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Lateral parabrachial nucleus mediates shortening of expiration and increase of inspiratory drive during hypercapnia

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Abstract

We have previously shown that unilateral or bilateral lesions of the lateral parabrachial nucleus (LPBN) in anesthetized, vagotomized rats markedly and selectively attenuate the shortening of expiratory duration (T_E) during hypoxia without appreciably affecting all other hypoxic response components (Song and Poon, *Respir. Physiol. Neurobiol.*, 165(1):1-8, 2009). Here, we report that unilateral LPBN lesion by kainic acid in the same group of animals not only abolished normal T_E -shortening during central chemoreceptors activation by hyperoxic hypercapnia, but led to paradoxical T_E -prolongation and corresponding decrease of respiratory frequency. Furthermore, LPBN lesion significantly attenuated the increase in phrenic activity during hyperoxic hypercapnia, without appreciably affecting the corresponding shortening of inspiratory duration (T_I). These findings provide the first evidence indicating that central chemoafferent inputs are organized in parallel and segregated pathways that separately modulate inspiratory drive, T_I , and T_E in conjunction with similar parallel and segregated central processing of peripheral chemoafferent inputs reported previously

Keywords

Lateral parabrachial nucleus; central chemoreceptor; respiratory control; expiratory duration; inspiratory drive

1. Introduction

The hypercapnic ventilatory response in humans is highly stereotyped (Cunningham et al. 1986) and is comprised of increases of inspiratory drive or tidal volume, increases of respiratory frequency (f), and decreases in inspiratory and expiratory durations (T_I , T_E) (Gardner 1980; Poon 1989a; Poon 1989b; Jubran et al. 1997). In adult animals hypercapnia typically elicits increases in inspiratory drive and tidal volume, but corresponding responses in f , T_I and T_E may vary depending on experimental conditions and animal species. In anesthetized or awake dogs, cats and rats with intact or severed vagi, hypercapnia generally causes increases of f and shortening of T_E , with slight decrease or no change in T_I (Clark et al. 1972; Gautier 1976; Ledlie et al. 1981; Oliven et al. 1985; Coles et al. 2002). However, hypercapnia reportedly may

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also lead to a paradoxical prolongation of T_E in decerebrate cats (St John 1979; Clanton et al. 1984) and similar post-hypercapnia prolongation of T_E in awake rats (Coles et al. 2002). The central pathways mediating these varied effects during central and/or peripheral chemoreceptors activation by hypercapnia are unclear.

In a companion report (Song et al. 2009) we have shown that unilateral or bilateral lesions of the lateral parabrachial nucleus (LPBN) in anesthetized, vagotomized rats markedly and selectively attenuate the shortening of T_E during hypoxia without appreciably affecting all other hypoxic response components (such as short-term potentiation and depression), suggesting an important role of this dorsolateral pontine structure in modulating the peripheral chemoafferent inhibitory control of T_E . In addition, baseline T_E under hyperoxia is significantly increased after bilateral LPBN lesions without appreciable changes in baseline T_I , indicating that the LPBN also mediates the shortening of T_E by other inputs. In the present study, we used a similar preparation to test the hypothesis that the LPBN also mediated T_E -shortening during hyperoxic hypercapnia, a stimulus that preferentially activates the central but not peripheral chemoreceptors (Fitzgerald et al. 1971; Lahiri et al. 1975). To our surprise, we found that central chemoreceptor inputs not only promoted T_E -shortening but also an increase of inspiratory drive via the LPBN, as well as a paradoxical T_E -prolongation effect via other pathways.

2. Methods

Experiments were performed on 6 urethane-anesthetized, pancuronium-paralyzed, vagotomized and ventilated adult Sprague-Dawley rats (330–380 g, Charles River Laboratories). All experimental protocols had been reviewed and approved by the M.I.T. Committee on Animal Care in accordance with published guidelines. Experimental methods and materials are as described in (Song et al. 2009) except as follows. Hyperoxic hypercapnia (O_2CO_2) was applied by abruptly switching the hyperoxic ventilation gas (40% O_2 balance N_2 medical-grade) to carbogen (5% CO_2 balance O_2) for 1 min. Thirty minutes after unilateral microinjection of kainic acid (KA, concentration at $1\mu g/\mu l$ in ACSF) into the LPBN, another O_2CO_2 test was performed. In this group of animals a hypoxia test was also performed (see (Song et al. 2009)) at 15 min after each O_2CO_2 test, when the animal's end-tidal PCO_2 and PO_2 and blood pressure had returned to corresponding pre-test baseline levels. T_I , T_E and f were measured for each respiratory cycle from recorded phrenic activity (Phr). Inspiratory drive was measured as the peak of the integrated phrenic signal ($\int Phr$). These values were normalized against their corresponding average pre-test baseline values in control or lesioned conditions. Data are expressed as means $\pm$ SE. Each animal served as its own control for all statistical analyses.

3. Results

In the six animals that received unilateral KA injection, the injection sites as marked by post-injection electrolytic lesions were verified to lie at the central or external-lateral subnucleus of the LPBN (Fig. 1). Baseline respiratory rhythm variables (f , T_E , T_I) under hyperoxia were not significantly different before and after lesion (Table 1, see also (Song et al. 2009)).

Before LPBN lesion, O_2CO_2 caused progressive shortening of T_E and increase in f (Fig. 2). The shortening of T_E reached maximum ($-9.7\pm 2.2\%$ below baseline; $P<0.01$, two-tailed t test) at ~ 30 sec into O_2CO_2 before gradually relaxing toward baseline, resulting in a decrementing-incrementing response pattern. The increase in f also reached maximum ($8.4\pm 1.5\%$ above baseline; $P<0.01$) at ~ 30 sec into O_2CO_2 but remained relatively stable afterward. Following the O_2CO_2 test both variables gradually recovered.

After LPBN lesion, O₂CO₂ paradoxically caused progressive prolongation of T_E and decrease of *f* (Fig. 2), in opposite to the control effects. The maximal prolongation of T_E (8.6±1.8% above baseline; P<0.01, two-tailed *t* test) and decrease in *f* (-3.9±1.6%) occurred ~40 sec into O₂CO₂. Following the O₂CO₂ test both variables gradually returned to baselines. The response curves in both variables were significantly different after lesion compared with control (P<0.01, two-way ANOVA with repeated measures).

In contrast to T_E, T_I showed similar progressive shortening during O₂CO₂ as well as similar recovery before and after lesion (Fig. 3). In two animals the shortening of T_I appeared to develop more rapidly after lesion compared with control. However, two-way ANOVA (with repeated measures) for the group as a whole showed no significant differences in the response curves before and after lesion (P>0.1).

In both control and lesioned conditions, $\dot{V}_{Phr}$ amplitude showed progressive increases during O₂CO₂ and gradual return during recovery (Fig. 3). However, the increases were significantly smaller after lesion compared with control (P<0.01, two-way ANOVA with repeated measures). For example, the maximal increase was 36.1±6.4% above baseline in control condition, but only 20.6±3.6% after lesion (P<0.01, paired *t* test).

4. Discussion

In accord with our previous finding that the LPBN plays an important role in mediating the shortening of T_E by peripheral chemoreceptor and other inputs in anesthetized vagotomized rats (Song et al. 2009), the present results confirm that the LPBN also mediates T_E-shortening by central chemoreceptor inputs in the same group of animals. These findings suggest that the LPBN is a critical structure that integrates central and peripheral chemoreceptor inputs to shorten T_E. Furthermore, recent studies using time-resolved decomposition of the respiratory response to carotid sinus nerve stimulation have shown that the effects of carotid chemoafferent inputs on T_I, T_E and $\dot{V}_{Phr}$ amplitude are not reflex-driven as traditionally thought but are secondary to resultant short-term potentiation of some tonic inputs, in the form of discrete neural integrators or differentiators (low-pass and high-pass filters) that exert distinct influences on these variables in parallel (Young et al. 2003; Song et al. 2009). Taken together, these observations raise the possibility that the LPBN may be the site of the putative fast integrator (with very short time constant) (Young et al. 2003; Song et al. 2009) whereby central chemoafferent inputs are potentiated by peripheral chemoafferent inputs to shorten T_E (Fig. 4).

Surprisingly, our data show that LPBN lesion not only abolished normal T_E-shortening during O₂CO₂ but led to paradoxical T_E-prolongation and corresponding decrease of *f*. This finding suggests that central chemoreceptor inputs not only can shorten T_E via the LPBN but may also prolong it via a separate, normally recessive pathway. Such counteracting control mechanisms are also indicated by the seeming decrementing-incrementing response pattern in T_E during O₂CO₂ (Fig. 1). The central chemoreceptors-mediated T_E-prolongation component presently demonstrated in anesthetized, vagotomized rats is consonant with a recently reported post-hypercapnia prolongation of T_E and decrease of *f* in intact or carotid sinus-denervated conscious rats (Coles et al. 2002). These paradoxical effects are reminiscent of similar T_E-prolongation that is characteristic of carotid chemoafferent short-term depression and post-hypoxia frequency decline (Dick et al. 2000; Poon et al. 2000; Young et al. 2003; Song et al. 2009), an effect that has been ascribed to short-term depression of the ventrolateral pontine A5 region (Dick et al. 2000). In light of this similarity, and the presently suggested integration of central and peripheral chemoreceptor inputs at the LPBN to shorten T_E, it is tempting to speculate that central chemoreceptor inputs may also interact with peripheral chemoreceptor inputs at the ventrolateral pons to prolong T_E via a similar integrator mechanism (Fig. 4).

We have previously reported (Song et al. 2009) that neither the baseline T_I under hyperoxia nor the shortening of T_I during hypoxia were affected by lesions at the LPBN. In the present study, we found that T_I -shortening during O_2CO_2 was not significantly affected by LPBN lesion either. These findings indicate that T_I is controlled by pathways other than LPBN.

However, the present data show that the increase of $\dot{V}_{Phr}$ amplitude during O_2CO_2 was significantly attenuated after LPBN lesion, in contrast to the reported lack of effect of similar lesion on changes in $\dot{V}_{Phr}$ amplitude during hypoxia in the same group of animals (Song et al. 2009). These findings taken together suggest that: (1) central chemoreceptor inputs may interact with other inputs (except peripheral chemoreceptor inputs) at the LPBN to augment inspiratory drive; (2) although both central and peripheral chemoafferent inputs converge at the LPBN, their integration to augment inspiratory drive occurs elsewhere (Fig. 4). Possible loci for the latter include inspiratory premotor or motor neurons, where some form of short-term potentiation of phrenic activity has been reported (McCrimmon et al. 1997).

In conclusion, we have shown that the control of T_E by central chemoreceptor inputs in anesthetized, vagotomized rats comprises two counteracting mechanisms acting in parallel – one tending to shorten T_E (dominant effect) via the LPBN, the other tending to prolong it (recessive effect) via other pathways. Central chemoreceptor inputs also promote augmentation of inspiratory drive via the LPBN and shortening of T_I via other pathways. Together with the reported effects of LPBN lesions on hypoxic respiratory response in the same group of animals (Song et al. 2009), the present results suggest that central and peripheral chemoreceptor inputs may interact at the LPBN to shorten T_E , and any interactions between these inputs to prolong T_E , shorten T_I and augment inspiratory drive must occur elsewhere. These findings provide the first evidence indicating that central chemoafferent inputs are organized in parallel and segregated pathways that separately modulate inspiratory drive, T_I , and T_E in conjunction with similar parallel and segregated central processing of peripheral chemoafferent inputs reported previously (Young et al. 2003; Song et al. 2009).

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Fig. 1. Histological section showing LPBN lesion in a representative animal

Left panel: Montage photomicrograph showing the kainic acid injection site. The center of injection (*) was at the central LPBN. *Right panel:* Camera-lucida drawing of the histological section (rectangle with broken lines) on standard plate (Plate 52) of Paxinos rat brain atlas (Paxinos et al. 1986). KF, Kölliker-Fuse nucleus; scp, superior cerebellum peduncle. See also (Song et al. 2009).

Fig. 2. Paradoxical decrease of respiratory frequency and prolongation of expiration during hyperoxic hypercapnia after unilateral lesion at the LPBN

Responses in normalized respiratory frequency (f) and expiratory duration (T_E) to hyperoxic hypercapnia (shaded areas) in control state (Ctr.) and after LPBN lesion. Data are means $\pm$ SE (n=6).

Fig. 3. Effects of unilateral LPBN lesion on changes in inspiratory duration and inspiratory drive during hyperoxic hypercapnia

Fig. 4. Hypothetical model showing parallel central pathways that mediate various components of the hypercapnic respiratory response

Open and filled triangles indicate excitatory and inhibitory effects. Broken lines indicate hypothetical pathways. The pathway mediating the shortening of T_I by central and peripheral chemoafferent inputs is not shown. LPBN, lateral parabrachial nucleus; vl-pons and dl-pons, ventrolateral and dorsolateral pons; central chemoaff., inputs from central chemoreceptors; peripheral chemoaff., inputs from peripheral chemoreceptors; E and I, expiratory and inspiratory rhythm generators; Phr.m/pre-m phrenic motoneuron/premotoneuron. This model complements previous models of central processing of peripheral chemoafferent inputs proposed in (Young et al. 2003; Song et al. 2009).

Table 1

Effects of unilateral LPBN lesion on baseline respiratory patterns.

		Before	After
Unilateral Lesion (n=6)	$f(\text{min}^{-1})$	39.2±1.8	43.0±3.3
	T_E (sec)	1.15±0.06	1.05±0.12
	T_I (sec)	0.40±0.03	0.42±0.03