Point:Counterpoint

Pacemaker activity imbues the respiratory network with the plasticity essential for eupnea. The extreme view, i.e., that pacemakers play no role in eupnea, would require that all bursting properties are suppressed in normoxia. For this notion, there is as little evidence as for the proposal that the pre-Bötzinger complex is suppressed during eupnea. Endogenous neuromodulators are essential for bursting during well-oxygenated conditions (i.e., normoxia). Blockade of endogenously activated serotonin receptors (5-HT2A) abolishes bursting in Cd-insensitive pacemakers, but retains action potential generation. At the network level, 5-HT2A blockade dramatically affects regularity and frequency, which coincides with the blockade of bursting in Cd-insensitive pacemakers (8). Moreover, the remaining network becomes dependent on ICAN. Thus the persistence of network activity does not indicate that rhythm-generating mechanisms are unaffected (18). The same is true following blockade of INaP. While this manipulation does not abolish respiratory rhythm generation in normoxia (2, 7, 10), rhythm generation is altered and destabilized in presence of norepinephrine (19).

If eupnea characterizes breathing with all its plasticity and ability to adapt to changes in environmental and metabolic conditions, then these experiments are consistent with the notion that pacemaker properties are essential for generating eupnea and gasping. Neuromodulators and synaptic mechanisms are capable of continuously regulating the contribution of different types of bursting mechanisms, which stabilizes network activity and imbues the network with the enormous plasticity that characterizes eupnea. By reducing some of these properties, the respiratory rhythm may persist, but the persisting rhythm is limited in its adaptive capability, which is one of the hallmarks of eupnea.

REFERENCES


COUNTERPOINT: MEDULLARY PACEMAKER NEURONS ARE ESSENTIAL FOR GASPING, BUT NOT EUPNEA, IN MAMMALS

For more than 80 years, the brain stem mechanisms that might underlie the neurogenesis of automatic ventilatory activity have been debated. Mechanisms proposed have included the discharge of pacemaker neurons, inhibitory synaptic interactions within a neuronal circuit, or a combination of these processes. Inherent to these discussions has been the question as to whether there are state-dependent changes in the mechanisms for respiratory rhythm generation such as occur during the transition from eupnea to gasping.

Eupnea and gasping differ in multiple aspects, with a primary difference being the rate of rise of inspiratory motor activity. In eupnea, phrenic activity increases gradually. In gasping, phrenic discharge reaches a peak almost immediately after onset and has a decrementing pattern (Fig. 1). During gasping, the temporal dispersion of cranial (X and XIIth nerves) vs. spinal motor outflows is lost as they synchronize, and laryngeal adductor activity (post-inspiratory discharge) is abolished (12, 19–21, 29; Fig 1). Therefore, we propose that multiple, simultaneously recorded motor outflows are necessary to assign behavioral terms to respiratory motor patterns.

Rhythms in vitro. Although an in vitro en bloc preparation was introduced more than 20 years ago (26) and slice preparations soon thereafter (17), the rhythms generated by these
in vitro preparations remain poorly defined. For en bloc preparations of the neonatal rat, the discharges of its cranial and spinal nerves have a similar invariant decrementing pattern that is initiated coincidentally and contains little post-inspiratory discharge (15–17). As en bloc preparations, activity recorded from the hypoglossal nerve of slice preparations from neonatal rats and mice can have a decrementing discharge (2, 3, 16, 17). Some continue to opine that this decrementing pattern represents a variant of eupnea (6) and that age and state-dependent changes account for the difference between the incrementing neural discharge, recorded in neonatal and adult in vivo and in situ preparations and the decrementing discharges of en bloc and slice preparations. However, other work indicates fundamentally similar respiratory patterns in immature and mature rats (4, 7, 21, 24, 29).

Another confounding finding is that, in addition to the decrementing pattern, two other rhythmic patterns have been described for thick in vitro slices obtained from the medulla of mouse (9, 13, 27, 28). The reason for multiple rhythms from thick slices and a single rhythm from thin (350 μm) sections from the neonatal rat is unclear. However, thin sections are largely limited to the medullary “pre-Bötzinger complex,” the region for rhythm generation in vitro, whereas thick slices probably include other varying amounts of the ventral medullary respiratory column and are therefore different and cannot be compared easily.

Neurogenesis of gasping by medullary pacemakers. Since the work of Lumsden in 1923 (10), the concept that gasping is generated by mechanisms intrinsic to the medulla has been well accepted. We acknowledge that the medulla can also generate “non-gasping” rhythms, but the mechanisms and physiological relevance of these elude us at present.

Gassing is irrevocably eliminated following ablations in either the “gasping center” of the lateral tegmental field or the adjoining pre-Bötzinger complex. Both regions contain elements of the same neurons (18, 20).

In eupnea, neurons in the pre-Bötzinger complex discharge during neural inspiration, expiration, or across both phases. In hypoxia-induced gasping, a subset of neurons begin to discharge in late neural expiration, prior to onset of the phrenic burst (12, 23). Some of these “pre-inspiratory” neuronal activities have the capacity for intrinsic rhythmic bursting that continues following a blockade of fast inhibitory and excitatory synaptic transmission. These rhythmic bursts, as well as gasping in situ and in vivo preparations, are eliminated by blockers of persistent sodium channels (23). Similar burster neurons that are sensitive to riluzole have been identified in the pre-Bötzinger complex in vitro (13, 27).

In vitro rhythms and gasping of in situ preparations are little altered by a blockade of inhibitory synaptic transmission, which is consistent with the hypothesis that the discharge of intrinsic bursting neurons may be essential for both rhythms (22). In contrast, a similar blockade markedly distorts eupnea of in vivo or in situ preparations, implying that inhibitory neuronal circuits are critical for this rhythm to be expressed (8, 11, 22).

Neurogenesis of eupnea by intrinsically bursting neurons? A more fundamental question than whether the discharge of medullary burster neurons can generate eupnea is whether medullary mechanisms alone, be they pacemakers or a neuronal circuit, can generate eupnea.

If eupnea is generated by the discharge of medullary bursters, a number of criteria must be fulfilled. First, a unique region that is critical for the neurogenesis of eupnea must be identified. Although ablation of many brain stem regions distorts eupnea, no region has been identified as a “noeud vital” for the neurogenesis of eupnea (20, 21, 24). Likewise, optical recordings of respiratory neuronal activities in a fetal mouse preparation failed to identify a specific medullary region in which the respiratory rhythm commenced (5).

A second criterion is that blockers of intrinsic burster neurons should eliminate eupnea. One group of these is dependent on conductance through persistent sodium channels, with blockers of these channels eliminating some in vitro rhythms...
and hypoxia-induced gasping in vivo and in situ (13, 27). Also eliminated was a “non-gasping” rhythm of an in situ preparation having a brain stem transection at the pontomedullary junction (14). This non-gasping rhythm differs dramatically from eupnea recorded in a preparation having an intact pons because there is a marked alteration in the shape and amplitude of the inspiratory burst as well as a loss of postinspiratory discharge (1, 24, 25). For in situ preparations having an intact pons or in conscious rats, blockade of persistent sodium channels did not eliminate eupnea (25).

Parenthetically, evaluations following brain stem transections are leading to additional confusion in that all non-gasping medullary rhythms are proposed to be variants of eupnea (6, 14). This proposal is premature and probably incorrect as shown by the differences in motor patterns and the response of medullary rhythms and pontomedullary rhythms to blockers of persistent sodium channels.

A second group of intrinsic bursters, dependent on a conductance through calcium channels, has been identified using thick slice in vitro preparations of neonatal mouse (13, 27, 28). Neurons with similar characteristics have not yet been recorded in situ or in vivo.

Finally, some laboratories rejected an essential role for any pacemakers even in the genesis of in vitro rhythms (2, 3, 6). This conclusion is based on the observation that an in vitro rhythm activity can be restored following a blockade of pacemaker discharge involving both persistent sodium and calcium conductances. Respiratory rhythm generation is considered to result from interactions among neurons in a localized medullary neuronal circuit. The nature of rhythm generation by this circuit is undefined. Synaptic inhibition is only considered essential for coordination with another medullary region in which expiratory activities are generated independent of those generating inspiratory discharge. Others consider that the in vitro rhythms that can be induced following a blockade of pacemaker discharge are not related to either the neurogenesis of eupnea or gasping (28).

In summary, a critical problem is determining what the rhythms generated in vitro actually are and how they relate to adequately defined motor behaviors (e.g., eupnea, gasping) in vivo and in situ. Differences between in vitro findings, compared with those in vivo or in situ, may not reflect “plasticity” or “transformations” but rather fundamentally different mechanisms of rhythm generation.

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