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PROJECTIONS FROM THE INSULAR CORTEX TO PAIN-RECEPTIVE TRIGEMINAL CAUDAL SUBNUCLEUS (MEDULLARY DORSAL HORN) AND OTHER LOWER BRAINSTEM AREAS IN RATS

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Abstract—This study examined the projections from the rat insular cortex (Ins) to lower brainstem areas which are possibly involved in orofacial pain processing. We first examined distributions of Ins neurons projecting directly to the trigeminal caudal subnucleus (Vc, medullary dorsal horn) and oral subnucleus (Vo) which are known to receive orofacial nociceptive inputs. After injections of a retrograde tracer, Fluorogold (FG), into the medial part and lateral part of laminae I/II of Vc, many neurons were labeled bilaterally with a contralateral predominance in the rostral level of granular Ins (GI) and dysgranular Ins (DI) and the caudal level of GI/DI, respectively, but none in the agranular Ins (AI). After FG injections into laminae III–V of Vc, no Ins neurons were labeled. After FG injections into the Vo, many neurons were labeled bilaterally with a contralateral predominance in the rostral and caudal GI/DI, but none in the AI. We then examined descending projections from the GI/DI to the lower brainstem. After injections of an anterograde tracer, biotinylated dextranamine (BDA), into the rostral GI/DI, many BDA-labeled axons and terminals were seen bilaterally with a contralateral predominance in the medial part of laminae I/II of Vc, dorsomedial Vo, juxtatrigeminal region, rostral ventromedial medulla (RVM), and nucleus of the solitary tract, and with an ipsilateral predominance in the parabrachial nucleus (Pb), Kölliker–Fuse nucleus (KF) and trigeminal mesencephalic nucleus. After BDA injections into the caudal GI/DI, they were seen bilaterally with a contralateral predominance in the lateral part of laminae I/II of Vc, ventrolateral Vo, juxtatrigeminal region and RVM, and with an ipsilateral dominance in the lateral zone (PAGl) of periaqueductal gray, Pb and KF. These results suggest that orofacial nociceptive processing of Vc and Vo neurons may be regulated by GI/DI directly or indirectly through brainstem nuclei such as PAGl, Pb, KF and RVM.

Key words: insula, descending inhibition, trigeminal, pain, orofacial.

INTRODUCTION

Recent clinical findings in patients with asymbolia (Berthier et al., 1988; Augustine, 1996) and pseudothalamic pain syndrome (Schmahmann and Leifer, 1992) have strongly suggested the importance of the insular cortex (Ins) in pain processing. In patients, electrical stimulation of the posterior Ins elicits painful sensations (Ostrowsky et al., 2002; Afif et al., 2008; Mazzola et al., 2009) and a lesion on the Ins impairs pain perception in various body areas (Greenspan et al., 1999; Bowsher et al., 2004). Furthermore, functional imaging studies in humans have shown that Ins neurons are activated in acute or chronic pain states (Coghill et al., 1994; Ploghaus et al., 1999; Hofbauer et al., 2001; Peyron et al., 2004; Lorenz and Casey, 2005), and functional magnetic resonance imaging studies in rats have revealed that Ins neurons can be activated by electrical and chemical nociceptive inputs (Hess et al., 2007; Shih et al., 2008a,b; Westlund et al., 2009). These studies indicate that the Ins plays an important

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Abbreviations: 7N, facial nerve; ABC, avidin–biotin-peroxidase complex; AI, agranular insular cortex; Am, ambiguus nucleus; AP, area postrema; BDA, biotinylated dextranamine; Cl, claustrum; cont, contralateral; cp, cerebral peduncle; Cu, cuneate nucleus; DI, dysgranular insular cortex; dm, dorsomedial part; DR, dorsal raphe nucleus; ECu, external cuneate nucleus; FG, Fluorogold; GI, granular insular cortex; GiA, alpha part of gigantocellular reticular nucleus; Gr, gracile nucleus; IC, inferior colliculus; III, oculomotor nucleus; Ins, insular cortex; IO, inferior olive; IP, interpeduncular nucleus; ipsi, ipsilateral; KF, Kölliker–Fuse nucleus; l, lateral part of laminae I/II; lfp, longitudinal fasciculus of the pons; m, medial part of laminae I/II; MG, medial geniculate nucleus; MnR, median raphe nucleus; PAG, periaqueductal gray; PAGdl, dorsolateral zone of PAG; PAGdm, dorsomedial zone of PAG; PAGl, lateral zone of PAG; PAGvl, ventrolateral zone of PAG; Pb, parabrachial nucleus; PB, phosphate buffer; Pbl, lateral part of Pb; Pbm, medial part of Pb; PBS, phosphate-buffered saline; Pn, pontine nuclei; PnR, pontine raphe nucleus; PnV, ventral part of the pontine reticular nucleus; py, pyramidal tract; R, red nucleus; RMg, raphe magnus nucleus; ROb, raphe obscurus nucleus; RPa, raphe pallidus nucleus; RVM, rostral ventromedial medulla; S1, primary somatosensory cortex; S2, secondary somatosensory cortex; SC, superior colliculus; scp, superior cerebellar peduncle; SN, substantia nigra; SO, superior olive; Sol, nucleus of the solitary tract; TSNC, trigeminal sensory nuclear complex; Vc, trigeminal caudal subnucleus; Ve, vestibular nucleus; VI, abducens nucleus; Vi, trigeminal interpolar subnucleus; Vidm, dorsomedial part of Vi; VII, facial nucleus; Vint, intertrigeminal region; Vivl, ventrolateral part of Vi; Vjuxt, juxtatrigeminal region; vl, ventrolateral part; Vmes, trigeminal mesencephalic nucleus; Vmo, trigeminal motor nucleus; Vo, trigeminal oral subnucleus; Vodm, dorsomedial part of Vo; Vor, rostro-dorsomedial part of Vo; Vovl, ventrolateral part of Vo; Vp, trigeminal principal nucleus; Vsup, supratrigeminal nucleus; Vtr, trigeminal spinal tract; XII, hypoglossal nucleus.

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role in pain processing not only in humans but also in rats. However, the exact location of the pain processing-related areas of the Ins and how the pain processing-related Ins actually regulates pain processing are still poorly understood.

Studies in rats have suggested that the rostral agranular insular cortex (AI) plays a key role in the cortical modulation of pain processing (Burkey et al., 1996, 1999; Jasmin et al., 2003; Coffeen et al., 2008, 2011). Jasmin et al. (2004) have demonstrated that the rostral AI of the rat sends efferent fibers to brainstem areas involved especially in pain modulation (inhibition or facilitation), which include the dorsal raphe nucleus, periaqueductal gray (PAG) and parabrachial nucleus (Pb), suggesting that projections from the rostral AI to these brainstem areas are most likely to contribute to the descending pain-modulatory control. However, Jasmin et al. (2004) have also reported that there are no direct projections from the rostral AI both to the spinal cord and the trigeminal sensory nuclear complex (TSNC) which includes the laminae I, II and V of the trigeminal caudal subnucleus (Vc; the medullary dorsal horn) and the trigeminal oral subnucleus (Vo), where orofacial nociceptive primary afferents principally terminate (e.g., Hu et al., 1981, 1992; Dallel et al., 1990; for review, see Dubner and Bennett, 1983; Bereiter et al., 2000; Sessle, 2000). On the other hand, Yasui et al. (1991) have shown that the dysgranular insular cortex (DI) at the level that coincides with the crossing of the anterior commissure sends efferent fibers directly to the contralateral Vc, and Desbois et al. (1999) have shown that the dorsomedial part of the Vc receives direct, contralateral projections from the granular insular cortex (GI) as well as the DI. However, detailed features of the direct projections from the entire Ins to all parts of the Vc remain unclear. It is essential that this be addressed, in order to understand the neuronal mechanisms underlying the direct influence of the Ins to orofacial pain processing. Therefore, in the present study, we first examined the distribution of Ins neurons directly projecting to laminae I and II, or lamina V of Vc, and to the Vo by means of a retrograde tract tracing method, and, then, examined features of the projections from the Ins to the Vc and Vo by means of an anterograde tract tracing method.

Descending inhibitory or facilitatory influences on orofacial pain processing can be elicited by electrical or chemical stimulation of several central structures (for review, see Dubner and Bennett, 1983; Sessle and Dostrovsky, 1992; Sessle, 2000; Dubner and Ren, 2004; Ren and Dubner, 2011). The modulatory influences from especially the PAG and rostral ventromedial medulla (RVM) have been extensively studied (e.g., Sessle et al., 1981; Chiang et al., 1994, 1995; Meng et al., 2000; for review, see Dubner and Ren, 2004; Ren and Dubner, 2011). The Pb also exerts predominantly inhibitory effects on the Vc nociceptive neurons (Chiang et al., 1991, 1994, 1995; Meng et al., 2000). However, little is known about how the Ins is related to the orofacial pain processing through these descending modulatory systems. Therefore, we also examined features of the descending projections from the Ins, which sends direct projections to the Vc and Vo, to the brainstem areas including the above-described descending pain-modulatory areas.

EXPERIMENTAL PROCEDURES

Animals and tracer injections

A total of 58 male Wistar rats in the weight range 280–330 g were used in the present study. All experimental procedures were approved by the Osaka University Graduate School of Dentistry Intramural Animal Care and Use Committee in accordance with the guidelines of NIH, USA, and all efforts were made to minimize the number of animals used. Surgery was performed under sodium pentobarbital anesthesia (55 mg/kg, i.p.) with supplementary doses of sodium pentobarbital (10 mg/ml) at such a level that neither corneal reflex nor spontaneous eye movements were apparent. All wound margins were anesthetized using small injections of lidocaine hydrochloride. Rectal temperature was maintained between 36 °C and 38 °C with a heating pad. An electrocardiogram was continuously monitored. The atlas of the rat brain by Paxinos and Watson (1998) was chiefly used for the delineation of brain structures and determination of coordinates for stereotaxic tracer injection.

In experiment 1 in 31 rats, the head of each animal was fixed to a stereotaxic apparatus. For injections into the Vc, the atlantooccipital membrane was exposed by reflecting the dorsal neck muscles laterally and its small part was cut to expose the dorsal surface of the Vc at the obex level and a little more caudal level on the right side. For injections into the Vo, a small burr hole was made by reference to the brain atlas on the occipital part of the skull, in order to expose the dura overlying the cerebellum on the right side, and a small part of the dura was cut to expose the cerebellar surface. For both injections, a glass micropipette (tip diameter 10–15 μm) filled with a retrograde tracer, 1% Fluorogold (FG, Fluorochrome, Englewood, CO, USA) in 0.1 M sodium acetate buffer was used. By reference to the brain atlas, the micropipette was inserted at a 20° angle into the right side of Vc or into the right side of Vo through the cerebellum. Single FG injections were made iontophoretically (positive pulses, 2 μA, 300 ms, 2 Hz, 20–30 min) into the medial part of laminae I/II of Vc in six rats, the lateral part of laminae I/II of Vc in 12 rats, the deep layers (laminae III–V) of Vc in eight rats, and the Vo in five rats.

In experiment 2 in 27 rats, the head of each animal was also fixed to a stereotaxic apparatus, but a small burr hole was made by reference to the brain atlas on the temporal part of the skull overlying the Ins, in order to expose the dura on the left side. A small part of the dura was cut to expose the Ins surface. The platform on which the animal was placed was rotated, so that the exposed Ins surface was horizontal. A glass microelectrode (tip diameter 10–15 μm) filled with an anterograde tracer, 4% biotinylated dextranamine (BDA, 10,000 MW, Molecular Probes, Eugene, OR, USA) dissolved in saline was inserted perpendicularly 1000–1400 μm from the exposed Ins surface. Single injections of BDA were made iontophoretically with 2-μA driving currents (application time 15–30 min) into almost all rostrocaudal levels of the GI and DI.

After FG injections in experiment 1 or BDA injections in experiment 2, all wounds were sutured closed, an antibiotic (cefotiam hydrochloride, 66 mg/kg) and an analgesic (flurbiprofen axetil, 3.3 mg/kg) were given i.p., and the animals were allowed to recover from anesthesia in their cages. During 7–8 days of postinjection survival, the animals were monitored on a daily basis to assess body weight, general behaviors, and any postoperative complications such as bleeding or inflammation.