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CENTRAL SEROTONIN 3 RECEPTORS PLAY AN IMPORTANT ROLE IN THE MODULATION OF NOCICEPTIVE NEURAL ACTIVITY OF TRIGEMINAL SUBNUCLEUS CAUDALIS AND NOCIFENSIVE OROFACIAL BEHAVIOR IN RATS WITH PERSISTENT TEMPOROMANDIBULAR JOINT INFLAMMATION

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Abstract—The role of central serotonin 3 receptors on neural activities recorded from superficial laminae of trigeminal subnucleus caudalis/upper cervical spinal cord junction region was investigated using rats with (Complete Freund's Adjuvant day 7 group) or without (non-Complete Freund's Adjuvant group) persistent temporomandibular joint inflammation evoked by Complete Freund's Adjuvant for 7 days. We identified two types of units, Deep-wide dynamic range units and Skin-wide dynamic range units from extracellular recordings. Deep-wide dynamic range units have mechanoreceptive fields in the deep craniofacial tissues including masseter muscle but do not have cutaneous mechanoreceptive fields. Deep-wide dynamic range unit discharges evoked by the formalin injection into masseter muscle were significantly enhanced in the late phase in Complete Freund's Adjuvant day 7 group. Discharges of Skin-wide dynamic range units evoked by the noxious pinch stimulation to facial skin in Complete Freund's Adjuvant day 7 group were significantly enhanced compared with those in non-Complete Freund's Adjuvant group. Topical administration of central serotonin 3 receptor antagonist, tropisetron, onto trigeminal subnucleus caudalis/upper cervical spinal cord junction region significantly reduced both formalin-evoked Deep-wide dynamic range unit and pinch-evoked Skin-wide dynamic range unit discharges in non-Complete Freund's Adjuvant and Complete Freund's Adjuvant day 7 groups significantly. The inhibitory effects of tropisetron on pinch-evoked Skin-wide dynamic range unit discharges were prolonged in Complete Freund's Adjuvant day 7 group compared with those in non-Complete Freund's Adjuvant group. The role of central serotonin 3 receptors in trigeminal subnucleus caudalis/upper cervical spinal cord junction region was also tested by orofacial formalin test in Complete Freund's Adjuvant day 7 group. Intracisternal administration of tropisetron decreased the orofacial nocifensive behavior in the late phase evoked by the injection of formalin into the masseter muscle. These results suggest that central serotonin 3 receptors in trigeminal subnucleus caudalis/upper cervical spinal cord junction region are involved in mediating pronociceptive effects in both superficial and deep craniofacial tissues nociception during persistent temporomandibular joint inflammation.

Key words: pain, masseter muscle, tropisetron, inflammation, electrophysiology, orofacial formalin test

The diagnostic criteria for temporomandibular disorders (TMD) include a heterogenous group of symptoms as well as pain in the temporomandibular joint (TMJ) and associated masticatory muscles such as masseter muscles (Dworkin and LeResche, 1992; Sarlani and Greenspan, 2003; Sarlani et al., 2004). Although the origin of TMD pain might be varied, many of the signs and symptoms are consistent with central neural dysfunction. Several clinical studies have demonstrated that a decrease in the efficacy of endogenous pain control systems has been implicated in the pathophysiology of chronic TMD pain (Maixner et al., 1995). For example, Kashima et al. (1999) reported that a submaximal effort tourniquet procedure induced a smaller attenuation in pressure pain sensitivity in the hand of patients with chronic masticatory myalgia than healthy controls. An ischemic pain task, which engages pain regulatory systems and diminishes experimental and acute dental pain in healthy subjects (Sigurdsson and Maixner, 1994), failed to suppress clinical muscle pain in TMD patients (Maixner et al., 1995).

It is well known that the neurotransmitter serotonin (5HT) plays important roles in modulating spinal nociceptive transmission. Activation of the descending serotonergic bulbospinal system modulates behavioral and dorsal horn neurons responses to noxious stimuli (Mayer et al., 1971; Zemlan et al., 1980). Most evidence indicates that spinal cord as well as trigeminal subnucleus caudalis (Vc) are the major sites affected by 5HT, released from descending fibers from rostroventral medial medulla or administered intrathecally in experimental studies (Beitz, 1982; Clatworthy et al., 1988; Fields et al., 1991). Depending on the type of noxious stimulation, the dose and route of administration, 5HT might either inhibit or facilitate nociceptive transmission (Oyama et al., 1996; Suzuki et al., 2004). However, controversy remains as to which types of 5HT receptor are involved in mediating serotonergic anti-

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Abbreviations: ANOVA, analysis of variance; CFA, Complete Freund's Adjuvant; NS, nociceptive specific; PNS, peripheral nervous system; RFs, mechanoreceptive fields; TMD, temporomandibular disorder; TMJ, temporomandibular joint; Vc, trigeminal subnucleus caudalis; Vc/C2 region, trigeminal subnucleus caudalis/upper cervical spinal cord junction region; WDR, wide dynamic range; 5HT, serotonin

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or pro-nociceptive effects. The dual effects of 5HT on modulating nociceptive transmission can be due to the possibility that different 5HT receptors mediate different effects.

The 5HT3 receptor, the only ligand-gated ion channel of the 5HT receptor subtypes (Richardson and Engel, 1986), is located focally within the CNS (Morales et al., 1998; Zeitz et al., 2002) and on peripheral nerves (Giordano and Rogers, 1989; Zeitz et al., 2002; Okamoto et al., 2004). However, there is controversy about the precise role of the spinal 5HT3 receptors in nociceptive processing. In the spinal cord, 5HT3 receptors are likely to serve both pro- (Ali et al., 1996; Green et al., 2000; Zeitz et al., 2002) and antinociceptive (Glaum et al., 1988; Alhaider et al., 1991; Bardin et al., 2000; Sasaki et al., 2001) functions.

Recently, converging lines of evidence indicate that the trigeminal subnucleus caudalis/upper cervical junction region (Vc/C2 region) plays a significant role in craniofacial nociceptive processing (Hu et al., 1992, 1997; Iwata et al., 1999; Zhou et al., 1999: Takeshita et al., 2001; Okamoto et al., 2003, 2005b). Several results demonstrated that nociceptive afferent inputs from masseter muscle and TMJ are relayed by the Vc/C2 region (Nishimori et al., 1986; Shigenaga et al., 1988; Cairns et al., 2001, 2002). 5HT-immunoreactive axon terminals are observed in laminae I–II of the rat Vc (Li et al., 1997), and 5HT3 receptors are expressed in the Vc (Gehlert et al., 1991) and spinal cord (Zeitz et al., 2002). Therefore, it is possible that neural activities in the Vc/C2 region in the orofacial pain model are modulated by the endogenous serotonergic systems related to 5HT3 receptors. It has been found that formalin-induced peripheral inflammation induces release of 5HT from descending 5HT fibers (Puig et al., 1992). Although a few experiments revealed the role of spinal 5HT3 receptors on orofacial pain (Seo et al., 2002), to our knowledge, no study has been performed to explore how 5HT3 receptors in the Vc/C2 are involved in the modulation of neural activity or orofacial nocifensive behavior evoked by the noxious stimuli to deep craniofacial tissues.

We performed *in vivo* extracellular electrophysiological approach and orofacial formalin behavioral test (Clavelou et al., 1989; Raboisson and Dallel, 2004; Okamoto et al., 2004, 2005a) using rats with persistent TMJ inflammation evoked by the injection of Complete Freund's Adjuvant (CFA) for 7 days. Inflammation of TMJ and surrounding masticatory muscles likely contributes to pain sensation in chronic TMD (Denucci et al., 1996; Lobbezzo et al., 2004), and markers for inflammation are elevated in samples of TMJ synovial fluid from chronic TMD patients (Kopp, 1998).

The aim of this study was to investigate (1) the roles of 5HT3 receptors of Vc/C2 region in neural activities recorded from superficial laminae of Vc/C2 region evoked by chemical stimulation to masseter muscle or noxious mechanical stimulation to facial skin, and (2) those in orofacial nocifensive behavioral activities evoked by the injection of formalin into masseter muscle during TMJ inflammation.

EXPERIMENTAL PROCEDURES

Animals

Experiments were conducted in accordance with International Association for the Study of Pain (Zimmermann, 1983) and approved by the institutional Animal Care and Use Committee. All efforts were made to minimize the number of animals used for experiments and their suffering. Adult male Sprague–Dawley rats (SLC Japan, Shizuoka, Japan) were used. Animals were housed in cages (two rats per cage), with free access to food and water. Cages remained in climate- and light-controlled protected units (25±2 °C, 12-h light/dark cycle with light on at 7:00 AM) for at least 5 days before the induction of left TMJ inflammation.

Induction of TMJ inflammation

Rats were anesthetized with sodium pentobarbital (60 mg/kg, Abbott Laboratories, Chicago, IL, USA) via i.p. injection. CFA (*Mycobacterium tuberculosis*, Calbiochem, La Jolla, CA, USA) suspended in an oil:saline (0.9% sodium chloride, WAKO, Osaka, Japan) (1:1) emulsion was used as the inflammatory agent. Rats received the injection of CFA (0.05 ml, 0.025 mg) into left TMJ region with a 27 gauge needle (Zhou et al., 1999) and were allowed to survive for 7 days (CFA day 7 group) after TMJ inflammation. We have demonstrated that formalin-evoked orofacial nocifensive behavioral activities were enhanced at the time point of 7 days after the onset of CFA-evoked TMJ inflammation (Okamoto et al., 2004, 2005a). Although the peak inflammation of the deep craniofacial tissues has passed at this time point (Zhou et al., 1999), this period is more likely to reflect chronic rather than acute pain condition (Zhou et al., 1999; Hutchins et al., 2000; Imbe et al., 2001; Okamoto et al., 2004, 2005a), since clinically, the major symptom in TMD is chronic orofacial pain in TMJ region and masticatory muscles (Denucci et al., 1996; Lobbezzo et al., 2004). Naïve rats served as controls (non-CFA group).

Electrophysiology

Animal preparation. Male rats weighing 200–350 g were anesthetized initially with pentobarbital sodium (60 mg/kg, i.p.). The left side of face, head and neck were shaved. After tracheotomy, animals were respired artificially with oxygen-enriched room air and catheters were placed in the right jugular vein. Anesthesia was maintained by continuous infusion of pentobarbital sodium (10–20 mg/kg/h) via jugular vein, and switched to a mixture of pentobarbital sodium (10–20 mg/kg/h) and a paralytic agent, gallamine triethioide (15–25 mg/h, MP Biomedicals Inc., Aurora, OH, USA) after completion of all surgical procedures and just prior to the electrophysiological recording session. The wound margins were covered with 2% lidocaine gel. Adequate depth of anesthesia was confirmed by the absence of corneal and hindlimb withdrawal reflexes just prior to gallamine triethioide and by fully constricted pupils throughout the experiment. The experiments did not exceed one hour, since only one unit was recorded from each animal preparation. In preliminary experiments, we have determined that withdrawal reflex was not induced by the noxious pinch stimulation to the hindpaw under the continuous infusion of pentobarbital sodium (10–20 mg/kg/h) alone.

The animal was placed in a stereotaxic frame and the C1 and the portion of C2 vertebrae were removed to expose the upper cervical dorsal horn. The brainstem surface was bathed in warm paraffin oil. Body temperature was maintained at 37–38 °C with heating blanket and thermal probe.

Identification of units. The caudal portion of Vc and upper cervical (C1–C2) spinal cord, 3–7 mm caudal to the obex was explored. A tangential approach (44° off vertical, 60° off midline) was used to record single units extracellularly with a tungsten-in-