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Research Report

Glutamatergic Kölliker–Fuse nucleus neurons innervate hypoglossal motoneurons whose axons form the medial (protruder) branch of the hypoglossal nerve in the rat

Shigefumi Yokota[a], *Jian-Guo Niu*[a,b], *Toshiko Tsumori*[a], *Tatsuro Oka*[a], *Yukihiko Yasui*[a,*]

[a]*Department of Anatomy and Morphological Neuroscience, Shimane University School of Medicine, Izumo 693-8501, Japan*
[b]*Department of Anatomy, Ningxia Medical University, Yinchuan 750004, PR China*

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ABSTRACT

This study was performed to understand the anatomical substrates for Kölliker–Fuse nucleus (KFN) modulation of respiratory-related tongue movement. After application of cholera toxin B subunit (CTb) to the medial branch of the hypoglossal nerve (HGn) and injection of biotinylated dextran amine (BDA) into the KFN ipsilaterally, an overlapping distribution of BDA-labeled axon terminals and CTb-labeled neurons was found in the ventral compartment of the hypoglossal nucleus (HGN) ipsilateral to the application and injection sites. At the electron microscopic level, the BDA-labeled terminals made asymmetrical synaptic contacts predominantly with dendrites of the HGN neurons, some of which were labeled with CTb. Using retrograde tracing combined with *in situ* hybridization, we demonstrated that almost all the KFN neurons sending their axons to the HGN were positive for vesicular glutamate transporter (VGLUT) 2 mRNA but not glutamic acid decarboxylase 67 mRNA. Using a combination of anterograde and retrograde tracing techniques and immunohistochemistry for VGLUT2, we further demonstrated that the KFN axon terminals with VGLUT2 immunoreactivity established close contact with the HGN motoneurons whose axons constitute the medial branch of the HGn. The present results suggest that glutamatergic KFN fibers may exert excitatory influence upon the HGN motoneurons sending their axons to the medial branch of the HGn for the control of protruder tongue muscles contraction to maintain airway patency during respiration.

1. Introduction

The tongue participates in a wide variety of motor tasks, including respiration (Lowe, 1980), and it is important to understand the respiratory control of hypoglossal nucleus (HGN) motoneurons because they are involved in maintaining airway patency during normal breathing, as well as in the pathogenesis of obstructive sleep apnea (Fregosi and Fuller, 1997; Remmers et al., 1978). However, little is known about the neuroanatomical substrates for network modulation of respiratory-related hypoglossal motor output.

The extrinsic and intrinsic tongue muscles are innervated by motoneurons in the HGN, where motoneurons innervating the protruder and retractor tongue muscles have been revealed to exist in the ventral compartment and in the dorsal compartment of the nucleus, respectively by retrograde labeling studies

* *Corresponding author*. Fax: +81 853 20 2105.
E-mail address: yyasui@med.shimane-u.ac.jp (Y. Yasui).

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(Krammer et al., 1979; O'Reilly and FitzGerald, 1990; Uemura et al., 1979). Efferent fibers from the HGN form the hypoglossal nerve (HGn), and those innervating the protruder (genioglossus and geniohyoid) and retractor (styloglossus and hyoglossus) tongue muscles form medial and lateral branches of the HGn, respectively (Krammer et al., 1979; Sawczuk and Mosier, 2001). Neuroanatomical studies have further identified brainstem premotor inputs to HGN motoneurons by using retrograde tracing technique (Borke et al., 1983; Cunningham and Sawchenko, 2000; Roda et al., 2004; Takada et al., 1984; Takeuchi et al., 1980; Travers and Norgren, 1983), as well as by using transsynaptic labeling with pseudorabies virus (Chamberlin et al., 2007; Dobbins and Feldman, 1995; Fay and Norgren, 1997). These studies indicate that premotor neurons of the HGN are distributed predominantly in the lower brainstem regions, such as the raphe nuclei, sensory trigeminal nuclei and lateral tegmental field including the lateral reticular formation of the medulla oblongata as well as the supratrigeminal region, parabrachial nucleus (PBN) and Kölliker–Fuse nucleus (KFN) of the pons.

The PBN region including the KFN, which is deeply involved in respiratory control, is called the pontine respiratory group (Feldman, 1986) or used to be called the pneumotaxic center (Cohen and Wang, 1959; Lumsden, 1923). Glutamate injection into the KFN region at mid to rostral levels of the nucleus causes the most intense inspiratory facilitatory response (Chamberlin and Saper, 1994). Furthermore, Kuna and Remmers (1999) demonstrated that electrical stimulation of discrete areas within the KFN elicits a burst of action potentials in the HGn. They also indicated electrophysiologically that HGN motoneurons innervating protruder tongue muscles receive a selective premotor input from the KFN neurons. Protruder tongue muscles have been known to maintain airway patency (Harper and Sauerland, 1978), and the primary extrinsic protruder muscle, genioglossus muscle, is phasically active during inspiration not only in the anesthetized rat (Andrew, 1955), but also in the quietly sleeping rat (Megirian et al., 1985). Taken together, these findings suggest that there exists a KFN-protruder motoneurons pathway for maintaining airway patency during inspiration. However, there have been no studies to examine the potential contacts between the KFN fibers and the HGN motoneurons innervating protruder tongue muscles.

With respect to the neurotransmitter of the PBN neurons, our previous study (Yokota et al., 2007) indicated that most of the PBN neurons express vesicular glutamate transporter (VGLUT) 2 mRNA that is a marker for glutamatergic neurons, whereas small numbers of them express glutamic acid decarboxylase (GAD) 67 mRNA that is a marker for GABAergic neurons; however, the KFN contains large numbers of VGLUT2 mRNA-positive as well as GAD67 mRNA-positive neurons. Furthermore, we demonstrated that almost all the KFN neurons projecting to the rostral ventral respiratory group (rVRG) and phrenic nucleus (PhN) are VGLUT2 mRNA-positive but not GAD67 mRNA-positive (Yokota et al., 2007). As far as we know, however, there have been no studies to examine whether the KFN-HGN projection is glutamatergic or GABAergic.

In the present study, we first demonstrate the existence of a monosynaptic pathway from the KFN to the HGN motoneurons supplying the medial branch of the HGn by using a combined anterograde tracing with biotinylated dextran amine (BDA) and retrograde tracing with cholera toxin B subunit (CTb), and then examine whether HGN-projecting KFN neurons are glutamatergic or GABAergic by using a combined retrograde tracing with CTb and *in situ* hybridization for VGLUT2 mRNA and GAD67 mRNA. Finally, we examine whether or not the KFN axon terminals with VGLUT2 immunoreactivity are in contact with the HGN motoneurons supplying the medial branch of the HGn by using a combination of anterograde transport of BDA, retrograde transport of CTb and immunohistochemistry for VGLUT2.

2. Results

2.1. *Combined anterograde and retrograde tracing*

2.1.1. *Light microscopic study*

In this set of experiments, 5 of 15 operated rats received successful application of CTb to the medial branch of the HGn and injection of BDA into the KFN (Fig. 1A). CTb-labeled neurons were identified by the presence of brown-colored diaminobenzidine (DAB) reaction product in their cell bodies and dendrites (Fig. 2). In these rats, CTb-labeled neurons were distributed in the ventral compartment of the HGN throughout its rostrocaudal extent (Fig. 1B–F). At the level of the rostral pole of the HGN, neurons in the lateral subcompartment were labeled with CTb (Fig. 1B), and more caudally, neurons in the medial and lateral subcompartments as well as in the centrolateral subgroup just dorsal to the lateral subcompartment were labeled (Fig. 1C). At the midlevel of the HGN where the area postrema was seen, neurons not only in the medial and lateral compartments, and centrolateral subgroup, but also in the centromedial subgroup were labeled with CTb (Fig. 1D). In more caudal sections, CTb-labeled neurons in the medial and lateral subcompartments, as well as in the centromedial subgroup were still found (Fig. 1E). At the level near the caudal pole of the HGN, many neurons in the medial subcompartment were labeled with CTb, and additionally some labeled neurons were found in the lateral accessory subcompartment just ventrolateral to the confines of the HGN (Fig. 1F). Many CTb-labeled dendrites were seen within the ventral compartment, and some of them extended dorsally toward the dorsal compartment and dorsomedially toward the dorsal surface or the central canal. Dendritic bundles of the CTb-labeled neurons also extended laterally toward the adjacent reticular formation. On the other hand, BDA-labeled fibers and terminals stained black were distributed in and around the HGN as well as the dorsal vagal complex, bilaterally with a slight ipsilateral predominance (Fig. 1B–F). The ventral compartment of the HGN contained moderate numbers of BDA-labeled fibers with bouton-like varicosities throughout its rostrocaudal extent. The dorsal compartment showed light to moderate labeling; the labeling in the caudal part was slightly denser than that in the rostral part. In addition, moderate labeling was seen in the midline areas medial to the HGN, as well as in the ventral marginal area of the dorsal motor nucleus of the vagus nerve with a few labeled axons in the core of the nucleus. Heavy labeling was also found in the nucleus of the solitary tract (Fig. 1E, F). Within the neuropil in and around the HGN, bouton-like varicosities labeled with BDA were frequently in close apposition to dendrites and occasionally to somata of the CTb-labeled neurons (Fig. 2).