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Respiratory Physiology & Neurobiology 147 (2005) 145–157
RESPIRATORY PHYSIOLOGY & NEUROBIOLOGY
www.elsevier.com/locate/resphysiol

Determinants of inspiratory activity

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Accepted 6 May 2005

Abstract

In vitro and in vivo studies have identified the pre-Bötzinger complex as an important kernel for the generation of inspiratory activity. The mechanisms underlying inspiratory rhythm generation involve pacemaker as well as synaptic mechanisms. In slice preparations, blockade of pacemaker properties with blockers for the persistent Na^+ current, and the Ca^{2+}-activated inward cationic current, abolishes respiratory activity. Here we show that blockade of the persistent Na^+ current alone is sufficient to abolish respiratory activity in the in situ preparation. Although pacemaker neurons may be critical for establishing the basic respiratory rhythm, their rhythmic output is modulated by many elements of the respiratory network. For example, levels of synaptic inhibition control whether they burst or not, and endogenously released neuromodulators, such as serotonin and substance P modulate their intrinsic membrane currents. We hypothesize that the balance between synaptic and intrinsic pacemaker properties in the respiratory network is plastic, and that alterations of this balance may lead to dynamic reconfigurations of the respiratory network, which ultimately give rise to different activity patterns.

Keywords: Pacemaker neurons; Pre Bötzinger complex; In vitro studies; In situ preparation; Brainstem; Rhythm generation; Synaptic inhibition

1. Introduction

The majority of repetitive rhythmic motor activities are generated by neuronal networks located within the central nervous system. These neuronal networks, also referred to as central pattern generators (CPGs) (Alford et al., 2003), can generate a basic rhythmic activity even after isolation from their natural afferent inputs. The neuronal networks that generate the rhythmic activity patterns for breathing, swallowing, coughing and vomiting are no exception (Lindsey, 2001; Salmoiraghi and Burns, 1960a). Major progress has been made in understanding the mechanisms of rhythm generation in the CPG for breathing. Isolated from afferent inputs and efferent outputs, the pre-Bötzinger complex (Smith et al., 1991) is capable of generating various inspiratory activity patterns that are thought to contribute to the generation of different forms of respiratory behaviors including fictive eupneic activity, fictive sigh and fictive gasping activity (Lieske et al., 2000, 2001; Ramirez and Lieske, 2003). The importance of the pre-Bötzinger complex for breathing in the in vivo animal has been clearly demonstrated with various experimental approaches (Gray

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doi:10.1016/j.resp.2005.05.003

et al., 2001; Solomon, 2002, 2003; Wenninger et al., 2004a, 2004b). The ability of central pattern generators to continue to generate rhythms upon isolation from other parts of the CNS has facilitated research into the cellular mechanisms that underlie rhythm generation. For this reason, the respiratory network has become a model system for rhythm generation in general. Insights gained in this mammalian neuronal network may have implications for better understanding rhythm generating networks in other parts of the mammalian nervous system. Thus, although we will focus in the present review on the neuronal control of inspiratory activity, we would like to emphasize that similar mechanisms could be found in other motor systems as well. In particular, the important interplay of pacemaker and synaptic properties in rhythm generation is a recurrent theme in many neuronal networks, not just the respiratory network (Arshavsky, 2003; Ramirez et al., 2004). In the present study, we will review published data and include novel results obtained in an in situ preparation of the medulla that in contrast to slice preparations, maintains portions of the circulatory system. These findings as well as the methods associated with these experiments are clearly marked as "Results" to avoid confusion with the discussion of previously published work.

Three main points will be discussed in this review: (1) the importance of pacemaker neurons in respiratory rhythm generation; (2) the role of synaptic inhibition in respiratory rhythm generation; and (3) the modulation of pacemaker properties by neuromodulators. We will conclude that much of the neuronal circuitry necessary to generate inspiratory activity is localized within the medulla, specifically within the pre-Bötzinger complex.

We would like to emphasize that recent studies suggest that the respiratory network contains an additional kernel for respiratory rhythm generation: a network located rostral to the pre-Bötzinger complex, which appears to generate expiratory activity (Onimaru and Homma, 2003; Janczewski et al., 2002; Ballanyi et al., 1999; Mellen et al., 2003). An important implication stemming from these studies is that the pre-Bötzinger complex may be specifically the kernel for *inspiratory* rhythm generation as opposed to a kernel important for *respiratory* rhythm generation in general.

2. The generation of the inspiratory rhythm

Our current understanding of inspiratory rhythm generation has been advanced by two key findings: Firstly, the pre-Bötzinger complex, located in the ventrolateral medulla is capable of generating rhythmic inspiratory activity when isolated in transverse slice preparations (Smith et al., 1991). Secondly, the same medullary area is capable of generating not only one type of inspiratory activity pattern, but three distinct activities that are defined as fictive eupneic, sigh and gasping activity (Ramirez and Lieske, 2003; Lieske et al., 2000, 2001).

3. Role of pacemaker neurons in generating respiratory activity

As is the case for most central pattern generating networks, the respiratory rhythm emerges through a combination of synaptic and intrinsic membrane properties that include bursting pacemaker properties (Ramirez et al., 2004; Butera et al., 1999; Del Negro et al., 2002a; Smith et al., 2000). The potential contribution of synaptic mechanisms and pacemaker properties to the process of respiratory rhythm generation has already been postulated in the late 19th and early 20th century (Marckwald, 1887; Winterstein, 1911; Salmoiraghi and Burns, 1960a, 1960b), but it took more than a century to routinely record from respiratory pacemaker neurons. This has led to a better understanding of how pacemaker neurons contribute to respiratory rhythm generation. Pacemaker neurons are coupled via glutamatergic synaptic excitation (Koshiya and Smith, 1999) and are highly influenced by synaptic inhibition as will be discussed below. Studies aimed at unraveling the mechanisms underlying burst generation in respiratory pacemaker neurons suggest that pacemaker bursting depends on the activation of the persistent sodium current. In a computational model of the activation and inactivation properties, the persistent sodium current was able to explain much of pacemaker behavior in respiratory pacemaker neurons (Butera et al., 1999). Thus, the persistent sodium current has long been hypothesized to be the principal ion channel generating pacemaker activity in the respiratory network (Rekling and Feldman, 1997; Butera et al., 1999; Smith et al., 2000; McCrimmon et al., 2001; Del Negro et al.,