TRPV1 Receptor Mechanisms Involved in Capsaicin-Induced Oedema in the Temporomandibular Joint Region

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Purpose: Recent evidence indicates that capsaicin (CAP), the active and pungent ingredient of hot red peppers, can induce inflammation and pain when it is applied to peripheral tissues. It has been shown to excite small-diameter afferent nerve fibers supplying the tissues by acting on a specific membrane receptor, the TRPV1 (or VR1) receptor. Since it is unclear how CAP application produces an inflammatory action in temporomandibular joint (TMJ) tissues, the aim of this study was to determine if CAP application to the rat TMJ region induces inflammatory changes such as oedema through an action on the TRPV1 receptor.

Materials and Methods: In eight groups of anaesthetized rats (each group, n=8), CAP (0.001%, 0.01%, 0.1%, or 1%) was injected into the TMJ region and was preceded by injection of vehicle or the TRPV1 receptor antagonists capsazepine or ruthenium red. Oedema was monitored by expansion of the TMJ tissues.

Results: Compared with vehicle controls, CAP 1%, 0.1% and 0.01% induced significantly greater oedema ($p<0.05$, ANOVA) in a dose dependent manner. The oedema became apparent as early as 15 minutes after the CAP injection and lasted over 120 minutes. Both the competitive antagonist capsazepine and the non competitive antagonist ruthenium red could significantly reduce the CAP induced oedema.

Conclusions: These findings indicate that CAP can induce a significant inflammatory response within the TMJ region in a dose-dependent fashion, and that this effect is mediated, at least in part, by TRPV1 receptor mechanisms.

Key words: temporomandibular joint, oedema, capsaicin

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INTRODUCTION

We have previously investigated a rat model of acute inflammatory injury of the temporomandibular joint (TMJ) region in which the application to the TMJ region of the small fibre excitant and inflammatory irritant mustard oil results in extensive TMJ tissue inflammation (Yu et al, 1996; Fiorentino et al, 1999; Wong et al, 2001) as well as reflex increases in the electromyographic (EMG) activity of jaw muscles (Cairns et al, 1998; Yu et al, 1996). Capsaicin (CAP), the active and pungent ingredient of hot red peppers, is another inflammatory irritant that can induce inflammation when it is applied to various orofacial tissues. CAP application to the skin of the mouse (Inoue et al, 1993, 1995; Blazso and Gabor, 1994) or rabbit (Buckley et al, 1990) or to the tooth pulp of the rat (Sunakawa et al, 1999) induces signs of inflammation such as oedema, and local application of CAP produces vasodilatation in the rat or pig nasal mucosa (Bari et al, 1994; Stjarne et al, 1994). The receptor mechanisms responsible for mustard oil-evoked jaw muscle activity and mustard oil-induced inflammation remain poorly understood and appear to require co-activation of several different peripheral receptor subtypes (Cairns et al, 1998; Banvolgyi et al, 2004; Jordt et al, 2004; Yu et al, 1996). In contrast, the effects of CAP are thought to be mediated through activation of the vanilloid receptor 1 (VR1 or TRPV1; for review see Jancso et al, 1967; Jancso and Jansco-Gabor, 1968; Holzer, 1991; Buck and Burks, 1986; Inoue et al,

1995; Caterina et al, 1997; Caterina and Julius, 2001; Julius, 2003). The excitatory effects of CAP can be blocked by the specific competitive TRPV1 receptor antagonist capsazepine (CAPZ) or the non-competitive antagonist ruthenium red (RR) (e.g. Maggi et al, 1988; Bevan et al, 1992; Inoue et al, 1993; Bandell et al, 1994; Bari and Jancso, 1994 Szallasi and Blumberg, 1999).

As a result of the well-characterized mechanism of action of CAP, we have begun to investigate the utility of CAP injection into the TMJ region as an acute inflammatory joint injury model. It has recently been found that injection of CAP into the TMJ region results in activation of nociceptive jaw muscle reflexes and the development of oedema (Lam et al, 2003; Tang et al, 2004) similar to injection mustard oil into this joint (Yu et al, 1996; Cairns et al, 1998; Fiorentino et al, 1999; Wong et al, 2001). There is some evidence that mechanisms other than TRPV1 receptor activation may play a role in the consequences of CAP injection into the TMJ region. For example, jaw muscle activity evoked by the TMJ region injection of CAP is attenuated by co-injection of NMDA receptor antagonists, which suggests a role for the activation of peripheral NMDA in this effect of CAP (Lam et al, 2003). Furthermore, pre-injection of the local anaesthetic bupivacaine, at a concentration sufficient to suppress CAP-evoked jaw muscle activity, has no effect on CAP-induced TMJ inflammation, which may indicate that the development of TMJ oedema after intraarticular injection of CAP is due to non-neurogenic mechanisms, i.e. potentially independent of TRPV1 receptor activation (Tang et al, 2004). Nonetheless, TRPV1 receptors have been documented on the terminal endings of small-diameter afferent fibres within the TMJ (Ichikawa et al, 2004), which appears to support the idea that these effects of CAP may be mediated through activation of this receptor subtype. These findings led us hypothesize that activation of the TRPV1 receptors does play a role in the development CAP-induced inflammation of the TMJ region. To address this hypothesis, we examined whether pre-injection of the TRPV1 receptor antagonists CAPZ or RR would attenuate the development of CAP-induced oedema in the rat TMJ region.

Some of these data have been previously presented in abstract form (Fiorentino et al, 2000).

MATERIALS AND METHODS

The experimental procedures in animals were approved by the University of Toronto Animal Care Committee in accordance with the regulations of the Ontario Animal Research Act (Canada). Eighty-four male Sprague Dawley rats weighing between 250 and 450g each were housed in light and dark cycles of 12-hour duration and had free access to food and water. Each rat was prepared for the continuous measurement of one dimensional tissue expansion, as previously described (Fiorentino et al, 1999; Wong et al, 2001; Tang et al, 2004). Therefore, only a brief description of the methodology follows.

Each rat was weighed immediately before anaesthesia, which was induced and maintained by intraperitoneal injection of a mixture of urethane (1000mg/kg) and α-chloralose (50mg/kg; Hu, 1990; Fiorentino et al, 1999). Depth of anaesthesia was determined periodically and was considered adequate if there was a lack of spontaneous movements by the animal, a lack of autonomic responses (e.g. increased heart rate or change in pupil size) to noxious pinching the paw, and the presence of a constricted pupil. Additional anaesthetics were administered as required to maintain an adequate depth of anaesthesia. The left side of the face was shaved, a tracheal tube inserted and the right femoral vein cannulated. Throughout each experiment, the core body temperature was maintained within physiological levels (37-37.5°C) by a feedback thermal blanket, and heart rate remained stable between 350 and 400 bpm.

Each rat was placed in a stereotaxic apparatus and two screws were inserted into the exposed dorsal surface of the skull. As previously described for tissue expansion measurements (Fiorentino et al,1999; Wong et al, 2001), a perpendicular bar connected to the stereotaxic apparatus was fixed to the screws with dental cement, and a moulded plaster block compound (Mounting Plaster, Whip Mix Corporation, Louisville, KY, USA.) was applied to the right side of the head, so that the rat's head was fixed in place, allowing the left ear bar to be removed. The plaster compound contacted an area of approximately 7.5cm^2 of the right side of the posterior face (which represented approximately 50% of the rat's ipsilateral facial area). At 30 minutes prior to the commencement of the tissue expansion measurements, a double barrel cannula consisting of two 27 gauge dental needles, glued together and connected by polyethylene tubing to two 25μL Hamilton syringes, was inserted into the left TMJ region posterior to the posterior infero lateral aspect of the zygomatic arch. One needle delivered the pre-load agent (10μL), either vehicle or TRPV1 receptor antagonist, and five minutes later the other needle delivered CAP (10μL, at one of several at different concentrations; see below). Tissue expansion was first measured 20 minutes prior to injection of chemicals, and subsequently every five minutes for the next 80 minutes and then every 10 minutes for the next 40 minutes; a final measurement was made at 150 minutes after the injection.

After the tissue expansion measurements, Evans blue dye (10mg/mL, 6mg/kg) was injected intravenously. Ten minutes later, the rat was euthanised with an injection of T-61 and perfused transcardially with warm

physiological saline. A postmortem dissection was performed by the retraction of the skin and superficial muscles (masseter and temporalis) for the exposure of both the ipsilateral and contralateral TMJ. Both joints along with the surrounding tissues were visually examined, and the presence of obvious blue staining of the ipsilateral TMJ disk and capsule signified the correct placement of the catheter and provided the indication of plasma extravasation of the dye into the TMJ region. If the ipsilateral TMJ region lacked blue staining, the acquired expansion data were discarded.

Experimental Design and Analyses

Five groups of rats (n=8 in each group) were first used to test for the effects of CAP (0.001%, or 0.01%, or 0.1%, or 1%; in 10% absolute alcohol, 10% Tween80, and 80% saline; 10μL; Calbiochem, USA) or vehicle (10% absolute alcohol, 10% Tween80, and 80% saline; 10μL) without any pre-load agent other than vehicle. We tested an additional 3 groups of rats (n=8 in each group) with pre-treatment with either the specific competitive antagonist CAPZ (0.5%, in 10% absolute alcohol, 10% Tween80, and 80% saline; 10μL; Research Biochemicals International, USA), followed by 0.1% CAP injection into the TMJ region five minutes later, or the non-competitive antagonist RR (0.1%, in 10% absolute alcohol, 10% Tween80, and 80% saline; 10μL; Aldrich, USA) followed by 1% or 0.1% CAP five minutes later. In a preliminary series of experiments, we also tested a small number of rats (n=3) with RR pre-treatment followed by an even lower concentration of CAP (0.01%) and found the results were similar to the vehicle + vehicle group.

The tissue expansion data were analysed in two ways. The time courses of tissue expansion were plotted out and the data statistically analysed with a one way analysis of variance (ANOVA) and post-hoc Tukey test between the vehicle + vehicle group and the other experimental groups at each time point of interest. For dose-response functions, a two way analysis of variance (2 way ANOVA) and post-hoc Tukey tests were performed on the value of the area under the time course curve of tissue expansion. Data are presented as means ± SE. $P < 0.05$ was considered to indicate statistical significance.

RESULTS

The application of CAP to the TMJ region induced oedema, as indicated by tissue expansion, and post-mortem dissection revealed Evans blue dye extravasation in the ipsilateral temporomandibular articular or periarticular tissues in animals receiving CAP 1% or CAP 0.1%. Fig 1 illustrates the magnitude and time course of the expansion for the four vehicle pre-treated experimental groups (Vehicle + CAP 0.001%, 0.01%, 0.1%, 1%) and the vehicle + vehicle group. There was a dose dependent increase in the magnitude of the CAP-induced expansion. Mean baseline expansion caused by the insertion of the catheter was similar among all five vehicle pre-treated groups (1-way ANOVA, Tukey test, $p>0.05$). At the lowest CAP concentration (0.001%),

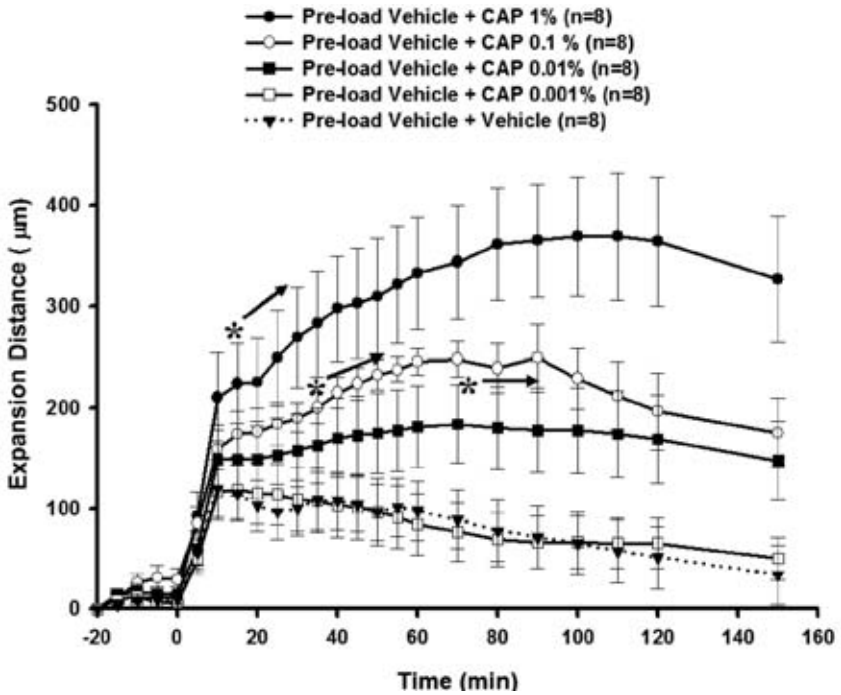

Fig 1 Time course of changes in expansion distance induced by application of CAP at different concentrations. Compared with vehicle controls, CAP induced significantly greater oedema, which became apparent 65 min after CAP 0.01%, at 35 min after CAP 0.1% and at 15 min after CAP 1%, respectively ($p<0.05$, ANOVA, Tukey tests). The notation * → indicates that tissue expansion was significantly increased from these time points onwards to 150 min. In contrast, there was no significant oedema induced by CAP 0.001%. Each data point represents mean tissue expansion ± SE for a certain time point over the pre- and post-CAP injection time course in eight rats.

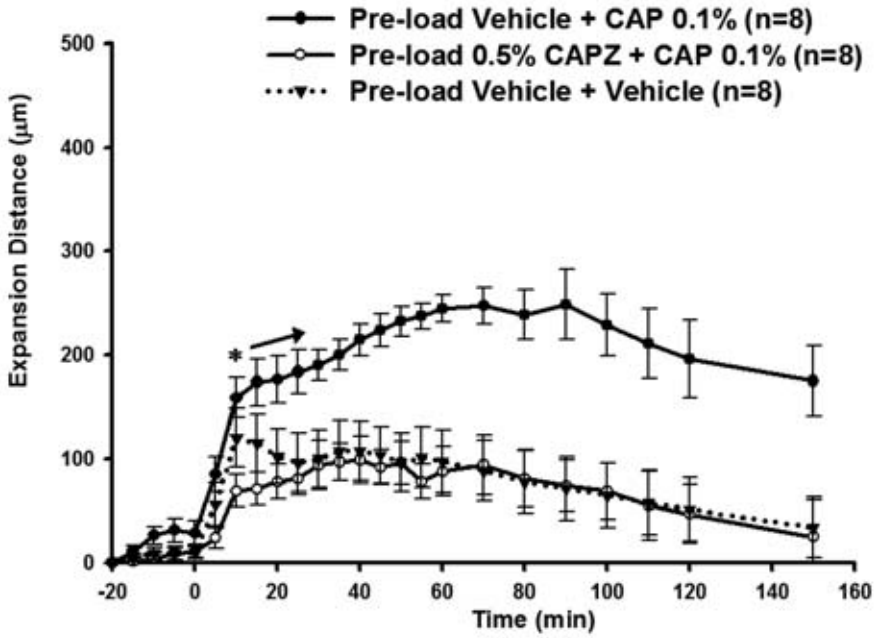

Fig 2 Time course of changes in expansion distance induced by CAP 0.1% following pre-treatment by CAPZ 0.5%. Compared with vehicle + CAP controls, pre-treatment with CAPZ resulted in a significant decrease in the mean expansion distance which became apparent 10 min after CAP application ($p<0.05$, ANOVA, Tukey tests). The notation * → indicates that tissue expansion was significantly increased from this time point onwards to 150 min. Each data point represents mean tissue expansion ± SE for a certain time point over the pre- and post-CAP injection time course in eight rats.

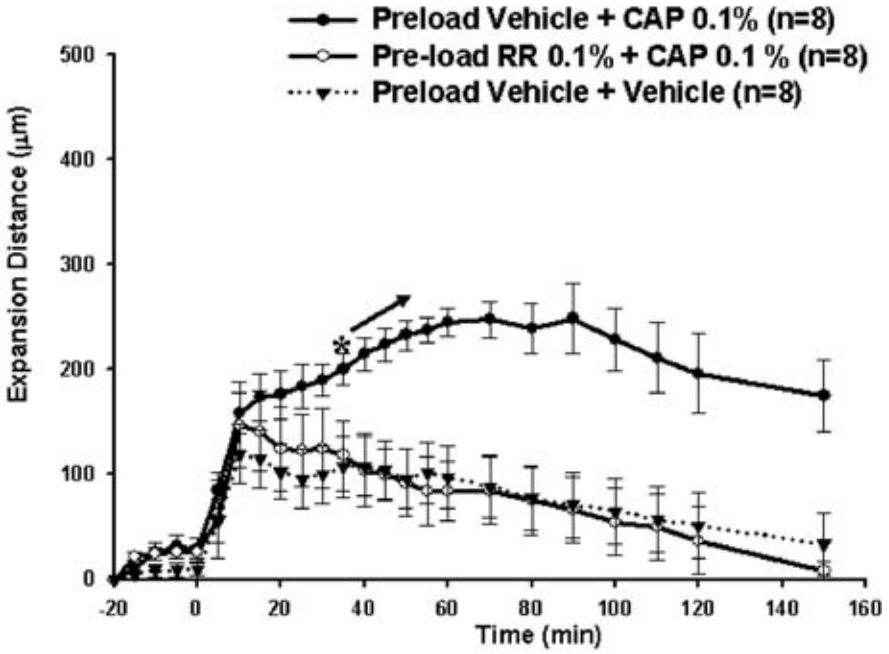

Fig 3 Time course of changes in expansion distance induced by CAP 0.1% following pre-treatment by RR 0.1%. Compared with vehicle + CAP controls, pre-treatment with RR resulted in a significant decrease in the mean expansion distance which became apparent 35 min after CAP application ($p<0.05$, ANOVA, Tukey tests). The notation * → indicates that tissue expansion was significantly increased from this time point onwards to 150 min. Each data point represents mean tissue expansion ± SE for a certain time point over the pre- and post-CAP injection time course in eight rats.

the tissue expansion curve was similar to that of the vehicle + vehicle group and there was no significant expansion at any time point. For the 0.01% CAP group, expansion was larger than that of the vehicle + vehicle group and reached statistical significance at time point 70 minutes and beyond after the CAP injection ($p<0.05$). Tissue expansion was even larger and significantly different from the vehicle + vehicle group at time point 35 minutes and beyond for the 0.1% CAP group, and at time point 15 minutes and beyond for the 1% CAP group. The expansion reached peak values between 90-110 minutes in these two groups and then decreased gradually after time point 120 minutes (Fig 1); the peak expansion values were 361±56μm in the 1% CAP group and 239±21μm in the 0.1% CAP group.

Pre-treatment with the competitive antagonist 0.5% CAPZ significantly attenuated the expansion effect of 0.1% CAP (1-way ANOVA, Tukey test, $p<0.05$) as shown in Fig 2. At all time points, the CAPZ pre-treated group showed no significant difference in expansion

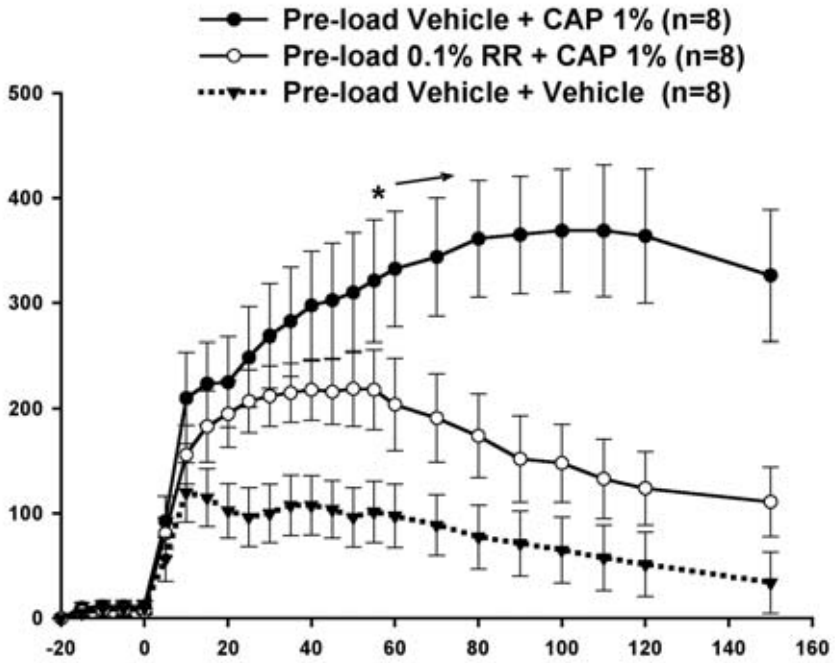

Fig 4 Time course of changes in expansion distance induced by CAP 1% following pre-treatment by RR 0.1%. Compared with vehicle + CAP controls, pre-treatment with RR resulted in a significant decrease in the mean expansion distance which became apparent 60 min after CAP application ($p<0.05$, ANOVA, Tukey tests The notation * → indicates that tissue expansion was significantly increased from this time point onwards to 150 min. Each data point represents mean tissue expansion ± SE for a certain time point over the pre- and post-CAP injection time course in eight rats.

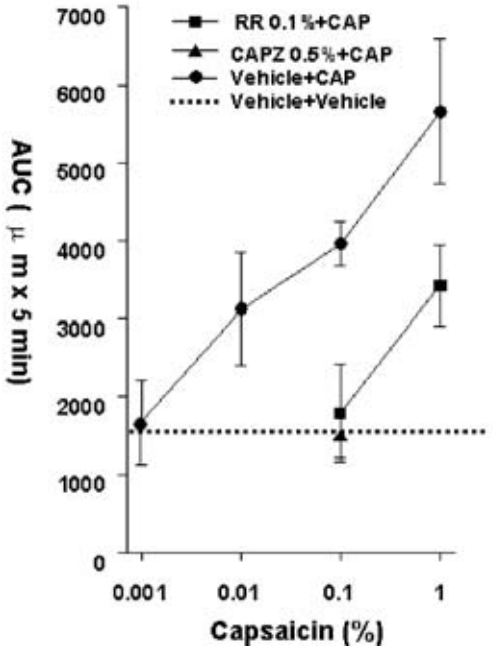

Fig 5 CAP induced cumulative tissue expansion with or without pre-treatment byTRPV1 receptor antagonists. Mean cumulative tissue expansion evoked by injection of various concentrations of CAP or vehicle control (dotted line) into the left temporomandibular joint region pre-treated with vehicle (•) or CAPZ (▲) or RR (■) pre-load. Each data point represents the mean ± SE value of the area under the tissue expansion curve (AUC). The horizontal dotted line indicates the mean data of the vehicle + vehicle group.

from that of the vehicle + vehicle group ($p>0.05$). Pre-treatment with the non-competitive antagonist 0.1% RR also significantly reduced the 0.1% CAP-induced expansion (Fig 3), and at all time points, the RR pre-treated group showed no significant difference in expansion from that of the vehicle + vehicle group ($p>0.05$). The expansion induced by 1% CAP was also significantly attenuated by 0.1% RR pre-treatment, but the effect was less marked than the attenuation of the 0.1% CAP-induced expansion (Fig 4). The group data were also analysed by evaluating the sum of cumulative tissue expansion: each tissue expansion curve was calculated as a single value reflecting the area under the curve, with a bin size of five minutes. A two-way ANOVA revealed a significant 'dose' effect ($p<0.05$) both for the CAP-induced expansion and for the attenuation of the expansion produced by the pre-treatment with the antagonist RR (Fig 5). More importantly, the interaction between the dose of CAP and the presence of antagonist was not significant ($p>0.05$), indicating a

parallel shift to the right of the dose-response curve with the antagonist pre-treatment.

DISCUSSION

The finding of the current study that application of CAP to the TMJ region induces a significant tissue expansion reflecting oedema in the TMJ region is consistent with other findings in our laboratory (Tang et al, 2004), but we have additionally revealed a dose-response relationship over the range of 0.001-1 % CAP. Furthermore, this effect may in part involve peripheral TRPV1 receptor mechanisms since this study has also documented that the CAP-induced inflammatory response could be markedly attenuated by pre treatment with either the specific competitive receptor antagonist CAPZ or the non-competitive antagonist RR.

Recent studies in our laboratory have shown that injection of the inflammatory irritant mustard oil into the TMJ region may induce significant tissue expansion and oedema when compared to controls or to non inflammatory substances such as glutamate or normal saline (Fiorentino et al, 1999; Wong et al, 2001). The advantages and the limitations of our expansion distance model with respect to other methods for monitoring oedema have been discussed recently (Fiorentino et al, 1999). Using this model, the current study has revealed that CAP induces a dose-dependent tissue expansion that our earlier studies documented are indicative of oedema. The effectiveness of CAP application in inducing TMJ oedema is in agreement with other findings in the TMJ region (Tang et al, 2004) and in other craniofacial tissues such as the ear skin of the mouse (Inoue et al, 1993, 1995; Blazso and Gabor, 1994) and the rabbit ear (Buckley et al, 1990) and nasal mucosa (Bari and Jancso, 1994) and the tooth pulp tissues (Sunakawa et al, 1999) of the rat. Following the local application of CAP, oedema and plasma extravasation have also been elicited within the larynx (Lidegran et al, 1998), trachea (Morimoto et al, 1989) and hind paw of rats (Blazso and Gabor, 1994) and mice (Caterina et al, 2000). Intra articular injection of CAP into the knee joint of rats also induces inflammatory changes in the joint tissues (Mapp et al, 1996), and its injection into the skin of the human forearm induces a flare response (Schmelz et al, 1997), hyperalgesia and allodynia (LaMotte et al, 1991; Torebjork et al, 1992). In the present study, the CAP induced expansion first became significantly increased as early as 15 minutes post-injection, reached its peak around 90-110 minutes, and then showed a gradual decrease over the remainder of the 150-minute post-injection period. In the study by Tang et al (2004), the CAP-induced TMJ tissue expansion also became obvious within 30 minutes post-injection, and was still significantly increased up to the 180 minutes after the CAP injection. Earlier investigations of the time course of CAP induced oedema in the ear skin of the mouse, as reflected in increases in the thickness of the ear, documented maximal values after 30-45 minutes (Inoue et al, 1993, 1995), but the dissimilarity of the experimental design, measurement method, doses and species used may explain the difference in the time course between the studies.

Capsaicin is a ligand for the TRPV1 receptor (Caterina et al, 1997; Caterina and Julius, 2001; Julius, 2003) although it can also activate the mustard oil-sensitive and extreme cold-sensitive (<10°C) TRPA1 (alias ANKTM1) receptors (Banvolgyi et al, 2004; Jordt et al, 2004; Story et al, 2003). Capsazepine is a specific competitive TRPV1 antagonist, but it can also operate at both voltage-gated calcium channels (Docherty et al, 1997) and nicotinic acetylcholine receptors (Liu and Simon, 1997). On the other hand, RR is capable of blocking the calcium ion influx and is considered a non-specific and non-competitive TRPV1 antagonist that may affect other receptors, such as TRPV1, TRPV2 (VR-1L), TRPV3 and TRPV4 (Benham et al, 2003). The use of both competitive and non-competitive antagonists in the current study thus represents an important step to relate CAP-induced effects in TMJ tissues to TRPV1 receptor mechanisms. Our findings that both the specific competitive antagonist CAPZ and the non competitive antagonist RR could significantly reduce and, in the case of RR, produce a right shift the dose-response curve of the expansion induced by the CAP injection into the TMJ region suggest that the CAP induced oedema is mediated, at least in part, by TRPV1 receptor mechanisms. These findings are supported by the recent documentation of TRPV1 receptors in rat TMJ tissues (Ichikawa et al, 2004). Also, studies in "knock-out", TRPV1-null, mice have revealed a reduction in inflammation and swelling induced by intraplantar injection of CAP, demonstrating that TRPV1 receptors may be critical for mediating the inflammatory effects of CAP (Caterina et al, 2000; Caterina and Julius, 2001).

There is evidence that CAP can act through TRPV1 receptor mechanisms to cause the release from small diameter afferent endings of pro inflammatory peptides which trigger plasma extravasation and vasodilatation (for review, see Jancso et al, 1967; Jancso, 1968; Holzer, 1991; Buck and Burks, 1986; Inoue et al, 1995; Caterina and Julius, 2001; Julius, 2003). However, Tang et al (2004) and Wong et al (2001) have shown that injection of local anaesthetic into the TMJ region could not block the inflammation induced by CAP or mustard oil injected into the rat TMJ tissues. This led Tang et al (2004) to question whether CAP is acting through, or only through, a neurogenic mecha-

nism. They suggested instead that CAP may evoke the direct release of inflammatory mediators from neuronal terminals without the conduction of action potentials along the axon, or CAP may predominantly act in a non-neurogenic fashion when applied to the TMJ region due to its inherent inflammatory nature or the activation of TRPV1 receptors located on other cellular components in the peripheral tissues. In this regard, research indicates that TRPV1 receptors are principally found on the terminal endings of small-diameter TMJ afferent fibres, many of which appear to also contain calcitonin gene related peptide, a potent vasodilatory neuropeptide which becomes elevated during TMJ inflammation and may contribute to the development of joint oedema (see Kopp, 2001; Hutchins et al, 2002; Ichikawa et al, 2004). Although CAP is an important pharmacological tool used to distinguish a subset of nociceptive sensory neurons, CAP may also cause the release non-neurogenic inflammatory factors. For example, an in vitro investigation has localized TRPV1 receptors on mast cells (Biro et al, 1998). In the event that mast cells may degranulate with the binding of CAP to TRPV1 receptors, there is the possibility that the release of histamine may mediate inflammation by its direct action on the vasculature (Bunker et al, 1991; Kiernan et al, 1971). 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 (McMahon et al, 1989). Therefore, a CAP induced inflammatory response may be activated regardless of functional nerve conduction.

Thus, while our findings that CAP-induced tissue expansion in TMJ tissues can be attenuated by TRPV1 receptor antagonists are consistent with the involvement of TRPV1 receptors in CAP-induced inflammation in these tissues, the mechanisms underlying these effects are still unclear and represent an important avenue of future investigation. It is important to understand these mechanisms underlying inflammation of TMJ tissues because TMJ inflammation is a process involved in a number of clinical conditions, e.g. TMJ arthritis, post-traumatic disorders, and some temporomandibular disorders (Mock, 1999; Zarb and Carlsson et al, 1999; Kopp, 2001). Several chemical mediators have been identified in TMJ tissues and implicated in TMJ inflammatory processes (Kopp, 2001; Lobbezoo et al, 2004). Our documentation of TRPV1 receptor mechanisms in TMJ tissues may be important in the elucidation of the processes underlying TMJ inflammation, and in the development of specific markers and diagnostic and prognostic tools and therapies.

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