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Research Paper

Catecholaminergic neurons in synaptic connections with pre-Bötzinger complex neurons in the rostral ventrolateral medulla in normoxic and daily acute intermittent hypoxic rats

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ABSTRACT

The rostral ventrolateral medulla (RVLM) contains cardiovascular-related catecholaminergic neurons and respiratory-related pre-Bötzinger complex (pre-BötC) neurons, which are intermingled and functionally connected for coordinating cardiorespiratory activities. Daily acute intermittent hypoxia (dAIH) is known to elicit respiratory plasticity. However, it is unclear if the catecholaminergic neurons directly synapse onto pre-BötC neurons, and if the local circuitry exhibits plasticity when exposed to dAIH. The present study was aimed to determine the synaptic phenotypes between dopamine-β-hydroxylase (DβH)-immunoreactive (ir) catecholaminergic neurons and neurokinin 1 receptor (NK1R)-ir pre-BötC neurons, and the effect of dAIH on the neuronal network. Immunofluorescence histochemistry was used to reveal immunoreactivities of DβH and NK1R in the RVLM of normoxic and dAIH rats. Synaptic phenotypes were examined with double-labeling immunoelectron microscopy. We found that DβH immunoreactivity was expressed in somata and processes, some of which were in close apposition to NK1R-ir pre-BötC neurons. DβH-ir gold particles were localized to somata, dendrites, and axonal terminals. DβH-ir terminals formed asymmetric synapses, and occasionally, symmetric synapses in the pre-BötC, featuring the local circuitry. Of the synapses, 28% in normoxic and 29.6% in dAIH groups were apposed to NK1R-ir dendrites. Significant increases in DβH expression and NK1R-ir processes were found in the dAIH group. Moreover, the area and number of processes in close appositions were significantly elevated, strongly suggesting that dAIH induced plasticity with increased connections and interactions between the cardiovascular- and respiratory-related neurons in the local circuitry. In conclusion, asymmetric synapses are predominant in the crosstalk between catecholaminergic and pre-BötC neurons in the RVLM, elaborating excitatory transmission driving the coupling of cardiorespiratory activities. The neural network manifests plasticity in response to dAIH challenge.

1. Introduction

The rostral ventrolateral medulla (RVLM) is composed of a heterogeneous population of neurons that elaborate local circuit network devoted mainly to respiratory and cardiovascular sympathetic functions. Cardiovascular- and respiratory-related neurons form two adjacent cell columns, with some intermingling among them (Arata et al., 1990; Ellenberger et al., 1990; Pilowsky et al., 1990). The close vicinity between these two groups of neurons indicates possible crosstalk for subtle control of cardiorespiratory homeostasis (Spyer and Gourine, 2009). Indeed, in vitro studies of slice and *en bloc* preparations in neonatal and young mice suggest that catecholamines are endogenously released from A1/C1 neurons to facilitate respiratory rhythm via α2

Abbreviations: CVLM, caudal ventrolateral medulla; dAIH, daily acute intermittent hypoxia; DβH, dopamine-β-hydroxylase; 5-HT_{2A}R, 5-hydroxytryptamine 2A receptor; ir, immunoreactive; IVLM, intermediate ventrolateral medulla; LTF, long-term facilitation; NK1R, neurokinin 1 receptor; PNMT, phenylethanolamine *N*-methyl transferase; PB, phosphate buffer; PBS, phosphate buffered saline; pre-BötC, pre-Bötzinger complex; PKC, protein kinase C; RVLM, rostral ventrolateral medulla.

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adrenoceptors (Zanella et al., 2006). Selective photostimulation of C1 neurons in the RVLM increases breathing in anesthetized and conscious mice (Abbott et al., 2013). Genetic mutants of the *Mecp2* gene that controls the formation of catecholaminergic neurons during development exhibited respiratory deficits during the prenatal and postnatal periods (Viemari et al., 2005). Catecholamines normally enable stabilization of respiratory network activity (Viemari and Ramirez, 2006), and a disturbance of these neuromodulators leads to breathing deficits such as reported in Rett syndrome (Viemari et al., 2005; Viemari, 2008).

The pre-Bötzinger complex (pre-BötC), residing in the RVLM, is proposed to be the respiratory rhythm generator and is critical for inspiratory rhythm oscillation (Smith et al., 1991; Ramirez and Richter, 1996; Feldman and Del Negro, 2006; Smith et al., 2013). Neurons in the pre-BötC are heterogeneous and express high levels of neurokinin 1 receptors (NK1R) (Gray et al., 1999, 2001; Wang et al., 2001). Bilateral destruction of most pre-BötC NK1R-immunoreactive (ir) neurons leads to an ataxic rhythm during wakefulness and apnea during sleep in adult rats (Gray et al., 2001; McKay et al., 2005). Therefore, NK1R immunoreactivity has been used as a marker for the pre-BötC (Gray et al., 1999, 2001; Guyenet and Wang, 2001; Wang et al., 2001; Schwarzacher et al., 2011). Neuromodulation of synaptic interactions between neurotransmitters and receptors is essential for neuronal network functioning. While previous tracing studies have reported on the close proximity between respiratory-related and catecholaminergic neurons in the RVLM (Ellenberger et al., 1990; Pilowsky et al., 1990), a deeper understanding of their synaptic relationship is still lacking. We hypothesize that the two groups of neurochemically-distinct and functionally-specific neurons engage in local synaptic circuits in the pre-BötC. As a first step to test our hypothesis, we aimed to provide the structural basis for a coupling of cardiovascular and respiratory activities. Furthermore, we hypothesized that the local neural network manifests plasticity in response to daily acute intermittent hypoxic (dAIH) challenge, a moderate hypoxic paradigm that enables phrenic and hypoglossal nerves to express respiratory metaplasticity known as long-term facilitation (LTF) (Wilkerson and Mitchell, 2009; Lovett-Barr et al., 2012; Doi and Ramirez, 2010). Also, the coupling of sympathetic and respiratory activities is enhanced in animals and human repeatedly exposed to intermittent hypoxia (Dick et al., 2014; Moraes et al., 2014). In this study, we aimed to investigate changes in the synaptic connections between the catecholaminergic and pre-BötC neurons.

2. Materials and methods

All experiments were performed on adult male Sprague-Dawley rats (230–250 g). Animals were housed in controlled environment at a constant temperature (22 ± 2 °C) and 12 h light/dark cycle, with food and water ad libitum. Protocols were approved by the Northwest China Committee of Experimental Animal Care and the Committee on the Use of Live Animals in Teaching and Research of The University of Hong Kong, and their regulations were in accordance with NIH guidelines.

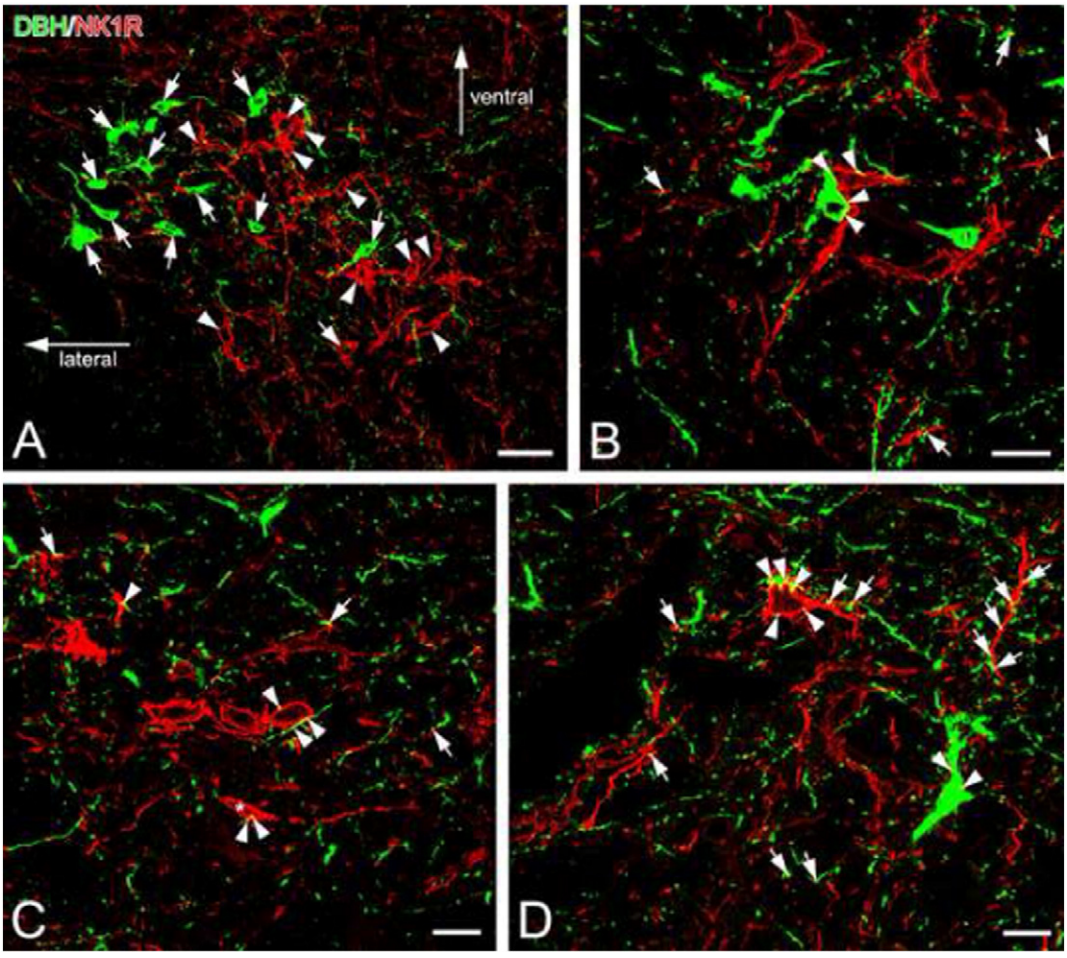

Fig. 1. Immunofluorescent micrographs showing dopamine-β-hydroxylase (DβH) and neurokinin 1 receptor (NK1R) double-labeling in the pre-Bötzinger complex (pre-BötC) in the normoxic group. DβH immunoreactivity is visualized with Alexa 488 (green) and NK1R immunoreactivity with Texas Red (red). DβH immunoreactivity recognizes catecholaminergic (C1) neurons (arrows in A) and NK1R immunoreactivity outlines pre-BötC neurons (arrowheads in A). Arrowheads in B–D present the close appositions of two different fluorophores with the somata. Arrows in B–D show DβH-immunoreactive (ir) varicosities in close associations with NK1R-ir processes. Asterisk in C illustrates a small fusiform NK1R-ir neuron. Scale bars = 50 μm (A), 25 μm (B–D).