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Neural mechanisms underlying respiratory rhythm generation in the lamprey

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ABSTRACT

The isolated brainstem of the adult lamprey spontaneously generates respiratory activity. The paratrigeminal respiratory group (pTRG), the proposed respiratory central pattern generator, has been anatomically and functionally characterized. It is sensitive to opioids, neurokinins and acetylcholine. Excitatory amino acids, but not GABA and glycine, play a crucial role in the respiratory rhythmogenesis. These results are corroborated by immunohistochemical data. While only GABA exerts an important modulatory control on the pTRG, both GABA and glycine markedly influence the respiratory frequency via neurons projecting from the vagal motoneuron region to the pTRG. Noticeably, the removal of GABAergic transmission within the pTRG causes the resumption of rhythmic activity during apnea induced by blockade of glutamatergic transmission. The same result is obtained by microinjections of substance P or nicotine into the pTRG during apnea. The results prompted us to present some considerations on the phylogenesis of respiratory pattern generation. They may also encourage comparative studies on the basic mechanisms underlying respiratory rhythmogenesis of vertebrates.

1. Introduction

The vertebrate nervous system is organized in a similar way throughout vertebrate phylogenesis, although the level of complexity increases. Lampreys are jawless vertebrates known as cyclostomes. They have changed comparatively little during evolution, and became separated from the main vertebrate line 560 million years ago (Kumar and Hedges, 1998). The lamprey central nervous system can be regarded as a vertebrate prototype, with the experimental advantage that it has fewer neurons than higher vertebrates and can be maintained *in vitro*. Studies on neuronal networks of the lamprey proved to be highly useful to provide insights into the basic mechanisms of central pattern generators (CPGs) of rhythmic activities, such as locomotion and respiration (Grillner, 2003, 2006). The lamprey model has been used for many years to identify the cellular mechanisms involved in the generation and control of locomotion in extreme detail and, more recently, to investigate the neural mechanisms underlying respiratory rhythm generation. The basic features of the neural organization as well as those of rhythmogenic networks have been conserved throughout vertebrate evolution (Grillner, 2003; Mutolo et al., 2007, 2010; Robertson et al., 2007; Kinkead, 2009; Ericsson et al., 2011, 2013; Stephenson-Jones et al., 2011, 2012a, 2012b; Cinelli et al., 2013).

This review examines the main characteristics of the lamprey respiratory network and describes recent results concerning respiratory rhythm generation and the relevant role of some neurotransmitters and neuromodulators. We also consider the characteristics of respiratory CPGs during vertebrate evolution and possible evolutionary trends in respiratory rhythm generation.

2. General features of the lamprey respiratory system

In the adult lamprey, breathing is produced by synchronous contractions of the branchial muscles that force water out of the gill openings; the inhalation phase is passive and is produced by the elastic recoil of cartilaginous baskets surrounding the gill sacs (Rovainen, 1977, 1979). The isolated brainstem of the adult lamprey spontaneously generates respiratory neuronal activity *in vitro*; this activity closely resembles that underlying the respiratory behavior of intact animals and persists after transections of the brain at both the obex and isthmus level (Rovainen, 1977, 1983; Thompson, 1985; Russell, 1986). Thus, both the neural network responsible for respiratory rhythm generation and respiratory motoneurons are located within the brainstem. The results obtained in this preparation contribute to improve current knowledge on the synaptic transmission within the respiratory network of the lamprey and,

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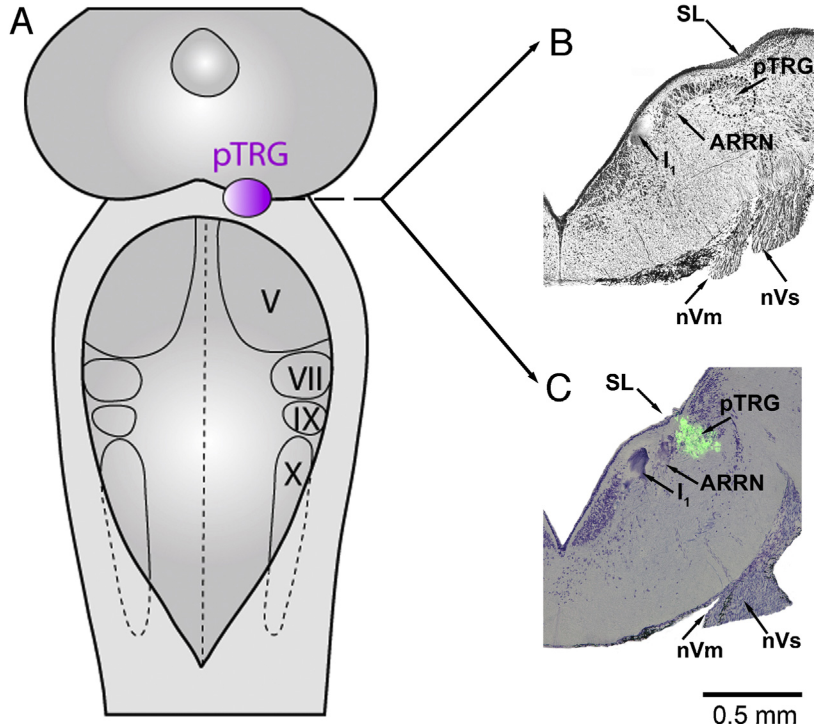

Fig. 1. Localization of the pTRG. (A) Schematic illustration of a dorsal view of the lamprey mesencephalon/rhombencephalon showing the levels of the coronal sections illustrated in B and C (arrows) and the location of the pTRG (pink area). (B) Photomicrograph of a transverse section of the rostral rhombencephalon showing the location of an horseradish peroxidase injection into the pTRG. (C) Photomicrograph of a transverse sections of the rhombencephalon showing the location of fluorescent beads microinjected into the pTRG (green). ARRN, anterior rhombencephalic reticular nucleus; I_1, isthmic Müller cell; nVm, motor root of the trigeminal nerve; nVs, sensory root of the trigeminal nerve; pTRG, paratrigeminal respiratory group; SL, sulcus limitans of His. V, trigeminal motor nucleus; VII, facial motor nucleus; IX, glossopharyngeal motor nucleus; X, vagal motor nucleus. B and C adapted from Mutolo et al. (2007) and Cinelli et al. (2014), respectively.

possibly, to obtain new hints for further investigations on the basic neural mechanisms operating in the respiratory network of higher vertebrates, including mammals. The vast majority of respiratory motoneurons are located in the facial, glossopharyngeal and, especially, vagal nuclei, while the neural aggregate responsible for respiratory rhythm generation appears to be located in a region rostrolateral to the trigeminal motor nucleus (Rovainen, 1977, 1979, 1983, 1985; Thompson, 1985; Russell, 1986; Bongianni et al., 1999, 2002, 2006; Guimond et al., 2003; Martel et al., 2007; Mutolo et al., 2007). Mutolo et al. (2007) reported that opioids have a modulatory role in the respiratory network and, in particular, that microinjections of the μ-opioid receptor agonist DAMGO at sites rostrolateral to the trigeminal motor nucleus abolish the respiratory rhythm. These apneic responses support the hypothesis that this specific opioid-sensitive region likely has a pivotal role in respiratory rhythmogenesis. Mutolo et al. (2007) proposed to name this area the paratrigeminal respiratory group (pTRG). The results on the depressant effects of opioids on the lamprey respiratory activity also imply that the inhibitory role of opioids on respiration is present at an early stage of vertebrate evolution. Respiration-related neurons with different firing patterns are present in the pTRG (Mutolo et al., 2007, 2010), thus corroborating our hypothesis on the involvement of the pTRG in respiratory rhythm generation. The different discharge patterns encountered in the pTRG may suggest different neuronal functions, but at present any attempt to ascribe a specific role to each type of neurons is only speculative.

3. Anatomical and functional characterization of the pTRG

An anatomical and functional characterization of the pTRG region has been recently provided (Cinelli et al., 2013, 2014). By retrograde labeling, we found neurons located in the isthmic periventricular cell layer with axonal projections to the vagal motoneuron region. Projecting neurons can be easily identified by anatomical landmarks, *i.e.* they are located in a dorsal aspect of the anterior rhombencephalic reticular nucleus, at the level of the isthmic Müller cell I_1, close to the sulcus limitans of His. This region corresponds closely to the pTRG as defined in our previous studies (Mutolo et al., 2007, 2010, 2011). Neurons located in the pTRG project to the ipsilateral and contralateral vagal motor nucleus as well as to the contralateral pTRG (Rovainen, 1985; Thompson, 1985; Russell, 1986; Gariépy et al., 2012; Cinelli et al., 2013). The results obtained with microinjections of several neuroactive drugs, such as DAMGO, substance P (SP), acetylcholine (ACh), glutamate or GABA receptor agonists and antagonists, exactly into this region (Mutolo et al., 2007, 2010, 2011; Cinelli et al., 2013, 2014) help to identify and characterize the pTRG and to support the notion that it corresponds to the respiratory CPG (see below). A schematic representation of a dorsal view of the lamprey mesencephalon/rhombencephalon showing the respiration-related areas along with photomicrographs of transverse sections illustrating the localization of the pTRG is reported in Fig. 1.

4. Glutamatergic mechanisms in the respiratory rhythmogenesis

Endogenously released excitatory amino acids play a crucial role in the lamprey respiratory rhythmogenesis acting on ionotropic receptors (Bongianni et al., 1999) and exert a modulatory role on respiratory activity via metabotropic receptors (Bongianni et al., 2002). The suppression of respiratory activity caused by bath application of ionotropic glutamate receptor antagonists (Bongianni et al., 1999; Mutolo et al., 2011) is mimicked by microinjections