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Pharmacological analysis of excitatory and inhibitory synaptic transmission in horizontal brainstem slices preserving three subnuclei of spinal trigeminal nucleus

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Abstract

Spinal trigeminal nucleus (Vsp) consists of three subnuclei: oralis (Vo), interpolaris (Vi) and caudalis (Vc). Previous anatomical studies using antero-/retro-grade tracers have suggested that intersubnuclear ascending/descending synaptic transmissions exist between subnuclei. However, pharmacological properties of the intersubnuclear synaptic transmission have not been studied yet. Since three subnuclei are located in Vsp along rostro-caudal axis, it will be necessary to prepare horizontal brainstem slices to perform pharmacological analysis of the intersubnuclear synaptic transmission. We here show horizontal brainstem slices retaining three subnuclei, and that, using blind whole-cell recordings in the slices, synaptic transmission may be abundantly retained between subnuclei in the horizontal slices, except for the transmission from Vo to Vc. Finally, pharmacological analysis shows that excitatory and inhibitory synaptic responses, respectively, are mediated by AMPA and NMDA receptors and by $GABA_A$ and glycine receptors, with a differential contribution to the synaptic responses between subnuclei. We therefore conclude that horizontal brainstem slices will be a useful preparation for studies on intersubnuclear synaptic transmission, modulation and plasticity between subnuclei, as well as, further, other brainstem nuclei.

Keywords: Spinal trigeminal nucleus; Horizontal brainstem slice; Whole-cell patch-clamp recording; Synaptic transmission; EPSC; IPSC

1. Introduction

Somatosensory signals are frequently generated in oral and perioral tissues during food intake. Trigeminal sensory neurons primarily relay the signals from the periphery to second-order neurons existing in trigeminal sensory nuclei (Vsn). Vsn comprises mesencephalic nucleus, principal sensory nucleus (Vp) and spinal trigeminal nucleus (Vsp); the Vsp is further subdivided, from rostrally to caudally, into oralis (Vo), interpolaris (Vi) and caudalis (Vc) (Olszewski, 1950). Vsn integrates the somatosensory signals, including pain, and further transmits the signals into the higher brain areas via projection neurons in Vp and Vsp (Waite and Tracey, 1995). In Vsp, primary afferents associated with oral receptive fields including tooth pulp are preferentially terminated in the rostral nuclei above the obex such as Vp, Vo and Vi, while afferents with facial receptive fields primarily in Vc (Arvidsson and Gobel, 1981; Marfurt and Turner, 1984; Shigenaga et al., 1986; Takemura et al., 1991, 1993, 2006). Since all or part of the somatosensory signals from different peripheral areas are processed and integrated in the Vsn before being transmitted into the higher brain centers, the signals assigned in various areas of Vsn, particularly subnuclei of the Vsp, should be intimately interrelated (Takemura et al., 2006; Woda, 2003).

Abbreviations: AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; D-AP5, D-(−)-2-amino-5-phosphonopentanoic acid; EPSC, excitatory postsynaptic current; GABA, γ-amino-butyric acid; IPSC, inhibitory postsynaptic current; NMDA, *N*-methyl-D-aspartate; SG, substantia gelatinosa; Vc, caudalis; Vh, holing potential; Vi, interpolaris; Vo, oralis; Vp, principal sensory nucleus; Vsn, trigeminal sensory nuclei; Vsp, spinal trigeminal nucleus

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Interconnections supporting the notion for the intimate interrelationship between subnuclei of Vsp have been suggested by various early observations using histological (Gobel and Purvis, 1972; Khayyat et al., 1975) and antero- and retro-grade tracing methods (Falls, 1984a,b; Ikeda et al., 1982, 1984; Jacquin et al., 1990; Li et al., 2000; Lovick and Wolstencroft, 1983; Nasution and Shigenaga, 1987; Panneton and Burton, 1982; Voisin et al., 2002). In addition, anatomical (Voisin et al., 2002) or functional (Chiang et al., 2002; Dallel et al., 1998; Hu et al., 1981; Park et al., 2001; Woda et al., 2001) studies have recently demonstrated an ascending connection from Vc to Vo which may be required for C-fiber-mediated nociceptive transmission from Vc to Vo (Pajot et al., 2000). In spite of these studies above, pharmacological analysis of the intersubnuclear synaptic transmission have not been tried yet.

For pharmacological analysis of the synaptic transmission in Vsp, it is necessary to perform whole-cell voltage-clamp recordings in a live slice preparation that would retain all three subnuclei and allow visual guidance of the recording patch pipettes on each subnucleus targeted. Although transverse or horizontal slices of rat trigeminal nucleus have been used for electrophysiological recordings of, especially, Vc neurons (Grudt and Williams, 1994; Hamba and Onimaru, 1998; Hamba et al., 2000; Jennings, 2001; Jennings et al., 2003, 2004, 2006; Liang et al., 2004; Onodera et al., 2000; Wang et al., 2001), it has not been tried to prepare a single brainstem slice that preserves all three subnuclei because of their anatomical separation along the rostro-caudal axis. Therefore, we attempted to produce live slices that could retain all three subnuclei of Vsp by horizontally cutting brainstem tissue in a rostro-caudal direction, and performed blind whole-cell voltage-clamp recordings to investigate the pharmacological properties of excitatory and inhibitory synaptic transmission in the Vsp.

2. Methods

2.1. Dissection and preparation of horizontal brainstem slice

Experiments were approved by Institutional Animal Care and Use Committee of School of Dentistry, Kyungpook National University, and were carried out in accordance with the National Institute of Health guide for the Care and Use of Laboratory Animals. Sprague Dawley rats (6–14 day-old; male or female) were deeply anaesthetized by intraperitoneal injection of urethane (1.5 g/kg), and decapitated. To prepare live slices, the brain and a part of spinal cord were rapidly removed and immersed in ice-cold Krebs' solution (composition in mM: NaCl 117, KCl 3.6, $CaCl_2$ 2.5, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 25, and glucose 11; pre-oxygenated with 95% O_2/5% CO_2 at pH 7.4). After the removal of the cerebral cortex and the cerebellum, the remaining brain tissue block, that includes brainstem (up to 1–2 mm below obex), was glued, with the dorsal face down (Fig. 1A), with cyanoacrylate adhesive to the upper surface of a hard mounting cube block that was pre-fixed to the bottom of a slicing chamber. Then, the slicing chamber was placed on the stage of Vibratome 1000^+ (Vibratome, St. Louis, MO, USA), and was filled with ice-cold Krebs' solution. The first cut with razor blade was made to discard the ventral aspect of the brainstem. The blade was then lowered 400 μm, and usually two slices were cut and transferred to a glass beaker containing oxygenated Krebs' solution at room temperature (23–25 °C). After an incubation period lasting 60 min at room temperature, one side (either right or left) of the horizontal brainstem slice was transferred into a recording chamber, where it was submerged and immobilized with nylon strands drawn taut across a C-shaped silver wire (∼0.5 mm o.d.), and was continuously perfused (2–3 ml/min) with oxygenated

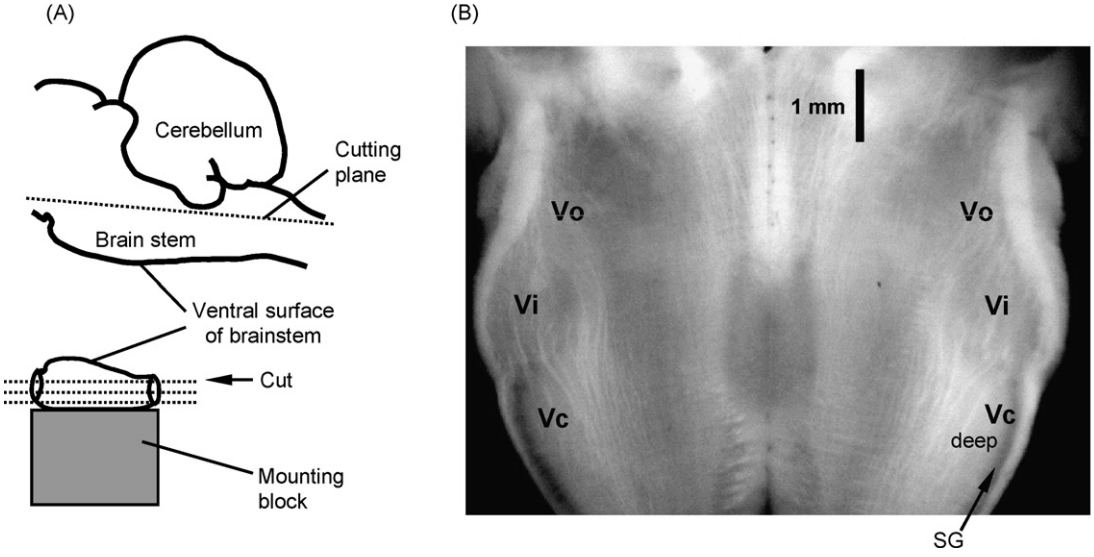

Fig. 1. Preparation of horizontal brainstem slice and identification of subnuclei of Vsp. Diagrams (A) show the horizontal cutting plane in the brainstem (upper diagram), and the trajectory (dot lines) of the razor blade during the cutting process of a brainstem block that was glued on a mounting block (lower diagram). (B) The locations of three subnuclei (Vo, Vi and Vc) of Vsp in a horizontal brainstem slice was identified in a digital photograph, and the SG area and deep layer of Vc are indicated.