier, Weese-Mayer et al. [36] saw no effect of almitrine on genioglossal activity in sleeping cats with their upper airway and vagi intact. However, in the same experiments, it was demonstrated that vagotomy unmasks an excitatory effect of almitrine on genioglossus EMG activity in anaesthetized cats [36]. The authors concluded that it was the elimination of the inhibition by pulmonary stretch receptors on genioglossus activity due to vagotomy rather than the effects of anaesthesia which evoked the response. In preliminary experiments in the present work, we observed that almitrine also excited geniohyoid muscle activity in anaesthetized, vagus-intact animals. However, since the volume-related increase in vagal afferent activity is markedly reduced during the apnoea caused by airway occlusion, almitrine may have a greater role in restoring upper airway patency during airway occlusion.

In conclusion, the present results demonstrate that the ventilatory response to almitrine is partly mediated by SLN afferents and that almitrine preferentially excites upper airway compared with diaphragm muscle activity.

ACKNOWLEDGMENTS
This work was supported by the Wellcome Trust, Health Research Board (Ireland) and Royal College of Surgeons in Ireland. We thank Servier Laboratories Ltd for the gift of almitrine. K.D.O’H. was supported by a Health Research Board (Ireland) Student Scholarship and A.K.C. by a Royal College of Surgeons in Ireland Student Scholarship. We thank T. Dowling and J. Slattery for technical assistance.

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