

TNF α Mechanically Sensitizes Masseter Muscle Nociceptors by Increasing Prostaglandin E₂ Levels

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Hakim AW, Dong X, Cairns BE. TNF α mechanically sensitizes masseter muscle nociceptors by increasing prostaglandin E₂ levels. *J Neurophysiol* 105: 154–161, 2011. First published October 27, 2010; doi:10.1152/jn.00730.2010. TNF α induces mechanical sensitization of rat masseter muscle nociceptors, which takes 2–3 h to manifest and is mediated through activation of P55 and P75 receptors. This study was undertaken to determine whether TNF α induces nociceptor mechanical sensitization through the release of other algogenic substances such as glutamate, prostaglandin E₂ (PGE₂), and/or nerve growth factor (NGF), which have been shown to induce mechanical sensitization of muscle nociceptors. Masseter muscle homogenate levels of PGE₂ and NGF were measured 3 h after injection of TNF α (1 μ g) or vehicle control using commercially available kits. Interstitial glutamate concentration was measured after injection of TNF α or vehicle control using a glutamate-selective biosensor probe. Diclofenac, a cyclooxygenase inhibitor that blocks the synthesis of PGE₂, D-2-amino-5-phosphonovaleric acid (APV), a competitive N-methyl-D-aspartate (NMDA) receptor antagonist, and a tyrosine kinase A (TrkA) receptor antibody, which blocks NGF-induced masseter muscle nociceptor sensitization, were used to assess the contribution of PGE₂, glutamate, and NGF to TNF α -induced nociceptor sensitization. PGE₂ and glutamate concentrations were significantly elevated 3 h after TNF α injection into the masseter muscle. Injection of diclofenac partially reversed the TNF α -induced decreases in the mechanical threshold (MT) of masseter muscle nociceptors, whereas vehicle control, APV, and TrkA antibody did not significantly alter nociceptor MT. These results suggest that TNF α -induced mechanical sensitization of masseter muscle nociceptors is mediated in part by increased PGE₂ levels. The findings of this study support the hypothesis that TNF α induces a delayed mechanical sensitization of masseter muscle nociceptors indirectly by the release of PGE₂.

INTRODUCTION

The mechanisms responsible for chronic muscle pain in conditions such as myofascial temporomandibular disorders and fibromyalgia are not known (Cairns 2010; Dworkin and LeResche 1992; Hedenberg-Magnusson et al. 1997; Lelebicic et al. 2007). However, an association between elevated serum and plasma levels of TNF α , a pro-inflammatory cytokine, and chronic muscle and joint pain has been previously reported (Bazzichi et al. 2007; Nordahl et al. 2000; Shah et al. 2008; Wang et al. 2008). In addition, many patients with chronic muscle pain have so-called myofascial “trigger points,” which are areas of focal muscle hypertonicities that, when palpated, reproduce their muscle pain, and TNF α levels seem to be significantly elevated in these regions (Shah et al. 2008). It is thought that TNF α acts on P55 (TNFR1) and P75 (TNFR2) receptors to produce its pro-nociceptive effects in various

tissues (Vandenabeele et al. 1995). Both P55 and P75 receptors are expressed by trigeminal ganglion neurons that innervate the masseter muscle as well as in the masseter muscle itself (Hakim et al. 2009). We have recently shown that intramuscular injection of TNF α causes a prolonged mechanical sensitization of masseter muscle nociceptors, which takes 2–3 h to manifest and is mediated via activation of peripheral P55 and P75 receptors (Hakim et al. 2009). However, it is not known whether some of the sensitizing effects of TNF α are mediated indirectly through the release of other nociceptive substances such as nerve growth factor (NGF), prostaglandin E₂ (PGE₂) and glutamate that have been shown to induce mechanical sensitization of muscle nociceptors (Cairns et al. 2002; Dong et al. 2009; Mense 1981; Murase et al. 2010; Svensson et al. 2010).

In other tissues, such as the skin, animal studies have shown that intraplantar injection of TNF α acts directly by exciting cutaneous afferent fibers (Junger and Sorkin 2000; Sorkin et al. 1997) and indirectly by inducing the release of other sensitizing substances (Cunha et al. 1992, 2005; Russell et al. 2009; Woolf et al. 1997). Behavioral studies have shown that intramuscular injection of TNF α causes a delayed mechanical sensitization of skeletal muscle that seemed to be associated with elevated levels of PGE₂, NGF, and neuropeptides, although a cause-effect relationship was not shown in these studies (Schafers et al. 2003). In the CNS, TNF α also induces release of glutamate through activation of P55 receptors (Hermann et al. 2005; Youn et al. 2008) and enhances α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) and N-methyl-D-aspartate (NMDA) receptor currents, as well as phosphorylation of the NR1 subunit of NMDA receptor (Wei et al. 2008). A 2–3 times increase in muscle interstitial glutamate concentration results in significant sensitization of masseter muscle nociceptors (Cairns et al. 2007). These results support the concept that TNF α could be mediating sensitization of masseter muscle nociceptors by inducing release of other algogenic substances.

The purpose of this study was to further explore the mechanism of TNF α -induced delayed mechanical sensitization of masseter muscle nociceptors. It was hypothesized that TNF α acts indirectly by inducing the release of NGF, PGE₂, and/or glutamate and that these nociceptive mediators are responsible for TNF α -induced masseter muscle sensitization. To test this hypothesis, glutamate concentration was measured using glutamate-selective biosensor probes, and NGF and PGE₂ levels were measured by ELISA after injection of a mechanically sensitizing dose of TNF α (1 μ g). Electrophysiology experiments were performed to assess whether these substances play a functional role in TNF α -induced mechanical sensitization of muscle nociceptors. Diclofenac, a cyclooxygenase inhibitor that blocks

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synthesis of PGE₂ (Cashman 1996; Gotzsche 2000), D-2-amino-5-phosphonovaleric acid (APV), a competitive NMDA receptor antagonist, and a tyrosine kinase A (TrkA) receptor antibody, which blocks NGF-induced masseter muscle nociceptor sensitization, were injected 3 h after intramuscular injection of TNF α to determine whether these antagonists could reverse TNF α -induced mechanical sensitization.

METHODS

Animals

A total of 70 adult male Sprague-Dawley rats (300–400 g) were used in this study. All experiments were done in accordance with the Canadian Council on Animal Care and were approved by the University of British Columbia Animal Care Committee.

ELISA

These experiments were done to determine masseter muscle concentration of PGE₂ and NGF. TNF α (1 μ g) or vehicle control ($n = 5$ in each group) was injected into rat masseter muscle, and after 3 h, the rat was killed with a high dose of pentobarbital sodium (120 mg/kg). Approximately 1 cm² of masseter muscle tissue was harvested from around the injection site, which was marked on the overlying skin with a black marker. Muscle tissue was placed on dry ice and stored at -70°C . Tissues were weighed and homogenized using homogenization buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton, 0.1% sodium dodecyl sulfate, 0.5% sodium deoxycholate). The average weight of muscle tissue in the TNF α and PBS groups was 0.20 ± 0.03 and 0.25 ± 0.05 g, respectively. Samples were centrifuged at 4°C for 15 min at 15,000 rpm. The supernatant of the homogenate was collected, and muscle protein concentration was determined using the Bradford method (Bradford 1976).

PGE₂ concentrations

PGE₂ level in muscle tissue homogenate was measured by enzyme immunoassay (Assay Design, Ann Arbor, MI) according to the manufacturer's instructions. The sensitivity of kit was 8.26 pg/ml. Samples and standards were run in duplicate and were averaged. The concentration of PGE₂ was determined per gram of muscle.

NGF concentrations

NGF level in the muscle homogenate was measured by ELISA (Promega, Madison, WI) according to the manufacturer's instructions. The minimum sensitivity of the kit was 7.8 pg/ml. Both samples and standards were run in duplicate. The concentration of NGF was determined per milligram of muscle protein.

Glutamate concentration

Glutamate biosensor probes (Pinnacle Technology) were used to measure interstitial glutamate concentration in the masseter muscle (Cairns et al. 2007). Glutamate probes were calibrated in vitro according to the manufacturer's instructions. The glutamate biosensor probe was inserted through a guide cannula, which was affixed to a catheter needle (3 mm between probe and needle tip), into the masseter muscle of isoflurane anesthetized adult male Sprague-Dawley rats ($n = 8$). Blood pressure, core body temperature, and heart rate were continuously monitored throughout the experiment. After a 60–90 min stabilization period, the baseline glutamate concentration was measured over 10 min, and subsequently, TNF α or vehicle control (10 μ l, $n = 4$) was injected into the masseter muscle near to glutamate biosensor probe. Masseter muscle glutamate concentration was measured each hour for 3 h.

Electrophysiological recording of muscle nociceptors

Adult male Sprague-Dawley rats (300–400 g, $n = 45$) were used for acute in vivo recording of extracellular action potentials from trigeminal ganglion neurons that innervate the masseter muscle (Cairns et al. 2002; Dong et al. 2007; Hakim et al. 2009). Isoflurane (2–2.5%) was used to anesthetize rats. A rectal thermometer was used to measure temperature. The trachea was cannulated, and the rat was given artificial respiration using a rodent ventilator. The carotid artery was cannulated to monitor blood pressure and to inject pentobarbital sodium (100 mg/kg) to kill the rat at the end of the experiment. Rat core body temperature, expired CO₂, heart rate, and blood pressure were monitored throughout the experiment and were kept within the range of 36.8–37.1 $^{\circ}\text{C}$, 20–50 mmHg, 300–400 beat/min, and 60–80 mmHg, respectively. The rat's head was placed into a stereotaxic frame. The skin, muscle, and dura overlying the caudal brain stem were reflected to allow stimulation of brain stem with stimulating electrode. A parylene-coated tungsten-recording electrode was lowered into the trigeminal ganglion to record action potentials from single trigeminal ganglion neurons. A blunt probe was used to mechanically stimulate the masseter muscle as a search stimulus to identify trigeminal ganglion neurons whose afferent fibers had receptive fields within the masseter muscle (Cairns et al. 2001, 2002). The skin overlying the masseter muscle was pulled, and pinch and pressure were applied to it to rule out innervation of the skin (Cairns et al. 2001, 2002). Masseter afferent fibers projecting to caudal brain stem were identified by the antidromic collision technique. Antidromic action potentials were evoked by stimulation of caudal brain stem with constant electric current. Masseter afferent fibers were mechanically stimulated to evoke orthodromic action potentials. Collision of antidromic and orthodromic action potentials was used to confirm the projection of masseter muscle afferent fibers to the caudal brain stem (Cairns et al. 2001, 2002). The conduction velocity of afferent fibers was calculated by dividing the distance between stimulating and recording electrodes by the latency of the antidromic action potential. To confirm that the mechanoreceptive field of each fiber was in masseter muscle, fibers were required to discharge in response to either injection of TNF α or hypertonic saline (HS; 1 M), which was injected into the masseter muscle at the end of the experiment. Afferent fibers were excluded from further analysis if they failed to discharge in response to injection of at least one of the substances injected into the masseter muscle. The relatively high MT of most afferent fibers coupled to their response to HS led us to identify these afferent fibers as putative nociceptors.

Electrophysiology experiments

These experiments were carried out to study the role of glutamate, NGF, and PGE₂ in TNF α -induced masseter muscle nociceptor sensitization. Nociceptors were assigned randomly to receive an injection of either vehicle control (10 μ l PBS, $n = 10$), the competitive NMDA receptor antagonist APV (10 μ l, 10 or 50 mM; $n = 10$ per concentration), the nonsteroidal anti-inflammatory drug (NSAID) diclofenac (0.1 mg/ml, 10 μ l; $n = 7$), or an antibody against the high affinity NGF TrkA receptor (2 μ g/ml, 10 μ l; $n = 8$) 3 h after TNF α injection. The investigator (A.H.) was unaware of the content of the injections. Baseline line mechanical threshold (MT) of nociceptors was measured using an electronic von Frey hair (model 160IC, IITC) every minute for 10 min before insertion of the catheter needle that contained TNF α (Hakim et al. 2009). The baseline MT was determined by averaging 10 consecutive mechanical stimuli. TNF α (Sigma; 1 μ g in 10 μ l PBS, $n = 10$) was injected into the receptor field of the identified fiber, and MT was measured after every hour for 10 min over 3 h. Ongoing discharge was measured by counting the number of action potentials over 1 min immediately before the baseline MT assessment, the injection of TNF α , and each hour for 3 h 1 min before each MT assessment (Bove and Dilley 2010). Three hours after injection of

TNF α , one of the test substances was injected, and MT was measured once each minute for 30 min. At the end of MT recoding, HS was injected into the mechanoreceptive field of masseter nociceptor. Rats were killed by injection of pentobarbital sodium.

Data analysis

The glutamate biosensor probe was connected to a four-channel potentiostat (model 3104), and the input signal from the probe was analyzed with pinnacle Acquisition Laboratory software (Pinnacle Technology). The change in glutamate concentration after injection of TNF α or PBS was determined by subtracting baseline glutamate concentration from the concentration of glutamate obtained after injection of TNF α or PBS.

The average nociceptor MT at each time point was calculated. To account for the large variability in the baseline MT of individual fibers in each treatment group, relative MTs were calculated by dividing the mean MT at each time point by the mean baseline MT. This permitted us to compare changes in MT over time between treatment groups. To determine whether TNF α injections increased nociceptor discharge, cumulative discharge was calculated by subtracting the number of action potentials recorded for 10 min before injection from the number recorded for 10 min after injection. Change in ongoing discharge was calculated by subtracting the number of action potentials recorded before injection of TNF α from the number recorded after injection at each hour. Positive numbers indicated an increase in ongoing discharge, and negative numbers indicated a decrease in ongoing discharge.

Statistical analysis

The Pearson product moment test was used to determine whether correlations were significant. Two-way repeated-measures ANOVA and Holm-Sidak post hoc test were used to determine the differences in interstitial glutamate concentration over time after injection of TNF α and PBS. A Student's *t*-test was used to compare mean levels of NGF between vehicle control and TNF α . One-way ANOVA was used to compare mean levels of PGE₂ between TNF α , vehicle control, and diclofenac injections. A logarithmic transformation of PGE₂ concentrations was necessary to produce equal variance before running the ANOVA. The MTs after injection of various treatments (PBS, APV, NGF antibodies, or diclofenac) were compared with the MT 3 h after injection of TNF α through the use of one-way repeated-measures ANOVA and Holm-Sidak post hoc test. Differences were considered statistically significant when $P < 0.05$. Unless otherwise indicated, data in the text are given as mean $\pm$ SE.

RESULTS

Effect of TNF α

In vivo single unit extracellular recording of 52 nociceptors was made. Seven nociceptors, which were not mechanically sensitized by TNF α 3 h after injection, were excluded from further analysis. The conduction velocity (CV) of the remaining 45 nociceptors was in the A δ (CV = 2–12 m/s) range. The mean baseline MT of these nociceptors was 38 ± 5 g. HS was injected as positive control to confirm that the receptor field of the afferent fiber recorded was in masseter muscle and that all afferent fibers were nociceptors (Cairns et al. 2003b; Kumazawa and Mizumura 1977; Mense 1977; Paintal 1960). TNF α evoked discharge in 12 of 45 nociceptors examined [median (interquartile range) cumulative discharge: 23(5–42) action potentials; Fig. 1]. Eight of 45 nociceptors had ongoing activity before baseline MT assessment. Insertion of the needle into the masseter muscle caused 4 additional nociceptors to begin ongoing dis-

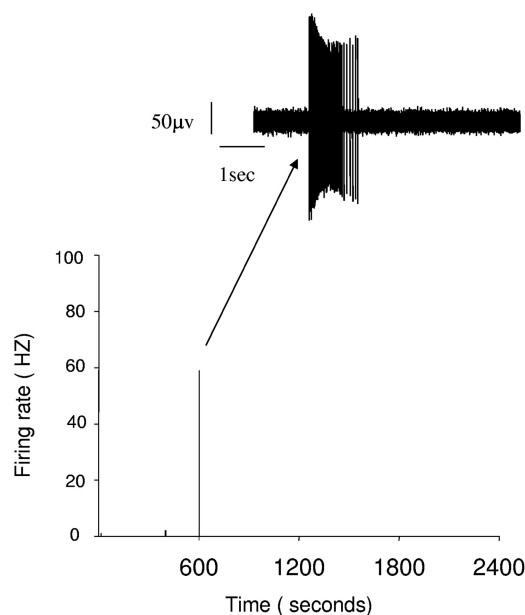


FIG. 1. The histogram shows an example of TNF α -evoked A δ fiber [conduction velocity (CV) = 3.4 m/s, mechanical threshold (MT) = 24 g] masseter muscle afferent discharge. Baseline activity was recorded for 600 s, and at 600 s, TNF α was injected into the mechanoreceptive field of masseter muscle afferent fiber, which evoked afferent fiber discharge.

charge; thus a total of 12 nociceptors exhibited some degree of ongoing activity (average, 0.23 Hz; range, 0.02–1.58 Hz) before TNF α injection. Three hours after injection, only 2 of the original 12 nociceptors having ongoing discharge had increased their discharge rate, whereas the rate of discharge had decreased or stopped altogether in the other 10 nociceptors. However, six previously nondischarging nociceptors developed ongoing discharge by 3 h after TNF α injection. Therefore over the 3 h period after injection, TNF α increased ongoing or induced novel ongoing discharge in a total of 8 of 45 nociceptors recorded.

Three hours after injection, TNF α (1 μ g) significantly decreased the MT of masseter muscle nociceptors compared with the preinjection baseline MT. The mean relative MT (%) of the 45 nociceptors that showed mechanical sensitization 3 h after TNF α injection was $39 \pm 3\%$. There was no significant correlation between baseline MT or CV and TNF α -induced mechanical sensitization.

Role of PGE₂ in TNF α -induced mechanical sensitization

To study whether TNF α might be acting to increase prostaglandin levels or alter NMDA receptor activation, we tested the effect of the NSAID diclofenac on TNF α -induced mechanical sensitization at a concentration 0.1 mg/ml, a concentration that also competitively inhibits peripheral NMDA receptors (Dong et al. 2009) and prostaglandin synthesis (Cashman 1996; Gotzsche 2000). This concentration of diclofenac also inhibits prostaglandin synthesis, as shown by our finding that TNF α injection significantly elevated levels of PGE₂ compared with vehicle control and that this TNF α -induced elevation in muscle PGE₂ level was attenuated by diclofenac (Fig. 2A).

Vehicle control injected at 3 h after TNF α injection had no significant effect on TNF α -induced mechanical sensitization at 10, 20, or 30 min after injection (Fig. 2B). Diclofenac, how-

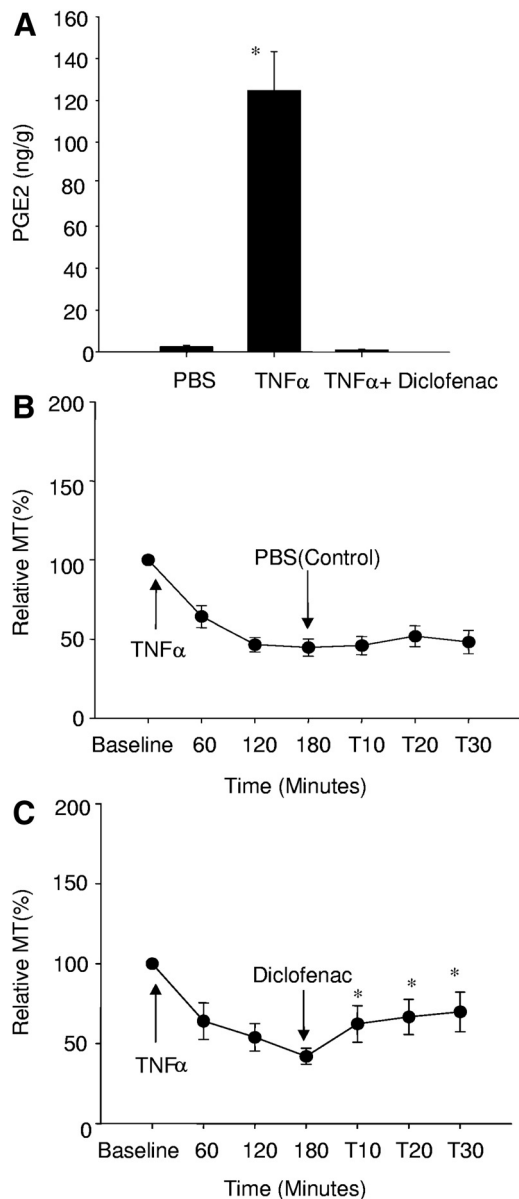


FIG. 2. A: the bar graph shows the levels of prostaglandin E₂ (PGE₂) after injection of PBS (vehicle control), TNF α , and TNF α with diclofenac (0.1 mg/ml). Injection of TNF α significantly elevated the levels of PGE₂ compared with vehicle control ($n = 5$). Injection of diclofenac at 3 h after TNF α injection significantly decreased the levels of PGE₂ ($*P < 0.05$, 1-way ANOVA, Holm-Sidak post hoc test). B: the line and scatter plot shows the change in relative MT after injection of PBS at 10 (T10), 20 (T20), and 30 (T30) min after injection. TNF α significantly decreased the MT 3 h after injection, and this TNF α -induced decrease in MT was not significantly changed by injection of PBS. C: the line and scatter plot shows the change in MT after injection of diclofenac (0.1 mg/ml). TNF α significantly decreased the MT at 3 h after injection, and this decrease in MT was partially reversed by injection of diclofenac ($*P < 0.05$, 1-way repeated-measures ANOVA; $n = 7$). Error bars indicate SE.

ever, when injected 3 h after TNF α , partially reversed TNF α -induced mechanical sensitization of masseter muscle nociceptors. A significant effect of diclofenac on relative MT was seen at 10, 20, and 30 min after its injection (Fig. 2C). These results suggested that an increase in PGE₂ levels contributes to TNF α -induced mechanical sensitization; however, because the concentration of diclofenac used also inhibits NMDA receptor

activation, additional experiments were undertaken to examine the role of glutamate and peripheral NMDA receptor activation in TNF α -induced mechanical sensitization.

Role of glutamate and peripheral NMDA receptors in TNF α -induced mechanical sensitization

The mean baseline concentration of glutamate in the masseter muscle was $44 \pm 16 \mu\text{M}$ ($n = 8$). Two-way repeated-measures ANOVA showed a significant effect of treatment ($P < 0.05$) but not time on glutamate concentration, and there was no significant interaction between time and treatment ($P = 0.1$). Post hoc tests showed that TNF α significantly elevated the concentration of glutamate compared with vehicle control (Fig. 3). However, as seen in Fig. 3, the source of the difference between the two treatment groups was principally caused by a decline in interstitial glutamate concentration after injection of vehicle rather than an increase in glutamate after injection of TNF α .

Injection of the competitive NMDA receptor antagonist APV (10 or 50 mM) 3 h after injection of TNF α did not significantly change the MT of masseter muscle nociceptors at 10, 20, and 30 min after injection ($P > 0.05$, 1-way repeated-measures ANOVA; Fig. 4, A and B). NMDA receptor activation did not seem to contribute significantly to the mechanism of TNF α -induced mechanical sensitization.

Role of NGF and TrkA receptors in TNF α -induced mechanical sensitization

TNF α injection did not significantly elevate the level of NGF compared with vehicle control (Fig. 5A). Injection of TrkA antibody 3 h after TNF α injection did not significantly reverse TNF α -induced masseter muscle nociceptor sensitization at 10, 20, or 30 min after injection (Fig. 5B). This result shows that NGF does not play an important role in TNF α -induced masseter muscle nociceptor sensitization.

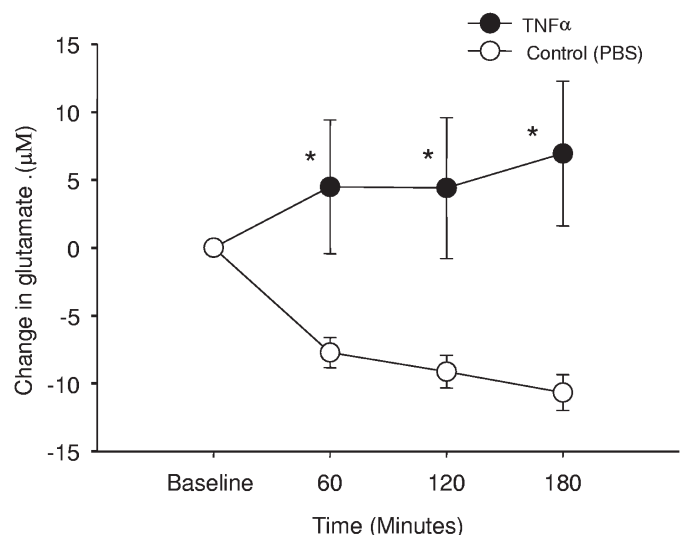


FIG. 3. The line and scatter plot shows the mean change in rat masseter muscle glutamate concentration at various time points after injection of TNF α ($n = 4$) or vehicle control ($n = 4$). Overall, the change in glutamate concentration after injection of TNF α was significantly higher than after injection of vehicle control. ($*P < 0.05$, 2-way repeated-measures ANOVA). Error bars indicate SE.

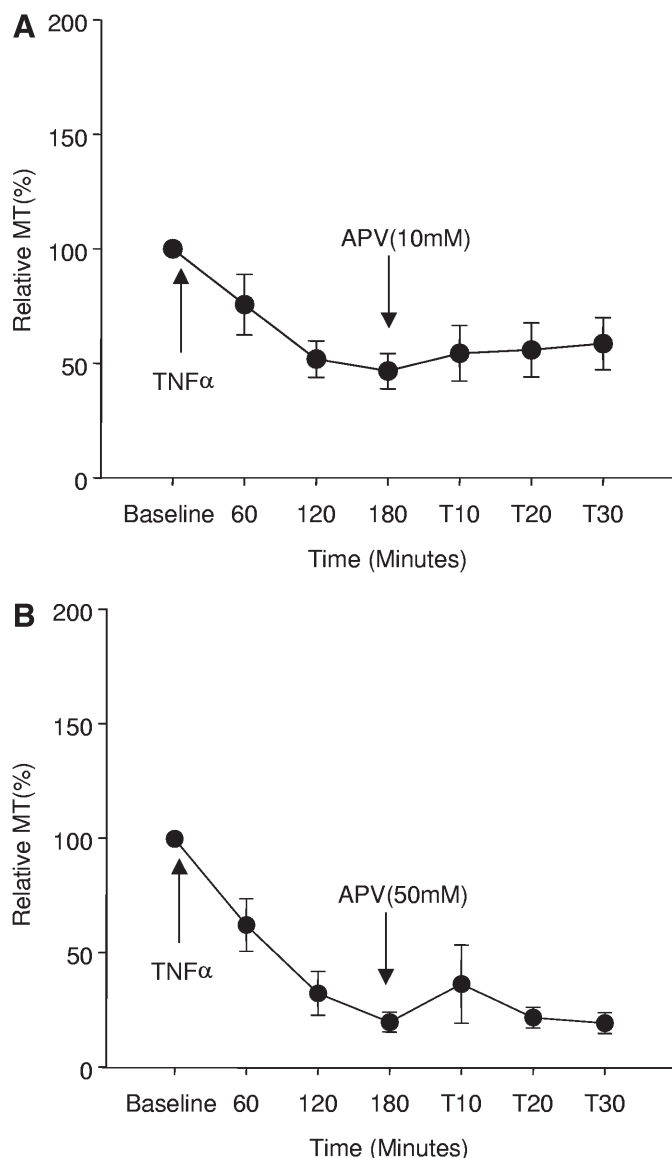


FIG. 4. The line and scatter plots show the change in relative MT 10 (T1), 20 (T2), and 30 (T3) min after injection of APV (A: 10 mM; B: 50 mM). The TNF α -induced decrease in relative MT was not significantly altered by injection of either concentration of APV. Error bars indicate SE.

DISCUSSION

Injection of TNF α had no effect on ongoing discharge but did produce mechanical sensitization of masseter muscle nociceptors that was associated with an increase in muscle concentrations of PGE $_2$ and glutamate. Diclofenac, but not the NMDA receptor antagonist APV, partially reversed TNF α -induced sensitization of masseter muscle nociceptors. TrkA receptor antibody, which binds to the high-affinity NGF receptor and has been previously shown to attenuate masseter muscle nociceptor mechanical sensitization induced by exogenously administered NGF (Svensson et al. 2010), did not reverse TNF α -induced mechanical sensitization of masseter muscle nociceptors. These results suggest that TNF α -induced sensitization of masseter muscle nociceptors is mediated, at least in part, by PGE $_2$.

PGE $_2$ is a pro-nociceptive cytokine that sensitizes muscle nociceptors in both animals and humans (Dong et al. 2009;

Mense 1981; Rukwied et al. 2007). In humans, PGE $_2$ potentiates acid-induced muscle pain (Rukwied et al. 2007) and has been suggested to contribute to the development of masseter muscle pain in fibromyalgia patients (Hedenberg-Magnusson et al. 2001). Furthermore, PGE $_2$ in combination with bradykinin (BK), histamine, and serotonin has a sensitizing effect on human muscle (Mork et al. 2003). In animals, PGE $_2$ injection has been shown to cause mechanical sensitization of rat gastrocnemius and masseter muscle nociceptors (Dina et al. 2008; Dong et al. 2009) and enhances the sensitizing response of BK on cat gastrocnemius muscle nociceptors (Mense 1981). The concentration of PGE $_2$ measured in rat masseter muscle in this study after vehicle control is similar to that previously reported for human skeletal muscle (Trappe et al. 2001). These findings indicate that an increase in the concentration of PGE $_2$, for

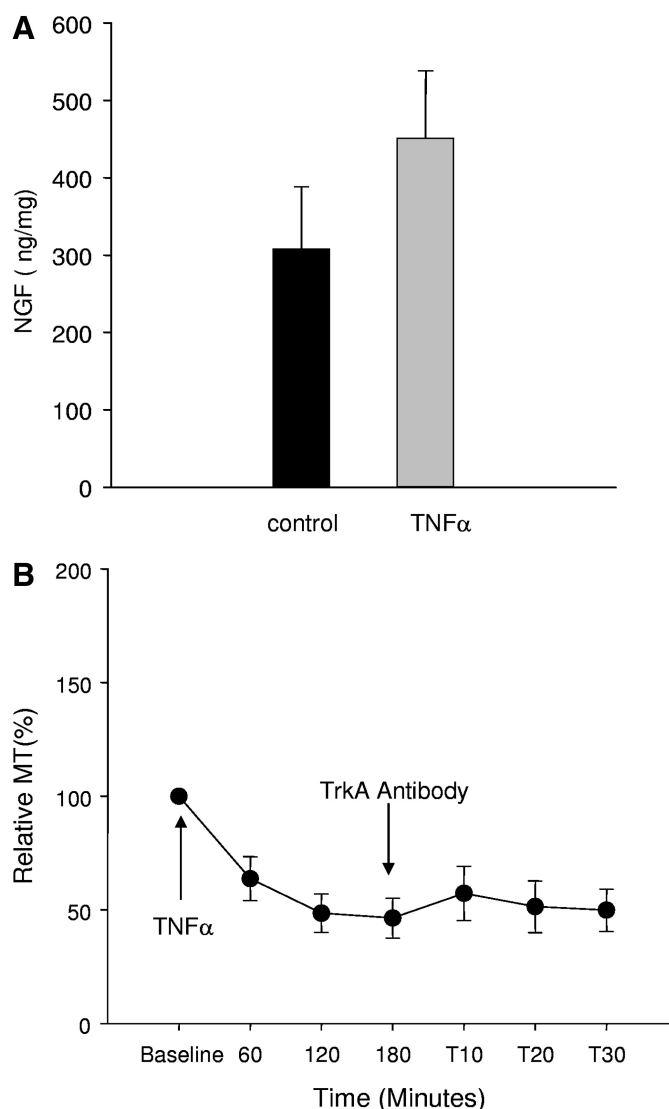


FIG. 5. A: bar chart shows the level of nerve growth factor (NGF) in the masseter muscle after injection of TNF α or vehicle control. Injection of TNF α did not significantly elevate NGF level compared with vehicle control (*t*-test, *n* = 5). B: the line and scatter plot shows the change in MT 10 (T1), 20 (T2), and 30 (T3) min after injection of tyrosine kinase A (TrkA) antibody. TNF α significantly decreased MT at 3 h of its injection, and this decrease in MT was not significantly reversed by injection of TrkA antibody (1-way repeated-measures ANOVA, *n* = 8).

example, caused by tissue injury, contributes to the development of muscle pain through nociceptor sensitization (Graven-Nielsen and Mense 2001; Mense 1981; Tegeder et al. 2002). PGE₂-induced sensitization is mediated by PGE₂ receptors EP2 and EP3 (Lin et al. 2006). These receptors are expressed on muscle tissue and trigeminal ganglion neurons (Graven-Nielsen and Mense 2001; Patwardhan et al. 2008). Studies have shown that TNF α treatment of dorsal root ganglion cell culture or injection into rat gastrocnemius muscle significantly elevated the level of PGE₂ (Fehrenbacher et al. 2005; Schafers et al. 2003). Our results are consistent with previous cutaneous animal experiments that have shown that TNF α -induced sensitization is mediated through prostaglandins (Cunha et al. 2005; Russell et al. 2009).

We recently showed that TNF α induces delayed mechanical sensitization of masseter nociceptors via activation of peripheral P55 and P75 receptors (Hakim et al. 2009). Activation of either TNF α receptor (P55 or P75) stimulated the release of PGE₂ in synovial and gingival fibroblasts (Butler et al. 1994; Taylor 1993). In vitro, activation of P55 and P75 receptors by TNF α leads to the activation of P38 MAP kinases, which in turn activate phospholipase A2 to liberate arachidonic acid, the precursor for prostaglandin synthesis (Ji and Woolf 2001). In this study, PGE₂ concentration was significantly elevated 3 h after TNF α injection, and diclofenac reversed the elevated level of PGE₂, which supports the concept that TNF α acts through P55/P75 receptor mechanisms to increase the synthesis of PGE₂ in vivo. However, because diclofenac only partially attenuated the effect of TNF α , although it completely reversed the TNF α -induced increase in PGE₂ levels, it is conceivable that other algogenic substances also contribute to TNF α -induced mechanical sensitization of masseter muscle nociceptors.

Glutamate is an excitatory amino acid that causes sensitization of rat masseter muscle nociceptors through activation of the peripheral NMDA receptor (Cairns et al. 2003b, 2007). A 200–300% increase in interstitial glutamate concentration of rat masseter muscle from baseline concentration is required to induce sensitization of masseter muscle nociceptors (Cairns et al. 2007). In this study, TNF α increased glutamate concentrations on average by about 6 μ M (~15%), which suggests that the increase in glutamate concentration induced by TNF α was likely not great enough to contribute to mechanical sensitization, at least through activation of peripheral NMDA receptors. It should be noted that the significant difference in glutamate concentration between TNF α and PBS injections mostly reflected a decrease in glutamate concentration after PBS injection, which we suggest was a result of local dilution of glutamate concentrations by the injectate (Cairns et al. 2003a). Nevertheless, it is possible that these small increases in interstitial glutamate concentrations could have activated non-NMDA receptors such as metabotropic glutamate receptors (mGluRs) or AMPA-activated ionotropic glutamate receptors (GluRs), which have been shown to contribute to the development of nociception after inflammatory injury (Bhave et al. 2001; Dogrul et al. 2000; Walker et al. 2001). mGluR5 protein is expressed in the masseter nerve and in trigeminal ganglion and activation of mGluR5 induces mechanical sensitivity in the masseter muscle (Lee and Ro 2007). In addition, both GluR1 and GluR2 subtypes are expressed in trigeminal ganglion neurons (Chun et al. 2008), and injection of AMPA into

masseter muscle both excites and mechanically sensitizes masseter muscle nociceptors (Dong et al. 2009). Although there is a potential that these other glutamate receptors might have been activated by the TNF α -mediated increase in interstitial glutamate concentration, previous findings that glutamate-induced mechanical sensitization of masseter muscle nociceptors can be completely reversed by NMDA receptor antagonists (Cairns et al. 2007) suggest that mechanisms other than activation of glutamate receptors are more important for TNF α -induced nociceptor sensitization.

NGF has also been shown to induce mechanical sensitization when injected into muscles (Andersen et al. 2008; Mann et al. 2006; Nie et al. 2009; Svensson et al. 2003, 2008a, 2010). Injection of NGF into the masseter muscle causes prolonged (~14 day) mechanical sensitization in healthy human subjects (Svensson et al. 2003, 2008a,b). In rats, the onset of mechanical sensitization of masseter muscle nociceptors by exogenously administered NGF into rat masseter muscle occurs within 30 min of injection and is mediated through activation of the TrkA receptors (Svensson et al. 2010). In this study, TNF α (1 μ g) injection into the masseter muscle did not significantly elevate the level of NGF, although in a previous study, TNF α injection into rat gastrocnemius muscle did significantly elevate the level of NGF compared with vehicle control (Schafers et al. 2003). This discrepancy in results could be caused by a higher dose of TNF α (10 μ g) injected into the rat gastrocnemius muscle in the study of Schafers et al. 2003. In addition, the concentration of NGF in our vehicle control-treated masseter muscle was higher than baseline concentrations of NGF in other rat skeletal muscles (Wu et al. 2009), which may indicate that there are higher basal concentrations of NGF in the masseter muscle. These high baseline concentrations may have made it more difficult to detect a significant change in NGF levels by TNF α in the masseter muscle. However, even with this caveat, we also found no significant attenuation of TNF α -induced mechanical sensitization of masseter muscle nociceptors with the same concentration of a TrkA receptor antibody that we have previously shown inhibits NGF-induced mechanical sensitization (Svensson et al. 2010). Taken together, these results suggest that NGF does not play an important role in the mechanism of TNF α -induced mechanical sensitization of the masseter muscle.

TNF α modulates a variety of ion channels. For example, TNF α enhances TTX-resistant sodium currents, an effect that is mediated by the P38 mitogen-activated protein kinase pathway through P55 receptor activation (Jin and Gereau 2006). TNF α has also been shown to reduce outward potassium currents in retinal ganglion neurons (Diem et al. 2001) and to inhibit potassium currents in small dorsal root sensory neurons (Liu et al. 2008). Furthermore, increases in PGE₂ levels act to further enhance the inhibitory effects of TNF α on potassium currents (Liu et al. 2008). These results suggest that downstream modulation of sodium and/or potassium channel function by TNF α could also contribute to TNF α -induced mechanical sensitization of masseter muscle nociceptors. These additional mechanisms of nociceptor sensitization may explain why we were only able to partially attenuate TNF α -induced mechanical sensitization with diclofenac.

Clinical relevance

Myofascial temporomandibular disorders are characterized by masticatory muscle pain and localized muscle tenderness, which some clinicians call “trigger points” but are more accurately described by the term “focal muscle hypertonicities” (Cairns 2010; Friction 2007). Focal muscle hypertonicities are tender nodules within taut bands of skeletal muscle, which are painful on palpation and refer pain to other body parts (Lavelle et al. 2007). Although focal muscle hypertonicities are not associated with tissue damage or inflammation (Cairns 2010), elevated levels of a number of pro-inflammatory compounds such as bradykinin, ATP, substance P, calcitonin gene-related peptide (CGRP), neurotrophins, protons, ILs and importantly TNF α have been found in them (Shah et al. 2008), which suggests that some degree of tissue injury and/or inflammation could be occurring at these sites. Although, NSAIDs are a drug of choice for the treatment of myofascial temporomandibular disorder-related pain conditions, in the small number of clinical studies undertaken to date, it has been difficult to show the efficacy of these agents for the treatment of muscle pain in these conditions (Cairns 2010; Friction 2007). We previously found that TNF α mechanically sensitizes masseter muscle nociceptors without gross inflammation and in this study, we showed that TNF α induces mechanical sensitization of muscle nociceptors that was partially attenuated by the NSAID diclofenac. We propose that injection of TNF α into skeletal muscles could be useful to model focal muscle hypertonicities and may prove useful to study mechanisms of NSAIDs for the treatment of masticatory muscle pain.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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