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Central distribution of nociceptive intradental afferent nerve fibers in the rat

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Abstract

The central distribution of intradental afferent nerve fibers was investigated by combining electron microscopic observations with a selective method for inducing degeneration of the A delta- and C-type afferent fibers. Degenerating terminals were found on the proprioceptive mesencephalic trigeminal neurons and on dendrites in the neuropil of the trigeminal motor nucleus after application of capsaicin to the rat's lower incisor tooth pulp. The results give anatomical evidence of new sites of central projection of intradental A delta- and C-type fibers whereby the nociceptive information from the tooth pulp can affect jaw muscle activity.

Keywords: Tooth pulp afferents; Capsaicin; Mesencephalic trigeminal nucleus; Trigeminal motor nucleus

1. Introduction

Experimental and clinical researches have shown that the nociceptive primary afferents from craniofacial tissues have a prominent role in controlling the motor function of jaw muscles (Pertovaara et al., 1986; Narhi et al., 1989; Kemppainen et al., 1997; Andersen et al., 1998; Wang et al., 1999; Sunakawa et al., 1999; Svensson et al., 2000). However, the neuronal pathway underlying this control is not fully understood.

The trigeminal sensory nuclear complex has traditionally been viewed as an essential brain-stem relay site of nociceptive information. Therefore, the anatomical studies on the central distribution of the nociceptive trigeminal ganglion neurons innervating the oral and facial territories have been focused on the subdivisions of this complex (Azerad and Woda, 1976; Ardvisson and Gobel, 1981; Marfurt and Turner, 1984; Henry et al., 1987; Sugimoto et al., 1988; Tsuru et al., 1989; Clements et al., 1991; Takemura et al., 1991, 1993; Bae et al., 2004).

On the other hand, other brain stem structures involved in the control of the trigeminal motoneuron activity, such as the mesencephalic trigeminal nucleus (Vmes), have been neglected. The Vmes contains the cell bodies of jaw muscle spindle afferents which are intermingled with those of periodontal receptors in the caudal portion of the nucleus (Szentagothai, 1948; Passatore et al., 1983; Honma et al., 2001). The Vmes neurons do receive synaptic inputs on their somata (Hinrichsen and Larramendi, 1970; Lucchi et al., 1972; Liem et al., 1992; Lazarov, 1996, 2000; Honma et al., 2001) and on their terminals (axo-axonic synapses) on trigeminal motoneurons (Bae et al., 1996; Kishimoto et al., 1998). Contacts on the Vmes neurons may arise from various sources. Some afferent connections to the Vmes neurons have been verified (Manni et al., 1982; Nagy et al., 1986; Rokx et al., 1988; Copray et al., 1991; Minkels et al., 1991; Ter Horst et al., 1991; Lazarov and Chouchkov, 1995;

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Buisseret-Delmas et al., 1997), while others remain conjectural. Moreover, the synaptic terminals on the Vmes neurons utilise a broad variety of transmitters and neuroactive substances, including glutamate and substance P (Lazarov, 2000, 2001), which are found in nociceptive primary sensory afferents (Hokfelt et al., 1975; Lieberman, 1976).

These data, taken together, suggest that the jaw muscle proprioceptive afferent transmission onto the involved trigeminal motoneurons can be modulated presynaptically by divergent inputs through different mechanisms. Therefore, the pathway of the proprioception could play some role in mediating the influence of pain from craniofacial tissues on trigeminal motoneuron activity. On the other hand, the activation of jaw muscle nociceptors significantly affects the proprioceptive properties of jaw muscle spindles (Capra and Ro, 2000; Svensson et al., 2000; Ro and Capra, 2001). Moreover, we have demonstrated by electrophysiological and anatomical researches that fatigue signals carried by small muscle afferent fibers from the masseter muscle of the rat may influence the muscle spindle activity through direct synapse on the somata of mesencephalic trigeminal neurons and on dendrites of neurons within the trigeminal motor nucleus (Brunetti et al., 2003).

It is well known that noxious stimulation of the tooth pulp can produce a facilitation of the jaw-opening reflex (Pertovaara et al., 1986). Thus, it seems reasonable to consider the mesencephalic trigeminal proprioceptive neurons as possible sites of termination of the nociceptive trigeminal ganglion cells which supply the tooth pulp. In this study we have investigated the presence of degenerating terminals, on the soma of the Vmes proprioceptive neurons and on their terminals within the trigeminal motor nucleus (Vmot) after the application of capsaicin to the tooth pulp. In fact the intradental fibers are capsaicin-sensitive (Ikeda et al., 1997; Chaudhary et al., 2001) and it is well known that local application of capsaicin can provoke the functional exclusion and finally the degeneration of small diameter afferent fibers (Jancso et al., 1985; Jancso and Lawson, 1990; Lynn, 1990; Della Torre et al., 1996; Brunetti et al., 2003).

2. Materials and methods

The "Principles of laboratory animal care" (NIH publication No 86-23, revised 1985) were followed. The ethical committee on animal experimentation of Bologna University approved the following protocol.

Six adult Wistar rats (700–800 g b.w.) were anaesthetized (1 ml ketamine + 0.25 ml xilazine I.P.) and the pulp chamber of the left lower incisor tooth was exposed with a high-speed dental drill. In three animals capsaicin (20 μl, 10 mM, Sigma) dissolved in a vehicle (10% ethyl alcohol, 10% Tween and 80% normal saline) was slowly injected into the pulp chamber by a Hamilton microsyringe while in three rats the vehicle only was injected; thereafter the opening was sealed with dental cement. After postoperative survivals of 12–15 days the animals, deeply anesthetized, were perfused through the ascending aorta. The perfusion was initiated by a fixative containing 1% paraformaldehyde and 1% glutaraldehyde in 0.1 M phosphate buffer pH 7.2 at room temperature followed by a cooled fixative containing 4% paraformaldehyde and 2.5% glutandehyde in the same buffer. The brain stem was removed, immersed in the second fixative mixture (3–4 h) and rinsed in 0.1 M phosphate buffer pH 7.2. Transverse serial sections of 300–400 μm thickness through the mesencephalon, the pons and the oblongata were cut on a vibratome and collected in cooled 0.1 M phosphate buffer; one section (40–50 μm thick) every fifth section was free floating stained with hematoxylin-eosin and examined under light microscope in order to localize the Vmes, the Vmot and the trigeminal sensory nuclear complex. The serial sections containing these nuclei (ipsilaterally and contralaterally to the injected tooth) were postfixed in 1% OsO_4 in 0.1 M Na cacodylate buffer, pH 7.4, dehydrated in ethanol, infiltrated with uranyl acetate and flat embedded in Durcopan ACM.

The neurons of the Vmes, Vmot nuclei and of the trigeminal sensory nuclear complex were identified in 1 μm thick toluidine-blue stained sections examined under light microscope. Ultrathin sections adjacent to the toluidine-blue stained sections were collected on formvar-coated grids, stained with uranyl acetate and lead citrate and examined with Zeiss 109 electron microscope.

The Vmes, the Vmot of the three capsaicin- and three vehicle-injected rats were observed ipsilaterally and contralaterally to the injected site. In each animal, 10 ipsilateral and 10 contralateral pseudounipolar Vmes cells located along the lateral edge of the periaqueductal gray, in which only the cell bodies of muscle spindle afferents are located, were examined. Each neuron was observed in five series of five to seven ultrathin sections taken at different levels of the soma. The Vmot was observed in five series of five to seven sections; each series was randomly taken through the rostrocaudal extension of the nucleus and 10 ipsilateral and 10 contralateral motoneurons sectioned at the level of the nucleolus were examined. The neurons of the trigeminal sensory nuclear complex of the three capsaicin-injected rats were examined in random sections collected at the levels of the principal trigeminal nucleus and of the oral subdivision of the trigeminal spinal nucleus ipsilaterally to the injected tooth.

3. Results

In the semithin sections cut along the rostrocaudal extension of the Vmes ipsilateral to the injected tooth, the neurons did not show any chromatolytic reaction. In the series of ultrathin sections, no degenerative features were found in the Vmes and in the Vmot of the vehicle-injected animals, as well as in those contralaterally to the capsaicin-injected site. On the contrary, capsaicin-sensitive terminals were present both in the Vmes and in the neuropil of the Vmot ipsilaterally to the injected site. Six axonal terminals showing degenerating fea-