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CENTRAL SENSITIZATION IN THALAMIC NOCICEPTIVE NEURONS INDUCED BY MUSTARD OIL APPLICATION TO RAT MOLAR TOOTH PULP

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Abstract—We have recently demonstrated that application of mustard oil (MO), a small-fiber excitant and inflammatory irritant, to the rat maxillary molar tooth pulp induces central sensitization that is reflected in changes in spontaneous activity, mechanoreceptive field (RF) size, mechanical activation threshold, and responses to graded mechanical stimuli applied to the neuronal RF in trigeminal brainstem subnucleus caudalis and subnucleus oralis. The aim of this study was to test whether central sensitization can be induced in nociceptive neurons of the posterior thalamus by MO application to the pulp. Single unit neuronal activity was recorded in the ventroposterior medial nucleus (VPM) or posterior nuclear group (PO) of the thalamus in anesthetized rats, and nociceptive neurons were classified as wide dynamic range (WDR) or nociceptive-specific (NS). MO application to the pulp was studied in 47 thalamic nociceptive neurons and found to excite over 50% of the 35 VPM neurons tested and to produce significant long-lasting (over 40 min) increases in spontaneous activity, cutaneous pinch RF size and responses to graded mechanical stimuli, and a decrease in threshold in the 29 NS neurons tested; a smaller but statistically significant increase in mean spontaneous firing rate and decrease in activation threshold occurred following MO in the six WDR neurons tested. Vehicle application to the pulp did not produce any significant changes in six VPM NS neurons tested. MO application to the pulp produced pronounced increases in spontaneous activity, pinch RF size, and responses to mechanical stimuli, and a decrease in threshold in three of the six PO neurons. In conclusion, application of the inflammatory irritant MO to the tooth pulp results in central sensitization of thalamic nociceptive neurons and this neuronal hyperexcitability likely contributes to the behavioral consequences of peripheral inflammation manifesting as pain referral, hyperalgesia and allodynia.

Key words: thalamic ventroposterior medial nucleus, posterior nuclear group, trigeminal, nociceptive specific neurons, wide dynamic range neurons.

In recent years great progress has been made in the study of central sensitization of nociceptive neurons in the spinal cord (for review, see Salter, 2004; Dubner, 2005; Woolf and Salter, 2006). Much less is known about sensitization in the trigeminal (V) brainstem nucleus but recent studies have documented that neurons in the subnucleus caudalis (Vc), which is the V homologue of the spinal dorsal horn and is known as the medullary dorsal horn, also undergo marked central sensitization following peripheral noxious stimuli, inflammation or nerve damage. For example, application of the inflammatory irritant mustard oil (MO) to the tooth pulp (Chiang et al., 1998, 2002, 2005b) as well as other orofacial tissues (Hu et al., 1992; Yu et al., 1993) induces Vc central sensitization reflected in increases in the spontaneous activity, cutaneous mechanoreceptive field (RF) size and responses of Vc nociceptive neurons and decreases in their cutaneous mechanical activation threshold; these changes are dependent on brainstem glutamatergic (*N*-methyl-D-aspartic acid, NMDA) and purinergic P2X receptor mechanisms (Chiang et al., 1998, 2005b; Hu et al., 2002). In the inferior alveolar nerve injury model comparable but more prolonged changes also occur in Vc and in a chronic pulp inflammation model sensitization in Vc has been reported in association with nociceptive behavior (Iwata et al., 2001; Tsuboi et al., 2006).

The Vc plays a major role in mediating central sensitization at the V brainstem level as evidenced by findings that the central sensitization of nociceptive neurons in subnucleus oralis (Vo) and accompanying increased jaw muscle electromyographic activity are dependent on the functional integrity of Vc (Cairns et al., 2001; Chiang et al., 2002; Hu et al., 2002). However, limited information is available on central sensitization of nociceptive neurons in the posterior thalamus. The few studies at the thalamic level have largely concentrated on the ventroposterior lateral (VPL) nucleus of the ventrobasal thalamus. Several studies have shown that following experimentally induced inflammation, the responses of many thalamic nociceptive neurons are increased and their thresholds reduced and they may display other altered properties (Guilbaud et al., 1987; Dostrovsky and Guilbaud, 1988, 1990). Similar changes in the properties on VPL nociceptive neurons have also been observed in the sciatic nerve chronic constriction injury (CCI) model of neuropathic pain (Guilbaud et al., 1990, 1995; Bordi and Quartaroli, 2000). Alterations in

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Abbreviations: CCI, chronic constriction injury; MinO, mineral oil; MO, mustard oil; NMDA, *N*-methyl-D-aspartic acid; NS, nociceptive-specific; PO, posterior nuclear group of the thalamus; RF, mechanoreceptive field; RM ANOVA, repeated measures analysis of variance; S-R, stimulus-response; V, trigeminal; Vc, subnucleus caudalis; Vo, subnucleus oralis; VPL, ventroposterior lateral nucleus; VPM, ventroposterior medial nucleus; WDR, wide dynamic range.

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response properties including central sensitization of VPL thalamic neurons have also recently been reported in association with central neuropathic pain, e.g. following spinal cord injury, lesions to ascending pathways, or block of glycinergic inhibition in the spinal cord (Sherman et al., 1997; Weng et al., 2000, 2003; Gerke et al., 2003). These changes appear not to be simply a reflection of changes occurring at the spinal level since the spinal cord injury results in an upregulation of Nav1.3 voltage-gated sodium channel subunits in VPL (Hains et al., 2005) and block of NMDA receptors in VPL thalamus reduces the hyperalgesia produced by peripheral inflammation (Kolhekar et al., 1997).

There have been two previous studies on the possible role of the thalamus in mediating central sensitization of nociceptive processing arising from the orofacial region. Vos et al. (2000) reported changes in neuronal properties including increased spontaneous activity in the ventroposterior medial nucleus (VPM) of the ventrobasal thalamus in the model of CCI of the infraorbital nerve, and Kaneko et al. (2005) reported increased responses to electrical stimulation of the pulp and decreased mechanical threshold of the cutaneous RF after MO pulpal application. Previous studies indicate that a major input in the rat VPM as well as the posterior nuclear group (PO) of the thalamus arises from Vc (Peschanski, 1984; Gauriau and Bernard, 2004; Guy et al., 2005) and we recently provided details of the somatotopic organization and response properties of nociceptive neurons in the rat VPM (Chiang et al., 2005a). The aim of this study was to test whether central sensitization can be induced in nociceptive neurons of the VPM as well as the PO by MO application to the pulp. The findings are compared with those we have detailed previously in Vc and Vo (Chiang et al., 1998; Park et al., 2001; Hu et al., 2002). Preliminary findings have been reported briefly (Hu et al., 2001).

EXPERIMENTAL PROCEDURES

Animal preparation

This study was carried out in 63 Sprague–Dawley adult male rats weighing 290–420 g. The methods used for animal preparation, stimulation, and neuronal recording and classification were similar to those detailed previously (Chiang et al., 1998, 2002, 2005b; Park et al., 2001; Hu et al., 2002) and so will only be briefly outlined. The animal was anesthetized by an i.p. injection of a mixture of urethane (1 g/kg) and alpha-chloralose (50 mg/kg). The trachea and the left external jugular vein were cannulated. To expose the pulp of the right maxillary first molar, an occlusal cavity was prepared with a low-speed dental drill and temporarily filled with a cotton pellet soaked with saline. The animal was then placed in a stereotaxic apparatus, and a craniotomy was performed to expose the contralateral (left) cerebral cortex overlying the thalamus. A supplemental dose of urethane (200–400 mg/kg, i.v.) was administered 1 h after surgery and during the recording session. The animal was immobilized with gallamine triethiodide (initial dose, 42 mg/kg; maintenance dose, 14 mg/h; i.v.) and artificially ventilated throughout the experimental period. An adequate level of anesthesia was confirmed periodically by the lack of spontaneous movements and responses to pinching the paw when the gallamine-induced muscle paralysis was allowed to wear off. In addition, pupil size and heart rate were also routinely monitored to ensure their stability when noxious pinch stimuli were applied. The expired % CO_2 and rectal temperature were also continuously monitored and maintained at physiological levels of 3.5–4.5% and 37–38 °C, respectively. All surgeries and procedures were carried out with the intent of minimizing the number of animals used and any suffering, and were approved by the University of Toronto Animal Care Committee in accordance with the regulations of the Ontario Animal Research Act (Canada) and the guidelines of the Canadian Council on Animal Care and the American Physiological Society.

Recording and stimulation procedures

Single neuronal activity in the contralateral thalamus was recorded extracellularly by means of an epoxy-resin-coated tungsten microelectrode (15 mega-ohm; FHC, Bowdoinham, ME, USA) held by a microdrive. As the microelectrode was advanced perpendicularly through the cortex into the thalamus, low and high threshold mechanical stimuli were applied to the orofacial tissues to search for neurons receiving an orofacial mechanoreceptive input. The VPM and immediately adjacent PO were dorsoventrally explored 2.1–3.2 mm lateral to the midline and between frontal planes P2.6 and P4.3 referred to Bregma (Paxinos and Watson, 1986). These neurons were assumed to be thalamocortical neurons although not identified by antidromic activation, since in the rat all neurons in this region project to cortex (Sherman and Guillery, 2001). Neuronal activity was amplified, displayed on oscilloscopes and also led to a window discriminator connected to an A/D converter (CED 1401plus, CED, UK) and a personal computer.

A wide range of mechanical (brush, pressure and pinch) stimuli was applied to the orofacial skin or intraoral mucosa to classify each neuron as low-threshold mechanoreceptive (LTM), wide dynamic range (WDR) or nociceptive-specific (NS) (Chiang et al., 1998, 2005b; Park et al., 2001; Hu et al., 2002). Noxious thermal heat was sometimes used to help identify nociceptive neurons. Only WDR and NS neurons were characterized in detail and included in the study.

The spontaneous activity, RF size, mechanical activation threshold and responses to graded (from subthreshold to suprathreshold) mechanical stimuli were assessed at the time intervals specified below. After the recorded neuron's firing became stable, its spontaneous (baseline) activity was measured over a period of 1 min. As detailed in our previous reports (Chiang et al., 1998, 2002, 2005b; Park et al., 2001; Hu et al., 2002), the RF of each neuron was determined through the use of a brush, blunt probe and a pair of forceps. The extent of the cutaneous RF was manually defined by brushing (in the case of a tactile RF) or pinching (in the case of a pinch RF) the skin; as previously described (Chiang et al., 1994, 2005a,b). The noxious stimulation was used sparingly at the same spot so as to avoid damage to the skin or mucosa and cause neuronal sensitization. A burst response consisting of at least two spikes during each stimulus trial (touch or pinch) was accepted as the criterion for the RF boundary of the neuron tested. A neuron was considered to have a deep RF when the neuron responded to stimulation applied by a blunt probe to the skin overlying muscle, bone, tendon, or temporomandibular joint (TMJ) and had a mechanical threshold above 5 g but no response could be evoked by the wide range of cutaneous stimuli used (Iggo, 1960; Schaible and Schmidt, 1983; Yu et al., 1993). For orofacial and/or intraoral as well as body RF measurement, 14 designated parts throughout the orofacial region and body, including intraoral (upper and lower intraoral mucosa, and tongue), perioral (upper and lower lips), face, nose, ears, forepaws and hindpaws, and tail were tested as previously described (Chiang et al., 1998, 2005b; Park et al., 2001; Hu et al., 2002). The extent of the neuronal RF was assessed in terms of the number of parts from which the response was evoked by light touch or firm pressure applied with a dental explorer or pinch with a constant force forceps (200 g). The activation threshold to a mechanical stimulus applied to the orofacial RF was assessed by a pair of