Release your inhibitions: the role of postinhibitory rebound and synaptic inhibition in the generation of expiratory activity

Robert TR Huckstepp[2] Gregory D Funk[1]

[1] Department of Physiology, University of Alberta, Edmonton, Canada

[2] School of Life Sciences, University of Warwick, Coventry, UK

Breathing in mammals is a fundamental behaviour produced by movements generated and controlled by the central nervous system. The formation of the diaphragm in mammals has led to a unique phenomenon, inspiratory led breathing. In fact, in most mammals at rest, only inspiration is active – expiration is passive. As such research into mechanisms of respiratory rhythm generation has focussed on the inspiratory oscillator, the preBötzinger Complex (preBötC). Whilst we now understand a large number of fundamental properties underlying the behaviour of the preBötC, much less is known about its expiratory counterpart that generates active expiration under conditions of increased respiratory drive; e.g., exercise.

At birth, the expiratory oscillator, referred to as the parafacial respiratory group (pFRG), is comprised of at least two neuronal subpopulations that discharge during the late expiratory phase (active expiration). One subpopulation is indistinguishable from chemosensitive neurons in the retrotrapezoid nucleus (RTN); they are CO_2 sensitive, glutamatergic and have the same molecular profile; e.g., they express NK1R, Phox2b, and Galanin but do not express TH[1-3]. The other subpopulation is CO_2 insensitive, inhibitory and does not express NK1R, Phox2b, or Galanin[3] - clearly distinct from chemosensitive RTN neurons. During development the expiratory oscillator changes dramatically: its activity becomes suppressed at rest[4]; it loses its chemosensitivity; the Phox2b positive subpopulation of neurons appears to be lost; and it contains both glutamatergic and GABAergic neurons[4,5]. Rhythmic respiratory activity of the chemosensitive neurons also disappears. Based on these differences, the adult expiratory oscillator is referred to as the lateral parafacial region (pF_L). The mechanisms underlying this transformation are unknown, as the majority of studies into active expiratory behaviour have focussed on the motor output and defining the conditions that recruit the pF_L, including REM sleep, elevated CO_2 and reduced O_2.

In this issue of the *Journal of Physiology* Magalhães *et al*., have performed the first electrophysiological characterization of pF_L neurons and unmasked the synaptic and intrinsic membrane properties that shape their expiratory discharge. Single cell PCR revealed not only a lack of markers of RTN neurons, but also the absence of TASK2 or GPR4 that have, somewhat controversially, been linked to the intrinsic chemosensitivty of RTN neurons. These data provide molecular evidence that the pF_L and RTN are functionally distinct, and may explain the CO_2/pH insensitivity in pF_L neurons[6].

Most importantly, the impressive 3 hour long whole-cell recordings in an *in situ* preparation performed by Magalhães *et al*. have revealed that the rhythmic bursting behaviour of pF_L neurons during hypercapnia is determined by an interaction between intrinsic membrane properties and changes in inhibitory synaptic input. pF_L neurons lack persistent sodium channels (I_{NaP})[6], which underlie the intrinsic oscillatory properties of many neurons, including respiratory neurons. They share many conductances with cardiac pacemakers, including TREK-1, HCN, NALCN and Cav3.1/3.2, where oscillatory bursting is induced by a TREK-1 hyperpolarising current[7], which activates HCN[8], which works in conjunction with NALCN[9,10] to generate a slow membrane depolarisation that activates Cav3.1/3.2 and triggers the burst. Importantly, while these conductances may facilitate oscillatory firing of pF_L neurons during active expiration, their relative densities do not support intrinsic oscillatory behaviour. When synaptically isolated, pF_L neurons fire tonically, perhaps explaining why abdominal activity becomes tonic in the absence of network activity. Thus, recruitment of active, rhythmic expiration by elevated CO_2 does not result from the activation of intrinsic oscillatory properties, but from changes in the pattern of synaptic inhibition. The

expiratory oscillator is silent at normal levels of CO_2 (normocapnia) because the membrane potential of pF_L neurons remains subthreshold due to post-synaptic GABAergic and glycinergic inhibition. While always subthreshold, their membrane potential varies throughout the respiratory cycle - they are most hyperpolarised during inspiration, more depolarised during post-inspiration as GABAergic inhibition is released and depolarised even further during phase 2 expiration when glycinergic inhibition is also removed[6]. When CO_2 levels increase, pF_L neurons hyperpolarize even further during inspiration due to increased frequency of GABA and glycinergic iPSCs. Release from this inhibition results in a greater than normal post inspiratory depolarization (and discharge in some cycles) due to a Cav3.2 T-type Ca2+ channel-mediated postinhibitory rebound, which is enhanced due to greater inhibition during the inspiratory phase and reductions in post inspiratory glycinergic iPSCs. As expiration proceeds inhibitory signalling is further diminished[6] and pF_L neurons continue to depolarize and discharge during phase 2 expiration.

These data of Magalhães *et al*., significantly advance understanding of the regions and mechanisms underlying active expiration. The proposed model also differs markedly from the contemporary hypothesis that active expiratory firing of pF_L neurons is driven by a decrease in inhibitory input coupled to increased excitatory input from RTN neurons that have a strong local projection to the pF_L[5]. These data also suggest that excitatory input into the pF_L does not play a major role in the generation of active expiration. Glutamatergic sEPSCs were observed during all phases of the respiratory cycle and were not altered by hypercapnia. Moreover, release from fast synaptic excitation did not alter pF_L neuronal firing[6]. Thus, excitatory input may simply provide a tonic depolarising input that works in conjunction with release from inhibitory input and PIR to induce rhythmic pF_L behaviour[6]. An important caveat of the study is that Magalhães *et al*., only recorded from glutamatergic neurons, though we know local GABAergic interneurons are also present[6]. It is therefore possible that excitatory RTN neurons project to GABAergic pF_L neurons, that in turn provide inhibitory drive to pF_L neurons, from which excitatory pF_L neurons may rebound. This would provide a source of GABAergic input that could work in conjunction with glycingeric inputs that may come from the BotC as proposed by Magalhães *et al*.,[6] or from inhibitory preBotC neurons[11].

In summary, this exciting study by Magalhães *et al*.[6] has inverted our thinking about the inner workings of the expiratory oscillator. Rather than a tonically supressed oscillator that escapes inhibition when respiratory drive is high, it now appears that inhibition is necessary to supress active expiration at rest, and that increases in inspiratory phase inhibition and decreases in expiratory phase inhibition are necessary to drive active expiration under conditions of elevated CO_2.

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