Temporomandibular Joint Nociception: Effects of Capsaicin on Substance P–like Immunoreactivity in the Rabbit Brain Stem

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Aims: *To specify the regions of the brain stem that are characterized by changes in substance P (SP)-like immunoreactivity following activation of capsaicin-sensitive afferents innervating temporomandibular joint (TMJ) tissues in New Zealand rabbits.* ***Methods:*** *Capsaicin, an activator of small-diameter unmyelinated and thinly myelinated nociceptive afferent fibers, was administered unilaterally to the right TMJ of experimental animals. Another group received vehicle solution and served as controls. The animals were sacrificed 6 hours post-treatment through transcardial perfusion. Their brain stems were removed and sectioned, and SP-like immunoreactivity was assessed in serial horizontal sections.* ***Results:*** *A decrease in brain stem SP-like immunoreactivity occurred ipsilateral to capsaicin application. This reduction was primarily localized in brain stem regions that correspond to the trigeminal main sensory nucleus, as well as subnucleus oralis, interpolaris, and caudalis of the trigeminal spinal tract nucleus.* ***Conclusion:*** *The present study revealed central nervous system changes following TMJ capsaicin treatment in rabbits.*
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Key words: temporomandibular joint, nociception, substance P, immunocytochemistry, rabbit

Nociceptive innervation to the temporomandibular joint (TMJ) is mainly provided by the auriculotemporal nerve.[1] Aδ and C-fibers, whose cell bodies are located in the trigeminal ganglion, project distally and terminate as non-encapsulated free nerve endings dispersed throughout the posterolateral part of the TMJ capsule,[2–5] the posterior band of the meniscus, and the posterior attachment.[6–9] Centrally, the majority of these fibers terminate at the trigeminal sensory nuclei located in the brain stem, the main and spinal tract nuclei.[10–12] Utilizing electrical, mechanical, and chemical (histamine, bradykinin) stimulation of TMJ nociceptive nerve fibers in the cat, Broton et al[13] classified neurons in subnucleus caudalis of the trigeminal spinal tract nucleus as low-threshold mechanoreceptive, wide dynamic range (WDR), and nociceptive-specific (NS). The TMJ afferent inputs to WDR and NS caudalis neurons were considered to be primarily nociceptive based on their electrophysiologic characteristics.

Similar results were reported by Kojima,[14] who studied the electrophysiologic behavior of caudalis neurons following electrical stimulation of the TMJ capsule in the rat.

On the basis of its cytoarchitecture, the subnucleus caudalis resembles the dorsal horn of the spinal cord and indeed is now often termed the medullary dorsal horn.[15] Olszewski[16] proposed that its subdivision includes 3 layers: the marginal layer, the substantia gelatinosa, and the magnocellular layer. More recently, Dubner and Bennet[17] proposed that the marginal layer corresponds to Rexed's lamina I, the substantia gelatinosa to lamina II, and the magnocellular layer to laminae III and IV. It is well established that small nociceptive afferent fibers (C and Aδ) originating from orofacial structures terminate in the superficial laminae of the medullary dorsal horn, where they synapse with second-order sensory neurons and are involved in the central processing of nociceptive input.[18,19]

Capsaicin acts primarily on small-diameter Aδ and C-fibers. In neonatal rats, systemic application of capsaicin largely results in destruction of C-fibers, accompanied by significant loss of small sensory ganglion cells.[20–24] By contrast, capsaicin administration to adult animals does not elicit destruction of fibers, but rather causes selective depolarization of primary afferent C-fibers, by acting on polymodal receptors. Prolonged exposure (days or weeks) of adult animals to capsaicin can produce a subsequent desensitization or neuroinhibition.[25–29] Substance P (SP) has been localized, along with other neuropeptides, in small-diameter afferent nerve fibers terminating centrally at the dorsal horn of the spinal cord.[30,31] Capsaicin has been shown to evoke the release of SP in the spinal cord in vitro[32,33] as well as in vivo,[34,35] leading to depletion of SP in the activated nerve fibers.[31]

To specify the regions of the brain stem that are characterized by changes in SP-like immunoreactivity following activation of capsaicin-sensitive afferents innervating TMJ tissues, we evaluated the central nervous system (CNS) changes following TMJ injection of capsaicin in New Zealand rabbits. Our results indicate that specific brain stem regions, which correspond to the trigeminal sensory nuclei, show changes in SP-like immunoreactivity following capsaicin application to the TMJ.

Materials and Methods

A total of 8 young adult (6-month-old) female New Zealand white rabbits *(Oryctolagus cuniculus)* purchased from Haselton Laboratories were included in this study. Five animals received surgically assisted injection of capsaicin in the right TMJ (experimental animal group), and 3 animals received vehicle injections (control animal group). In all animals, the left TMJ was untreated and served as an internal control for our analysis. In brief, the animals were anesthetized via administration of ketamine (Ketamine HCL, Abbott Laboratories, 15 mg/kg intramuscular) and xylazine (Rompun, Bayer, 5 mg/kg intramuscular). The right TMJ was approached with an anteroposterior incision between the posterior end of the zygomatic arch and the ear cartilage, followed by a blunt dissection to expose the zygomatic arch and posterior margin of the articular eminence. The superior joint space was not exposed during this surgical procedure. The posterior margin of the articular eminence was located by palpation, and a 1-mL tuberculin syringe with a 27-gauge needle was used to inject the experimental solutions into the upper joint space; this surgically assisted intra-articular injection technique was utilized to minimize leakage or spreading of the irritant solution beyond the articular space. The experimental animals received 50 μL of a 1% capsaicin solution (Sigma) in 5% *chemophor el* (Sigma) plus 5% ethanol in sterile saline, whereas the control animals received vehicle only (5% *chemophor el* plus 5% ethanol in sterile saline). Six hours post-treatment, the animals were deeply anesthetized as described above and sacrificed through transcardial perfusion of 500 mL sodium nitrite solution (0.5% in phosphate-buffered saline [PBS], pH = 7.4), followed by 1,000 mL of 4% paraformaldehyde solution in PBS (pH = 8.0).The brain stems were dissected and post-fixed for 3 hours in the same fixative, followed by overnight incubation in 30% sucrose in PBS (pH = 7.4).The tissue was then frozen over dry ice and stored at –80°C. All protocols were approved by the University Committee on Animal Resources and met all guidelines of the US National Institutes of Health for the use of research animals.

Immunocytochemistry

For SP-like immunocytochemistry (ICC), we used a protocol modified from Hancock[36] and also described in Kyrkanides et al.[37,38] In brief, brain tissue was cut on a freezing sliding microtome in 40-μm-thick sections, and every third section was stored in cryoprotectant solution at –20°C until processed. Every third section was included in the ICC protocol. Tissue sections were washed free-

floating in PBS, incubated in 10% normal goat serum (NGS) for 30 minutes, and then incubated overnight with a rat anti-SP (human) monoclonal antibody (Chemicon; 1:1,000 dilution) plus 0.4% Triton-X and 1.5% NGS at 4°C. Next, the tissue was washed in PBS, incubated in 10% NGS for 30 minutes, and incubated in goat anti-rat IgG secondary antibody for 90 minutes. Tissue was washed in PBS and incubated with avidin-biotin-peroxidase complex (Vector) for 60 minutes. The peroxidase reaction was developed for 8 minutes in 0.05% 3,3-diaminobenzidine (DAB) and 0.01% hydrogen peroxide in PBS solution with nickel enhancement. The above protocol gives a Ni-DAB reaction that is linear for 12 minutes. Sections were mounted, dehydrated, and cover-slipped. Control sections for antibody specificity were processed simultaneously in the absence of primary antibody. All tissue was processed simultaneously, all photographs were taken using identical illumination and shutter speeds, and prints were processed identically.

Image Analysis

SP-like immunoreactivity in control and experimental animals was measured by an Axioplan microscope (Carl Zeiss) to which a Dage-MTI series 68 videocamera was attached.[39] The output of this camera was sent to an Image Based Analysis System (IBAS; Carl Zeiss). One investigator (SK), who was blinded as to treatment allocation, performed the measurements. The system consists of a dedicated host computer and array processor and a video board for real-time image processing. The software allowed for SP-like immunoreactivity area measurements.

Data were recorded as the number of SP-like immunoreactive pixels per microscopic field. The change in SP-like immunoreactivity in brain stem sections of control (vehicle-injected) and experimental (capsaicin-injected) animals was expressed as the relative change in immunoreactivity recorded in the right (ipsilateral to TMJ injected with capsaicin or vehicle) versus the left (no treatment) side in every section studied (left-right/left). Anatomic designations of the different regions examined in reference to the trigeminal sensory nuclei examined were assigned on the basis of published specifications.[40–42] Averages were then calculated for each level of the brain stem for the animals receiving capsaicin (experimental animal group) or vehicle solution (control animal group). Comparison of the 2 animal groups was performed by 1-way analysis of variance (ANOVA) for each brain stem level, with alpha = .05. Post hoc analysis was accomplished with the Bonferroni multiple comparisons test. A P value < .05 was considered significant.

Results

To identify the regions of the brain stem characterized by changes in the levels of SP-like immunoreactivity, serial brain stem sections were analyzed immunocytochemically following unilateral application of capsaicin solution (experimental group) or vehicle only (control group) to the right TMJ. SP-like antigenicity was primarily immunolocalized in fibers in the superficial laminae of subnucleus caudalis (medullary dorsal horn laminae I and II). Representative examples of brain stem sections derived from an experimental animal are depicted in Figs 1a to 1j. Specifically, at the level of subnucleus caudalis, SP-like immunoreactivity was primarily localized at the dorsomedial part of this subnucleus (Figs 1g and 1h). SP-like immunoreactivity was also localized at the level of subnucleus interpolaris, at the dorsal/dorsolateral portion of the subnucleus (Figs 1e and 1f). At the level of subnucleus oralis, SP-like immunoreactivity was seen demarcating the dorsal boundary of this subnucleus (Figs 1c and 1d). At the level of the trigeminal main sensory nucleus, SP-like immunoreactivity was primarily noted at the dorsomedial portion of this nucleus (Figs 1a and 1b).

As previously described, changes in SP-like immunoreactivity were expressed as the relative change in immunoreactivity recorded in the right side (ipsilateral to the TMJ injected with capsaicin) versus the left side (no treatment) in every section studied (left-right/left). Averages were then calculated for each level of the brain stem for the animals receiving capsaicin (experimental group) or vehicle (control group), and are depicted in Fig 2. It appears that 5 brain stem regions were mainly affected by capsaicin treatment: *(1)* from 6.6 mm to 5.4 mm rostral to the obex, *(2)* from 4.3 mm to 3.6 mm, *(3)* from 2.4 mm to 2.1 mm, *(4)* from 0.9 mm rostral to obex to –0.6 mm caudal to obex, and *(5)* from –1.8 mm to –2.7 mm caudal to obex (Figs 1 and 2). These regions corresponded to the levels of *(1)* the main sensory nucleus, *(2)* to subnucleus oralis, *(3)* subnucleus interpolaris, *(4)* subnucleus caudalis, and *(5)* the level of the first cervical nerve in the spinal cord, respectively.[40–42] Only minimal changes were noted in control animals.

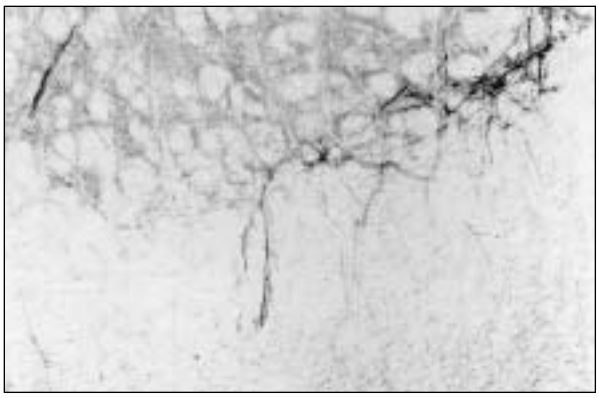

Fig 1a Experimental side, trigeminal main sensory nucleus.

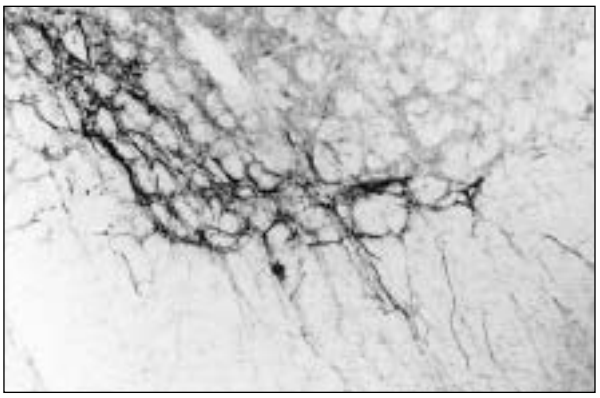

Fig 1b Contralateral side, trigeminal main sensory nucleus.

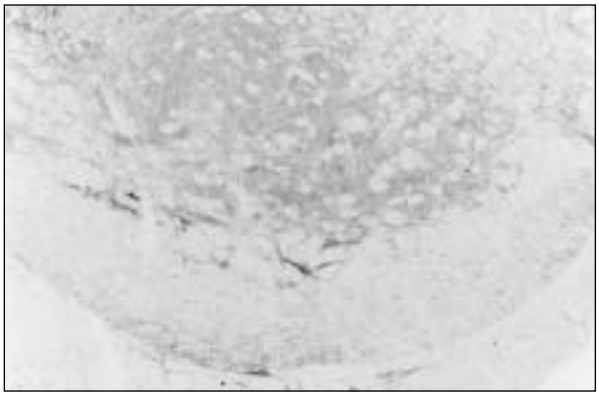

Fig 1c Experimental side, subnucleus oralis.

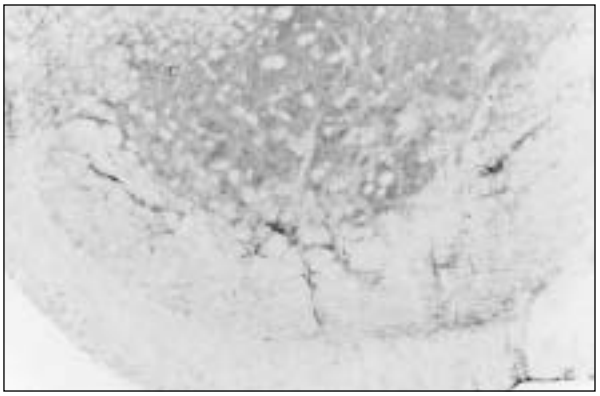

Fig 1d Contralateral side, subnucleus oralis.

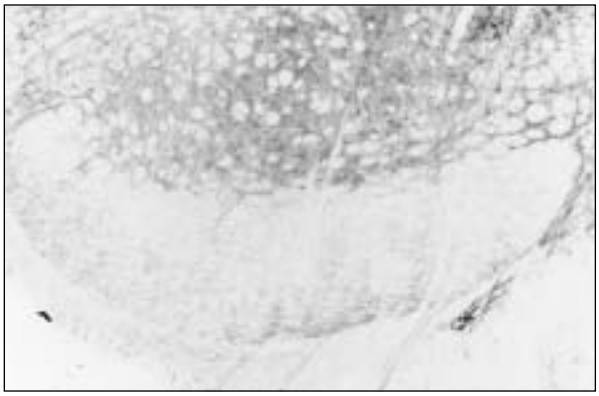

Fig 1e Experimental side, subnucleus interpolaris.

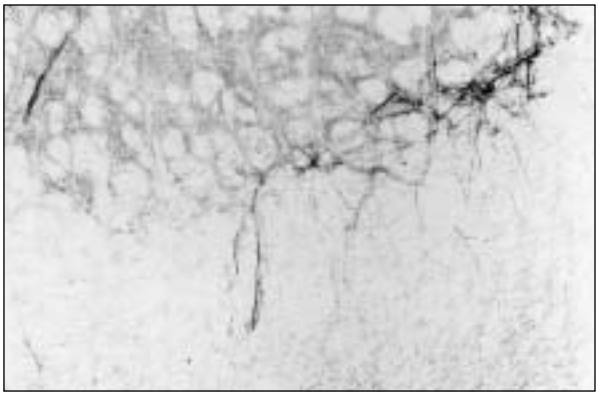

Fig 1f Contralateral side, subnucleus interpolaris.

Figs 1a to 1j SP-like immunoreactivity was significantly reduced in the experimental side of the trigeminal main sensory nucleus (a) versus the contralateral side (b), subnucleus oralis (c and d), subnucleus interpolaris (e and f), and subnucleus caudalis (g and h). However, the capsaicin had no effect on SP-like immunoreactivity in region C1 (i and j) (original magnification ×10). In each of figures 1a to 1j, the orientation is such that the lateral brain stem is shown at the bottom part of each micrograph.

Discussion

Local application of capsaicin-induced changes in the levels of SP-like immunoreactivity in the brain stem. These changes were noted in specific regions of the brain stem in a reproducible manner between animals; these regions appear to correspond to various trigeminal sensory nuclei, including the subnuclei caudalis, interpolaris, and oralis, as well as the main sensory nucleus. SP-like

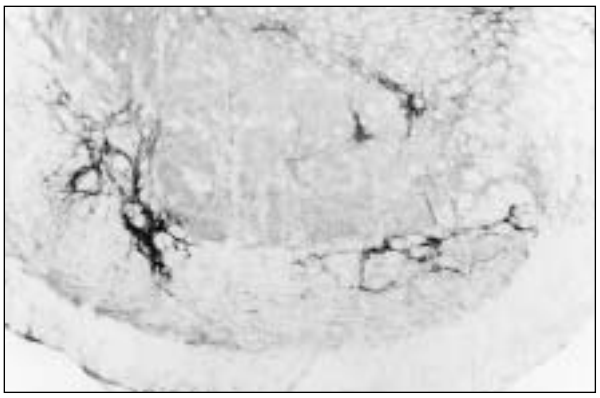

Fig 1g Experimental side, subnucleus caudalis.

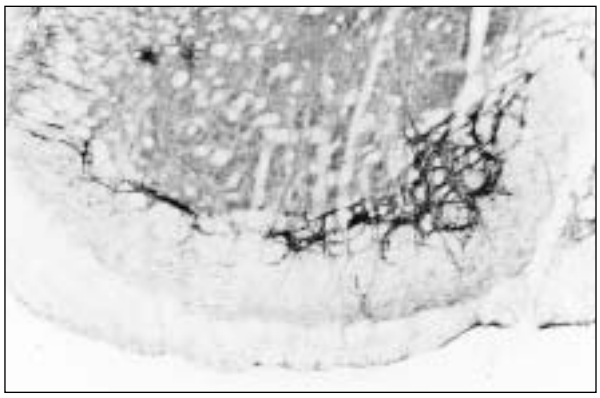

Fig 1h Contralateral side, subnucleus caudalis.

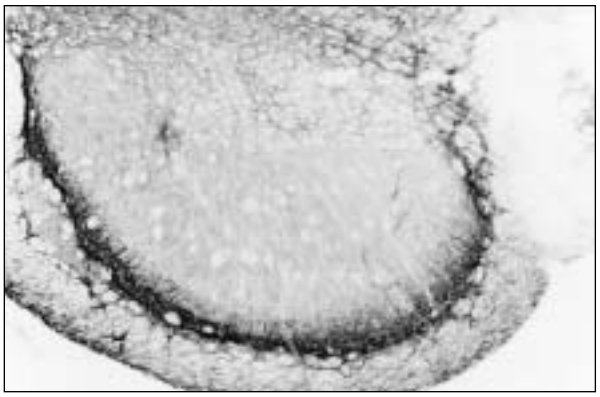

Fig 1i Experimental side, C1.

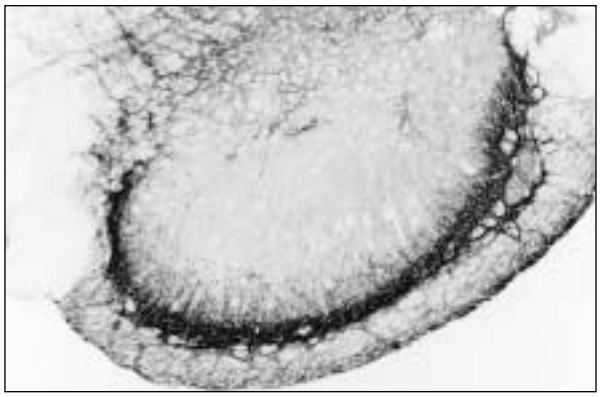

Fig 1j Contralateral side, C1.

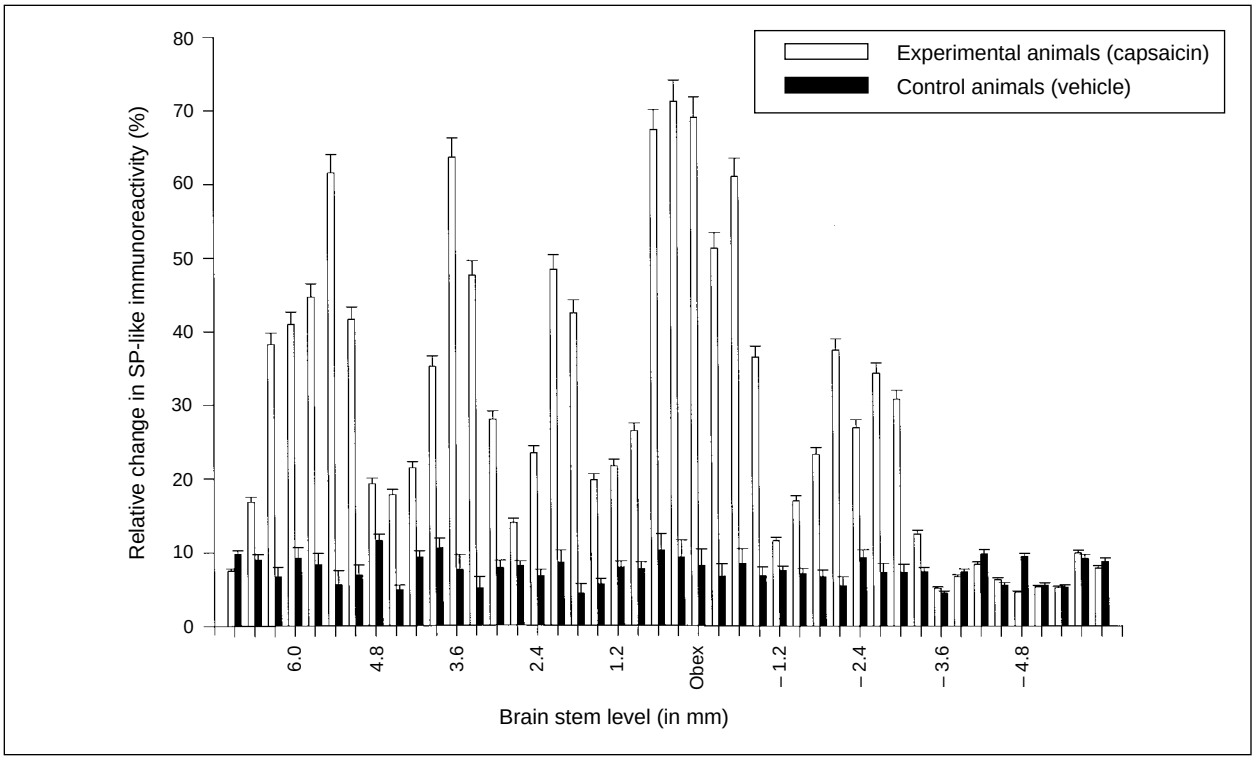

Fig 2 Relative changes in SP-like immunoreactivity in serial brain stem sections of experimental (capsaicin-injected) and control (vehicle-injected) animals. Specific regions of the brain stem were characterized by a remarkably greater degree of reduction in SP-like immunoreactivity in experimental animals compared to control animals.

immunoreactivity was primarily localized in laminae I and II of subnucleus caudalis. In fact, our findings are in agreement with previous reports on SP immunolocalization in the ferret,[43] including the subnucleus caudalis (dorsomedial part), interpolaris (dorsal/dorsolateral part), and oralis (demarcating its dorsal boundary) of the trigeminal spinal tract nucleus. At the level of the trigeminal main sensory nucleus, SP-like immunoreactivity was primarily noted at its dorsomedial portion.

The classical view of the functional organization of the trigeminal sensory nuclei depicts subnucleus caudalis as the primary recipient of small-diameter nociceptive afferent fibers from the orofacial structures. In comparison, the main sensory nucleus has been related to tactile sensation.[44] Small-diameter nociceptive afferents are believed to selectively project into the trigeminal spinal tract nucleus and not the trigeminal main sensory nucleus.[1,17,45,46] Hathaway et al,[47] for example, administered mustard oil, an irritant that activates small afferent fibers, to examine the areas of the brain stem receiving sensory input from the TMJ, on the basis of mustard oil–induced c-fos expression in the rat brain stem. They reported that c-fos induction was localized primarily in the subnucleus caudalis as well as the caudal portion of subnucleus interpolaris. However, previous studies utilizing retrograde tracers reported that the central processes of afferent fibers from the TMJ extend to the rostral portion of the trigeminal sensory nuclei as well as the subnucleus caudalis.[10–12] It has also been shown that some primary afferent fibers in the sensory root of the trigeminal nerve bifurcate into ascending and descending branches at the level of the caudal pons and project to the main sensory nucleus and the trigeminal spinal tract nucleus, respectively.[48] Li et al,[48] by employing a combination of fluorescent retrograde double labeling and immunofluorescent histochemistry, found that some neurons in the trigeminal ganglion in the rat project by way of axon collaterals to both the caudal parts of the trigeminal spinal nucleus and the main sensory nucleus, and that about 40% of these neurons showed SP-like immunoreactivity. Furthermore, several studies have provided evidence for the existence of trigeminal inter-nuclear connections.[49] Hence, it should be no surprise that exposure of TMJ sensory afferents to capsaicin resulted in rostral as well as caudal trigeminal nuclear SP-like immunoreactivity changes, including changes within the main trigeminal sensory nucleus.

The role of SP in the central processing of pain has been previously documented.[50–53] This neuropeptide is reportedly present in the small-diameter unmyelinated or thinly myelinated afferent nerve fibers (C- and Aδ fibers, respectively). These fibers are involved in conveying nociceptive input to spinal cord dorsal horn neurons.[30] Previous studies have documented the release of SP in the dorsal horn following electrical stimulation of the dorsal roots,[54] as well as from dissociated neurons in culture.[55] Capsaicin is a chemical irritant known to act primarily on nociceptive C-fibers, as well as Aδ nerve fibers. It evokes the release of SP in cultured spinal cord slices in vitro.[32,33,56] Moreover, Yaksh et al[34] documented the in vivo release of SP in rat spinal cords following chemical stimulation of sensory neurons with capsaicin. In a similar animal study, Go and Yaksh[35] showed release of SP in the rat spinal cord following spinal perfusion with capsaicin, which subsequently resulted in neuronal desensitization accompanied by reduction of SP spinal levels. In our study, exposure of TMJ afferents to capsaicin resulted in a decrease of SP-like immunoreactivity 6 hours following treatment. It appears that capsaicin successfully stimulated TMJ afferents and resulted in release of SP centrally. This relatively short period of time would not allow for any de novo replenishment of cellular SP.[57] Based on the methods employed in this study, this extracellular release of SP from the primary afferent fibers is detected as a decrease in SP immunoreactivity within the fibers themselves, a finding consistent in all experimental animals.

Clinically, capsaicin has been used in the management of pain associated with diabetic neuropathy.[58] Application of capsaicin-containing cream resulted in significant pain attenuation compared to placebo treatment. Furthermore, use of capsaicin-containing ointment successfully relieved pain associated with osteoarthritis and psoriasis. In another study,[59] topical capsaicin application was evaluated in managing joint pain associated with rheumatoid arthritis or osteoarthritis in a 4-week, double-blind, placebo-controlled, randomized trial. It was reported that capsaicin significantly reduced joint tenderness and pain. Similar results were reported by Deal et al.[60]

The present study found CNS changes in SP levels following TMJ capsaicin treatment. These effects appear to be specific and well-localized in brain stem regions involved in the central processing of orofacial pain.

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