Pain and allodynia/hyperalgesia induced by intramuscular injection of serotonin in patients with fibromyalgia and healthy individuals

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Abstract

The aim of this study was to investigate the effect of injection of serotonin (5-HT) into the masseter muscle on pain and allodynia/hyperalgesia. Twelve female patients with fibromyalgia (FM) and 12 age-matched female healthy individuals (HI) participated in the study. The current pain intensity (CPI) and the pressure pain threshold (PPT) of the superficial masseter muscles were assessed bilaterally. 5-HT in one of three randomized concentrations (10⁻³, 10⁻⁵, 10⁻⁷ M) or isotonic saline was then injected into either of the two masseter muscles in a double-blind manner. After the injections the CPI and PPT were recorded ten times during 30 min. The injections were repeated twice with the other concentrations of 5-HT after 1 and 2 weeks, respectively. In the FM-group there was a non-significant increase of CPI after injection that lasted during the entire 30-min period irrespective of whether 5-HT or saline was injected. Neither did the PPT change significantly. In the HI-group pain developed significantly after injection irrespective of whether 5-HT or saline was injected, but significantly more so after 5-HT at 10⁻³ M than saline injection. CPI decreased quickly and then remained on a very low level for most of the experiment. 5-HT at both 10⁻⁵ M and 10⁻³ M caused a significantly greater decrease of PPT than saline. In conclusion, our results show that 5-HT injected into the masseter muscle of healthy female subjects elicits pain and allodynia/hyperalgesia, while no such responses occur in patients with fibromyalgia.

Keywords: Double-blind method; Fibromyalgia; 5-Hydroxytryptamine; Pain threshold; Visual analogue pain scale

1. Introduction

Fibromyalgia (FM) is a musculoskeletal pain condition that has gained a large interest in research over the past 15–20 years. FM is mainly affecting women in their mid-age and is characterized by widespread pain, tenderness, pronounced fatigue, muscle stiffness, and sleep disturbances (Wolfe et al., 1990, 1997a; Russell, 1998a). The disability associated with this condition is increasingly recognized (Bennett, 1996) and many of them are forced to take long periods of sick leave (Wolfe et al., 1997b). For research purpose the criteria according to the American College of Rheumatology (ACR) are most often used for diagnosis (Wolfe et al., 1990). These include pain from all four body quadrants and pain upon digital palpation of at least 11 of 18 bilateral points. Since these points are located not only over muscle tissue it has been suggested that FM-patients suffer from an increased sensitivity to pressure in general (Scudds et al., 1987; Kosek et al., 1996). None of the points is located in the orofacial area. However, several studies have shown that patients with FM frequently suffer from orofacial pain and tenderness (Eriksson et al., 1988; Hedenberg-Magnusson et al., 1997).

The pathophysiology behind fibromyalgia is largely unknown, but probably involves both systemic and peripheral mechanisms (Yunus, 1992; Russell, 1993; Russell, 1998b). In peripheral tissue, it has been suggested that a decreased microcirculation is involved (Henriksson and Bengtsson, 1991). The decreased microcirculation leads to a release of algogenic substances, such as serotonin (5-HT), histamine and prostaglandins, which may sensitize muscle nociceptors by lowering the firing threshold to mechanical stimuli (Mense, 1993). These substances may also be released due to microtrauma (Mense, 1993). We have in a previous study investigated the level of 5-HT in the masseter muscle in patients with FM (Ernberg et al., 1999). The results indicated that patients with FM release more 5-HT after needle provocation than healthy individuals, and that high levels of 5-HT in the masseter muscle are associated with pain and allodynia/hyperalgesia.

5-HT is known to be released from platelets due to tissue
2. Material and methods

2.1. Subjects

Twelve female patients with FM and a mean age (±SD) of 45.9 (±7.2) years participated in the study. All patients had participated in a special program for FM-patients in the Department of Rehabilitation Medicine at the Karolinska Hospital, Stockholm, where the diagnosis of FM according to the ACR criteria (Wolfe et al., 1990) was determined. They were then referred to the Department of Clinical Oral Physiology for voluntary participation in this study. The patients were first assigned to a brief examination in order to evaluate if they fulfilled the inclusion criteria. These were pains from the masseter region for at least 3 months and tenderness to digital palpation of the superficial masseter muscle. Exclusion criteria were general inflammatory connective tissue disease (e.g. rheumatoid arthritis) or symptoms that could be referred to disease in other parts of the orofacial region (e.g. temporomandibular joints, toothache, neuralgia). Four of the patients took antidepressant drugs (three citalopram and one sertraline), two anxiolytics (buspirone), nine analgesics (one tramadol, seven paracetamol and one salicylic acid) and two muscle relaxants (clorzoxazon).

A group of 12 healthy females (HI) with a mean age (±SD) of 45.6 (±10.4) years who participated on a voluntary basis were also included in the study. They were age-matched to the FM-patients and had no pain from the orofacial region. Minor tenderness to palpation of the masticatory muscles was accepted. No healthy subject received medication.

The methods and selection of patients were approved by the local ethical committee at Huddinge Hospital, Karolinska Institutet, Stockholm (368/96). All individuals had given their verbal consent.

2.2. Methods

2.2.1. Pain assessment

A 100-mm visual analogue scale (VAS) with end-points marked by ‘No pain’ and ‘Worst pain ever experienced’ was used to assess the current pain intensity (CPI) from the masseter region shortly before the injections. It was assessed for the right and left side separately, when at rest (CPIrest) as well as at maximum voluntary mouth opening (CPIMVM). The CPI was assessed again immediately after injection and then every second minute during the first 10 min and every 5th min during 20 min. The individual median pain intensity over the first 10 min and over the whole experimental period (30 min) was calculated and used for statistical analyses, as were the change in pain intensity over time, and pain duration. The latter was defined as the time period after which the pain had returned to within 10% of the individual maximum pain level. If the pain did not return to this point within 30 min the subjects were asked to report at what time it finally did.

2.2.2. Most tender point

For the FM-patients the most tender point by external digital palpation over the superficial masseter muscle was determined and recorded on an overhead sheet, where the borders of the ear, mandible and chin were outlined (Fig. 1). For the healthy individuals a standardized point was used, which was located at the midpoint of the superficial masseter muscle 2 cm above the mandibular base (Fig. 1). These points were used for the investigation concerning the effects of the test substances.

2.2.3. Assessment of pressure pain thresholds

The pressure pain threshold (PPT) was assessed (kPa) with an electronic algometer (Somedic Sales AB, Sollentuna, Sweden) with a blunt rubber recording tip of 10 mm in diameter. The algometer was held perpendicular to the skin surface and the pressure was increased slowly with a pressure rate of 50 kPa/s. The subjects were instructed to press a button as soon as the sensation of pressure changed to pain. This was first done over the soft tissue close to the base of the thumb on the dorsal side of the hand, in order to accustom the subject to the procedure. Recordings of PPT were then made over the two superficial masseter muscles as well as over a reference point on the forehead (glabella, frontal bone). The PPT was recorded at each site three times 2 min apart before injection. The mean of the three recordings was
used as pre-injection level. Immediately after the injection the PPT was assessed again and then every second minute during the first 10 min and every 5th min during 20 min (single recordings). For each recording after injection the percentage difference of PPT from pre-injection level was calculated. The individual mean of the PPT differences during the first 10 min and during the whole experimental period (30 min) as well as the change in PPT over time were used for statistical analyses.

2.2.4. Blood sampling

Venous blood was collected (2 ml) in a tube without additives for analysis of the serum level of 5-HT (S-5-HT). This blood sample was left in room temperature for 1 h to coagulate and then cold-centrifuged (+4°C, 1700 × g) for 30 min. Approximately 200 μl of the supernatant was then pipetted into polystyrene tubes and kept frozen at −22°C until analysis. To exclude any risk of interference with the analysis of S-5-HT, the subjects were asked to avoid tryptophan-rich food (e.g. banana, pineapple, tomato and chocolate) for 24 h before the examination. Due to diurnal variation of 5-HT (Candito et al., 1990) all blood samples were collected in the afternoon.

2.2.5. Substances

Sterile 5-HT stock solution (Sigma, St. Louis, MO, USA) with a concentration of 10⁻³ M was prepared at the Huddinge Hospital Pharmacy Department. Part of the stock solution was diluted with sterile isotonic saline (0.9 mg/ml, Pharmacia and Upjohn, Solna, Sweden) to concentrations of 10⁻⁵ and 10⁻⁷ M under sterile conditions. The solutions were divided into portions of 0.3 ml and frozen (−80°C). To avoid oxidation of the 5-HT all samples were covered by aluminum foil during dilution and the samples were frozen in light protected sterile Eppendorph tubes. Sterile isotonic saline was used as comparison.

2.2.6. Injections

All subjects received all three concentrations of 5-HT (10⁻³, 10⁻⁵ and 10⁻⁷ M) and isotonic saline in a randomized controlled double-blind manner. A laboratory technician prepared the syringes shortly before injections in time to allow the solution to become room-tempered, labeled them ‘right’ and ‘left’ and then protected them with an aluminum cover. 0.2 ml of test substance or saline was injected over 5 s into the selected points of the masseter muscles. Injections were made with a 19-mm long needle (diameter 0.4 mm) from a 1-ml syringe. The injections were repeated twice with the other concentrations of 5-HT 1 week apart.

2.2.7. Analysis of 5-HT

After thawing, the 5-HT samples were analyzed by a commerically available competitive EIA (No 0642, Immu-
3.2.1. FM-group

The median (IQR) changes in CPI for each time interval (10 and 30 min) are shown in Table 2.

3.2.1. FM-group

There was no significant change regarding CPIrest for any concentration of 5-HT or saline compared to baseline (Fig. 2A) or any significant difference between the response to 5-HT and saline for this variable.

CPIMVM increased significantly after injection of 5-HT at 10^{-5} M (P < 0.001), while there was no significant change for the other 5-HT concentrations or for saline compared to baseline (Fig. 3A). There was no significant difference between the response to 5-HT and saline.

When the patients who were under medication with antidepressant drugs were excluded, still no differences between the response to 5-HT and saline regarding CPI were found.

3.2.2. HI-group

Pain at rest (CPIrest) developed significantly after injection of all concentrations of 5-HT (10^{-7} M: P < 0.01, 10^{-5} and 10^{-3} M: P < 0.001), but also after injection of saline on the contralateral side corresponding to all concentrations of 5-HT (10^{-7} M: P < 0.01, 10^{-5} M: P < 0.001 and 10^{-3} M: P < 0.01) (Fig. 2B). The CPIrest developed significantly more after injection of 5-HT at 10^{-3} M than saline (10 and 30 min: P < 0.05), while there was no difference between the other concentrations of 5-HT and saline.

CPIMVM developed significantly after injection of all concentrations of 5-HT (10^{-7} M: P < 0.01, 10^{-5} M: P < 0.001 and 10^{-3} M: P < 0.05), but also after injection of saline contralateral to the injection of 5-HT at 10^{-5} M (P < 0.05) (Fig. 3B). The CPIMVM developed significantly more after injection of 5-HT at 10^{-3} M than saline (10 and 30 min: P < 0.05), while there was no difference between the other concentrations of 5-HT and saline.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>FM</th>
<th>HI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPIrest Median (IQR)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>34 (37)</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Left</td>
<td>35 (40)</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CPIMVM Median (IQR)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>38 (37)</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Left</td>
<td>43 (37)</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PPT Mean (±SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>103 (±44)</td>
<td>219 (±44)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Left</td>
<td>108 (±48)</td>
<td>204 (±55)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Reference</td>
<td>163 (±77)</td>
<td>329 (±91)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>S-5-HT Mean (±SD)</td>
<td></td>
<td></td>
<td>&gt;0.1</td>
</tr>
<tr>
<td></td>
<td>568 (±438)</td>
<td>817 (±432)</td>
<td></td>
</tr>
</tbody>
</table>

* CPIrest, degree of resting pain; CPIMVM, degree of pain during maximum voluntary mouth opening in the masseter region assessed with a 100-mm Visual Analogue Scale; PPT, pressure pain threshold over the superficial masseter muscle (kPa) and over a reference point (glabella, frontal bone); S-5-HT, serum level of serotonin (nmol/l); IQR, interquartile range; SD, standard deviation; P, P-value (Mann–Whitney U-test or unpaired t-test).
Table 2
Changes in current pain intensity (CPI; mm) and pressure pain threshold (PPT; %) of the masseter muscle during 10 and 30 min after injection of 5-HT in different concentrations as well as isotonic saline in 12 patients with fibromyalgia (FM) and 12 healthy individuals (HI).a,b

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>FM</th>
<th></th>
<th></th>
<th>HI</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5-HT</td>
<td>Saline</td>
<td>5-HT</td>
<td>Saline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10−7 M</td>
<td>CPIRest median (IQR)</td>
<td>10</td>
<td>3.8 (15.5)</td>
<td>4.8 (21.2)</td>
<td>2.8 (10.4)</td>
<td>3.5 (10.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>5.5 (8.0)</td>
<td>5.5 (19.2)</td>
<td>2.2 (9.6)</td>
<td>2.2 (9.6)</td>
</tr>
<tr>
<td></td>
<td>CPIMVM median (IQR)</td>
<td>10</td>
<td>4.0 (9.5)</td>
<td>4.0 (16.0)</td>
<td>4.8 (11.0)</td>
<td>3.8 (11.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>2.5 (9.5)</td>
<td>3.0 (13.0)</td>
<td>2.8 (11.2)</td>
<td>3.2 (10.9)</td>
</tr>
<tr>
<td></td>
<td>PPT (%) mean (±SD)</td>
<td>10</td>
<td>−4.2 (±29.4)</td>
<td>−4.1 (±18.7)</td>
<td>1.0 (±17.3)</td>
<td>−8.7 (±14.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>−4.0 (±24.7)</td>
<td>−4.6 (±18.4)</td>
<td>−0.4 (±19.0)</td>
<td>−10.7 (±13.5)</td>
</tr>
</tbody>
</table>

| 10−5 M    | CPIRest median (IQR) | 10 | 8.8 (13.1) | 5.8 (13.6) | 2.8 (5.8) | 2.0 (4.2) |
|           |      | 30 | 5.2 (12.5) | 5.8 (19.8) | 1.0 (4.4) | 1.0 (2.4) |
|           | CPIMVM median (IQR) | 10 | 2.0 (16.0) | 2.0 (12.0) | 1.8 (7.4) | 1.8 (3.4) |
|           |      | 30 | 2.0 (10.5) | 3.0 (12.0) | 0.8 (6.2) | 0.8 (2.8) |
|           | PPT (%) mean (±SD) | 10 | −12.1 (±16.0) | 0.4 (±11.4) | −7.8 (±13.5) | 3.4 (±13.0) |
|           |      | 30 | −9.0 (±15.9) | 1.1 (±17.0) | −8.4 (±12.0) | 3.6 (±13.4) |

| 10−3 M    | CPIRest median (IQR) | 10 | 11.5 (12.0) | 9.8 (11.4) | 2.8 (10.1) | 0.5 (7.9) |
|           |      | 30 | 12.0 (17.8) | 5.8 (9.9) | 1.8 (7.9) | 0.5 (6.4) |
|           | CPIMVM median (IQR) | 10 | 3.0 (14.5) | 1.0 (15.3) | 2.0 (13.1) | 1.5 (9.8) |
|           |      | 30 | 2.5 (20.5) | 4.0 (12.5) | 1.5 (10.9) | 1.0 (8.5) |
|           | PPT (%) mean (±SD) | 10 | −3.2 (±23.9) | −1.5 (±19.4) | −10.9 (±13.3) | −4.9 (±12.4) |
|           |      | 30 | −6.1 (±22.7) | −0.6 (±19.8) | −11.1 (±10.6) | −3.4 (±14.2) |

a CPIRest, current pain intensity of resting pain; CPIMVM, current pain intensity during maximum voluntary mouth opening measured with a Visual Analogue Scale; IQR, interquartile range.
b Bold figures denote significant differences between 5-HT and saline (Mann–Whitney U-test or unpaired t-test; P < 0.05).

3.3. PPT after injection
The mean (±SD) changes in PPT for each time interval (10 and 30 min) are shown in Table 2.

3.3.1. FM-group
There was no significant change regarding PPT after injection of any concentration of 5-HT or saline compared to baseline or any significant difference between the response to 5-HT and saline (Fig. 4A). When the patients who were under medication with antidepressant drugs were excluded, still no differences between the response to 5-HT and saline were found. Neither was there any significant change during the experimental period regarding PPT over the reference point at any visit.

3.3.2. HI-group
The PPT decreased significantly more after injection of saline than 5-HT at 10−7 M (10 and 30 min: P < 0.05), and significantly more after injection of 5-HT at 10−5 (10 and 30 min: P < 0.05) as well as 10−3 M (30 min: P < 0.05) than saline (Fig. 4B). The change in PPT over time compared to baseline was not significant for any concentration of 5-HT or saline. Neither was there any significant change over time regarding the PPT over the reference point at any visit.

3.4. Pain duration
The median duration of pain after injection is shown in Table 3. The duration of pain was generally longer in the FM-group than in the HI-group, and the difference found between groups was statistically significant for CPIRest after injection of 10−3 M 5-HT and saline corresponding to 5-HT at 10−3 M (P < 0.05). Eight of the FM-patients reported that the increased pain from one or both of the masseter muscles remained after cessation of the experiment (30 min). For two of these patients pain returned to pre-injection level within a few hours, three reported increased pain for 5–6 h and two for more than 15 h. No healthy subject reported pain from the masseter muscles after cessation of the experiment. There was no relation between pain duration and the substance injected or between pain duration and concentration of 5-HT.

3.5. Pain radiation
Four of the patients and one of the healthy subjects reported pain radiation on the ipsilateral side after injection. One patient experienced pain in the cheek, two patients in the mandible and one patient in the eye. The healthy subject experienced pain in the lower molar teeth. Radiation of pain was only reported after injection of 5-HT 10−3 (three indi-
Fig. 2. Panels showing the difference to baseline in current pain intensity at rest (CPI_{rest}) of the masseter muscle after intramuscular injection of 5-HT in different concentrations and isotonic saline. (A) In 12 female patients with fibromyalgia (FM). (B) In 12 healthy female subjects (HI).

Fig. 3. Panels showing the difference to baseline in current pain intensity during maximum voluntary mouth opening (CPI_{MVM}) of the masseter muscle after intramuscular injection of 5-HT in different concentrations and isotonic saline. (A) In 12 female patients with fibromyalgia (FM). (B) In 12 healthy female subjects (HI).
4. Discussion

4.1. 5-HT vs. saline

The results of this study show that 5-HT, injected intramuscularly, elicits pain in the masseter muscle of healthy individuals, but not after saline injection.

Table 3

<table>
<thead>
<tr>
<th>Concentration (M)</th>
<th>Duration of Pain</th>
<th>FM</th>
<th>HI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PainRest</td>
<td>PainMaxM</td>
<td>PainRest</td>
</tr>
<tr>
<td>$10^{-7}$</td>
<td>30 (±15)</td>
<td>30 (±15)</td>
<td>12 (±28)</td>
</tr>
<tr>
<td>$10^{-5}$</td>
<td>30 (±22)</td>
<td>30 (±15)</td>
<td>25 (±28)</td>
</tr>
<tr>
<td>$10^{-3}$</td>
<td>30 (±23)</td>
<td>30 (±24)</td>
<td>8 (±22)</td>
</tr>
</tbody>
</table>

* PainRest, duration of resting pain; PainMaxM, duration of pain during maximum voluntary mouth opening; IQR, interquartile range.

* Bold figures denotes significant difference between FM and HI (Mann–Whitney U-test; *P* < 0.05).
pain parameters by injection of 5-HT $10^{-5}$ M into the temporal muscle. We used three different concentrations of 5-HT and found that a concentration of at least $10^{-3}$ M is necessary to exert a nociceptive effect, which may explain this difference between studies. The difference regarding PPT may also be due to anatomical or physiological differences between the masseter and anterior temporal muscle.

It has been shown previously that the dose of bradykinin required for sensitization of muscle nociceptors is lower than the dose required for excitation (Mense and Meyer, 1988). Our results show that this is valid also for 5-HT, i.e., the dose required for reduction of PPT (sensitization) was lower than for excitation (CPI). Our results further indicate that 5-HT receptors on afferent nerves in the human muscle are activated by 5-HT. Which subclasses of receptors that are activated are to be investigated in future studies.

It is remarkable that there was no difference in the response to 5-HT and saline in the FM-group. Therefore, 5-HT in the injected concentrations does not seem to affect pain or allodynia/hyperalgesia in FM-patients in a significant way. This seems not to be in agreement with our previous result, showing that patients with FM release more 5-HT after needle provocation than healthy individuals, and that high level of 5-HT in the masseter muscle is associated with pain and allodynia/hyperalgesia (Ernberg et al., 1999). One hypothetical explanation to this finding could be that there is a difference between endogenous and exogenous (injected) 5-HT with respect to the pain response, i.e., that only endogenously released 5-HT sensitizes and excites muscle nociceptors in FM-patients. However, this is not very likely, since our study has shown that exogenous 5-HT most probably sensitize and excite these nociceptors in healthy subjects. Still another explanation could be that the 5-HT receptors for pain and allodynia/hyperalgesia already are occupied by endogenous 5-HT in patients with FM and therefore not available for injected 5-HT. Some of the patients were on medication with serotonin re-uptake inhibitors, sedative, and analgesic drugs. Since this may also have influenced our results, we tested to exclude them. However, the result remained.

4.2. Pain duration

The duration of increased pain after injection was longer in the FM-group than the duration of pain in the HI-group, irrespective of the substance injected. The difference was statistically significant regarding CPI$_{\text{Rest}}$ for 5-HT at $10^{-3}$ M and after saline corresponding to 5-HT at $10^{-5}$ M. We have defined pain duration as the time until CPI had returned to a level of 10% of the individual maximum CPI difference. This was done since, in the HI-group pain developed quickly, then disappeared almost completely within 4–5 min, but remained at a very low level during the rest of the experimental period. In the FM-group the temporal pain pattern was different. The pain increased after injection, but then remained at a substantially increased level for a much longer time. For most patients in the FM-group the CPI had not returned to the defined level after 30 min when the experiment ended. In fact, some of the patients reported increased pain until the next day. However, self-reports of increased pain after cessation of the experiment were not included in the statistical analysis, since this was not estimated according to the definition above. Many FM-patients self-reported that they experienced more long lasting pain after trauma or pinching than before they had the disease. This might be due to central mechanisms at the brain stem or higher levels, e.g., wind-up or central sensitization and hyperexcitability (Kosek et al., 1996; Sörensen et al., 1998).
help with preparing the stock solution of 5-HT. This study was supported by grants from Faculty of Odontology at Karolinska Institutet, The Swedish Dental Society, The Swedish Medical Research Council (B94-24X-10416-02B) and Signe and Reinhold Sund Foundation.

References


