Phenotypic Properties and Intrinsic Currents of Neurons Involved in the Neural Generation of Mammalian Breathing

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Abstract

Breathing is essential for mammalian life. Although there is an emerging consensus that the inspiratory respiratory rhythm is generated in a lower brainstem region known as the preBötzinger Complex (preBötC), the mechanism of rhythmogenesis is still unclear. Additionally, the modulation of intrinsic currents within preBötC neurons has yet to be fully elucidated. This dissertation addresses both of these issues and relies on imaging, electrophysiological, and modeling techniques. The first chapter examines the size and composition of the preBötC. Previously, it has been shown that preBötC neurons expressing substance P (SP)-sensitive neurokinin receptors (NKR) are essential for normal breathing *in vivo*. Combined with an *in vitro* study that indicated that nearly all inspiratory neurons respond to SP, these data suggested that the critical rhythmogenic population of neurons are NKR^+. We show that ~40% of the putatively rhythmogenic population of neurons could be identified as NKR^+ *in vitro* whereas most of the neurons responded to SP. We also show how this disparity may be attributed to gap junctions between inspiratory neurons that transmit the response of SP to neighboring neurons. This may resolve much of the conflict between the previous *in vitro* and *in vivo* studies that reached widely disparate conclusions about the number of rhythmogenic neurons that express NKRs. Using additional data, we also offer a rough estimate of the size of the rhythmogenic population of neurons. The chapter also decribes the means by which SP excites the vast majority of preBötC neurons by illustrating the characteristics of the SP-activated current (I_{SP}) in these neurons. In the subsequent chapter, we characterize a voltage-dependent potassium current that is involved in maintaining stable rhythms during normal fictive breathing. This study shows that the majority of putatively rhythmogenic neurons exhibit a low-threshold, slowly-inactivating transient potassium current (I_A) and that blockade of I_A in the context of normal network activity has deleterious effects on the frequency and discharge pattern of inspiratory activity. Therefore, specific intrinsic currents like I_A play a key role in ensuring stable rhythmogenesis by keeping network activity synchronous and coherent. The third chapter presents a mathematical model of heterogeneous and rhythmogenic neurons that initiate network bursts. We show how this behavior relies on feedback synaptic connections within the network that reinforces activity, i.e., *recurrent-excitation*. We also compare model results to experimental data and make testable predictions. The experimental data includes applications of riluzole, which blocks the persistent Na^+ current while not preventing rhythmogenesis, and the aforementioned experiments that showed that destruction of NKR^+ neurons prevents normal breathing. The final chapter elaborates on the discussion of I_{SP} from the first chapter and presents evidence suggesting that a cyclic adenosine monophosphate (cAMP)-modulated non-specific cation channel may account for the depolarizing response in preBötC neurons from several neuromodulators. These channels may be a primary target of convergent mechanisms that alter the respiratory rhythm from different afferent projections. Altogether, this dissertation advances the field's understanding on several fronts. We have distinguished possible functional roles of neurons from electrophysiological characteristics, estimated the number of neurons necessary for rhythmogenesis, characterized I_{SP}, and clarified the distribution of NKRs in inspiratory neurons. We have identified and characterized a voltage-dependent potassium currrent important for inspiratory activity and analyzed its role. We have also described in detail how rhythmic bursts form from recurrent excitation and how this relates to experimental data. Finally, we have identified and begun characterizing a potentially important and novel mechanism for the modulation of membrane potentials in critical inspiratory neurons.