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Hypercapnia attenuates inspiratory amplitude and expiratory time responsiveness to hypoxia in vagotomized and vagal-intact rats

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ABSTRACT

A negative influence of central chemosensitivity on peripheral chemoreflex response has been demonstrated recently in a decerebrate-vagotomized rat preparation in situ with separate carotid body and brainstem perfusions. Here, we report similar negative influences of hypercapnia on the hypoxic respiratory response in anesthetized, spontaneously breathing rats before and after vagotomy and anesthetized, artificially ventilated rats after vagotomy. Baseline breathing patterns and responsiveness to hypercapnia and hypoxia varied widely between the three respiratory modes. Despite this, the responses in inspiratory amplitude and expiratory duration (and hence respiratory frequency and neural ventilation) to hypoxia varied inversely with the background CO_2 level in all three groups. Results demonstrate a hypoadditive hypercapnic–hypoxic interaction in vivo that resembles the hypoadditive central–peripheral chemoreceptor interaction in situ for these respiratory variables in the rat, regardless of differences in vagal feedback, body temperature and ventilation method. These observations stand in contrast to previous reports of hyperadditive peripheral-central chemoreceptor interaction.

1. Introduction

Central and peripheral chemoreceptor influences on breathing are traditionally modeled as additive for simplicity although many extant data in human subjects and animal models suggest the possibility of not only additive (Clement et al., 1992, 1995; Heeringa et al., 1979; Mohan and Duffin, 1997; StCroix et al., 1996; Swanson and Bellville, 1974; van Beek et al., 1983) but also hyperadditive (Adams et al., 1978; Cunningham et al., 1986; da Silva et al., 2011; Honda et al., 1981; Loeschcke et al., 1963; Robbins, 1988; Roberts et al., 1995; Tenney and Brooks, 1966; Teppema et al., 2010) or even hypoadditive interaction (Adams and Severns, 1982; Berger et al., 1978; Cragg and Drysdale, 1983; Eldridge et al., 1981; Gesell et al., 1940; Giese et al., 1978; Ou et al., 1976; Smith et al., 1984). A major confound on this issue is that hypercapnia and hypoxia are often used as physiological stimuli to activate the central and peripheral (carotid) chemoreflex loops, respectively, whereas hypercapnia may stimulate both and hence its effect is nonspecific. Another difficulty is that hypercapnic and hypoxic stimuli may interact at multiple sites in the chemoreflex loops and the interaction could vary with stimulus intensities and timing at different sites. For example, hypercapnia and hypoxia are well-known to stimulate carotid chemoreceptors in a multiplicative (i.e., hyperadditive) fashion in adult rats (Kumar, 2009; Pepper et al., 1995; Roy et al., 2000) as in cats (Fitzgerald and Parks, 1971; Lahiri and DeLaney, 1975) but the corresponding central interaction effects are unclear. It has been suggested that hypoxic and hypercapnic stimuli at sufficiently high intensities may cause saturation of neuronal circuits (Eldridge et al., 1981) or exert depressant effects on neuronal activity (Cherniack et al., 1970) especially when presented in combination. Hypoxia may also occasion multiple physiological changes such as decreased whole-body metabolism (Frappell et al., 1992), increased or decreased systemic arterial blood pressure (Kontos et al., 1965; Song and Poon, 2009b; Song et al., 2011) and improved pulmonary ventilation-perfusion matching (Alfaro et al., 2001), all of which may secondarily affect breathing and perturb the interaction of central and peripheral chemoreflexes in a complex manner.

Recently, several authors have tackled this problem by surgically isolating the central from the peripheral chemoreceptor contributions to the interaction. Day and Wilson (2009) using an in situ dual (brainstem and carotid chemoreceptor) perfused preparation in vagotomized-decerebrate rats showed that neural minute ventilation was more responsive to peripheral chemoreflex the lower the brainstem P_{CO_2}. This was achieved even when the latter was kept at hypocapnic levels, thus ruling out possible neuronal saturation or depressant effects that might confound the interaction at high CO_2 levels. They interpreted their findings

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as indicative of a hypoadditive (negative) interaction between central and peripheral chemosensitivity in modulating the peripheral chemoreflex. In contrast, Blain et al. (2010) employing a carotid body perfusion preparation in unanesthetized vagal-intact dogs reported that carotid body stimulation augmented central hypercapnic chemoreflex responses in both respiratory frequency and tidal volume, whereas carotid body inhibition (disfacilitation) produced the opposite effects. They concluded that peripheral chemoreceptor stimulation resulted in hyperadditive (synergistic) ventilatory responses to central hypercapnia. Both studies were designed to obviate possible confounds in previous studies of hypercapnic and hypoxic chemoreflex interaction, yet divergent outcomes emerged. A possible explanation of this surprising discrepancy is that the interaction of central and peripheral chemoreceptors may be non-commutative: the influence of central chemosensitivity on peripheral chemoreflex (central–peripheral chemoreflex interaction) (Day and Wilson, 2009) may differ from that of peripheral chemosensitivity on central chemoreflex (peripheral–central chemoreflex interaction) (Blain et al., 2010) depending on the sites of interaction. Another possibility is that the hypoadditive interaction that exists in a highly reduced preparation in rats (Day and Wilson, 2009) may morph into a hyperadditive one in the presence of some unknown factors only found in a more intact preparation or in dogs (Blain et al., 2010).

Peculiar to the in situ artificially perfused rat preparation is the relatively low respiratory frequency due to vagotomy and relatively low perfusion temperature (33–34 °C). Since the resulting hypoadditive central–peripheral chemoreflex interaction was reportedly confined to the response in respiratory frequency mainly owing to impaired peripheral chemoreflex responsiveness in expiratory duration (T_E) at higher brainstem P_{CO_2} levels, it is germane to inquire whether impaired vagal and temperature modulation of T_E and respiratory frequency might be the culprit behind the negative interaction. This is of interest since central and peripheral chemosensitivities are dependent on vagal feedback (Kiwull-Schone and Kiwull, 1979; Moreira et al., 2007; Richardson and Widdicombe, 1969; Tonkovic-Capin et al., 2000) and body temperature (Baker et al., 1996; Cummings and Frappell, 2009; Cunningham and O'Riordan, 1957; Kiley et al., 1984; Watanabe et al., 1996). Further, recent studies have shown that the T_E responses to acute hypoxia and hypercapnia are specifically modulated by distinct regions of the pneumotaxic center in dorsolateral pons, where central and peripheral chemoreceptor inputs are likely integrated along with vagal inputs (Ezure, 2004; Kubin et al., 2006; Mizusawa et al., 1995; Song and Poon, 2009a,b; Song et al., 2011; St John, 1975). Elucidation of the contribution of T_E responsiveness to hypercapnic–hypoxic interaction in vagotomized and vagal-intact animals may shed light on the mechanism of such interaction. However, to our knowledge there has been no study that examines the effects of vagal feedback and body temperature on central and peripheral chemoreflex interaction and the contribution of impaired T_E responsiveness to such interaction. Another interesting question is whether such interactions at the chemoreceptor level can be correlated to interactions of hypercapnic and hypoxic chemoreflex responses in animals and humans. This is a pertinent question because the ventilatory responses to hypoxia and hypercapnia are widely studied in animal models and in healthy subjects and patients, in whom artificial brainstem or carotid body perfusion is impracticable.

In the present study, we have therefore re-examined the influences of both hyper- and hypocapnia on the hypoxic respiratory chemoreflex response in both vagotomized and vagal-intact anesthetized but spontaneously breathing rats as well as vagotomized, anesthetized-paralyzed and artificially ventilated rats, all at normal body temperature. To allow direct comparison with Day and Wilson (2009) and to examine (i) how nonspecific central vs. peripheral effects of the hypercapnic stimulus might influence hypercapnic–hypoxic interaction and (ii) whether any saturation/depressant effects at high stimulation intensities might contribute to such interaction, we employed a hypercapnic and hypoxic stimulation and hypocapnic disfacilitation protocol that paralleled the brainstem and carotid chemoreceptor stimulation and disfacilitation sequence in Day and Wilson's study. We found that despite the widely different resting breathing patterns and responsiveness to hypoxia and hypercapnia in these preparations due to differences in vagal feedback and ventilation method, a hypoadditive influence of hypercapnia on hypoxic chemoreflex responses in T_E and respiratory frequency (hypercapnic–hypoxic interaction) prevailed in all cases. In addition, we found that such hypoadditive interaction also applied to the inspiratory amplitude (neural tidal volume) component. Our results are in agreement with Day and Wilson (2009) and run counter to Blain et al. (2010).

2. Methods

2.1. Animal preparation

Experiments were performed on 10 adult, male Sprague–Dawley rats (330–380 g, Charles River Laboratories, Wilmington, MA). Experimental protocols were reviewed and approved by the M.I.T. Committee on Animal Care in accordance with published guidelines. Animals were injected with atropine sulfate (0.025 mg, s.c.), then anesthetized with urethane (Sigma, 1.5 g/kg, i.p.). Trachea was cannulated. Lactated Ringer's solution was continuously infused (0.05–0.1 ml/min) through a femoral vein cannula. A femoral artery was cannulated and connected to a blood pressure monitor (BP-100, CWE, Ardmore, PA). Supplemental urethane was given as needed (1/10 original dosage, i.v. or i.p.). Body temperature was maintained at 36.5 ± 0.2 °C (TC-831, CWE, Ardmore, PA).

One group of animals ($n = 5$) breathed O_2-enriched (40%) medical air spontaneously. A separate group of animals ($n = 5$) were paralyzed with pancuronium bromide (Sigma, initial dose 0.5 mg, i.v., supplemented every hour at 0.1 mg, i.v.), vagotomized and artificially ventilated with the same gas using a mechanical ventilator (AVS-1, CWE, Ardmore, PA). The O_2 and CO_2 concentrations of the respired gas were monitored with a Gemini Respiratory Gas Analyzer (CWE, Ardmore, PA). Spontaneously breathing animals were studied before and after bilateral vagotomy. Ventilated animals were studied after bivagotomy only.

2.2. Electrophysiological recording

To monitor respiratory motor output in spontaneously breathing animals, diaphragmatic EMG was recorded by implanting two fine silver wires into the diaphragm. In ventilated animals, the phrenic nerve was isolated and mounted on a bipolar platinum wire electrode (FHC, Bowdoin, ME). The phrenic discharge or diaphragmatic EMG signal was amplified (CyberAmp 380, Axon Instruments, Union City), integrated with an analog Paynter filter (time constant 50 or 100 ms) and sampled (at 10 kHz) into a Dell PC with LabView (National Instruments, Austin, TX).

2.3. Hypoxia tests

Fig. 1 illustrates the experimental protocols for the hypoxia test under varying CO_2 backgrounds. In spontaneously breathing animals, the hypoxia was presented under either normocapnic or hypercapnic background. For normocapnic hypoxia, the animal was given 8% O_2 (balance N_2) for 30 s. For hypercapnic hypoxia, the animal was given 5% CO_2 (balance O_2) for 4–5 min and then 5% CO_2 and