Capsaicin-induced Joint Inflammation Is Not Blocked by Local Anesthesia

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The purpose of this study was to evaluate the effect of local anesthetic blockade of afferent innervation on the development of capsaicin-induced edema in the rat temporomandibular joint (TMJ) region and on reflex jaw muscle activity. Under halothane anesthesia, 64 male Sprague-Dawley rats were prepared for monitoring of edema development by lateral movement of a needle overlying the left TMJ region and for acute recording of electromyographic activity in ipsilateral digastric and masseter muscles. A double-barrel catheter was inserted into the TMJ region for delivery of saline or 0.5% bupivacaine from 1 needle, followed with the injection of 1% capsaicin, 0.1% capsaicin, or vehicle control from the other needle 5 minutes later. Application of capsaicin into the saline pretreated TMJ region led to dose-dependent edema development and reflex jaw muscle activity; however, only 1% capsaicin solution resulted in significant tissue expansion and muscle activity when compared with the vehicle control. Pretreatment of the rat TMJ region with bupivacaine failed to inhibit capsaicin-induced edema development, although successful blockade of nerve conduction was confirmed with the absence of reflex jaw muscle activity. Capsaicin-induced edema of the rat TMJ region developed independent of axonal conduction, suggesting neurogenic inflammation may arise regardless of functional nerve conduction.

Key Words: Neurogenic inflammation; Capsaicin; Local anesthesia.

Temporomandibular disorders (TMDs) encompass an array of problems involving the temporomandibular joint (TMJ) and/or the masticatory musculature. Although signs and symptoms have assisted in the identification of TMDs,[1] objective criteria for their diagnosis and indications for treatment remain controversial.[2,3] Despite the lack of information on biologic processes underlying TMJ adaptation to mechanical stress and subsequent pathophysiology, preliminary studies have suggested neurogenic inflammation to be one of the mechanisms of injury implicated in the development of TMDs.[3]

Neurogenic inflammation involves the antidromic stimulation of nociceptive primary afferents to release neuropeptides such as substance P to evoke an increase in vascular permeability and plasma extravasation (PE), leading to inflammatory effects on peripheral tissues.[5–7] Recent human studies revealed the presence of substance P in TMJ aspirates from TMD patients,[8,9] and similarly investigations on experimentally induced arthritic TMJ rats provided evidence to support a neurogenic component in the inflammatory process of TMDs.[10,11] Therefore, a neural blockade may theoretically prevent the release of these inflammatory neuropeptides and subsequently assist in the resolution of TMDs.

Capsaicin is a pharmacological tool used to evoke acute neurogenic inflammation[13,14] through the activation of specific vanniloid receptors, termed vanniloid subtype 1 (VR1) receptors,[15] located along primary sensory afferents.[16] Previously, our laboratory confirmed the application of capsaicin to the rat TMJ region elicits an inflammatory response, resulting in PE and edema development via the activation of VR1 receptors.[17] In addition, a dose-dependent, sustained, and reversible in-

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crease in electromyographic (EMG) activity of the ipsilateral masseter and digastric muscles was evoked.[18] However, the presence or absence of a capsaicin-induced neurogenic component in the TMJ inflammatory process remains unknown. Thus, the next logical step in the investigation of this potential neurogenic mechanism of injury in the TMJ region tends toward the evaluation of the effects of nerve conduction inhibition.

Therefore, the aim of this study was to investigate the effect of local anesthesia blockade of afferent innervation on the development of capsaicin-induced neurogenic edema in the rat TMJ region and capsaicin-induced reflex jaw muscle activity.

MATERIALS AND METHODS

The University of Toronto Animal Care Committee in accordance with the regulations of the Ontario Animal Research Act (Canada) approved the following protocol. Sixty-four male Sprague-Dawley rats weighing between 225 and 400 g each were prepared for the continuous measurement of one-dimensional tissue expansion, as previously described by Fiorentino et al[19] and Wong et al,[20] and for the acute recording of jaw muscle EMG activity as previously described by Cairns et al.[21] Therefore, only a brief description of the methodology follows.

The rats were divided into 6 different experimental groups. The left TMJ regions of rats in groups 1, 2, and 3 were pretreated with saline (10 μL), followed by the application of 1.0% capsaicin (10 μL); 0.1% capsaicin (10 μL); or vehicle control (ethanol, Tween 80, and saline in a 1 : 1 : 8 ratio, 10 μL), respectively. The left TMJ regions of rats in groups 4, 5, and 6 were pretreated with bupivacaine (0.5%, 10 μL), followed by the application of 1.0% capsaicin (10 μL); 0.1% capsaicin (10 μL); or vehicle control (ethanol, Tween 80, and saline in a 1 : 1 : 8 ratio, 10 μL), respectively.

Under surgical inhalational anesthesia (O_2, 0.3–0.4 L/min; N_2O, 0.6 L/min; halothane, 1.5–2%), a tracheotomy was performed and the rat was artificially ventilated for the duration of the experiment. Bipolar electrodes were fabricated out of 36-gauge Teflon-coated, single-stranded stainless steel wires and inserted unilaterally into the left digastric and masseter muscles for EMG recording. The rat's head was stabilized on a stereotaxic frame, and a double-barrel cannula consisting of two 27-gauge dental needles connected by polyethylene tubing to two 25-μL Hamilton syringes was inserted posterior to the posterior infero-lateral aspect of the zygomatic arch. One needle delivered the preload agent, either saline (10 μL) or bupivacaine (0.5%, 10 μL), and 5 minutes later capsaicin (1.0%/0.1%, 10 μL) or vehicle (10 μL) was administered from the other needle. A sharp tungsten needle was bent 90° at its distal tip, and its proximal end was stabilized with a drop of cyanoacrylate adhesive over the left TMJ region. The distal tip was allowed to rest passively on a paper support track fixed lateral to the left TMJ area, and this needle served as a physical marker of lateral tissue expansion. A second needle was fixed to a micromanipulator, and the tip of the first was matched with the tip of the second with the aid of a ×1.6 dissecting microscope. Therefore, realignment of the 2 needle tips with the use of the micromanipulator allowed the quantification of tissue expansion/edema in the TMJ region in micrometers. On completion of the surgery, the halothane level was gradually reduced (0.9–1.3%) until noxious pressure applied to the hind-paw toes produced a slight hindlimb withdrawal reflex, thus ensuring an adequate level of anesthesia was maintained for the duration of the experiment.[21]

Baseline edema measurements were taken every 5 minutes for 15 minutes prior to the injection of the preload agent and were denoted as $time_{-15}$, $time_{-10}$, and $time_{-5}$. Subsequently, the preload agent was injected at $time_0$, and measurements were taken in 1-minute intervals until $time_5$, at which time capsaicin or vehicle was delivered. Again, the amount of expansion was recorded in 1-minute intervals from $time_6$ to $time_{10}$, inclusive. From $time_{12}$ to $time_{70}$, the recording interval was increased to 2 minutes, and from $time_{75}$ to $time_{180}$, edema measurements were taken every 5 minutes. Therefore, the expansion distance was recorded for a total period of 180 minutes. If the injection of the preload agent did not result in a straight lateral movement of the edema measurement needle, the resultant data were excluded.

Concurrently, baseline EMG activities were observed for 15 minutes before the administration of the preload agent, and continuous EMG activities were recorded following the injection of the preload agent and capsaicin. The recording of EMG activities was terminated 30 minutes after the reflex activity induced by capsaicin returned to baseline levels. EMG activity was amplified (gain, ×500; bandwidth, 30–1000 Hz); fed into a computer equipped with a CED 1401 board and analysis software (Spike2); stored electronically; and analyzed off-line. For statistical analyses, EMG data were normalized relative to baseline values and expressed as cumulative EMG activity area under the curve measurements. If the injection of the preload agent evoked reflex jaw muscle activity that failed to return to baseline levels recorded prior to the injection of capsaicin/vehicle solution or the injection of capsaicin into the bupivacaine pretreated TMJ region led to increased EMG activity, the resultant data were excluded.

After tissue expansion measurements and EMG re-

cordings were collected, Evan's blue dye (10 mg/mL, 20 mg/kg) was injected intravenously for the evaluation of PE. The rat was sacrificed and a postmortem dissection consisting of the retraction of the skin and superficial muscles (masseter and temporalis) for the exposure of both the ipsilateral and contralateral TMJ was performed. Both joints along with the surrounding tissues were visually examined, and the presence of obvious blue staining of the disk and capsule signified the correct placement of the catheter and provided the indication of PE into the TMJ region.[22,23] If the TMJ region lacked blue staining, the acquired edema and EMG data were discarded.

Statistical analyses of the obtained data were evaluated with 1-way analysis of variance (ANOVA), 2-way analysis of variance (2-way ANOVA), and post hoc Tukey tests when appropriate. A *P*-value less than .05 was determined as statistically significant.

RESULTS

Among the 64 rats used in this experiment, only data obtained from 48 rats were deemed suitable for analysis. Twelve rats either responded to the injection of saline and the resultant increased EMG activity did not return to baseline levels prior to the injection of capsaicin/vehicle, or the application of the preload agent failed to induce a straight lateral movement of the edema measurement needle. In 2 rats, the application of 1% capsaicin to the bupivacaine pretreated TMJ region led to reflex jaw muscle activity, which indicated an unsuccessful nerve conduction blockade. Moreover, there was an absence of blue PE staining in the TMJ region of 2 other rats. Therefore, for the measurement of tissue expansion, each experimental group consisted of 8 rats each, and for the EMG recording of jaw muscle activity, groups 1 and 2 consisted of 6 rats, group 3 consisted of 10 rats, and groups 4–6 consisted of 8 rats each.

The dose-response curve demonstrating the relationship between tissue expansion and increasing capsaicin concentrations for the saline pretreated experimental groups (1, 2, and 3) is presented in Figure 1. Mean baseline expansion distances caused by the trauma induced by the insertion of the catheter were similar among the 3 saline-pretreated subgroups (ANOVA, $P > .05$). The capsaicin-induced edema development occurred in a dose-dependent manner; however, statistical analysis revealed that only 1% capsaicin-evoked tissue expansion was significantly different from the vehicle group at $time_{28}$ and onward (Tukey test, $P < .05$). In addition, the comparison of tissue expansion induced by 1% with that elicited by 0.1% capsaicin indicated statistical significance at $time_{48}$ and onward (Tukey test, $P < .05$). Therefore, in addition to the demonstration of a dose-dependent capsaicin-induced tissue expansion in the rat TMJ region, a difference in time point at which edema development was statistically significant between various concentrations of capsaicin was revealed.

To evaluate the effect of 0.5% bupivacaine pretreatment of the TMJ region on capsaicin-evoked edema, the saline preload groups were compared with their respective bupivacaine preload groups. Regardless of the type of preload utilized, there were no statistically significant differences in the resultant tissue expansion (2-way ANOVA, $P > .05$), which was indicated by the close superimposition of the 2 plots for the respective pairs of experimental groups. Therefore, 0.5% bupivacaine was clearly ineffective in reducing the capsaicin-induced tissue expansion in the TMJ region. A summary plot of the cumulative edema development of all 6 experimental groups is provided in Figure 2.

Furthermore, the injection of capsaicin or vehicle solution into the saline pretreated TMJ region at $time_5$ elicited a sustained and reversible reflex ipsilateral masseter and digastric EMG response. However, the injection of capsaicin or vehicle solution into bupivacaine pretreated TMJ regions failed to evoke an increase in reflex ipsilateral masseter and digastric EMG activity. Therefore, successful nerve conduction blockade by the pretreatment of TMJ tissues with bupivacaine was confirmed by the absence of reflex EMG activity in response to capsaicin.

Since the EMG data were similar for both the masseter and digastric muscles, only the summarized graph displaying the EMG activity for the digastric muscle is given in Figure 3. A 2-way ANOVA demonstrated significance between the increased EMG activity in both masseter and digastric muscles evoked by capsaicin in saline pretreated and bupivacaine pretreated rats ($F_{1,37} = 6.604$, $P < .05$; $F_{1,37} = 20.568$, $P < .001$, respectively). Furthermore, an interaction between the concentration of capsaicin and the type of preload agent administered on the resultant reflex EMG activity in masseter and digastric muscles ($F_{2,37} = 3.69$, $P < .05$; $F_{2,37} = 9.401$, $P < .001$, respectively) was revealed. Moreover, the reflex EMG activity of both muscles increased in a dose-dependent manner; however, only 1% capsaicin in the saline pretreated group evoked a significant increase in masseter and digastric EMG activity when compared with the vehicle control (Tukey test, $P < .05$).

Qualitative analysis of the postmortem dissection by the naked eye revealed an Evan's blue dye response in the ipsilateral temporomandibular articular and periarticular tissues in all experimental groups (data not shown). However, the 1% capsaicin demonstrated more intense staining compared with that produced by the

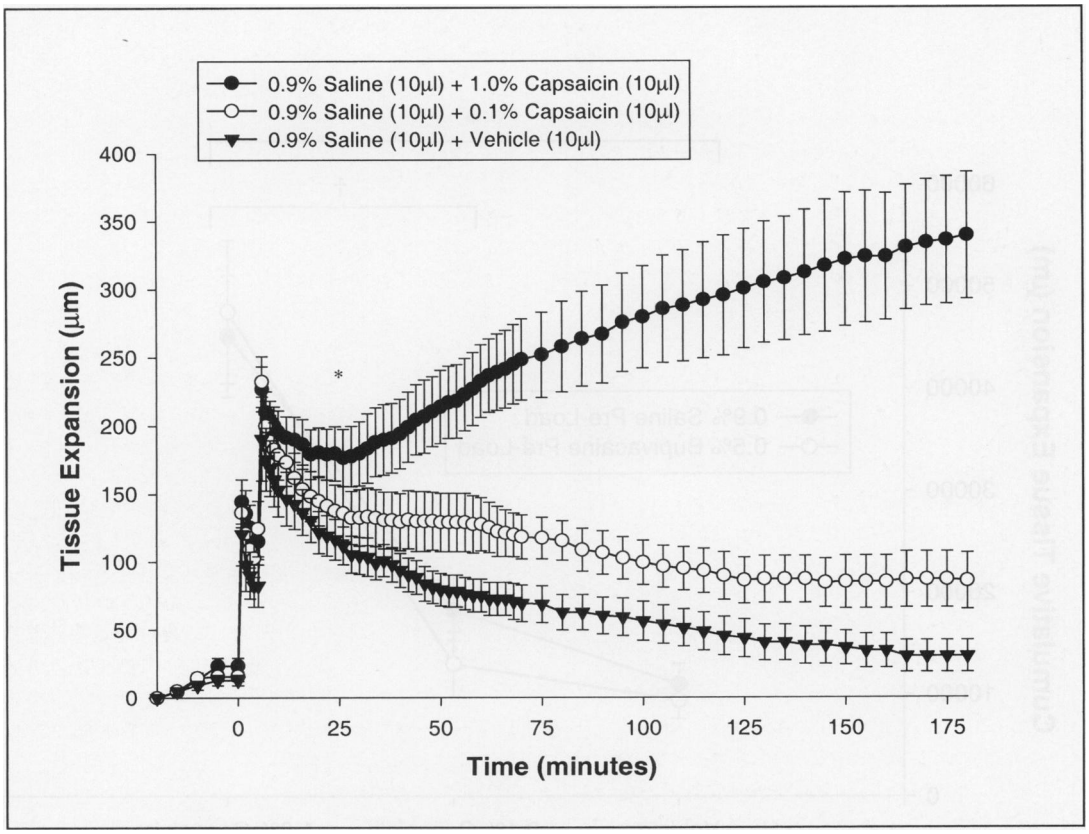

Figure 1. Dose-response curve: tissue expansion versus increasing capsaicin concentration. Mean changes in tissue expansion evoked by the injection of various concentrations of capsaicin or vehicle control into the left temporomandibular joint (TMJ) region. Each data point represents mean tissue expansion ± SE for a certain time point over the 180-minute time course in 8 rats. Asterisk (*) indicates tissue expansion of that 1% capsaicin data point $time_{28}$ and onward was significantly higher in comparison with that evoked by the vehicle control (ANOVA, $P < .05$). Small dagger (†) indicates that tissue expansion of that 1% capsaicin data point $time_{48}$ and onward was significantly higher in comparison with that evoked by the 0.1% solution (ANOVA, $P < .05$).

0.1% solution or vehicle control. In addition, examination of the contralateral rat TMJ revealed an absence of Evan's blue dye staining of the temporomandibular articular or periarticular tissues.

DISCUSSION

The application of capsaicin to the rat TMJ region resulted in an inflammatory reaction illustrated by the expansion of the periarticular tissue and plasma extravasation as demonstrated by Evan's blue dye staining in this area of interest. Since capsaicin binds to VR1 receptors located on primary sensory afferents innervating the rat TMJ region[17] to produce edema and plasma extravasation, the findings in this study suggest that acute inflammation of the rat TMJ region encompasses a neurogenic component. In addition, sustained and reversible increase in ipsilateral masseter and digastric muscle activities were evoked with the deposition of capsaicin into the rat TMJ region.

Subsequently, to understand the characteristics of the potential neurogenic mechanism in the development of acute inflammation in the TMJ region evoked by capsaicin, the evaluation of the consequences of nerve conduction inhibition was performed. Although a complete blockade of nerve conduction was confirmed with the lack of EMG response to capsaicin, the amount of edema and plasma extravasation generated by the application of different concentrations of capsaicin did not significantly differ among the saline pretreated and bupivacaine pretreated rats. Thus, this present study also demonstrated that the administration of local anesthetic prior to the injection of capsaicin failed to significantly reduce the resultant tissue expansion and plasma extravasation in the TMJ region; however, the reflex jaw muscle activity was abolished.

The plasma extravasation induced by capsaicin administration in the rat TMJ region suggests a neurogenic component in its inflammatory process; however, the absence of a significant reduction in capsaicin-induced edema in the local anesthetic pretreated TMJ region

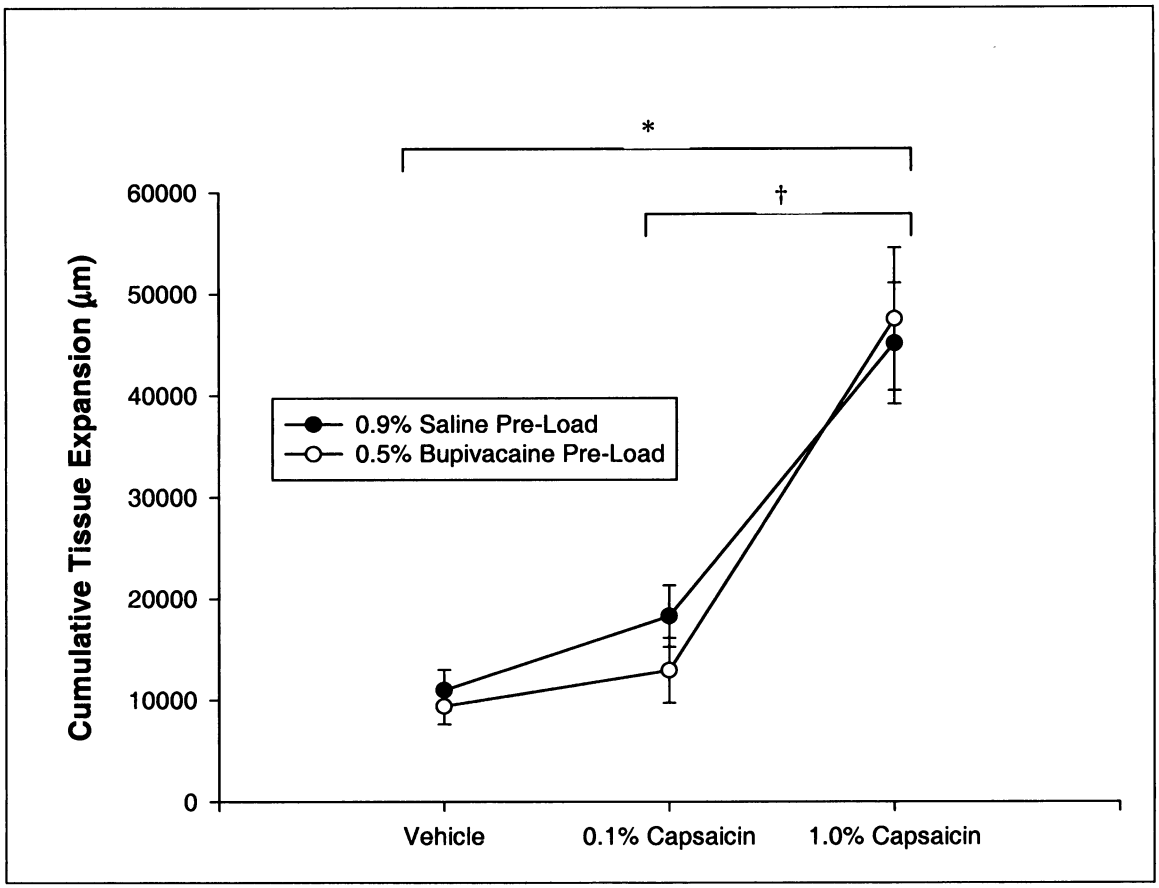

Figure 2. Capsaicin-induced cumulative tissue expansion. Mean cumulative tissue expansion evoked by injection of various concentrations of capsaicin or vehicle control into the left temporomandibular joint (TMJ) region pretreated with a saline preload or bupivacaine preload. Each data point represents the mean ± SE of normalized values relative to baseline tissue expansion in 8 rats. The horizontal dotted line indicates mean baseline cumulative tissue expansion distance. Asterisk (*) indicates that cumulative tissue expansion induced by 1% capsaicin was significantly higher in comparison with that evoked by the vehicle control (Tukey test, $P < .05$). Small dagger (†) indicates that tissue expansion of that data point was significantly higher in comparison with that induced by the 0.1% capsaicin solution (Tukey test, $P < .05$). No statistical difference was demonstrated between cumulative tissue expansion induced by various concentrations of capsaicin or vehicle control in saline pretreated and bupivacaine pretreated experimental groups.

questions the true mechanism underlying neurogenic inflammation and the pure neurogenic nature of the inflammatory irritant, capsaicin. The findings from this investigation suggest 2 possible interpretations. First, capsaicin may evoke the direct release of inflammatory mediators from neuronal terminals without the conduction of action potentials along the axon. Alternatively, capsaicin may predominantly act in a nonneurogenic fashion when applied to the TMJ region due to its inherent inflammatory nature or the activation of VR1 receptors located on other cellular components.

In the event that capsaicin is indeed purely acting in a neurogenic fashion, the lack of the necessity for nerve conduction disproves both the classical and current concepts of neurogenic inflammation. Past studies have argued that the axon reflex[24] and dorsal root reflex[25] are mechanisms underlying the neurogenic inflammatory process. In addition, both the central nervous system and autonomic nervous system have been implicated in the development of neurogenic inflammation.[26-28] However, the results in the present investigation suggest otherwise. In the absence of central and autonomic descending influences, spinal reflex loops and antidromic conduction from adjacent terminal nerve branches, the resultant capsaicin-induced neurogenic inflammatory reaction was equivalent to that produced in the presence of functional nerve conduction mechanisms. Therefore, these results lead to the hypothesis that inflammatory mediators can be directly released from afferent nerve terminals without the conduction of nerve impulses.

Unfortunately, the body of evidence supporting the above potential mechanism in the development of neurogenic inflammation is limited, and the findings from previous studies have been inconsistent. Earlier studies

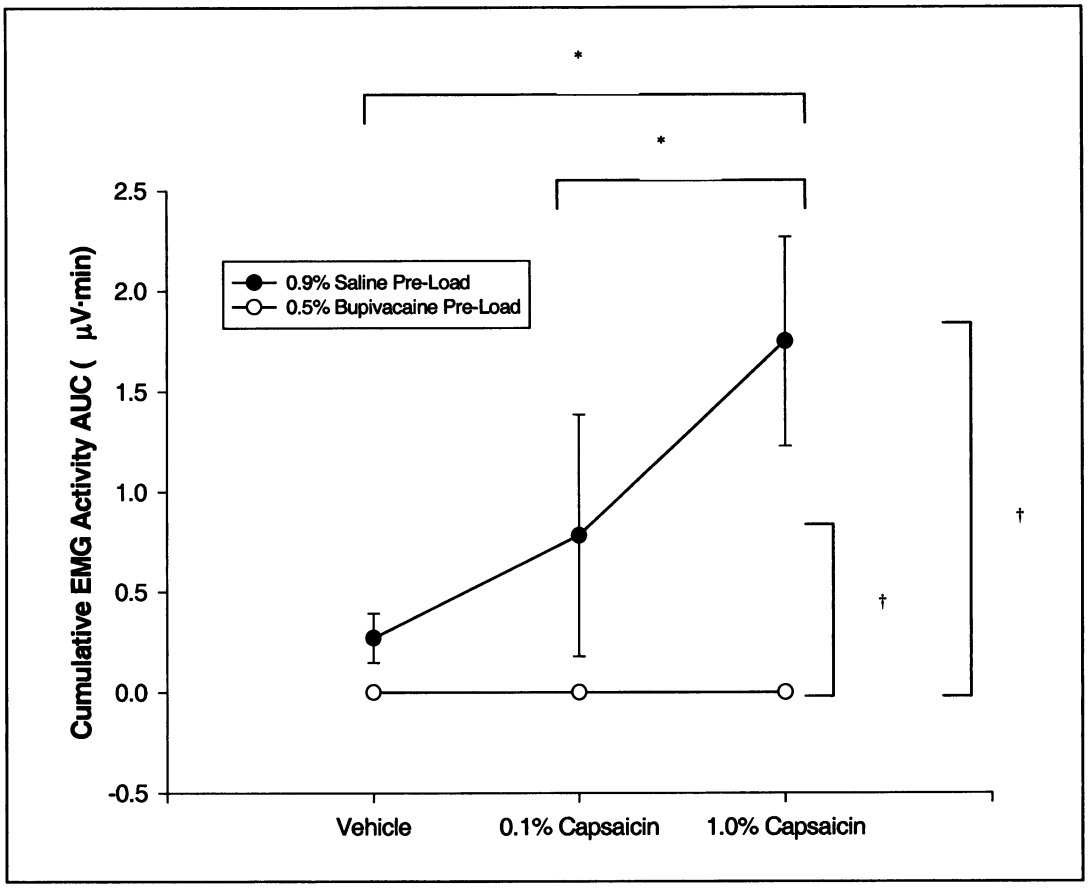

Figure 3. Capsaicin-induced cumulative digastric electromyographic (EMG) activity. Cumulative mean changes in EMG area in left digastric muscle evoked by injection of various concentrations of capsaicin or vehicle control into the ipsilateral temporomandibular joint (TMJ) region. Each data point represents mean ± SE of the normalized values relative to baseline EMG activity in each rat, and the horizontal dotted line indicates mean baseline activity. Asterisk (*) indicates cumulative EMG activity induced by 1% capsaicin was significantly higher in comparison with 0.1% capsaicin solution and vehicle control (Tukey test, $P < .05$). Small dagger (†) indicates statistical significance among EMG activity in digastric muscle evoked by capsaicin in saline pretreated and bupivacaine pretreated rats (2-way analysis of variance [ANOVA], $P < .001$). Also, the interaction between the concentration of capsaicin and type of preload agent administered on the resultant reflex EMG activity in the digastric muscle was statistically significant (2-way ANOVA, $P < .001$).

by Jancso et al[14] demonstrated that axonal conduction was not necessary for the capsaicin-induced plasma extravasation and edema development since prior local anesthetic treatment did not abolish the reaction. Similarly, Szolcsanyi et al[29] demonstrated the release of sensory neuropeptides such as substance P from the peripheral endings of sensory nerves innervating the rat trachea in the presence of local anesthesia, suggesting a direct mechanism for peptide release not mediated by an axon reflex or central descending influences. Recently, under a comparable protocol as the present study, Wong et al[20] revealed the ineffectiveness of local anesthetic pretreatment of the TMJ region on mustard oil–induced neurogenic inflammation. Thus, it was concluded that the direct release of inflammatory mediators from neuronal terminals is the potential mechanism underlying the neurogenic component of inflammation in the TMJ.

Contrary to the above findings, Lundberg and Saria[30] demonstrated the effectiveness of lidocaine in preventing neurogenic PE in the airway mucosa. Likewise, Dux et al[31] demonstrated the inhibition of mustard oil–induced neurogenic inflammation with the pretreatment of the skin with local anesthetic. Therefore, in contrast to the findings in this present investigation, axonal conduction blockade inhibited PE in a dose-dependent manner, thus suggesting that functional nerve conduction is necessary for the development of neurogenic inflammation. Nonetheless, the discrepancies on the theory of direct release of inflammatory mediators between various investigations may be due to tissue specificities and species differences.

Alternatively, the assumption that capsaicin may induce inflammation predominantly in a nonneurogenic fashion is paradoxical since this substance is known as

an important pharmacological tool used to distinguish a subset of nociceptive sensory neurons. However, since this agent is an inflammatory irritant, it is conceivable that a general inflammatory response may be evoked by the innate immune defense system due to its recognition of capsaicin as a foreign substance. Therefore, along with neuropeptides released from neuronal terminals, nonneurogenic inflammatory factors may be released by capsaicin. For example, an in vitro investigation has localized VR1 receptors on mast cells.[32] In the event that mast cells may degranulate with the binding of capsaicin to its VR1 receptors, there is the possibility that the release of histamine may mediate inflammation by its direct action on the vasculature. Furthermore, there is a possibility that the afferents innervating the TMJ region cannot elicit neurogenic inflammation, since one study has suggested that there may be some tissue-specific trophic influences on the development of neurogenic inflammation.[33] Therefore, a capsaicin-induced inflammatory response may be activated regardless of functional nerve conduction.

In summary, the pretreatment of the rat TMJ region with local anesthetic failed to inhibit capsaicin-induced tissue expansion. Thus, the edema developed independent of axonal conduction, suggesting the direct release of inflammatory mediators from primary afferent nerve terminals. Hence, contrary to traditional belief, this hypothesis leads to the assumption that central and autonomic descending influences, spinal reflex loops along with antidromic stimulation from adjacent nerve terminals, are not necessary for the development of neurogenic inflammation in the rat TMJ region. Alternatively, capsaicin may act in a nonneurogenic manner; however, the mechanism by which this irritant acts remains debatable. Therefore, to fully comprehend the complex inflammatory response in the TMJ region, further investigations such as the effect of depletion of mediators on TMJ inflammation are warranted.

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