Effects of Local Serotonin Administration on Pain and Microcirculation in the Human Masseter Muscle

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Aims: *To investigate whether exogenously administered 5-hydroxytryptamine (5-HT) at high or low concentration influences pain and microcirculation in the human masseter muscle.* ***Methods:*** *In 12 healthy female subjects, 5-HT in 2 concentrations (0.1 μmol/L and 1,000 μmol/L) and isotonic saline were injected into the masseter muscles in a randomized and balanced double-blind manner. The pain intensity after injections was recorded with Borg's rating scale, and intramuscular blood flow was monitored continuously during the experiment with a laser-Doppler technique. Nonparametric statistics were used for analyses.* ***Results:*** *Administration of 5-HT at 1,000 μmol/L induced significantly more pain than saline (Wilcoxon:* $P < .05$*), while there was no difference between 5-HT at 0.1 μmol/L and saline. The blood flow did not change significantly after injection of 5-HT at either concentration compared to saline. However, changes in pain intensity and blood flow were positively correlated after injection of 5-HT at 1,000 μmol/L (Spearman:* $P < .05$*).* ***Conclusion:*** *Intramuscular administration of 5-HT at 1,000 μmol/L into the human masseter muscle induced pain, but 5-HT did not have any effect on local blood flow at either concentration.* J OROFAC PAIN 2006;20:241–248

Key words: ischemia, laser-Doppler flowmetry, masseter muscle, pain, serotonin

Chronic musculoskeletal pain conditions are very common and disabling for the patient. They are also costly in terms of human suffering and sick leave. Orofacial pain of muscular origin (myalgia) is a frequent form of chronic musculoskeletal pain, with a prevalence of 5% to 10% in the general population.[1] Orofacial myalgia is often poorly localized and is commonly referred to the neck, face, teeth, or preauricular regions. It is frequently accompanied by headache and restricted mouth opening capacity.[2] The etiology and pathophysiology behind this localized myalgia are so far unclear, but it has for a long time been assumed that it develops because of microtrauma or muscle ischemia after physical overloading of muscles.[3,4] There are now several reports indicating that the microcirculation is disturbed in the affected

muscles of patients with localized myalgia, which suggests that local ischemia participates in the pathophysiology of muscle pain.[5,6] Ischemia may lead to a cascade of biochemical events, including release of pain mediators, that may activate and sensitize peripheral nerves and thereby cause pain.[2,7] Some of these pain mediators are also reported to induce neuroplastic changes in the brainstem, ie, central sensitization, which is believed to contribute to muscle allodynia and hyperalgesia[7–9] and to increase muscle activity by reflex pathways in animal studies.[10,11]

Serotonin, or 5-hydroxytryptamine (5-HT), is released peripherally from platelets because of tissue damage or ischemia and participates in pain mediation by activating peripheral sensory afferents. The present investigators[12] and others[13] have shown that a high intramuscular level of 5-HT is associated with local pain and allodynia as well as hyperalgesia in patients with chronic myalgia. Furthermore, intramuscular injection of 5-HT in healthy subjects has been reported to induce local pain and allodynia[14,15] mediated by the 5-HT_3 receptor.[16] 5-HT is also involved in the modulation of the peripheral microcirculation. It is suggested that 5-HT may redirect blood flow from nutritive capillaries to non-nutritive vessels, which leads to decreased oxygen uptake and aerobic contractile activity.[17] If decreased oxygen uptake is prolonged, this may lead to ischemia, which may cause pain.[7] Therefore, 5-HT may have also an indirect effect on muscle pain. From animal experiments it is evident that 5-HT causes either vasoconstriction of arterioles by acting on the 5-HT_2 receptor or vasodilatation by acting on the 5-HT_1 receptor, depending on the concentration of 5-HT.[18,19] Intramuscular infusion of 5-HT at 1 nmol/L into the rabbit masseter muscle causes vasoconstriction,[20] which can be reversed by the selective 5-HT_2-antagonist ritanserin.[21] In humans, intra-arterial infusion of 5-HT at 0.1 μmol/L increased forearm blood flow, while 80 μmol/L decreased the blood flow.[22] The present authors first hypothesized that endogenously released 5-HT at a sufficient concentration might be a cause of both pain and ischemia in patients with chronic localized myalgia. Secondly, it was hypothesized that exogenously administered 5-HT at high concentration would lead to muscle pain and decreased microcirculation but that 5-HT at low concentration would increase muscle blood flow without any effect on muscle pain. Thus, the aim of this study was to investigate whether intramuscular injection of 5-HT influences pain and microcirculation in the human masseter muscle.

Materials and Methods

Study Population

Twelve healthy female subjects with a mean (SD) age of 45 (11) years participated in the study on a voluntary basis. They had no pain in the orofacial region and no or only minor tenderness to palpation of the masticatory muscles.

The methods and selection of patients were approved by the ethics committee at Karolinska University Hospital at Huddinge, Sweden (368/96) and followed the guidelines of the Declaration of Helsinki. The subjects received both written and oral information about the study and gave their oral consent prior to inclusion.

Assessment of Pain

Borg's categorical rating scale for pain assessment (0 to 10) was used bilaterally for the masseter region during the experiment. This scale is a category rating scale with ratio properties and has been found to be reliable.[23] The scale was printed on a paper that was mounted in front of the subject. To avoid influencing blood flow recordings by head movements, the subjects used a laser pointer to score their pain intensity rather than speaking aloud. Pain intensity was assessed every fifth minute during the stabilization period (20 minutes) and every second minute after injection of the test substances (10 minutes).

Assessment of Local Capillary Blood Flow

Intramuscular blood flow in the masseter muscle was measured by a laser-Doppler flowmetry system technique (LDF; PeriFlux System; Perimed). Measurement was performed with a flexible microtip (diameter 0.5 mm, length 80 mm; MT B500-0, Perimed) connected to a laser light source and detector unit (PeriFlux 4001-2) by a master probe (PeriFlux 418-1). The flowmeter output signals were recorded continuously during the experiment and stored for later analysis by a computer running the Perimed software (PeriSoft for Windows; Perimed) at a sampling frequency of 4 Hz. The time constant was set to 0.2 second according to the manufacturer's recommendation for this type of application.

Test Substances

Sterile 5-HT stock solution (Sigma) with a concentration of 0.1 mol/L was prepared at the

Karolinska University Hospital Pharmacy in Huddinge, Sweden. The stock solution was diluted with sterile isotonic saline (0.9 mg/mL, Pharmacia & Upjohn) to concentrations of 0.1 μmol/L and 1,000 μmol/L under sterile conditions, divided into portions of 0.5 mL, and frozen (–80°C). To avoid oxidation of the 5-HT, all samples were covered by aluminum foil during dilution and were frozen in light-protected sterile Eppendorf tubes. Sterile isotonic saline was used as a placebo control.

Injections

The subjects received 2 injections on each side, 1 with 5-HT and 1 with saline. Thus, only 1 concentration of 5-HT was injected on any side, and this injection was followed or preceded by a saline injection. The order of substances and the sides to be injected were randomized in a balanced manner by a laboratory technician, who did not participate during the experiment. For example, if a subject was randomized to receive 5-HT at 0.1 μmol/L for the first injection on the right side, saline was used for the first injection on the left side. Saline was then used for the second injection on the right side, and 5-HT at 1,000 μmol/L was used for the left side. The injections were made in a double-blind manner; neither the subject nor the examiners knew which substance was being injected into which side. The laboratory technician prepared the syringes and labeled them with side and order (ie, R inj 1, R inj 2, L inj 1, L inj 2) shortly before injection. Enough time was allowed for the solution to become room-tempered.

Procedure

The subjects were placed in a supine position in a conventional dental chair and instructed to remain immobile; no movement of the head or talking was permitted. The most prominent point of the masseter muscles on both sides was chosen for the injection site. This point was determined by digital palpation while the subject clenched for a few seconds. Skin surface anesthesia over the injection site was achieved by application of an anesthetic cream (EMLA; Astra) for 20 minutes. A standard catheter with a diameter of 0.8 mm (Venflon 2; Omeda) was inserted into the masseter muscle perpendicular to the skin surface and guided by a 1-mm canal through a sterile 6-mm-thick acrylic plastic plate to a depth of 19 mm as measured from the surface of the acrylic plastic plate. The catheter was then withdrawn 1 mm. The LDF microtip was introduced into the muscle through the catheter until it protruded 1 mm outside the catheter. The depth (19 mm) was ensured by a tiny rubber disk attached to the LDF probe. A standard disposable needle with a diameter of 0.4 mm and a length of 19 mm (Neofly; BOC Ohmeda) was used for injection of the test substance, which was inserted into the muscle 8 mm distant from the LDF probe at an angle of approximately 70 degrees to the surface of the acrylic plastic plate and to its full length. It was guided by another 1-mm canal through the acrylic plastic plate. Thus, the tips of the injection needle and LDF probe were in close proximity in the muscle (Fig 1). To ensure that the LDF probe and injection needle would be in close proximity, this technique was first tested in vitro. Twenty minutes after muscle puncture, 0.5 mL of the first test substances was injected bilaterally over a period of 20 seconds (injection 1) from a 2-mL syringe. After a 10-minute rest period, 0.5 mL of the second test substances was injected bilaterally into the muscles over a period of 20 seconds (injection 2). A final 10-minute rest period terminated the experiment. This time period was chosen because previous studies have shown that injection of 5-HT causes a transient effect on blood flow and pain.[15,20]

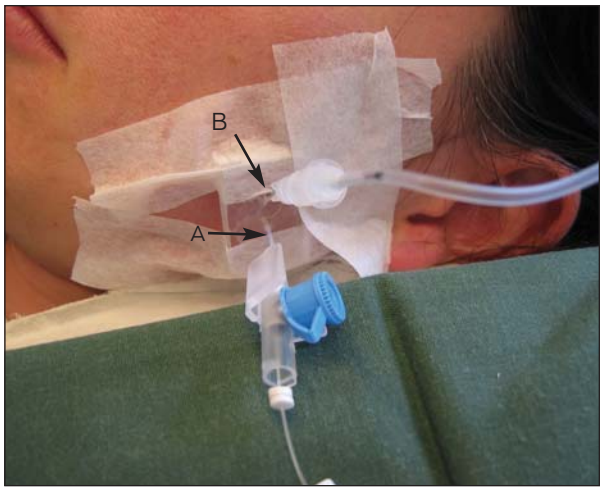


Fig 1 The experimental setup for blood flow measurements and injection of test substances in 12 healthy female subjects. An acrylic plastic plate was attached with surgical tape to the skin overlying the most prominent point of the masseter muscles localized during contraction. The laser-Doppler probe *(A)* was inserted into the masseter muscles perpendicular to the surface of the skin, while the injection needle *(B)* was inserted into the muscles at a 70-degree angle to the surface of the acrylic plastic plate. In this manner the tips of the LDF probe and the injection needle were localized in close vicinity in the muscles.

Data Analysis

The recorded capillary blood flow was calculated with the Perimed software (PeriSoft for Windows) by one of the investigators (HK) after all subjects had been examined. This investigator did not participate in data collection and had no access to the protocols. The blood flow was calculated in arbitrary perfusion units (PU) for the intervals of stabilization, baseline, and postinjection. For the stabilization period, mean blood flow was calculated for a 60-second interval during the first 0 to 2 minutes of the period (1), at 9 to 11 minutes into the period (10), and during the last 2 minutes of the period (20). Baseline refers to the mean blood flow during the 60 seconds immediately prior to injection of the test substances, and postinjection refers to the mean blood flow for 60 seconds during each of the intervals 0 to 1 minute (1), 4 to 6 minutes (5), and 8 to 10 minutes (10) after injection of test substances. Since the interindividual blood flow varied widely between subjects, the relative changes in percent blood flow from baseline for the different intervals were used in the statistical analysis. The reproducibility of the assessment of the mean blood flow values has previously been calculated and found to be acceptable.[24]

The pain levels corresponding to the blood flow values at the different time intervals after injection were calculated as follows. The baseline pain level was assessed immediately before injections, ie, 20 minutes after muscle puncture. The postinjection pain levels were the median pain levels 0 to 2 minutes (1), 4 to 6 minutes (5), and 8 to 10 minutes (10) after injection. The raw changes from baseline pain level for the different intervals (1, 5, and 10 minutes) were used in the statistical analysis.

Statistical Analysis

Nonparametric statistical methods were used. Friedman repeated measures analysis of variance (ANOVA) on ranks with the Student-Newman-Keuls method (SNK) as a post-hoc test was used to test the significance of the changes in pain intensity and blood flow during stabilization and after injection of test substances. The significance of the intraindividual differences in pain intensity and blood flow at the different time intervals were tested with the Wilcoxon signed rank test. Each muscle served as its own control, ie, the 5-HT injection on 1 side was compared to saline injection on the same side. The significance of the correlations between changes in pain intensity and blood flow values were tested by Spearman's product-moment rank correlation test. A significance level of $P < .05$ was used.

Results

Pain

Muscle puncture and insertion of the LDF probe induced pain at low levels on both sides; the pain decreased from 0 to 5 minutes and remained low during the stabilization period (Fig 2; Friedman: $P < .001$). There were no differences in pain levels between the sides at any time point during stabilization.

Pain intensity after injections is shown in Fig 3. Pain intensity increased significantly directly after injection of all 3 substances (Friedman: $P < .001$) and then quickly decreased. However, 5-HT at 1,000 μmol/L induced slightly more pain than 5-HT at 0.1 μmol/L and isotonic saline. The post-hoc test showed that the pain level at 1 and 5 minutes after injection of 5-HT at 1,000 μmol/L was significantly higher than at baseline (SNK: $P < .05$). Pain intensity differed significantly between 5-HT at 1,000 μmol/L and saline at 1 minute after injection (Fig 3). The pain level at 1 minute after injection of 5-HT at 0.1 μmol/L and saline was significantly higher compared to baseline (SNK: $P < .05$), but there were no differences in pain intensity between 5-HT at 0.1 μmol/L and saline at any time point.

Blood Flow

Blood flow decreased on both sides after probe insertion and stabilized over the 20-minute stabilization period (Fig 4). The decrease was significant on the left side (Friedman: $P = .031$) but not on the right side. There were no significant differences in blood flow between the right and left sides at any other time point during stabilization. Change in blood flow after injection is shown in Fig 5. There was a large interindividual variation in blood flow change, but the blood flow increased initially after all injections and then returned to baseline. Injection of 5-HT at 1,000 μmol/L induced the largest median increase of blood (114%, IQR = 218%; Fig 5a), but the increase was not significant (Friedman: $P = .334$). Injection of 5-HT at 0.1 μmol/L increased the median blood flow less (75%, IQR = 187%), but that increase was significant (Friedman: $P = .018$). The post-hoc test showed that blood flow was significantly higher 1 minute after injection compared to baseline as well as 5 and 10 minutes after injection

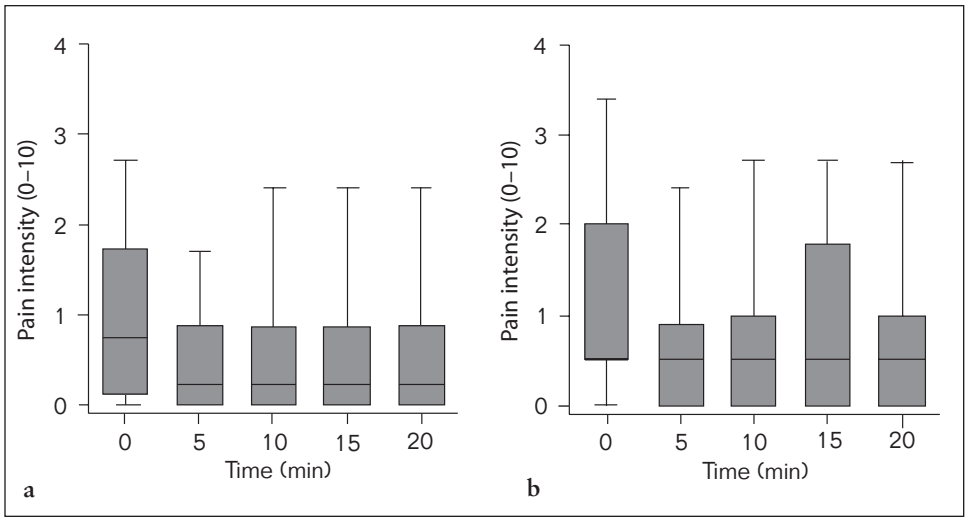


Fig 2 Box plot (10th, 25th, 50th, 75th, and 90th percentiles) showing the change in pain intensity (Borg's rating scale) of the masseter muscles during stabilization after muscle puncture on *(a)* the right side and *(b)* the left side. There was a minor but significant increase in pain intensity on both sides during this period (right side: $P = .013$; left side: $P = .024$).

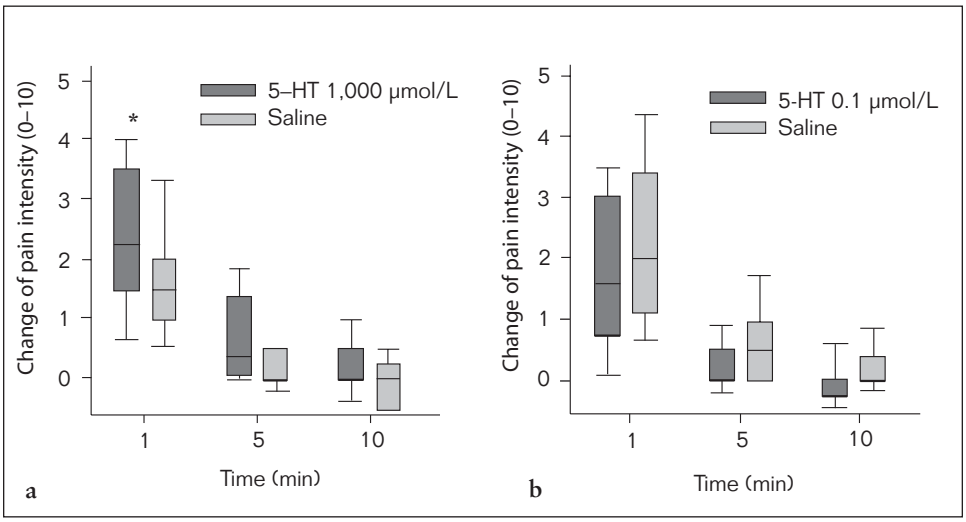


Fig 3 Box plot (10th, 25th, 50th, 75th, and 90th percentiles) showing the change from baseline in pain intensity (Borg's rating scale) of the masseter muscles after injection of 5-HT at *(a)* 1,000 µmol/L and *(b)* 0.1 µmol/L compared to isotonic saline. Injection of all substances significantly increased the pain intensity. * Significant difference between 5-HT and saline ($P < .05$).

(SNK: $P < .05$; Fig 5b). Blood flow did not change significantly after saline injections. When the median blood flow changes at the various time points were compared, no significant differences between 5-HT and saline could be detected.

Relationship Between Blood Flow and Pain

Blood flow change 1 minute after injection of 5-HT at 1,000 µmol/L was positively correlated to the change in pain intensity 5 minutes (rs = 0.80, n = 12, $P < .001$; Fig 6) and 10 minutes (rs = 0.67, n = 12, $P = .015$) after injection. Blood flow and pain changes were also correlated at 5 minutes after injection of 5-HT at 1,000 µmol/L (rs = 0.64, n = 12, $P = .022$). Finally, the pain intensity at 5 minutes after injection of 5-HT at 1,000 µmol/L was positively correlated to the blood flow at 10 minutes after injection (rs = 0.67, n = 12; $P = .015$). There were no correlations between 5-HT at 0.1 µmol/L or saline and pain.

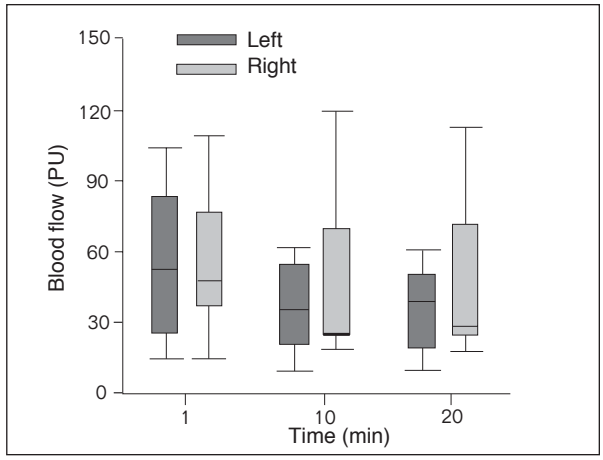
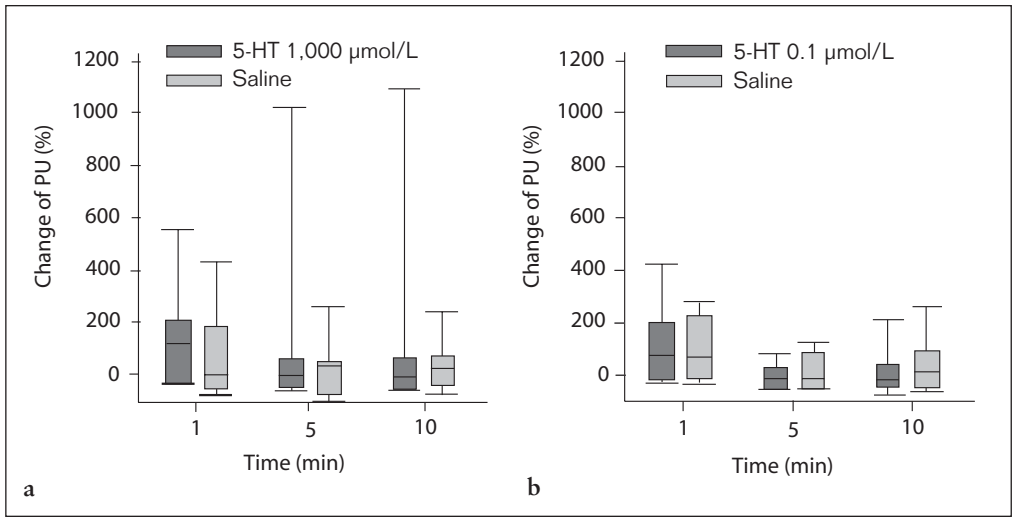


Fig 4 Box plot (10th, 25th, 50th, 75th, and 90th percentiles) showing the blood flow values (PU) during stabilization at 1, 10, and 20 minutes after bilateral muscle puncture of the masseter muscle. The blood flow decreased during stabilization on both sides, and there were no significant differences between the sides at any time point.



Figs 5a and 5b Box plot (10th, 25th, 50th, 75th, and 90th percentiles) showing the percentage change of blood flow (PU) from baseline after injection of 5-HT and isotonic saline into the masseter muscle at 1, 5, and 10 minutes after injections of *(a)* 5-HT 1,000 µmol/L and *(b)* 5-HT 0.1 µmol/L. Blood flow was significantly increased at 1 minute after injection of 5-HT at 0.1 µmol/L ($P < .05$), but there were no significant differences between 5-HT of any concentration and saline at any time point.

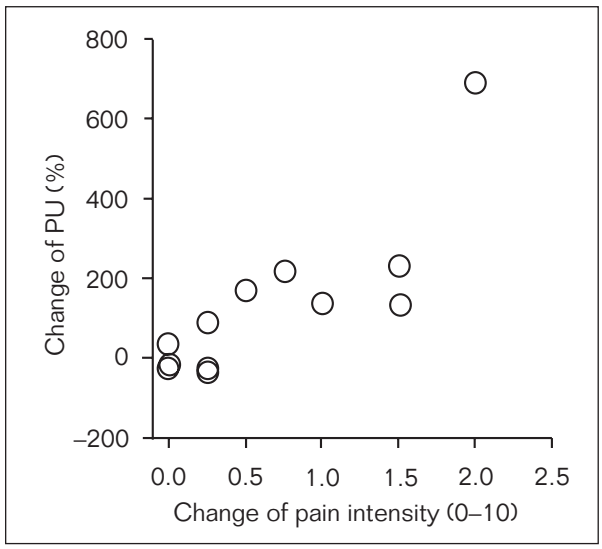


Fig 6 Scatter plot showing the correlation between the blood flow changes (%) at 1 minute after injection of 5-HT at 1,000 µmol/L and the change in pain intensity (0 to 10) at 5 minutes after the injection. There was a significant positive correlation ($r_s = 0.80$, n = 12, $P < .001$).

Discussion

The results of this study showed that injection of 5-HT at 1,000 µmol/L induced significantly more pain in the masseter muscle than isotonic saline but did not significantly alter blood flow. At 0.1 µmol/L, 5-HT did not induce pain or alter blood flow significantly compared to saline. However, blood flow and pain were correlated after injection of 5-HT at 1,000 µmol/L. Since there were no such associations after injection of 5-HT at 0.1 µmol/L or saline, this correlation suggests a relationship between blood flow and pain caused by injection of 5-HT at the higher concentration. It cannot be claimed to be a causal or direct effect, but it might be explained by activation of sensory afferents (axon reflex), which in some subjects is sufficient to induce an acute inflammatory response by the release of inflammatory mediators such as substance P and calcitonin gene–related peptide.

Effects of 5-HT on Pain

Injection of 5-HT at 1,000 µmol/L induced significantly more pain than isotonic saline shortly (1 minute) after injection, while there was no difference between 5-HT at 0.1 µmol/L and saline. This is in agreement with a previous study in healthy female subjects[15] and indicates that a rather strong concentration of 5-HT is needed to evoke pain. The pain is most probably due to activation of 5-HT_3 receptors on primary sensory afferents,[16] but

release of other mediators induced by 5-HT may also contribute to the pain. Since the blood flow was not significantly changed by injection of 5-HT at 1,000 μmol/L, these results indicate that pain was not caused by ischemia. However, the present experimental study was performed on healthy subjects, while in chronic pain patients ischemia may lead to release of 5-HT and other mediators that may activate and sensitize muscle nociceptors and thereby cause pain.[12,13,25] This possibility should be investigated in future experiments.

Effects of 5-HT on Microcirculation

Saline and 5-HT at any concentration did not differ significantly with respect to effect on blood flow, although 5-HT at 0.1 μmol/L significantly increased the blood flow. This effect is probably unspecific and not related to 5-HT. The finding that 5-HT at 1,000 μmol/L did not influence masseter muscle blood flow is not in agreement with previous results; it was previously shown that high nonintramuscular doses of 5-HT decrease muscle blood flow in the rat cremaster muscle and human forearm.[18,22] On the contrary, the tendency in the present study was the opposite, ie, there was a tendency toward an increase of masseter muscle blood flow. The vascular responses to 5-HT are complex, and there are several possible explanations to the diverging results. First, different species and muscles were studied, and previous studies have shown that the vascular responses to 5-HT vary between different muscles.[17,18] Furthermore, the distribution of 5-HT receptor subtypes is reported to vary between human arteries of the head[26]; the distribution also may differ between muscles. The position of the LDF probe within the muscle may also have influenced 5-HT effects. If the probe tip is located in the vicinity of more metabolically active muscle cells, 5-HT is more likely to cause vasoconstriction than if it is located close to tendons or connective tissue, where 5-HT is reported to cause vasodilation.[17]

The difference between studies could also be due to differences in methodology. In a study by Alsip and Harris,[18] changes of vessel diameter were analyzed visually, whereas Blauw et al[22] used venous plethysmography. The present study as well as the authors' previous studies in the rabbit[20,21] used the LDF technique, which is an indirect measure of capillary blood flow that generally is believed to measure tissue perfusion, ie, the speed and concentration of moving erythrocytes.[17]

There was considerable interindividual variation in blood flow for all injections, but the variation was especially large after injection of 5-HT at 1,000 μmol/L. This was mainly due to 1 subject who statistically was an outlier. When this subject was excluded, the result did not change, which was expected since nonparametric statistics based on ranks were used.

Relationship Between Blood Flow and Pain

Increased blood flow after injection of 5-HT at 1,000 μmol/L was associated with increased pain at different time intervals after injection. No such associations were found between blood flow and pain after injection of 5-HT at 0.1 μmol/L saline. Since blood flow did not increase significantly after injection of 5-HT at 1,000 μmol/L, the correlation does not indicate a direct or causal relationship. However, the results suggest that exogenously administered 5-HT causes complex responses with respect to pain and blood flow in the masseter muscle. Subjects with increased blood flow directly after injection of 5-HT at 1,000 μmol/L responded with increased pain some minutes later, while subjects with no or little change of blood flow responded with unchanged pain intensity. It is possible that the former subjects have a higher density of 5-HT receptors. A direct activation of vasodilative 5-HT_1 receptors by 5-HT in these subjects is also a possibility, although vasoconstriction would be expected after administration of such a strong concentration of 5-HT.[18,22] In addition, although the distribution of 5-HT receptor subtypes in the masseteric artery is unknown, it is likely that it is similar to the temporal artery. If so, vasoconstrictive 5-HT_2 receptors would dominate.[26] It is also possible that activation of 5-HT_3 receptors on sensory afferents by 5-HT, in combination with tissue trauma from the injection needle and LDF probe in these subjects, would be sufficient to induce an acute inflammatory response, including a neurogenic inflammation (axon reflex) by release of neuropeptides, such as substance P and calcitonin gene–related peptide from the activated sensory afferents.[27] Both of these mediators have strong vasodilator properties. The trauma may also activate the synthesis and release of prostaglandins and other vasoactive substances that further enhance inflammation. Because of plasma extravasation, 5-HT and other mediators from circulating blood cells could further increase the pain.

In conclusion, intramuscular administration of 5-HT at 1,000 μmol/L into the human masseter muscle induced pain, but 5-HT did not directly affect local blood flow at concentrations of either 0.1 μmol/L or 1,000 μmol/L.

Acknowledgments

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