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Effect of capsaicin-evoked jaw-muscle pain on intramuscular blood-flow

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Aim: To investigate effect of capsaicin-evoked masseter-muscle pain on intramuscular blood-flow (BF) at rest and during contractions. **Methods:** Eight healthy men (22-31 years) participated. BF was measured with Laser Doppler (Moor Instruments, UK) using a single-fibre probe inserted into the right masseter. Three BF probes were attached to the skin above right and left masseter and the right-middle finger. Subjects performed 30 sec isometric contractions at 5%, 15%, and 25% of maximal voluntary contraction. After the contractions, capsaicin (0.1 mL, 100 µg/mL) was injected into the right masseter close to the fibre probe. When the pain sensation had disappeared, the series of submaximal contractions were repeated. BF data were sampled continuously, processed in 10 sec bins, and analyzed with repeated-measures ANOVAs. **Results:** Intramuscular BF significantly increased immediately after capsaicin injection ($P<0.050$) and rapidly (30 sec) decreased to pre-injection values. A significant increase in cutaneous BF above the right masseter was observed ($P<0.050$) and lasted for 10 min, while a significant BF decrease in the finger ($P<0.050$) was observed. The contractions were associated with increases in intramuscular BF before and after the injection ($P<0.022$) and the contraction levels were also associated with increase in intramuscular BF before injection ($P=0.008$) but not after injection ($P=0.314$). **Conclusions:** This study demonstrated BF increased by muscle contraction but failed to show effects of contraction levels on BF in a muscle exposed to nociceptive stimuli. Neurogenic inflammation in muscles could possibly be mediated via antidromical effects and local release of vasoactive substances. The decreased BF in the finger could be due to involvement of central regulatory mechanisms.

Background

The most common complaint of patients with jaw-muscle disorders is a spontaneous deep ache often associated with tenderness to palpation or pain on movement (1, 2). Operationalized criteria for so-called myofascial temporomandibular pain (TMD) has been developed as described in the Research Diagnostic Criteria for TMD (3). Simons and Mense (4) hypothesised that the compromised blood flow (BF) plays an important role in the development of muscle pain and tenderness subsequently leading to an energy crisis with release of metabolites and neuroactive substances. One way to compromise the BF has been speculated to be due to low levels of continuous muscle contraction (5, 6). This hypothesis has gained some support in limb muscles (7-9) and in jaw muscles (10-13). Indeed, intramuscular BF during controlled isometric contraction in normal jaw muscles has been described (5, 6). These studies have indicated that BF during a sustained isometric contraction is insufficient to meet metabolic demand, resulting in relative local ischemia. Furthermore, the studies have also indicated the development of a strong reflex hyperaemia immediately after the contraction (5, 6). However, little is known about the BF during controlled levels of isometric contraction and in the post-contraction phase especially in painful jaw muscles.

Capsaicin, which is the pungent extract of chilli peppers, is a potent algescic substance and evokes pain, hyperalgesia, and neurogenic inflammation (flare) when applied topically or injected into the skin of humans by activation of A δ -mechano-heat polymodal and C fibres (14-24). While intramuscular injection of capsaicin seems to activate mechanical nociceptors with slowly conducting nerve fibres such as Group III and IV (25) and has been useful to induce experimental muscle pain and soreness (26-28), then neurogenic inflammation in muscles as manifested by

increased blood perfusion has not been studied so far. It has been reported that intramuscular injection of capsaicin causes longer-lasting pain (< 30 min) compared to other substances (e.g. hypertonic saline) (27, 29, 30). There is so far no description of BF changes in deep craniofacial tissues following nociceptive stimulation with capsaicin in contrast to abundant reports with increases in cutaneous BF, i.e., neurogenic inflammation (31-34) after topical or subcutaneous injection of capsaicin (14, 19, 20, 35-38).

The objective of this study was to investigate the effect of acute jaw-muscle pain evoked by intramuscular injection of capsaicin on BF changes in the painful muscle at rest and during different levels of submaximal contractions. The hypothesis was that intramuscular injection of capsaicin into the masseter muscle would cause local vasodilatation (neurogenic inflammation) due to antidromically activated axon branches. This reaction would be investigated in the capsaicin-injected masseter muscle as the results of increased intramuscular and cutaneous blood flow. Furthermore, decreased levels of skin blood flow from the non-painful sites (e.g. finger, non-injected masseter muscle) would be seen because sympathetic vasoconstrictor neurons, which are controlling skin blood flow, would be activated in order to compensate for the local vasodilatation. Finally, it was expected that these responses would be attenuated during submaximal muscle contractions.

Materials and methods

Subjects

Eight healthy men (mean age: 25.4 ± 3.7 years, range 22 to 31 years) participated in this study. All were University students and were in good health. The absence of painful muscle disorders and temporomandibular joint problems were checked in accordance with the Research Diagnostic Criteria (RDC) for TMD (3). Furthermore, none of the subjects took medication which could influence pain responses or vascular responses. Informed consent was obtained from each subject prior to inclusion, and the experimental protocol followed the Helsinki Declaration and had been approved by the local ethics committee.

Study Design

Fig. 1-A shows a flow chart illustrating the study design. All subjects performed a maximal voluntary contraction (MVC) by clenching their teeth as hard as possible. After five minutes rest, the subjects performed a series of submaximal contractions at different levels using visual feedback of the electromyographic (EMG) signal. The EMG activity was measured bilaterally from the masseter muscles. After the submaximal levels of masseter muscle contraction, the subjects received a bolus injection of capsaicin into the right masseter muscle. The intensity of the capsaicin-evoked masseter pain was assessed continuously by the subjects on an electronic 0-100 mm visual analogue scale (VAS). When the pain intensity had returned to 0, the submaximal levels of masseter muscle contraction were repeated. The BF responses in the right masseter muscle were measured throughout the experiment (Fig. 1-B). In addition, continuous skin temperature (Fig. 1-B) and intermittent heart rate (Fig. 1-A) were measured.

Two weeks after the capsaicin session, four out of eight subjects took part in a second session to determine the effect of a control injection (isotonic saline) on BF during rest.

EMG and bite force recordings

The skin was cleaned with ethanol, and bipolar disposable surface electrodes (Blue Sensor Type N-10-E, Medicotest) were placed with their long axis transverse to the main fibre direction of the right and left masseter muscles. Electrode placement was based on palpation of the muscles during full effort, as previously described (39). The inter-electrode distance was 10 mm. A saline-soaked grounded electrode was wrapped around the arm. The EMG signals were amplified differentially (5,000 to 20,000 times, Disa 1SC01), filtered (20 to 500 Hz), and sampled (1000 Hz).

A silicon coated U-shaped force transducer (7 mm high, 1.1 x 1.1 cm area, Aalborg University) (40) was placed on the right side between the first molars, and the subjects were asked to bite on the force transducer as hard as they could for 5 sec in order to obtain the MVC force. The EMG was measured simultaneously (MVC-EMG). Verbal encouragement was given to obtain the maximal effort. The subjects were then asked to bite at submaximal levels of 5%, 15%, and 25% of the MVC-EMG (target levels). The subjects increased the force up to the specified target level and held the contraction for 30 sec with the use of visual feedback from the surface EMG signals (EMG feedback). The contractions were followed by five minutes rest in order to avoid muscle fatigue by the submaximal contractions (6). A computer program automatically determined the peak MVC-force (Newton). The EMG activity at the different force levels was quantified as the mean root-mean-square (RMS) value.

Capsaicin and saline evoked masseter muscle pain.

In the capsaicin session (n = 8), a total of 0.1 mL (100 µg/mL) capsaicin was injected into the masseter muscles in accordance with previous descriptions (26). In the control session (n = 4), a single bolus injection of isotonic saline (0.1 mL, 0.9 %) was performed in a similar way into the right masseter muscle. The placement of capsaicin and saline injections was in the middle belly of the right masseter muscle in accordance with the previous studies (26, 27). Subjects were asked to score their pain intensity on the continuous 0-100 mm VAS with their right masseter muscle at rest. The left end of the VAS was labelled either “no pain” and the right end was labelled “most pain imaginable”.

Measurements of blood flow

Cardiovascular changes of masseter muscles during MVC, submaximal levels of masseter muscle contraction, and before, during, and after the experimental pain were monitored. Laser Doppler (Moor Instruments, UK) was used in this study with one probe for intramuscular and three probes for cutaneous recordings. The diameter of the skin probes were 7.0 mm, while the intramuscular probe was 0.8 mm. The subjects were seated in a dental chair. The surface BF probes were placed over the right and left masseter muscles and right middle finger (Fig. 1). The intramuscular probe was inserted perpendicular to the skin surface into the masseter muscle close to the injection site and fixed with tape in order to minimize artefacts from body movement. The absence of movement artefacts was ascertained before starting the BF recording. The temperature at the masseter muscles and finger were also monitored by the surface probes. The experimental room temperature was controlled at 23 degrees Celsius. The recordings were started when the BF values (units) became stable

(baseline). One min after the baseline recording, MVC and submaximal levels of masseter muscle contraction were performed. The signals were AD-converted and were stored in a personal computer. BF signals were sampled at 40 Hz and processed in 10 sec bins before, during, and after the injections and in 3 sec bins during the submaximal levels of masseter muscle contractions.

In addition to BF data, the heart rate (right index finger) and blood pressure (left arm) were measured to see any possible interaction between local and general cardiovascular changes (Agilent A3 Patient Monitor, Agilent Technologies, UK).

Statistical Analysis

The effect of capsaicin and saline injection on intramuscular masseter BF, surface masseter muscle BF, and finger BF were evaluated by 1-way analysis of variance (1-ANOVAs) with repeated measures followed by Dunnett's test. The variable was "time" and 10 sec before the injections were set as baseline for the post hoc tests. The baseline BF before each submaximal contraction in the condition of before and after the injections was compared with the use of 1-ANOVAs with repeated measures. The baseline BF was also compared between before and after the injections with the use of 2-way analysis of variance (2-ANOVAs). The variables were time (before and after the injection) and contraction level. The effect of submaximal contractions on intramuscular BF in the condition of before and after the injections was evaluated by 2-ANOVAs with repeated measures followed by Dunnett's test. The variables were contraction and contraction level. The BF during rest was set as baseline for the post hoc tests. Electrical masseter muscle activities (EMG-RMS) during submaximal contractions were evaluated by 2-ANOVAs with repeated measures. The variables were contraction and contraction level. The 2-ANOVA with repeated measures tests

were also performed on EMG-RMS before and after the injection. Finally, heart rate and blood pressure were evaluated by 1-ANOVAs with repeated measures followed by Dunnett's test. The variable was time. The values immediately after mounting the equipment were set as baseline for post hoc tests. Parametric statistics (mean \pm SD) were used to describe the data and the level of significance was set at $P < 0.050$.

Results

Effect of intramuscular injections with the jaw at rest

The capsaicin injection evoked moderate pain which was rated as 37.5 ± 13.9 on the VAS 5 min after the injection and lasted for a total of 14.9 ± 1.4 min. In contrast, the injection of isotonic saline was associated with no or very low levels of pain (5.0 ± 10.0) and only lasted 4.6 ± 1.7 min.

Immediately after the capsaicin and saline injections, BF in the right masseter muscle was significantly increased (Fig. 2AD. Dunnett's tests: $P < 0.050$). However, in both sessions the BF rapidly (about 30 sec) decreased to baseline values. A significant increase in cutaneous BF above the right masseter muscle was observed only following the injection of capsaicin (Fig. 2A. Dunnett's test: $P < 0.050$) and lasted 10 min. No significant changes were detected in cutaneous BF above the left masseter muscle (Fig. 2BE), but the capsaicin injection was associated with a significant decrease of cutaneous BF in the middle finger (Fig. 2C. Dunnett's test: $P < 0.050$).

Effect of submaximal masseter muscle contraction

The effect of submaximal masseter muscle contraction on intramuscular BF in the right masseter (Fig. 3) was analyzed with ANOVAs before and after the injection of capsaicin. Before the capsaicin injection, there were no significant differences between baseline BF in the right masseter muscle (1-ANOVA: $F = 1.446$, $P = 0.269$) although the BF before the 5% MVC contraction tended to be slightly higher than before the 15% and 25% MVC contractions (Fig. 3A). There was a significant main effect of contraction (2-ANOVA: $F = 5.079$; $P = 0.022$) with higher BF values during

the 25% MVC contraction compared to baseline BF (Dunnett's tests: $P < 0.050$). There was also a significant main effect of contraction level (2-ANOVA: $F = 7.003$; $P = 0.008$) with a tendency towards higher BF values during the higher levels of contractions, however, post-hoc analysis could not demonstrate this (Dunnett's tests: $F < 2.070$, $P > 0.163$)

After the pain from the capsaicin injection had vanished, another set of submaximal levels of masseter muscle contraction was performed. Again there were no significant differences in baseline BF in the right masseter (1-ANOVA: $F = 3.130$, $P = 0.075$) but contraction was associated with significant increases in BF (2-ANOVA: $F = 5.270$; $P = 0.020$). Post-hoc tests showed that there were contraction-related increases at 5% and 15% MVC (Dunnett's tests: $P < 0.050$) but not at 25% MVC. Although there was not a significant main effect of contraction level (2-ANOVA: $F = 1.259$; $P = 0.314$), the BF during the 25% MVC contraction showed significantly higher values compared to during the 5% MVC contraction (Dunnett's tests: $P < 0.050$) (Fig. 4).

Finally, the baseline BF values were directly compared before and after the capsaicin injection and revealed no significant differences (2-ANOVA: $F = 3.757$; $P = 0.094$).

The contraction levels were determined with the use of EMG feedback and as expected there were significant effects of contraction (Fig. 5, 2-ANOVAs: $F > 19.839$; $P < 0.003$) and contraction levels (2-ANOVA: $F = 33.133$; $P < 0.001$) on the RMS values. There were, however, no significant effects of the capsaicin injection (2-ANOVA: $F = 5.006$; $P = 0.056$).

Heart rate and blood pressure

Table 1 summarizes the heart rate and blood pressure data before and after the injections with capsaicin and isotonic saline. Five min after the capsaicin injection, diastolic blood pressure was significantly increased as compared to baseline values (Dunnett's tests: $P < 0.050$), while the heart rate was significantly decreased as compared to baseline (Dunnett's tests: $P < 0.050$). There were no other significant differences (1-ANOVAs: $F < 2.584$, $P > 0.114$).

Discussion

This study demonstrated immediately after the capsaicin injection, cutaneous BF increases over the masseter muscle on the injection side, and cutaneous BF decreases in the middle finger with no BF changes in the contralateral cutaneous BF over the masseter muscle. The results also showed intramuscular BF increases evoked by submaximal levels of masseter muscle contraction but failed to show effects of contraction levels on intramuscular BF in a painful muscle. This study provides some indications of a neurogenic inflammation in the masseter muscle based on the observed changes in masseter blood flow.

Intramuscular blood flow during submaximal contractions

Although chronic masseter muscle pain has been described and discussed in the field of orofacial pain and temporomandibular disorders since the 1930s (41), the underlying mechanisms of pain are still not fully understood. One of the earliest study, which investigated blood flow in human temporal muscle during clenching measured by Xenon clearance(43), observed increased BF and suggested that the pain induced by isometric contraction was not due to muscle ischemia. However, later studies, which measured intramuscular BF during controlled levels of isometric contraction and post-contraction phases, indicated that the BF may be arrested at certain low levels of sustained contraction. It was suggested that the insufficient BF causes relative local ischemia with the development of a strong reflex hyperaemia after the contraction had been completed, and thereby causing muscle fatigue and pain (5, 6). This post-contraction phase has been thought to be a critical factor for assessing and recognizing if the BF is sufficient to meet metabolic demand (44). The present study

showed that isotonic masseter muscle contractions were associated with increased intramuscular BF in the masseter muscle compared to that during rest. However, the masseter BF data did not show the reactive hyperaemia, which can normally be seen in limb muscles (45, 46). Larsson et al. (1996) (46) did not find intramuscular BF changes in forearm muscles from resting level during contractions at 10%, 20%, and 30% MVC, whereas they noted mean increases of 150% and 200% at 40% and 50% MVC. There is also evidence that, in limb muscles, the level of isometric contraction that can arrest intramuscular BF varies among different muscles from around 60% for the forearm (47) down to 20% for the quadriceps (48). The present study showed increased masseter intramuscular BF in response to increasing masseter muscle contraction levels. However, the present study did not show a peak level of BF for masseter muscle contraction and, unfortunately, could not decide the threshold of masseter muscle contraction to decrease BF. Further studies will be needed to pursue this issue with the use of higher contraction levels than 25% MVC.

Experimental neurogenic inflammation

Neurogenic inflammation is a biological phenomenon which includes arteriolar vasodilatation (flare) and oedema caused by extravasations of plasma from post-capillary venules triggered by the release of proinflammatory neuropeptides such as substance P and calcitonin gene-related peptide from sensory nerve terminals (31-34).

Capsaicin injection was used in this study to produce a neurogenic inflammation with burning spontaneous pain and thermal and mechanical hyperalgesia in the masseter muscle. Mechanical nociceptors associated with slowly conducting nerve fibres such as Group III and IV are likely to have been activated by the injection (25) and the subjects felt cramp-like masseter muscle pain. The duration

of pain, the area and duration of mechanical hyperalgesia, and the area of flare evoked by the capsaicin have been shown to be dose-dependent (15). Other merits of using capsaicin are that capsaicin is more nociceptive-specific compared to e.g. hypertonic saline (27, 29, 30), intramuscular injection of capsaicin causes long-lasting pain with increasing sensitivity compared to other substances, and the capsaicin can deliver different levels of pain in a reproducible manner (21). This study used 100 µg/mL 0.1 mL of capsaicin and the intensity of masseter muscle pain on VAS was 37.5 ± 13.9 in accordance with previous studies (26, 27).

This is the first report to describe intramuscular BF changes in masseter muscle following nociceptive stimulation such as capsaicin. The capsaicin injection evoked an immediate increase in the intramuscular BF which decreased within 30 sec. This reaction in the masseter muscle might be due to the injection flow itself since the same intramuscular BF reaction was seen when isotonic saline was injected. However, only the capsaicin injection was associated with a two-fold increase in surface masseter BF with a peak about 60 sec and lasting for about 10 min. Moreover, only the capsaicin injection was associated with a decrease in surface finger BF, and higher blood pressure for 5 min compared to the resting phase. A possible explanation for the increased surface masseter BF could be that local muscle tissue damage and the release of pain-producing vasoactive agents such as substance P, prostaglandins, and bradykinin (49) and resulted in flare or neurogenic inflammatory reactions in both muscle tissue and the overlying skin (15, 19, 50, 51). Furthermore, the decrease in surface finger BF and increased general blood pressure might occur as a part of compensatory vasodilatation and blood distribution in order to maintain adequate perfusion of the muscle. However, these kinds of reactions did not seem to occur on the contralateral side in accordance with other observations (see Kemppainen et al.

2003) (52).

An intramuscular probe, which was made by optical glass fibre, was used in this study to measure intramuscular BF during pain and submaximal contractions. Such contractions can be associated with movement artefacts which to some extent were minimized by filtering and the analysis procedure. Furthermore, there may be variations in BF due to the bleeding or twitched muscle fibres during the contractions. Due to the difficulties mentioned above, the present experimental was not conducted as a randomized clinical trial. Thus, we employed a fairly complex experimental study with an invasive technique to estimate masseter intramuscular BF with relatively fewer controls (with isotonic saline injection). Due to the small sample size, the study may have been under-powered but the study clearly shows that the magnitude of BF responses following noxious stimulation is modest. We also note that several previous studies on BF used relatively small sample sizes (5, 6, 43, 53, 54). Future studies need to improve this point, for example, with the use of non-invasive probes (55, 56).

One striking finding was that contractions at 25% MVC was associated with intramuscular BF increases before the capsaicin injection whereas after the capsaicin injection contractions at 5% and 15% MVC were able to increase the BF. This was demonstrated by the result that contraction levels were significant before but not after capsaicin injection. This could indicate minor changes, e.g. due to a neurogenic inflammation, in the BF response to muscle contractions.

It needs to be mentioned that the current study was not designed to investigate potential gender-related differences in nociceptive processing or physiological responses and only employed male subjects. Further studies preferably using non-invasive techniques for assessment of BF will be needed to address the question about gender-related differences. We would like to propose that studies on BF responses in

painful muscles during pain and various contractions may help to further the understanding of pathophysiologic mechanisms related to craniofacial pain conditions such as TMD and tension-type headache.

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