"Physiological and Morphological Characterization of Genetically Defined Classes of Interneurons in Respiratory Rhythm and Pattern Generation of Neonatal Mice"

Maria Cristina De Guzman Picardo

College of William & Mary, Department of Applied Science, 2012
Field: Neuroscience, Degree: Ph.D.

Advisor: Christopher A. Del Negro, Associate Professor of Applied Science

Abstract

Breathing in mammals depends on an inspiratory-related rhythm that is generated by glutamatergic neurons in the preBötzinger complex (preBötC), a specialized site of the lower brainstem. Rhythm-generating preBötC neurons are derived from a single lineage that expresses the transcription factor (TF) *Dbx1*, but the cellular mechanisms of rhythmogenesis remain incompletely understood. To elucidate these mechanisms we comparatively analyzed *Dbx1*-expressing neurons (*Dbx1*$^+$) and *Dbx1*$^-$ neurons in the preBötC in knock-in transgenic mice. Whole-cell recordings in rhythmically active newborn mouse slice preparations showed that *Dbx1*$^+$ neurons activate earlier in the respiratory cycle and discharge greater magnitude inspiratory bursts compared to *Dbx1*$^-$ neurons. Furthermore, *Dbx1*$^+$ neurons required significantly less input current to discharge spikes (rheobase) in the context of network activity. The expression of intrinsic membrane properties indicative of A-current (I_A) and hyperpolarization-activated current (I_h) was generally mutually exclusive in *Dbx1*$^+$ neurons, which may indicate rhythmogenic function. In contrast, there was no such relationship in the expression of intrinsic currents I_A and I_h in *Dbx1*$^-$ neurons. Confocal imaging and digital reconstruction of recorded neurons revealed dendritic spines on *Dbx1*$^-$ neurons, but *Dbx1*$^+$ neurons were spineless. *Dbx1*$^+$ neuron morphology was largely confined to the transverse plane whereas *Dbx1*$^-$ neurons projected dendrites to a greater extent in the parasagittal plane (rostrocaudally). A greater percentage of *Dbx1*$^+$ neurons showed contralaterally projecting axons whereas *Dbx1*$^-$ neurons showed axons projecting in the rostral direction, which were severed by transverse cutting of the slice. Our data suggest that the rhythmogenic properties of *Dbx1*$^+$ neurons include a higher level of intrinsic excitability that promotes burst generation in the context of network activity, which may be attributable to dendritic active properties that are recruited by excitatory synaptic transmission. Along with *Dbx1*, the TF *Math1* has been shown to give rise to neurons that have important respiratory functions, including a potential role in coordinating the inspiratory and expiratory phases. To evaluate this role, we performed physiological and morphological characterizations of *Math1*$^+$ neurons in transgenic mice and found that one out of six recorded *Math1*$^+$ neurons showed expiratory activity. The expiratory *Math1*$^+$ neuron appeared to be have a larger soma as well as a greater somatodendritic span in all axes (dorsal-ventral, medial-lateral and rostral-caudal) than the non-respiratory modulated *Math1*$^+$ neurons. This suggests that respiratory modulated *Math1*$^+$ neurons may be physiologically and morphologically specialized compared to non-rhythmic *Math1*$^+$ neurons. Their larger morphological span and rhythmic expiratory modulation could be indicative of a function in coordinating phasic activity between inspiratory and expiratory oscillators. Although our findings are still preliminary, the data thus far are consistent with a hypothesized respiratory network model wherein the *Math1*$^+$ neurons function in coordinating the pattern of inspiration and expiration. Identifying and characterizing hindbrain interneurons according to developmental genetic origins as well as physiological properties provides complementary information to help elucidate the cellular mechanisms underlying the generation and coordination of the respiratory rhythm.