Eur Respir J, 1996, 9, 2323–2327
DOI: 10.1183/09031936.96.09112323
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European Respiratory Journal
ISSN 0903 - 1936

Effect of upper airway cooling and CO_2 on diaphragm and geniohyoid muscle activity in the rat

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Effect of upper airway cooling and CO_2 on diaphragm and geniohyoid muscle activity in the rat. K.D. O'Halloran, A.K. Curran, A. Bradford. ©ERS Journals Ltd 1996.

ABSTRACT: Upper airway (UA) reflexes play an important role in regulating breathing and UA patency, but the effects of UA CO_2 and cooling on ventilation and UA muscle activity are controversial.

Diaphragm and geniohyoid electromyographic activities were recorded in anaesthetized rats, breathing spontaneously through a low-cervical tracheostomy. Warmed, humidified air containing 0 or 10% CO_2 and cooled, room humidity air were applied at constant flow to the UA through a high- cervical tracheostomy. Spontaneous tracheal airflow, UA airflow and temperature, blood pressure, and rectal temperature were recorded.

In all animals, the geniohyoid muscle had phasic inspiratory activity, which slightly preceded diaphragmatic activity. CO_2 had no effect on mean peak integrated diaphragmatic activity and variable effects on geniohyoid activity. The coefficients of variation of these activities were unaffected by CO_2. Similar results were obtained following bilateral mid-cervical vagotomy. Cool air decreased respiratory frequency (78±8%) (mean±SD % of control), peak inspiratory flow (78±5%) and diaphragmatic activity (77±4%), and increased geniohyoid activity (149±11%). Cutting the superior laryngeal nerves abolished these effects.

In conclusion, whilst moderate upper airway cooling inhibits breathing and excites geniohyoid muscle activity, upper airway carbon dioxide has minimal effect.
Eur Respir J., 1996, 9, 2323–2327.

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Keywords: Airway
CO_2
cool air
diaphragm
geniohyoid

Received: January 11 1996
Accepted after revision July 4 1996

Supported by the Wellcome Trust, Health Research Board (Ireland) and Royal College of Surgeons in Ireland. AKC was supported by a Royal College of Surgeons in Ireland Student Scholarship and KDO by a Health Research Board (Ireland) Student Scholarship.

Cooling of the upper airway (UA) stimulates laryngeal cold receptors [1] and inhibits laryngeal mechanoreceptors [2] running in the superior laryngeal nerve (SLN). These receptors are known to reflexly influence breathing and UA muscle activity, but the effects of cooling on UA muscle activity are controversial. In anaesthetized dogs, MATHEW *et al*. [3] found that laryngeal cooling (20–25°C) had no effect on posterior cricoarytenoid muscle activity but inhibited the muscle's response to UA occlusion, and this effect was abolished by SLN section. In anaesthetized cats, JAMMES *et al*. [4] reported that laryngeal cooling (8°C) inhibited laryngeal muscle activity (the identity of the muscle was not determined) but the effect of SLN section on this response was not examined. On the other hand, UKABAM *et al*. [5], in decerebrate, vagotomized cats, found that hypoglossal nerve activity was either excited or inhibited by laryngeal cooling (8–16°C) through a SLN-mediated reflex. The cause of these conflicting results may be due to whether the vagi were intact or cut, since vagal afferents greatly influence UA muscle activity [6], or else it may be related to the low and wide-ranging temperatures used. In anaesthetized rats, we have previously shown that more moderate cooling (25–30°C) of the UA causes a substantial fall in UA resistance, which is partly mediated through a SLN reflex [7]. The mechanism of this effect is unknown but we have speculated that it may be due to reflex activation of UA dilator muscles.

The application of CO_2 to the UA in cats has been shown to reflexly increase the activity of the genioglossus muscle, and it has been proposed that this effect functions in the control of UA patency [8] since the genioglossus is an important dilator of the UA. The geniohyoid (GH) muscle is also a major UA dilator [9], but the effects of UA CO_2 on this muscle have not been studied. UA CO_2 has also been shown to inhibit breathing in anaesthetized cats with intact vagi [8, 10]. In decerebrate cats, however, this effect is only obtained following vagotomy [11], and we have previously reported that UA CO_2 had no effect on breathing in anaesthetized rats with intact vagi [7].

Therefore, in the present experiments, we examine the effects of moderate cooling (29°C) of the UA and of CO_2 applied to the UA lumen on ventilation and on the activity of the GH muscle in intact and vagotomised, anaesthetized rats.

Material and methods

Experiments were performed on 20 male Wistar rats (500–600 g body weight) anaesthetized with 100 $mg \cdot kg^{-1}$ of alpha-chloralose and 1 $g \cdot kg^{-1}$ of 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. Atropine sulphate (0.5 $mg \cdot kg^{-1}$) was injected subcutaneously to reduce airway secretions. A jugular vein was

cannulated to administer supplemental anaesthetic as required. A common carotid artery was cannulated to record arterial blood pressure.

Animals breathed room air spontaneously through a cannula inserted into a low-cervical tracheostomy with the aid of a binocular microscope. Spontaneous tracheal airflow was recorded using a heated pneumotachograph and a differential pressure transducer placed in series with this cannula. A second cannula was inserted through a high-cervical tracheostomy and pushed cranially to just below the level of the cricoid cartilage. Care was taken to avoid damaging the recurrent laryngeal nerves and SLN. The SLN and vagus nerves were marked with threads for later section. A second heated pneumotachograph and differential pressure transducer were placed in series with the UA cannula to record UA airflow.

To record diaphragm (DIA) and GH electromyograms (EMG), bipolar, copper wire electrodes were placed into the GH muscle and into the DIA, the latter through an abdominal incision which was then closed. Electrode locations were verified at the end of each experiment. EMG signals were amplified, filtered and integrated. Raw and integrated EMG, together with spontaneous tracheal airflow, UA airflow and temperature, and arterial blood pressure signals were digitized and recorded on a microcomputer.

Warmed (37.4±0.2°C), saturated air containing 0 and 10% CO_2 and cooled (29.3±0.8°C), room humidity (70–80%) air were delivered at a constant flow of 10–20 $mL \cdot s^{-1} \cdot kg^{-1}$ (as measured from the pneumotachograph placed in series with the UA cannula) to the UA through the UA cannula, in an expiratory direction to exit through the nose and mouth (nose open) or through the mouth only, the nose having been sealed (nose sealed).

Experimental protocol

With the nose open, the effects of CO_2 were studied by switching from warm air to the test airflow and then back to the warm air. The nose was then sealed and trials with CO_2 and cool air carried out by again switching from warm air to the test airflow and then back to the warm air. Cool air was not applied with the nose open because we have previously shown in anaesthetized guinea-pigs that the ventilatory effects of UA cooling are the same with the nose open or closed [12], presumably because of the likelihood that cool air would be warmed and humidified by the time it reached the nasal cavity. A bilateral, mid-cervical vagotomy was performed and trials repeated. Finally, the SLN were cut bilaterally and trials repeated. In some experiments, only the effects of CO_2 or cool air were tested. Test airflows were applied for 30–60 s, and at least 3 min was allowed between trials for complete recovery.

Data analysis

Phasic inspiratory DIA and GH integrated EMG activity was quantified as the height of the peak integrated signal in arbitrary units. The variability of EMG activity was also quantified by calculating the coefficient of variation as (standard deviation/mean) × 100%. Mean peak integrated EMG activity, respiratory frequency, inspiratory and expiratory duration (measured from the tracheal airflow record) and peak inspiratory flow were calculated during warm airflow for the last 20–30 breaths before switching to test gases, for 20–30 breaths during the tests when effects were maximal, and for 20–30 breaths following complete recovery after warm air was reintroduced. All values are expressed as percentage change with respect to warm air controls ±SD. Responses to cool air and to warm air containing 10% CO_2 were compared to pretrial warm air controls using Student's t-test with a p- value of less than 0.05 considered significant.

Results

The effect of CO_2 on ventilation and EMG activity

In all animals, the GH muscle had phasic inspiratory activity which slightly preceded that of the DIA. With the nose open and vagi intact, the mean coefficients of variation for peak DIA and GH activity were 7.3 and 15.9%, respectively (fig. 1a). With the nose open or

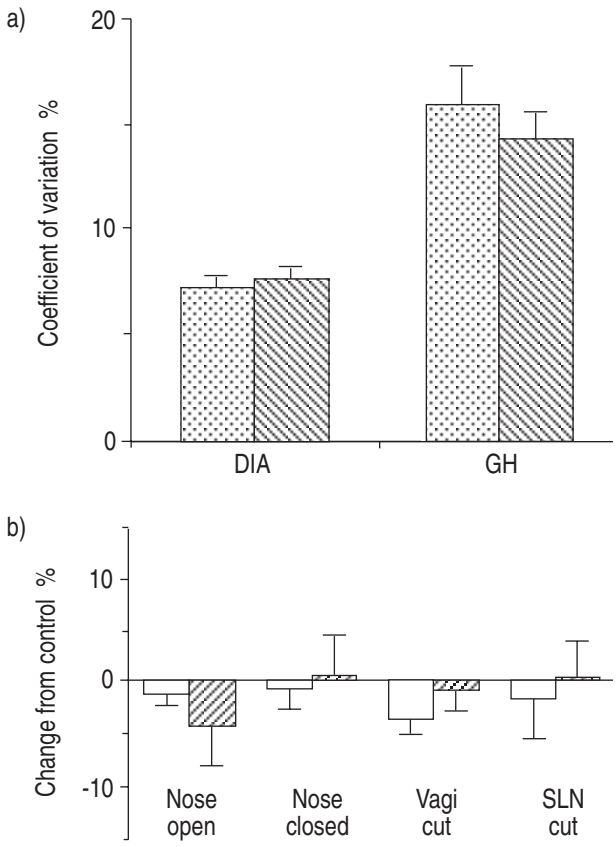

Fig. 1. – Effects of upper airway CO_2 on diaphragm (DIA) and geniohyoid (GH) muscle activity. a) coefficients of variation (expressed as a percentage ±SD) before (▭) and during (▬) CO_2 application with the nose open and vagi and superior laryngeal nerves (SLN) intact. b) Values (expressed as percentage change from air control ±SD) for peak integrated DIA activity (▭) and GH activity (▬).

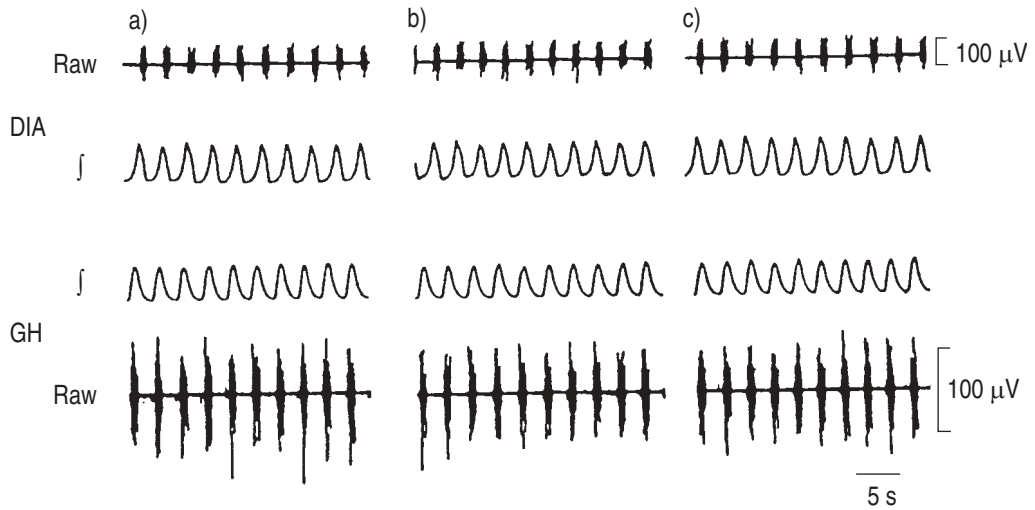

Fig. 2. – Simultaneous recordings (from top to bottom) of raw and integrated (∫) diaphragmatic (DIA) activity and integrated (∫) and raw geniohyoid (GH) activity: a) before; b) during; and c) after the application of 10% CO_2 to the upper airway with the nose open and the vagi sectioned.

closed, the addition of 10% CO_2 to the warm airflow had no effect on respiratory frequency, peak inspiratory flow and peak integrated DIA EMG activity; and had variable effects on peak integrated GH EMG activity, which when combined for all animals (55 trials in 15 animals) resulted in no significant change from warm air control (fig. 1b). The coefficients of variation for both muscle activities were also unaffected by CO_2 (fig. 1a). These results persisted following bilateral mid-cervical vagotomy and SLN section. Vagotomy itself had no effect on the coefficient of variation of DIA and GH activity (7.6 and 13.5%, respectively, following vagotomy) but caused typical decreases in respiratory frequency and increases in peak inspiratory flow and DIA and GH EMG activity. Figure 2 shows an example of raw and integrated DIA and GH EMG activity before, during and after application of CO_2 to the UA.

The effect of cool air on ventilation and EMG activity

Cooling of the UA had no effect on rectal temperature. With the nose sealed and vagi intact, reducing UA luminal temperature significantly decreased peak inspiratory flow and respiratory frequency (table 1). The effect on frequency was due to an increase in expiratory duration without any change in inspiratory duration. Cooling decreased mean peak integrated DIA EMG activity (15 out of 20 trials in 12 animals) but increased mean peak integrated GH EMG activity (17 out of the same 20 trials in the same 12 animals) (table 1). An example of the effects of cooling on DIA and GH EMG activity can be seen in figure 3. These responses were unaffected by bilateral mid-cervical vagotomy but were abolished following SLN section (table 1).

Discussion

The main findings of this study are that CO_2 in the UA has no effect on DIA muscle activity and variable effects on GH activity in anaesthetized rats, and that cool air in the UA inhibits DIA but excites GH activity. The effects of cooling were abolished by SLN section.

UA CO_2 has been shown to inhibit breathing in anaesthetized cats [8, 10, 11], conscious dogs [13], birds [14], amphibians and reptiles [15]. UA CO_2 may also inhibit breathing in preterm infants [16], although we have observed tachypnoea in neonatal guinea-pigs [12]. In contrast, UA CO_2 has been reported to have no effect on breathing in conscious ponies [17], and in anaesthetized dogs [18] and rabbits [14]. The lack of effect of UA CO_2 on DIA activity or on respiratory frequency or peak inspiratory flow in the rat in the present experiments confirms our previous findings that UA CO_2 does not affect spontaneous tracheal airflow [7]. Furthermore, this absence of an effect was not influenced by vagotomy, which has been shown to unmask the inhibitory response in the cat [11]. An artifactual insensitivity of the preparation cannot explain the absence of a CO_2 effect, since cool air evoked consistent reflex effects in the same preparation. Although laryngeal CO_2- sensitive receptors have been demonstrated in cats

Table 1. – Effect of cool air on ventilation and EMG activity

	f_R	t_I	t_E	$V'_{I,peak}$	∫DIA	∫GH
SLN intact (n=12) %	78±8*	104±5	190±28*	78±5*	77±4*	149±11*
SLN cut (n=9) %	96±2†	99±2	105±4†	93±4†	97±4†	104±5†

Values are expressed as mean±SD percentage of control for respiratory frequency (f_R), inspiratory duration (t_I), expiratory duration (t_E), peak inspiratory flow ($V'_{I,peak}$) and mean peak integrated diaphragm (∫DIA) and geniohyoid (∫GH) electromyographic (EMG) activity in animals with the nose sealed before (SLN intact) and after (SLN cut) superior laryngeal nerve (SLN) section. n=number of animals; *: $p<0.05$, compared to control (Student's t-test); †: $p<0.05$, compared to SLN intact (Student's t-test).

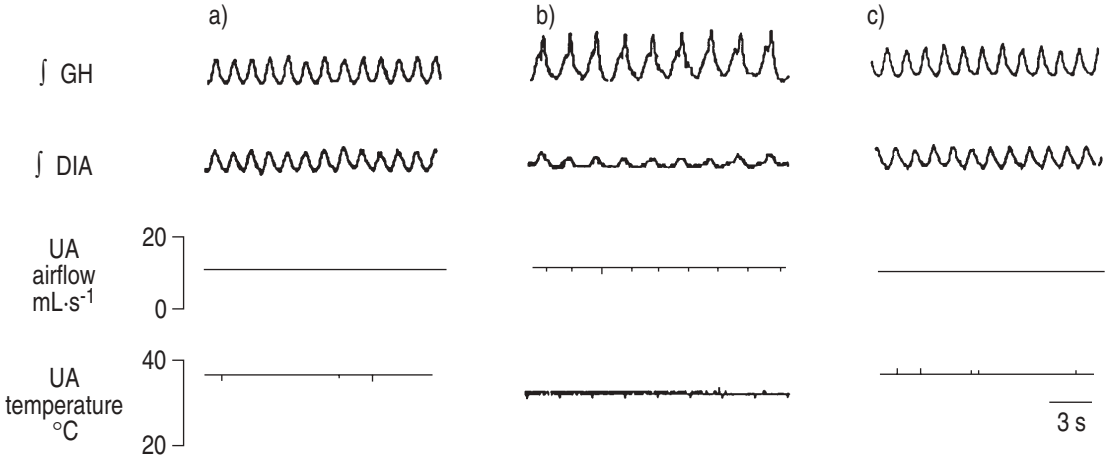

Fig. 3. – Simultaneous recordings of integrated (∫) geniohyoid (GH) and diaphragm (DIA) activity, upper airway (UA) airflow and UA temperature: a) before; b) during; and c) after the application of cool air to the UA with the nose closed and the vagi and superior laryngeal nerves intact.

[19, 20] and dogs [21], the responses of UA receptors to CO_2 have not been studied in the rat.

UA CO_2 has also been shown to excite UA muscle [8] and motor nerve activity [11] in cats, and this has been suggested to play a role in the regulation of UA patency [8]. We previously demonstrated [7] that supraglottic UA resistance was unaffected by CO_2 in rats but, in view of the responses reported in the cat, we suggested that CO_2 may still have reflexly excited UA muscles and stabilized the UA without actually dilating it. However, the present results show that CO_2 had no effect on the activity of the GH, an UA dilator. Furthermore, CO_2 had no effect on the coefficient of variation of either DIA or GH activity. These values are a measure of the breath-to-breath variability of muscle activity, and the present values were very similar to those reported for the same muscles in anaesthetized cats [22]. The present results show that peak GH activity has a much greater variability than that of DIA activity, a condition which may predispose to UA instability in unfavourable circumstances.

Cool air in the UA excites laryngeal cold receptors in dogs [1], cats [20], and rabbits [23]. Laryngeal cold receptors have not been studied in the rat, although nasal cold receptors have been demonstrated in this species [24].

UA cooling inhibits breathing in adult cats [5], guinea-pigs [25] and rats [7], and also in neonatal dogs [26] and guinea-pigs [12]. On the other hand, laryngeal cooling has no effect on breathing in adult dogs [3]; and JAMMES *et al*. [4] observed no significant changes in ventilation with laryngeal cooling in adult cats. BASNER *et al*. [27] reported that breathing was unaffected by UA cooling in adult humans. The present results show that respiratory frequency and peak inspiratory flow are decreased and that DIA activity is inhibited by moderate UA cooling in anaesthetized rats, and that these effects are abolished by SLN section. This is consistent with our previous findings in anaesthetized rats using the same techniques [7], which showed that breathing was inhibited through a SLN-dependent reflex. The difference in humidity between the warm and cool air in the present experiments is unlikely to have affected our results since the difference was slight, although it may have produced a small amount of additional airway cooling. In the previous study, we also demonstrated that cool air caused a marked reduction in UA resistance, which was attenuated by SLN section. We proposed that the SLN reflex component of this fall in resistance was due to a reflex effect on UA muscle activity. The present finding that cool air excites GH activity supports this proposal, especially since SLN section abolished the effect. Since the GH muscle is an UA dilator, we conclude that part of the fall in resistance caused by UA cooling is due to a SLN-mediated reflex excitation of UA dilator muscles. The inhibition of breathing caused by cool air would have raised arterial carbon dioxide tension (P_{a,CO_2}) and decreased arterial oxygen tension (P_{a,O_2}), conditions known to excite UA muscle activity [28]. However, the excitation of the GH muscle is probably not secondary to the inhibition of breathing, because it was rapid in onset and was observed in some cases when the breathing was unaffected.

In conclusion, these results show that moderate upper airway cooling depresses diaphragmatic activity and increases geniohyoid muscle activity through a superior laryngeal nerve reflex in anaesthetized rats. In contrast, airway carbon dioxide has no effect on ventilation and variable effects on upper airway muscle activity.

Acknowledgements: The authors wish to thank T. Dowling and J. Slattery for technical assistance and P. Balmer for secretarial assistance.

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