Studies of Respiratory Rhythm Generation Maintained in Organotypic Slice Cultures

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Field: Neuroscience Degree: Ph.D.

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Abstract

Breathing is an important rhythmic motor behavior whose underlying neural mechanisms can be studied *in vitro*. The study of breathing rhythms *in vitro* has depended upon reduced preparations of the brainstem that both retain respiratory-active neuronal populations and spontaneously generate respiratory-related motor output from cranial and spinal motor nerves. Brainstem-spinal cord *en bloc* preparations and transverse medullary slices of the brainstem have greatly improved the ability of researchers to experimentally access and thus characterize interneurons important in respiratory rhythmogenesis. These existing *in vitro* preparations are, however, not without their limitations. For example, the window of time within which experiments may be conducted is limited to several hours. Moreover, these preparations are poorly suited for studying subcellular ion channel distributions and synaptic integration in dendrites of rhythmically active respiratory interneurons because of tortuous tissue properties in slices and *en bloc*, which limits imaging approaches. Therefore, there is a need for an alternative experimental approach. Acute transverse slices of the medulla containing the preBötzinger complex have been exploited for the last 25 years as a model to study the neural basis of inspiratory rhythm generation. Here we transduce such preparations into a novel organotypic slice culture that retains bilaterally synchronized rhythmic activity for up to four weeks *in vitro*. Properties of this culture model of inspiratory rhythm are compared to analogous acute slice preparations and the rhythm is confirmed to be generated by neurons with similar electrophysiological and pharmacological properties. The improved optical environment of the cultured brain tissue permits detailed quantitative calcium imaging experiments, which are subsequently used to examine the subcellular distribution of a transient potassium current, I_A, in rhythmically active preBötC interneurons. I_A is found on the dendrites of these rhythmically active neurons, where it influences the electrotonic properties of dendrites and has the ability to counteract depolarizing inputs. These results suggest that excitatory input can be transiently inhibited by I_A prior to its steady-state inactivation, which would occur as temporally and spatially summating synaptic inputs cause persistent depolarization. Thus, rhythmically active interneurons are equipped to appropriately integrate the activity state of the inspiratory network, inhibiting spurious inputs yet yielding to synaptic inputs that summate, which thus coordinates the orderly recruitment of network constituents for rhythmic inspiratory bursts.