Point:Counterpoint: The parafacial respiratory group (pFRG)/pre-Bötzinger complex (preBötC) is the primary site of respiratory rhythm generation in the mammal

PURPOSE AND SCOPE OF THE POINT: COUNTERPOINT DEBATES

This series of debates was initiated for the Journal of Applied Physiology because we believe an important means of searching for truth is through debate where contradictory viewpoints are put forward. This dialectic process whereby a thesis is advanced, then opposed by an antithesis, with a synthesis subsequently arrived at, is a powerful and often entertaining method for gaining knowledge and for understanding the source of a controversy.

Before reading these Point:Counterpoint manuscripts or preparing a brief commentary on the content, the reader should understand that authors on each side of the debate are expected to advance a polarized viewpoint and to select the most convincing data to support their position. This approach differs markedly from the review article where the reader expects the author to present balanced coverage of the topic. Each of the authors has been strictly limited in the lengths of both the manuscript (1,200 words) and the rebuttal (400). The number of references to publications is also limited to 30, and citation of unpublished findings is prohibited.

POINT: THE PFRG IS THE PRIMARY SITE OF RESPIRATORY RHYTHM GENERATION IN THE MAMMAL

Optical recording studies (20) have determined that the distribution of respiratory neurons in the ventrolateral medulla extends further in the rostral medulla than determined in electrophysiological studies (1, 14). The rostral respiratory neuron group is referred to as the parafacial respiratory group (pFRG) based on its position relative to the facial nucleus (20); the pFRG is located lateral, ventral, medial, and caudal to the facial nucleus and consists predominantly of neurons that burst before inspiration [pre-inspiratory (Pre-I) neurons]. The medial portion of the pFRG may overlap with the retrotrapezoid nucleus (RTN), which has been identified as an area of origination for neurons with projections to the ventral respiratory group (VRG) (5, 25). The major neuron population of the pFRG appears to be lateral to the RTN. The caudal portion of the pFRG overlaps the most rostral portion of the VRG (Bötzinger complex), i.e., the ventral part of the retrofacial nucleus near the caudal end of the facial nucleus. This caudal portion of the pFRG corresponds to so-called rostral ventrolateral medulla (RVL) (1, 4, 14) where most Pre-I neurons have been recorded in previous electrophysiological studies.

Our hypothesis (4, 15) is that the respiratory rhythm generator (RRG) composed of Pre-I neurons in the pFRG periodically triggers the inspiratory pattern generator (IPG), which is composed of inspiratory neurons in the caudal ventrolateral medulla, including the pre-Bötzinger complex (preBötC) (24). Data supporting this hypothesis were found in early electrophysiological studies of brain stem-spinal cord preparations of newborn rats (14, 15, 18) as follows. First, under standard experimental conditions, C4 inspiratory activity is always preceded by the firing of Pre-I neurons, although Pre-I neuron activity occasionally is not followed by inspiratory activity. Second, single pulse stimulation in the RVL induces premature Pre-I neuron bursting and resets the phase of the respiratory rhythm, regardless of whether C4 inspiratory activity is induced. Third, bilateral lesions of the caudal ventrolateral medulla corresponding to the preBötC eliminate C4 inspiratory activity, whereas rhythmic activity of the Pre-I neurons in the RVL is preserved after the disappearance of transient inhibition corresponding to the inspiratory phase. Fourth, electrolytic lesioning of the RVL reduces the rate of C4 inspiratory activity or terminates it. This is consistent with findings that microinjection of excitatory amino acid antagonists into the RVL produces apnea in adult rats (26). More recently, we reported that partial bilateral lesioning of the pFRG area significantly reduced respiratory rate and induced changes in the spatiotemporal pattern of respiratory neuron activity (20). Fifth, after complete transection of the rostral medulla, including the pFRG, from the caudal medulla, including the preBötC, Pre-I neuron-like rhythmic activity persisted in the rostral block, whereas C4 inspiratory activity was abolished. Moreover, transection of the medulla at the intermediate level of the RVL consistently decreased C4 burst rate (12). These results (particularly points 4 and 5) indicate a strong rhythm-generating property of the pFRG/RVL and suggest that intrinsic burst generation of preBötC neurons (11) is probably not exerted in the intact brain stem-spinal cord preparation.

According to our hypothesis, excitatory synaptic connections from Pre-I neurons to inspiratory neurons are essential for rhythm generation. Analysis of postsynaptic potentials identified inspiratory neuron subtypes that receive excitatory postsynaptic potentials (EPSPs) or inhibitory postsynaptic potentials (IPSPs) during pre- and postinspiratory phases corresponding to the active phase of Pre-I neurons (19). Indeed, the presence of excitatory synaptic connections from Pre-I neurons to inspiratory neurons has been indicated by analysis of spike-triggered averaging (21).

Pre-I neurons possess strong intrinsic burst-generating properties. Approximately 50% of Pre-I neurons in the RVL of the brain stem-spinal cord preparation of newborn rats retain rhythmic bursting activity after blockade of synaptic transmission with low Ca2+/high Mg2+ solution (16, 17). In our hands, burst generation of all inspiratory neurons examined disappeared in low Ca2+/high Mg2+ solution. The intrinsic burst generation of Pre-I neurons was found to be voltage dependent (17) and was modified by various neuromodulators such as (nor)epinephrine (3), serotonin (22), NMDA (10), substance P (30), and H+ (16). cAMP is involved in the regulation of intrinsic bursting of Pre-I neurons; intracellular cAMP-elevating agents induce or enhance burst activity of Pre-I neurons in low Ca2+/high Mg2+ solution (2).

Although the RRG and IPG interact via mutual synaptic connections, it is possible that they comprise independent networks (4, 15). The frequency of inspiratory burst activity...
may be affected by modulation of properties of both the (Pre-I neuron-mediated) RRG and the (inspiratory neuron-mediated) IPG. According to our hypothesis, at least three mechanisms may be responsible for pharmacologic reduction in the frequency of inspiratory output activity of the respiratory network in the brain stem-spinal cord preparation (4). First, a decrease in the rate of Pre-I neuron bursts directly results in a frequency decrease. This mechanism would account for the effects of epinephrine (3), GABA (4), propofol (9), and the pontine inhibitory system (28). Second, a decrease in the intraburst firing frequency of Pre-I neurons results in decreased respiratory frequency via failure to trigger the IPG. This mechanism would explain the inhibitory effects of serotonin (22), neuromuscular blocking agents (23), and dopamine (6). Third, a direct (postsynaptic) or indirect (presynaptic) inhibition of the IPG without an inhibitory effect on Pre-I neurons decreases the frequency of the final inspiratory output. This mechanism may be responsible for the effects of vagal stimulation (18) and opiates (13, 27). In particular, μ-opiate agonists such as DAMGO inhibit inspiratory burst generation without a direct inhibitory effect on Pre-I neurons. Mellen et al. (13) confirmed that such an effect of DAMGO results in quantal slowing of the inspiratory burst rate. They also showed a quantal respiratory frequency decrease in juvenile rats in vivo after fentanyl treatment. Pace-making properties of Pre-I neurons under conditions of quantal slowing were confirmed in the in vivo preparation by monitoring abdominal muscle activity, which has been shown to reflect central Pre-I neuron activity (8). This quantal nature was also observed in adult rats (29).

Our hypothesis does not exclude the possibility that the IPG produces inspiratory bursts independent of Pre-I neuron activity under specific conditions such as lung deflation (7). In conclusion, in the in vitro brain stem-spinal cord preparation and under some conditions in vivo, Pre-I neurons in the pFRG determine the respiratory rhythm, and the intrinsic burst generating property of the preBöTC is masked and functions as the IPG.

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COUNTERPOINT: THE PreBo¨tC IS THE PRIMARY SITE OF RESPIRATORY RHYTHM GENERATION IN THE MAMMAL

The modern quest to locate the mammalian RRG began almost two centuries ago (14). We assert that this journey finally succeeded with the discovery of the preBo¨tC (5–7, 26). As various locations for RRGs were proposed only to be later discounted, why do we think the preBo¨tC is the real thing?

Lesions demonstrate necessity. Whereas compatible data contribute to the general acceptance of hypotheses, and simulations demonstrate plausibility, experiments designed at falsification are the most stringent test. If a site is essential for rhythm generation, its destruction by any means, e.g., genetic, molecular, pharmacological, surgical, mechanical, must lead to an irreversible and permanent (minutes or hours simply will not do), disruption of normal breathing. Experimental animals should be essentially intact, unanesthetized [anesthetics and analgesics can themselves disrupt normal breathing (15)], be at normal body temperature, and allowed sufficient time to recover from transient disturbance(s) produced by the procedure or the lesion itself. For full recovery hours of mechanical ventilation may be required. The true extent and precise location of the lesion must be verified, and adjacent areas and axons of passage must remain intact. The preBo¨tC is the only region that passes these tests; other regions have either failed or have not been adequately tested.

Pons and spinal cord are not essential RRGs. Under anesthesia in many mammals, midcorticobulbar transections do not affect breathing but midpontine transections produce apneustic breathing, suggesting a pontine RRG, particularly in the paramedian reticular formation and the retrotrapezoid nucleus (“pneumotaxic center”/pontine respiratory group-PRG). However, the PRG fails the lesion test because in unanesthetized mammals, eupneic breathing continues after midpontine transection (1) or even more caudal transections through the facial nucleus (VIIIn) (25). Thus the PRG or other structures rostral to the VIIIn are not essential RRGs; they likely play an important modulatory role and maybe even a rhythmogenic role under extraordinary circumstances. The spinal cord is also unlikely to contain a RRG (see discussion in Ref. 4). This leaves two medullary candidates for the primary RRG(s) in mammals: 1) preBo¨tC and 2) retrotrapezoid nucleus [RTN (3)]/parafacial respiratory group [pFRG/20]. (Whether RTN and pFRG are distinct, perhaps overlapping, regions or one functional group remains to be established; hence the term RTN/pFRG).

preBo¨tC passes the lesion tests. Bilateral ablation of >80% of preBo¨tC neurokinin-1 receptor (NK1R) expressing neurons in awake adult rats produces an ataxic breathing pattern resulting in abnormal blood gases and pH, which do not improve over weeks (9, 16); anesthesia produces apnea that persists even with mechanical ventilation (9). Substantial preBo¨tC lesions in adult goats produce similar effects (29); if mechanically ventilated, these goats maintain normal blood gases suggesting that circulation and other non-breathing regulatory functions are retained (29). Tonic drives (related to wakefulness) from outside the preBo¨tC must play an important role because lesion-induced apneas are exacerbated during sleep (16).

To be fair, several papers claim that lesioning/synaptic inactivation of the preBo¨tC only transiently affects breathing in anesthetized or decerebrate animals (8, 18, 22, 27). These papers are sometimes cited as the evidence that the preBo¨tC is not a RRG. However, insofar as the lesions were either insufficient in extent [preBo¨tC lesions must be substantial to produce disturbances in breathing (9, 16)] or anatomically misplaced, these studies do not meet the requirements of the lesion tests. First, only when injections in/near the preBo¨tC of 8-conotoxin are made instead with tetrodotoxin (TTX) do persistent apneas develop (22). Because TTX also blocks axons of passage, apneas could be due to effects on preBo¨tC inputs, not necessarily on the preBo¨tC itself. Second, careful examination of the figures in these papers show that the preBo¨tC was not substantially lesioned, because injection sites were often misplaced and/or verification of preBo¨tC neuronal damage was not fully assessed. We ask the reader to judge the matter by comparing Fig. 4A in Ref. 22, Fig. 4 in Ref. 27, and Fig. 7B in Ref. 18 with Fig. 1A in Ref. 9 and Fig. 2B in Ref. 23; see also the discussion in Refs. 10 and 23.

RTN/pFRG has not yet passed the lesion tests. Extensive RTN/pFRG lesions in en bloc preparations slow but do not abolish the rhythm, suggesting a role in rhythm modulation but inconclusive as to an RRG role (20). There are NK1R-labeled processes and neurons in the RTN/pFRG (19). Seventy-five percent of preinspiratory neurons respond to substance P in the presence of TTX, suggesting that they express NK1Rs (24). However, whereas lesions of RTN NK1R neurons in awake rats affect central chemoreception, the respiratory rhythm is unaffected (19); an important caveat is that these lesions only destroyed ~40% of the targeted neurons and more extensive lesions may be necessary (9). In addition, the inspiratory rhythm in juvenile rats continues after complete brain stem transection caudal to the major portion of the RTN/pFRG but rostral to the preBo¨tC (12). This transaction irreversibly eliminates active expirations, indicating that the RRG producing inspirations is located more caudally (preBo¨tC) than the RRG for active expirations (RTN/pFRG; see also Ref. 29).

Breathing in mice with presumptive RTN/pFRG anomalies. Observations in Hoxa1 [1] or Krox-20 - mice, whose phenotype include lesion/pathology in the region containing RTN/pFRG (see Ref. 2), support the preBo¨tC as an essential RRG. These mutant mice die within 24 hours after birth due to prolonged apneas (2). These data might suggest that the RTN/pFRG is an essential RRG. However, these neonatal mice can be rescued by injection of naloxone, an opiate antagonist. Why? Normally, the surge of opiates at birth depresses pre-Bo¨tC (10), but not RTN/pFRG (28), activity (see Ref. 6). At birth in normal mammals the opiate-resistant RTN/pFRG can provide the drive to maintain breathing until the opiate surge dissipates (~2 days); the RTN/pFRG acts as a “anti-apneic system” (2). However, in Hoxa1 [1] or Krox-20 - mice, the opiate-sensitive preBo¨tC RRG (10, 15, 17) is not aided by a functional opioid-insensitive RTN/pFRG, resulting in a fatal suppression of breathing that can be reversed by naloxone. Thus breathing may continue in the (presumed) absence of the RTN/pFRG, but not without a functional preBo¨tC.

Coexistence of two rhythm generators. We hypothesize that inspiratory and expiratory activity originates from different rhythm generators, with the preBo¨tC driving inspiratory rhythm (12). Because breathing in mammals is dominated by inspiration, we assert that the preBo¨tC is the essential RRG. We further hypothesize that the RTN/pFRG provides rhythmic expiratory drive to abdominal (13), hypoglossal (12), and facial (21) nerves. RTN/pFRG neurons may be rhythmic only when
there is active expiration. In many states, particularly in anesthetized rats, there may be little or no active expiration, so RTN/pFRG neurons may not be rhythmic under these conditions (11). The balance between the two oscillators may be different in other vertebrates species (30), especially when breathing is primarily driven by active expiration. In fact, given our proposal that the evolution of the preBotC is associated with the movement of vertebrates onto land and/or the emergence of the diaphragm in mammals (7, 17), we predict that the preBotC would not be present in fish. We have also proposed that lifetime degeneration of preBotC neurons leads to death during sleep (16). In conclusion, we assert that there is considerable evidence that the preBotC is an essential RRG; further investigation will be necessary to establish whether there are other essential RRGs.

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REBUTTAL FROM DRS. FELDMAN AND JANCZEWSKI

Recent advances in identifying sites for respiratory rhythm generation are largely based on observations first made in vitro preparations. Both supporters and critics of these in vitro preparations emphasize that they are different in some respects from more intact in vivo preparations (7, 8).

Both the in vitro and in vivo data cited here support the hypothesis that the preBotC and RTN/pFRG can each contribute to respiratory rhythm generation. If, for the sake of discussion, we stipulate this as fact, then what are their respective contributions under different conditions? Certainly, if one oscillator is suppressed (or absent), the other may dominate. In typical slices, the preBoC is the sole generator but its function is depressed due to low temperature, deafferentation, etc. (7); thus a sustained rhythm in slices requires a boost in excitability (typically by increased [K⁺]o).

Many conditions that appear to depress preBoC function, including opiates, spare RTN/pFRG function. Thus we agree with Onimaru and Homma that in en bloc in vitro preparations, where both putative oscillators are present in a low temperature and deafferented environment, the relatively more excitable RTN/pFRG may drive the rhythm (6). In vivo, particularly in...
behaving mammals, what conditions affect the relative excitability of these two oscillators? Opiates—endogenous (birth) or exogenous (substance abuse, analgesia)—depress the preBo¨tC but not the RTN/pFRG; this may result in a situation where the primary oscillator is the RTN/pFRG (1, 4). However, we suggest that at rest in juvenile and adult mammals when breathing is dominated by inspiration with little or no active expiration, conditions favor the preBo¨tC over the RTN/pFRG (3). Two observations support this conjecture. First, lesions of a key subset of preBo¨tC neurons produce an irreversible pathological breathing pattern in unanesthetized behaving adult rats (2, 5). If the RTN/pFRG is otherwise capable of generating inspiratory rhythm, it does not appear to do so in this case, although inspiratory activity can be observed (9). Second, transecting the brain stem to remove the RTN/pFRG abolishes expiratory but not inspiratory activity (4). Moreover, when the preBo¨tC is depressed by exogenous opiates in juvenile or adult rats, expiratory motor rhythm is unaffected, whereas inspiratory activity skips cycles, i.e., quantal breathing.

We suggest that whereas the RTN/pFRG may drive the rhythm when the preBo¨tC is depressed, in behaving, i.e., not anesthetized, not decerebrated, not drugged, not lesioned, not perfused, not hypothermic, etc., mammals the preBo¨tC rules!

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REBUTTAL FROM DR. ONIMARU AND HOMMA

Lesion experiments. Results of the preBo¨tC lesion indicate that the preBo¨tC is essential for inspiratory burst generation, but are also consistent with the pFRG-Pre-1 rhythm generator hypothesis in which the preBo¨tC is presumed to function as an inspiratory pattern generator. Moreover, lesions of preBo¨tC in the in vivo preparations may induce complex effects on rhythm generation of Pre-1 neurons, which receive inhibitory (and excitatory) synaptic inputs from inspiratory neurons (8). Effects of the pFRG lesion or complete elimination of the rostral structure on respiratory rhythm generation are inconsistent between different preparations (5, 7, 8), probably due to the different experimental conditions. Even if the caudal medulla produced rhythmic inspiratory activity after removal of the rostral structure including the pFRG, this does not mean that the preBo¨tC dominantly determines the respiratory rhythm in the intact preparation before the removal.

Mice preparations. Concerning gene-manipulated mice, it is not evident whether pFRG-Pre-1 neurons exist and function normally or not, without conducting a detailed neuron level analysis. Indeed, the abnormal respiratory rhythm in Krox 20–/– mice could be explained by deletion of excitatory inputs (and/or increase of inhibitory inputs) to the medullary rhythm generator from the pons, whereas the medullary rhythm generators (pFRG or preBo¨tC or both) might have been intact (4). It was suggested that such pontine excitatory and inhibitory systems affect Pre-1 neuron activity in rats (10).

Expiratory activity. In the brainstem-spinal cord preparation, active expiration (motor burst) is absent or weak at normal pH levels and appears in solutions of low pH (3), whereas pFRG-Pre-1 neurons generate rhythmic burst activity under normal pH conditions. Moreover, we have recorded a subtype of expiratory neuron that received inhibitory synaptic inputs from Pre-1 and inspiratory neurons (1). This type was clearly distinguishable from Pre-1 neurons and was thought to correspond to late expiratory (or E augmenting) neurons in adult mammals (2). Thus Pre-1 neuron activity is not simply reflected in motoneurons discharged as active expiration.

Currently, there is considerable evidence that the preBo¨tC is essential for inspiratory burst generation, whereas it would be necessary to show more direct evidence that preBo¨tC determines the primary respiratory rhythm in quiet breathing in vivo preparations with the intact medulla (and pons). However, there is clear evidence that pFRG-Pre-1 neurons generate the primary respiratory rhythm by which inspiratory bursts are entrained, at least, in the in vitro brain stem-spinal cord preparations of newborn rats and in some in vivo preparations (6, 8, 9).

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