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Laryngeal reflex apnea in neonates: Effects of CO_2 and the complex influence of hypoxia

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ABSTRACT

We have examined influence of hypocapnia, mild hypercapnia and hypoxia on the durations of fictive apnea and respiratory disruption elicited by injection of 0.1 ml of water into the laryngeal lumen—the laryngeal chemoreflex (LCR)—in 20 unanesthetized, decerebrate, vagotomized piglets aged 4–10 days that were paralyzed and ventilated with a constant frequency and tidal volume. The LCR was enhanced by hypocapnia and attenuated by hypercapnia as reported by others. The responses to laryngeal stimulation during hypoxia were varied and complex: some animals showed abbreviated responses during the tachypnea of early hypoxia, followed after 10–15 min by more prolonged apnea and respiratory disruption accompanying the reduction in ventilatory activity that commonly occurs during sustained hypoxia in neonates. We speculate that this later hypoxic enhancement of the LCR may be due to accumulation of adenosine in the brain stem.

1. Introduction

The larynx is not only the principal organ of speech and song, but also an important valve that both regulates the flow of respiratory gases and protects the lower respiratory tract from aspiration of harmful materials (Bartlett, 1989; Thach, 2010). In accordance with the latter function, the laryngeal mucosa is richly endowed with sensory nerve endings (Sant'Ambrogio et al., 1985), which, when stimulated chemically or mechanically, give rise to rapid and profound protective responses (Sant'Ambrogio and Mathew, 1986). Most laryngeal afferents travel ipsilaterally in the superior laryngeal nerve (SLN) through the nodose ganglion and enter the brain stem in the nucleus of the solitary tract (NTS); there they are distributed via interneurons to higher brain centers and to autonomic and motor nuclei that reflexly influence laryngeal, cardiovascular and respiratory control systems (Miflin, 1993).

The reflex responses to laryngeal stimulation undergo a major developmental shift during early postnatal life (Thach, 2001). An intralaryngeal drop of water or many other fluids in adults generally elicits coughing and only a brief interruption of breathing. The same stimulus in neonates usually produces little or no coughing, but instead apnea, often accompanied by bradycardia (Lee et al., 1977), and these responses may last for many seconds, leading to dangerous levels of arterial and tissue hypoxia (Downing and Lee, 1975). The long suspicion that the LCR may play a role in some cases of the Sudden Infant Death Syndrome (SIDS) (Downing and Lee, 1975; Johnson et al., 1975) is reinforced by the observation that it may elicit fatal apnea in anesthetized animals (Richardson and Adams, 2005). Factors that influence the reflex in neonatal animals have therefore been examined fairly extensively.

To result in apnea, an inhibitory reflex must overcome the individual's underlying drive to breathe. Thus it is not surprising that when that drive is reduced by hypocapnia (Lawson, 1982) or general anesthesia (Lee et al., 1977; Richardson and Adams, 2005), laryngeal reflex apnea may be profound, long-lasting and occasionally fatal. At the other end of the "drive" scale, animals that have their breathing stimulated by hypercapnia (Litmanovitz et al., 1994; Sasaki et al., 2009), exercise (Haouzi et al., 1997) or aminophylline (Lee et al., 1977) are relatively resistant to reflex respiratory inhibition: laryngeal stimulation only results in a brief period of apnea before breathing resumes.

The respiratory consequences of hypoxia are more complicated, reflecting the enhancement of breathing by stimulation of the peripheral chemoreceptors (Kumar, 2009) and the depression of brain function by the direct action of hypoxia centrally (Bissonnette, 2000). A further complication is the time dependent, biphasic respiratory response to sustained hypoxia, which is particularly prominent in neonates, including human infants (Cohen et al., 1997). The initial increase in breathing induced at the onset of hypoxia is followed within seconds or a few minutes by a reduction in ventilation, sometimes to levels below the normoxic baseline. This ventilatory "roll-off" is due—at least in part—to the central accumulation of adenosine (Darnall and Bruce, 1987; Koos, 2011). A final complexity of the interaction of hypoxia and the LCR is that hypoxia becomes progressively severe during the course of an

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apneic period; by contrast, the arterial PCO_2 only rises to approach the value in mixed venous blood (Sasse et al., 1996). After one circulation time, the mixed venous value begins to rise, but only slowly because of the large capacity for CO_2 storage (Eger and Severinghaus, 1961).

Beyond the multiple ways in which acute hypoxia can influence breathing, as outlined above, recent observations indicate that neonatal animals that have been exposed to chronic intermittent hypoxia *in utero* or in early postnatal life have altered responses to respiratory challenges (Reeves and Gozal, 2005). This finding is of uncertain consequence for the LCR and is not addressed in this study, but the question is of potential importance with regard to SIDS as some SIDS infants are known to experience episodes of apnea, bradycardia and hypoxia days or weeks before their demise (Meny et al., 1994).

We have examined the influence of hypocapnia, mild hypercapnia and hypoxia on the LCR in neonatal animals under conditions that control or minimize the influence of some of the variables discussed above. Thus, to avoid the effects of general anesthesia, time-varying blood gases and changes in mechanical ventilation, we studied unanesthetized, decerebrate piglets that were paralyzed and artificially ventilated at a constant frequency and tidal volume with controlled gas mixtures. We monitored phrenic nerve activity, and the animals were vagotomized to avoid reflex effects of lung inflation by the ventilatory pump.

We examined the influence of hypocapnia and hypercapnia to test whether the findings reported previously (Lawson, 1982; Litmanovitz et al., 1994) were directly related to respiratory drive rather than to secondary reflex effects of changes in ventilation and to determine whether the effects of CO_2 on the LCR could be shown in our reduced preparation. These hypotheses were confirmed.

The hypoxia study was carried out to test the hypothesis that LCR-induced apnea would be shortened during the early hyperpnea of sustained hypoxia, but would become prolonged during the time-dependent reduction in ventilatory activity during continued hypoxia in a way that might be important for the pathogenesis of SIDS. This hypothesis was confirmed for some animals, but the response pattern for the group of piglets was too variable for this finding to be statistically significant.

2. Methods

We studied 20 piglets (8 male, 12 female), ranging from 4 to 10 days in age and from 1.8 to 3.3 kg in body weight. The Institutional Animal Care and Use Committee of Dartmouth College approved all procedures.

2.1. Surgical preparation

Each piglet was anesthetized with 2–4% halothane in O_2. Femoral arterial and venous catheters were placed, and arterial pressure was monitored with a transducer. Rectal temperature was maintained at $38 \pm 1\,^{\circ}C$. by means of a thermostatically controlled heating pad. A cannula was inserted through a low cervical tracheotomy, and the animal was ventilated with O_2 at a rate and depth to maintain the end-tidal CO_2 concentration, monitored by an infrared analyzer, at ~5%. After surgical exposure of the carotid sinus regions, the internal and external carotid arteries were ligated to reduce blood loss during decerebration. The animal was placed in the prone position, with the head secured in a stereotaxic head holder. The scalp was incised, the skull was opened, and the brain stem was transected at the level of the superior colliculi; arterial bleeders were clipped, and all brain tissue rostral to the section was removed by suction. After decerebration, halothane was discontinued, and the animal was paralyzed with pancuronium bromide 1 mg/kg iv, supplemented as needed. A phrenic nerve was isolated and sectioned low in the neck; the central cut end was desheathed and positioned under viscous paraffin on a bipolar wire recording electrode to monitor respiratory activity. The phrenic signal was filtered (0.03–10 kHz), amplified and integrated as a moving time average with a time constant of 100 ms. The integrated phrenic signal, rectal temperature, end-tidal CO_2 concentration and blood pressure were recorded on a laboratory computer for later analysis.

A pharyngeal catheter (PE-90) was inserted through a nostril and positioned with its tip at the rostral border of the larynx. The catheter was filled with water, of which 0.1 ml was injected into the laryngeal lumen during phrenic expiration each time the LCR was elicited. As the volume of the catheter exceeded that of the injectate, the water reaching the larynx was approximately at body temperature. Previous experiments indicate that the laryngeal receptors responding to water are not appreciably temperature sensitive in the range we studied (Xia et al., 2006). After these preparations, the animal was left undisturbed for a period of at least 30 min.

2.2. Experiments with hypercapnia and hypocapnia

In 11 piglets, the inspired gas mixture was changed to ~2% CO_2 in O_2, and the ventilatory pump was readjusted to return the end-tidal CO_2 concentration to ~5%. Once a stable state was reached (3–5 min), The LCR was tested 3 times, separated by at least 5 min. Then, without changing the respiratory frequency or tidal volume provided by the ventilator, enough CO_2 was removed from the inspired gas to lower the end-tidal CO_2 concentration to ~4%, and after 5 min another 3 tests of the LCR were performed. Finally, the end-tidal CO_2 concentration was raised to ~6% by adding CO_2 to the inspired mixture, and the testing of the LCR was repeated. These maneuvers enabled us to assess the influence of CO_2 on the LCR without any confounding changes in mechanical stimulation by the pump.

2.3. Experiments with hypoxia

Nineteen piglets (10 of which were previously used in the hypercapnia/hypocapnia experiments) were studied during acute, sustained exposure to either 10% or 12% O_2 in the inspired air for 10–20 min. The initial experiments were with 10% O_2, but as this was associated with high mortality, we changed to 12% O_2. Ventilation was maintained at the control rate, and no significant change occurred in end-tidal CO_2 concentration. The LCR was elicited 3 times at 5-min intervals before the hypoxic exposure and was tested at intervals during the hypoxic period.

2.4. Analysis

We evaluated the LCR responses based entirely on time-related events, as we have done before (Curran et al., 2005; Duy et al., 2010). We defined the duration of the LCR as the time from the beginning of water-induced phrenic apnea until the onset of five regular fictive breaths; these breaths did not have to be identical in frequency or amplitude to those occurring before the reflex was elicited, but had to occur at regular intervals (variation < 20%). We also measured the longest fictive apnea duration occurring with each LCR trial; this response measure was more conservative and less subjective than the duration of the LCR. In order to compare the responses of different animals studied under similar conditions, we expressed each response of each piglet as a percentage of the average baseline value for that piglet. The data were not normally distributed, and we employed non-parametric statistical testing (Wilcoxon signed rank test) with $P > 0.05$ as the criterion to assess the significance of response changes with conditions. Because we sometimes had to