Effects of upper airway carbon dioxide on upper airway resistance and muscle activity in young guinea-pigs

A.K. Curran, K.D. O’Halloran, A. Bradford


ABSTRACT: The upper airway (UA) of adult animals is known to contain carbon dioxide-sensitive receptors and UA CO₂ reflexly affects breathing, UA dilator muscle activity and UA resistance. These effects may function in the control of UA patency. There is evidence that some UA reflexes are stronger in young than in adult animals, but it is not known whether CO₂-sensitive receptors are present in the UA of young animals, and the effects of UA CO₂ on UA resistance and on UA dilator muscle activity have not been investigated in young animals.

The responses of ventilation, UA resistance and geniohyoid muscle electromyo-graphic activity to warm air containing 10% CO₂ applied to the isolated UA were measured in anaesthetized, vagotomized young guinea-pigs breathing spontaneously through a low-cervical tracheostomy.

Upper airway carbon dioxide caused an increase in ventilation (46.7±16.3 to 49.9±16.8 mL·min⁻¹·100 g body weight⁻¹) and upper airway resistance (56.8±14.8 to 63.7±17.7 cmH₂O·L⁻¹·s⁻¹·kg body weight⁻¹). Similar effects were obtained following vagotomy. Geniohyoid activity became apparent following vagotomy and this activity was reduced by upper airway carbon dioxide. These responses were abolished by topical anaesthesia of the upper airway. This suggests that the reflexes seen are due to carbon dioxide-sensitive receptors in the upper airway.


A variety of carbon dioxide-sensitive receptors have been identified in the upper airway (UA) of adult cats [1] and dogs [2], and introducing CO₂ into the isolated UA in adult animals causes a number of reflex effects such as a reduction in ventilation [3, 4], an increase in UA muscle activity [3, 4] and an increase in laryngeal resistance [5]. These effects have been suggested to play a role in the regulation of UA patency [3, 5].

It is not known whether the UA contains CO₂-sensitive receptors in young animals, although it has been shown previously that passing CO₂ into the isolated UA causes tachypnoea in young guinea-pigs [6]. There is evidence that some UA reflexes are stronger in young animals compared to adults [7–9], suggesting that such reflexes may play a more important role in regulating UA patency in the young. The importance of studying such reflexes in the young is also relevant to their greater vulnerability to UA collapse and to obstructive sleep apnoea in infants and possibly sudden infant death [10]. However, the effects of UA CO₂ on UA resistance or on UA muscle activity have not been investigated in young animals.

Therefore, the present study was undertaken in order to test the hypothesis that applying CO₂ to the isolated UA affects UA resistance and UA dilator muscle activity in young guinea-pigs. Guinea-pigs are more mature when young compared to many species [11]. However, UA reflexes are very different in 10–14-day-old compared to adult guinea-pigs [12] and there are also maturational differences in local airway responses between 15-day-old and adult guinea-pigs [13].

Materials and methods

The methods used for UA isolation and application of UA airflow have been described previously [6, 14]. Briefly, 15 young guinea-pigs (aged 10–16 days; body mass 150–250 g) were anaesthetized with urethane (2 g·kg body weight⁻¹ i.p.) and placed supine on a thermostatically controlled heating blanket to maintain body temperature at 37 °C. A cannula was inserted into a low-cervical tracheostomy through which the animal breathed spontaneously. Tracheal airflow was continuously recorded using a heated pneumotachograph (Hans Rudolph, KS, USA) and a differential pressure transducer (Validyne DP 15, CA, USA) attached to this cannula, and the signal was integrated to give tidal volume. A common carotid artery was cannulated and connected to a pressure transducer (Validyne DP 215) to measure systemic arterial blood pressure. This procedure interferes with the blood flow to the ipsilateral carotid body and sinus. However, blood pressure remained stable throughout the experiments. A jugular vein was cannulated to administer supplemental doses of anaesthetic as required.

A cannula was inserted into a high-cervical tracheostomy, and was pushed cranially to lie just caudal to the level of the cricoid cartilage. A high-pressure source produced a steady flow of 5–10 mL·s⁻¹ of warmed humidified air or warmed humidified air containing 10% CO₂ which was delivered to the UA in an expiratory direction and passed over the larynx and pharynx to exit through the nose and mouth. Although the UA normally receives airflow of
alternating inspiratory and expiratory flow, the technique of continuous expiratory flow has been used extensively to study UA receptor and reflex function [2, 4–7, 9, 14–17]. In order to maximize any responses obtained 10% CO2 was used since the receptor and reflex responses to UA CO2 have been shown to be greater with 10% CO2 than with the more physiological level of 5% CO2 [4, 16, 17]. UA airflow was recorded using a pneumotachograph (Hans Rudolph) in series with the high-cervical tracheostomy cannula and connected to a pressure transducer (Validyne DP 15). The temperature of the UA airflow was monitored using a thermocouple microprobe (Physitemp, Gardens City, UK). The signal was band-pass filtered, rectified and amplified (Neurolog NL 104) before being integrated using a leaky integrator with a time constant of 50 ms (Neurolog NL 703). All signals were digitized and recorded using a commercial data acquisition system and stored for later analysis on a microcomputer.

Protocol

In all 15 animals, with the vagus nerves intact, variables were recorded continuously during application of warmed humidified air. Trials were performed by switching to warmed humidified air containing 10% CO2 which was applied for 30–60 s before switching back to air alone. The vagus nerves were cut at the mid-cervical level and the 10% CO2 trial repeated. Following this, in nine of the 15 animals, a solution of 2% Xylocaine was applied to the entire UA for 10 min, and the 10% CO2 trial repeated.

Data analysis

The values for respiratory variables, UA resistance and geniohyoid muscle electromyographic activity were averaged from ten control breaths prior to the application of 10% CO2 to the UA, from ten breaths during the trial period when the effect of the 10% CO2 was greatest and from ten breaths following the removal of the CO2. Since there was a slight fall in UA resistance during inspiration and an increase during expiration, UA resistance was calculated as the mean of inspiratory plus expiratory resistance. Phasic geniohyoid integrated electromyographic activity was quantified as the height of the peak integrated signal from end-expiratory level in arbitrary units. The breath-to-breath variability of geniohyoid muscle activity was also quantified as the coefficient of variation, i.e. SD/mean expressed as a percentage. This was carried out because geniohyoid electromyographic activity has been demonstrated to show marked breath-to-breath variability in adult cats [15] and cats [18]. Data are expressed as absolute values or as mean ± SD percentage change with respect to control. The data was analysed for statistical significance using analysis of variance and Fisher’s least significant difference test, with a p-value of <0.05 being taken as significant.

Results

With the vagus nerves intact, there was no phasic electromyographic activity in the geniohyoid muscle. Switching the flow through the UA from air to air containing 10% CO2 had no effect on UA airflow or temperature or on arterial blood pressure. The effects of UA CO2 on ventilation and UA resistance are shown in table 1. UA CO2 caused a significant increase in respiratory frequency (due to shortening of the expiratory time), minute ventilation and UA resistance.

Following section of the vagus nerves, phasic inspiratory geniohyoid electromyographic activity became apparent. The effects of UA CO2 following vagotomy were similar to those before vagotomy, i.e. there was an increase in respiratory frequency, minute ventilation and UA resistance (table 1, fig. 1). In addition, UA CO2 caused a decrease in peak integrated inspiratory geniohyoid electromyographic activity (fig. 1). This reduction in the activity of the geniohyoid, a UA dilator muscle, may have been associated with the increase in UA resistance. However, it was not possible to calculate the correlation of geniohyoid activity and UA resistance because the variances of the two sets of data were unequal. The increase in respiratory frequency, minute ventilation and UA resistance and the decrease in peak integrated inspiratory geniohyoid electromyographic activity were abolished by topical anaesthesia of the UA. The coefficient of variation of peak integrated geniohyoid electromyographic activity was unaffected by UA CO2 or by UA anaesthesia. Thus the coefficient was 20.4±7.4% in air and 20.5±7.1% with 10% CO2. Following UA anaesthesia, the coefficient was 21.6±3.9% in air and 22.7±9.2% with 10% CO2.

Discussion

The present results show that with intact vagus nerves applying 10% CO2 to the lumen of the UA causes an increase in respiratory frequency, minute ventilation and UA resistance. Following vagotomy, similar effects were obtained and phasic inspiratory geniohyoid muscle electromyographic activity became apparent. This activity was inhibited by UA CO2. The effects on ventilation confirm previous findings using the same preparation [14]. The absence of phasic geniohyoid muscle activity when the vagi were intact and its appearance when the vagi were cut also confirms previous observations [14], and is consistent with the fact that UA muscle activity is increased by vagotomy in adult animals [19, 20]. It is also consistent with the possibility that the Hering-Breuer inflation reflex has a greater inhibitory effect on UA muscle activity in young animals compared to adult.
animals since geniohyoid activity was completely absent in the presence of intact vagi in the present experiments.

Cutting the vagi had no effect on the ventilatory and UA resistance responses to UA CO2. Since vagotomy abolishes recurrent laryngeal afferent activity, this suggests that the responses are not due to afferents in the recurrent laryngeal nerves. The lack of effect of vagotomy on these responses may also suggest that Hering-Breuer inflation receptors have little effect on these reflexes. However, this interpretation must be viewed cautiously since vagotomy receptors have little effect on these reflexes. The effects of UA CO2 on UA resistance or muscle activity have not been investigated previously in young animals. UA CO2 caused an increase in UA resistance and a decrease in geniohyoid muscle activity. The coefficient of variation of geniohyoid muscle activity is a measure of its activity. Since the geniohyoid muscle is a UA dilator, an effect on the variability of this activity might have been anticipated. However, UA CO2 had no effect on the coefficient of variation of geniohyoid muscle activity. Since the geniohyoid muscle is a UA dilator, the increase in UA resistance may have been caused by the decrease in geniohyoid activity. A similar effect of UA CO2 on UA resistance in adult rats in which laryngeal but not supraglottic resistance was increased has been described previously. Furthermore, geniohyoid muscle activity is unaflected by UA CO2 in adult rats. Therefore, although the inhibition of geniohyoid activity may have contributed to the increase in UA resistance in the present experiments, other sites of resistance may have been involved since the overall resistance of the sub- and supraglottic airway was measured and since UA resistance was increased by UA CO2 in the absence of geniohyoid activity.

The inhibitory effect of UA CO2 on geniohyoid muscle activity is in contrast to the excitatory effect of CO2 on UA muscle and motor nerve activity observed in adult cats. The authors have previously suggested that this excitatory effect on a UA dilator may stabilize the UA since respiratory frequency is increased without increasing the collapsing pressure in the UA necessitated by an increase in tidal volume. This in turn might restore UA patency through reflex contraction of UA dilators. The presence of an opposite response of UA muscle activity to UA CO2 in young guinea-pigs may increase the vulnerability of the young to UA collapse.

Since UA CO2 reduced geniohyoid muscle activity, an effect on the variability of this activity might have been anticipated. However, UA CO2 had no effect on the coefficient of variation of geniohyoid muscle activity. Since the geniohyoid muscle is a UA dilator, the increase in UA resistance may have been caused by the decrease in geniohyoid activity. A similar effect of UA CO2 on UA resistance in adult rats in which laryngeal but not supraglottic resistance was increased has been described previously. Furthermore, geniohyoid muscle activity is unaffected by UA CO2 in adult rats. Therefore, although the inhibition of geniohyoid activity may have contributed to the increase in UA resistance in the present experiments, other sites of resistance may have been involved since the overall resistance of the sub- and supraglottic airway was measured and since UA resistance was increased by UA CO2 in the absence of geniohyoid activity.

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The absence of geniohyoid activity with intact vagi may suggest that this muscle and its response to UA CO2 is unimportant in the regulation of UA resistance and patency in intact animals. However, anaesthesia preferentially depresses UA muscle activity compared to the diaphragm. Furthermore, UA CO2 reduced geniohyoid activity following vagotomy, and such a response would be expected to occur during UA occlusion and apnoea. When volume-related vagal afferent activity would be greatly reduced.

### Table 1. Effect of upper airway carbon dioxide on ventilation and upper airway resistance

<table>
<thead>
<tr>
<th></th>
<th>VR breaths·min⁻¹</th>
<th>VT mL·100g⁻¹</th>
<th>VE mL·min⁻¹·100g⁻¹</th>
<th>h s</th>
<th>e s</th>
<th>RUA cmH2O·L⁻¹·s⁻¹·kg⁻¹</th>
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<tbody>
<tr>
<td>Vagus nerves intact</td>
<td></td>
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<tr>
<td>Control</td>
<td>35.1±21.1</td>
<td>1.1±0.4</td>
<td>46.7±16.3</td>
<td>0.7±0.4</td>
<td>1.9±1.4</td>
<td>56.8±14.8</td>
</tr>
<tr>
<td>10% CO₂</td>
<td>39.6±22.0*</td>
<td>1.1±0.4</td>
<td>49.9±16.8*</td>
<td>0.6±0.4</td>
<td>1.5±1.2*</td>
<td>63.7±17.7*</td>
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<tr>
<td>Vagi cut</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>21.3±10.1</td>
<td>1.6±0.7</td>
<td>40.8±14.6</td>
<td>1.0±0.5</td>
<td>2.4±1.2</td>
<td>84.8±42.0</td>
</tr>
<tr>
<td>10% CO₂</td>
<td>24.0±11.7*</td>
<td>1.5±0.6</td>
<td>43.8±15.4*</td>
<td>1.0±0.5</td>
<td>2.1±0.9*</td>
<td>93.6±42.2*</td>
</tr>
<tr>
<td>After Xylocaine</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>36.6±21.0</td>
<td>1.2±0.5</td>
<td>39.5±15.9</td>
<td>0.6±0.3</td>
<td>1.6±0.9</td>
<td>116.2±28.1</td>
</tr>
<tr>
<td>10% CO₂</td>
<td>36.3±20.7</td>
<td>1.2±0.4</td>
<td>39.3±15.8</td>
<td>0.5±0.2</td>
<td>1.6±0.9</td>
<td>117.5±25.9</td>
</tr>
</tbody>
</table>

Data are presented as mean±sd. All parameters were measured before (control) and during (10% CO₂) the application of 10% CO₂ to the isolated upper airway. VR: respiratory frequency; VT: tidal volume; VE: minute ventilation; h: inspiratory time; e: expiratory time; RUA: upper airway resistance. *: p<0.05 versus control.
All of these effects of UA CO₂ on ventilation, UA resistance and UA muscle activity were abolished by anaesthesia of the UA, suggesting that they are due to reflex effects from CO₂-sensitive receptors in the UA. A variety of UA CO₂-sensitive receptors have been identified in adult cats [1] and dogs [2], and UA CO₂ has been shown to exert reflex effects in adult rats [5] and cats [3, 4]. However, there is evidence that UA receptor function is immature in young animals [22] and it is not known whether UA CO₂-sensitive receptors are present in young animals. The present results are indirect evidence that such receptors are present in young guinea-pigs and that these receptors are functionally active.

It is not clear whether the effects on UA resistance and geniohyoid activity are specific reflex effects or whether they are secondary to the increase in ventilation. It is possible that the hypocapnia caused by the increase in ventilation may have reduced the drive to the UA dilator muscles including the geniohyoids, resulting in increased UA resistance.

To summarize, the present results show that upper airway carbon dioxide caused an increase in ventilation and upper airway resistance and a decrease in geniohyoid muscle activity in young guinea-pigs. All of these effects were abolished by topical anaesthesia of the upper airway, suggesting that they are reflex effects from carbon dioxide-sensitive upper airway receptors.

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**References**


