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Prolonged rhythmic gum chewing suppresses nociceptive response via serotonergic descending inhibitory pathway in humans

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Abstract

Serotonergic (5-HT) neurons are implicated in modulating nociceptive transmission. It is established that 5-HT neuronal activity is enhanced by rhythmic behaviors such as chewing and locomotion in animals. We thus hypothesized that 5-HT descending inhibitory pathways may be enhanced by rhythmic behavior of gum chewing in humans. To evaluate this idea, we examined nociceptive flexion reflex (NFR), while a subject chewed gum rhythmically for 20 min. NFR was elicited by electrical stimulation of the sural nerve, and the evoked potential was recorded from the biceps femoris muscle. Visual analogue scale (VAS) was also obtained. To assess 5-HT activity, we determined 5-HT levels quantitatively in platelet poor plasma (PPP) and whole blood (WB) using HPLC system. Both NFR area and VAS were significantly decreased at 5 min after the onset of chewing and these reductions persisted until cessation of chewing. There were no significant changes in NFR and VAS while resting without chewing. The PPP 5-HT level increased significantly just after cessation of chewing and had returned to the pre-chewing level by 30 min after cessation of chewing. The WB 5-HT level obtained 30 min after cessation of chewing was significantly greater than the pre-chewing level. Serotonin transporters have recently been discovered at the blood–brain barrier, suggesting that the rise in blood 5-HT may possibly reflect an increase in 5-HT level within the brain. The present results support our hypothesis that the rhythmic behavior of chewing suppresses nociceptive responses via the 5-HT descending inhibitory pathway.

Keywords: Serotonin (5-HT); NFR (nociceptive flexion reflex); descending inhibitory control

1. Introduction

Serotonergic (5-HT) neurons in the rostral ventromedial medulla (RVM) are important in modulating spinal nociceptive transmission (Basbaum and Fields, 1984; Millan, 2002). Three physiological classes of RVM neurons, ON, OFF, and neutral cells are known to have different nociceptive modulatory effects on spinal nocifensor reflexes. 5-HT neurons, belonging to RVM neutral cells, do not directly mediate the antinociceptive effects of supraspinal opioids in the rat, since opioid administration does not alter the firing of RVM neutral cells (Potrebic et al., 1994). It is important to note that 5-HT neurons in the RVM do not directly contribute to opioid analgesia.

Besides pain modulation, 5-HT neurons have been suggested to be closely linked with many functions including waking, attention, appetite, and affective modulation. Corresponding to these functions, 5-HT neurons project to various areas of the whole brain, including the cerebral cortex, the limbic cortex, the cerebellum, the brainstem and the spinal cord (Aprison et al., 1978; Gold et al., 1988; Jacobs and Fornal, 1993; McGinty and Harper, 1976; Melzter, 1989; Morley and Blundell, 1988).

Regarding activation of 5-HT neurons, Jacobs and Fornal, (1993) have demonstrated that the activity of 5-HT neurons is enhanced by voluntary rhythmic movements, which include mastication, locomotion and respiration. Because the earlier data were obtained in

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animal models, the present study was designed to demonstrate this unique feature of 5-HT neurons in humans. We focused the present study on mastication as one of the voluntary rhythmic movements.

Mastication is well known to be important for food intake. However, mastication is also involved in the systemic, mental and physical functions of the body (Nakata, 1998). Recently, several investigators have reported that mastication produces an antinociceptive effect (Kemppainen et al., 2001; Ogawa et al., 2003).

Based on these observations, we hypothesized that activation of 5-HT neurons by the rhythmic behavior of chewing might enhance the 5-HT descending inhibitory pathway and suppress nociceptive responses in humans.

To evaluate pain both subjectively and objectively, we used visual analogue scale (VAS) and the nociceptive flexion reflex (NFR), respectively. NFR is known to be an objective and stable measurement for pain assessment, and is a type of withdrawal reflex (Hugon, 1973; Skljarevski and Ramadan, 2002; Willer, 1977).

2. Methods

2.1. Subjects

Nine healthy young males and females, age range 26–32 years, voluntarily participated in this study. Subjects with psychiatric illness, systemic neuromuscular diseases and history of trauma were excluded. No dietary or exercise restrictions were requested. Oral and written informed consent was acquired from each subject. The study was approved by the Tokyo Medical and Dental University Ethics Committee and conducted in accordance with the Declaration of Helsinki. It was made clear to the subjects that they were free to terminate the study if they did not want it to continue. No explanation was given about the aim of this study or chewing-induced analgesia.

2.2. Pain induction and measurement (NFR and VAS)

The NFR recording and stimulating electrodes were attached to the subject. All recording and stimulation electrode sites were cleaned and gently abraded. The reflex was induced by transcutaneous electrical stimulation of the sural nerve at the right ankle while the subject reclined in a comfortable armchair with the lower legs bent to an angle of less than 150 degrees so as to achieve a state of muscular relaxation. For the NFR recording, a pair of electrodes was placed over the right biceps femoris muscle 10 cm superior to the popliteal fossa.

Electrical stimulation consisted of a train of 8 1 ms square wave pulses at 250 Hz. The electrical pulse was delivered from a constant current isolator system (SS104J, Nihon Kohden, Japan) controlled by a timing controller (SEN3301, Nihon Kohden). The stimuli were delivered randomly every 5–15 s during gum chewing to avoid anticipation and habituation to the stimuli. EMG signals were amplified by a bioelectric amplifier (Nihon Kohden EEG-4217) with a time constant of 0.03 s and a low-pass filter at 1kHz (frequency range: 0.5–120 Hz).

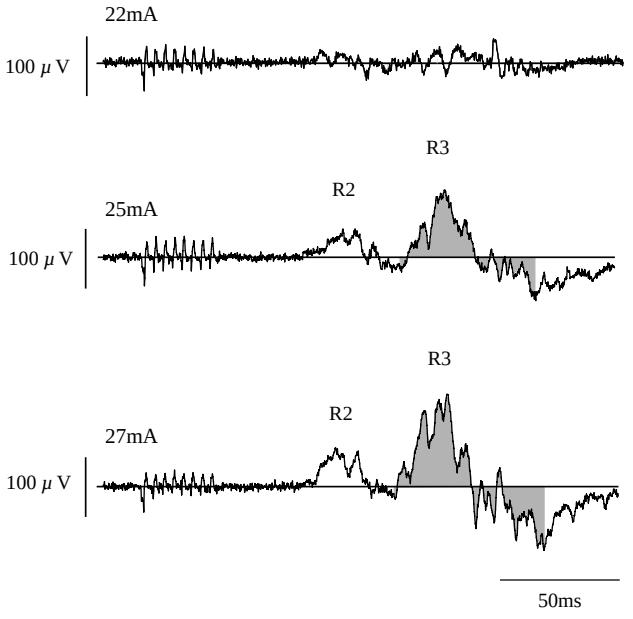

Fig. 1. Nociceptive flexion reflexes (NFR) recorded in biceps femoris muscle. The reflex was induced by transcutaneous electrical stimulation of the sural nerve at the right ankle. Electrical stimulation consisted of a train of 8 1 ms square wave pulses at 250 Hz. The three representative responses shown were obtained when the intensities of the electrical stimulation were 22 mA (top panel), 25 mA (middle panel), and 27 mA (bottom panel). Each NFR was assessed as the shaded area based on the shape of the response between 100 and 170 ms in this case. Note that a tactile reflex (known as R2) appeared at a latency of 72 ms following stimulation of the sural nerve (see bottom trace). For calculation of the NFR area, the NFR response (R3) was full wave rectified and integrated (see text for details).

As shown in Fig. 1, each NFR was assessed as the area based on the shape of the response between 100 and 170 ms. Considering the onset latency (101–125 ms) of nociceptive response (R3) described by Skljarevski and Ramadan (2002), we determined the onset of the NFR as the minimal negative point which appeared between 101 and 125 ms. Note that the tactile component (R2) occurred between 40 and 70 ms. The end of the NFR was determined as the minimal negative point following a large positive wave of R3 component.

The raw EMG signals including the NFR were digitized at a sampling rate of 5 kHz for a microcomputer-based analysis. The digitized EMG data were full wave rectified and 10 consecutive traces were averaged in the microcomputer system. Then, a shape of each NFR was determined as described above and the area of the NFR was calculated. The shaded area in Fig. 1 corresponded to the calculated NFR area.

To determine the intensity of the stimulus, we assessed the relationship between NFR and VAS prior to the experiment. As the intensity increased, the subject was asked to mark a 100 mm VAS with the subjective judgment of pain intensity for each stimulus. The end points of the VAS were labeled ‘no pain’ (left) and ‘extremely painful’ (right). The intensity of the stimulus was fixed at the level at which the subject obtained about half the tolerance threshold (the maximum intensity that he or she could bear) of VAS score. The mean intensity of the stimulus used in the present study was 23.9 mA, corresponding to 117% of NFR threshold; the mean NFR threshold was 20.6 mA. That level was maintained throughout the experiment.

The NFR threshold was determined at both the beginning and the end of the experiment to check the conditions of electrodes and to exclude the influence of habituation.

2.3. Experimental protocols

Each experiment was started at 2:00pm

A subject was asked to chew gum voluntarily and rhythmically for 20 min at a comfortable speed (task experiment). To ensure the intensity and rhythm of mastication, we monitored EMG of masseter muscle throughout gum chewing. The composition of gum (Xylish sugarless, Meiji Co., Tokyo, Japan) was gumbase, maltitol, xylitol and so on. The flavor was mint. The quantity of gum was up to the subject. The mean quantity was 4.3 ± 1.4 g.

As a control experiment, the subject rested without mastication for 20 min. Subjects were instructed not to clench during the NFR stimulus.

NFR was recorded every 5–15 s during each experiment. VAS was scored by the subject at the onset of each experiment and every 5 min during the experiment.

To assess 5-HT neuronal activity, we sampled blood and urine before and, immediately and 30 min after cessation of gum chewing (and also at 60 min after the onset of gum chewing for urine). Blood samples were obtained by venous puncture, using a cannula. The subject was asked to empty his/her bladder before the timed urine collections described above.

2.4. Analysis by HPLC

The samples were refrigerated as quickly as possible using the following procedures. Blood (5 ml), collected in heparin-sodium-salt-containing vacutainer tubes, was divided into 2 plastic tubes. One was for the analysis of platelet poor plasma (PPP), the other for analysis of whole blood (WB).

For PPP analysis, 90 μl of a 10% (weight per volume) solution of ascorbic acid in purified water were added to 3.5 ml of blood and the sample was centrifuged at $2000 \times g$ for 10 min at 4 °C. As the internal standard, *N*-methyl-serotonin (Sigma, MO, USA) was dissolved in 0.1 M acetic acid containing 100 mg/l of EDTA (Dojindo, Kumamoto, Japan) to give a final concentration of 10 ng/ml. Ten microliter of the internal standard were added to 1 ml of the supernatant. The PPP sample was stored frozen at −20 °C until the assay.

For WB analysis, we applied the method described in detail by Kremer et al. (1988). Half a milliliter of blood was suspended in 2.5 ml of water. Then, 30 μl of the internal standard and 10 μl of a 10% (weight per volume) solution of ascorbic acid in water were added to the suspended blood sample. The sample was then stored frozen at −20 °C until the assay.

For the urine sample analysis, 500 μl of 0.1 M acetic acid containing 100 mg/l EDTA were added to 500 ml of urine to maintain the pH and the sample was stored frozen at −20 °C in a plastic microtube.

HPLC analysis was conducted within 2 weeks after the experiment. Blood samples were thawed and methanol added to remove proteins. Then, 500 μl of methanol were added to a 500 μl PPP sample and 167 μl of methanol were added to 1 ml of WB sample.

Both PPP and WB samples were centrifuged at $4670 \times g$ for 10 min at 4 °C. The PPP supernatant was injected into the HPLC system. The 500 μl supernatant of the WB sample was suspended in 4.5 ml of mobile buffer. The mobile phase consisted of a phosphate buffer (Na^+, 0.1 M) containing 50 mg/L EDTA·2Na and an ion-pair (300 mg/L sodium-octyl-sulphate, nacalai, Kyoto, Japan) and 20% methanol at pH 6.0. The WB sample was injected into the HPLC system. Urine samples were thawed and injected into the HPLC system.

5-HT levels were determined using an HPLC-ECD (EICOM 300, EICOM, Kyoto, Japan) system. The working electrode was a graphite carbon electrode set at a detector potential of +400 mV against a $Ag/AgCl_2$ reference electrode. 5-HT was separated on a reversed phase column (EICOMPAK CA-5ODS). The flow rate was set at 0.22 ml/min and the analysis temperature was 35 °C.

2.5. Statistical analysis

Paired *t*-test, repeated measure one-way ANOVA and Fisher's protected least significant difference test for post-hoc determination were used. Pearson's correlation test was used for correlation analyses. All results are expressed as means ± SD.

Since the first six subjects showed no apparent changes in NFR, VAS, and 5-HT levels while resting without chewing, we did not obtain the control data from the remaining three subjects. Note that nine subjects were included in statistical analysis of task experiments (gum chewing). 5-HT data from one subject was lost due to technical error and was not included in statistical analysis of 5-HT.

3. Results

3.1. Masticatory movement

To monitor masticatory movement during the task of gum chewing, we recorded masseter EMG in every subject, as shown in Fig. 2(A). Fig. 2(B) and (C) shows the changes in the mean integrated area of masseter EMG and the mean frequency of masticatory movement at early, middle, and late periods during gum chewing for 20 min. There were no significant changes in intensity and rhythm of the masticatory movement throughout the task of gum chewing.

3.2. Changes in NFR

Fig. 3 shows the time course of change in the mean NFR area during gum chewing, with data expressed as percentages of the basal level. Each mean NFR area was calculated from 10 successive NFR areas during an analysis period marked by a numbered line in Fig. 3. Since 10 successive NFR stimuli were delivered randomly every 5–15 s, each analysis period was approximately 1.6 min. Gum chewing for 20 min consisted of 12 analysis periods in the task experiment. The mean NFR area showed a gradual decrease during gum chewing ($n=9$, $F=4.421$, $P<0.0001$). There was a significant post hoc difference between the first and the fourth analysis periods ($P<0.05$). The significant decrease in mean NFR areas ($P<0.05$, 0.01) was maintained until the end of gum chewing. The decreased

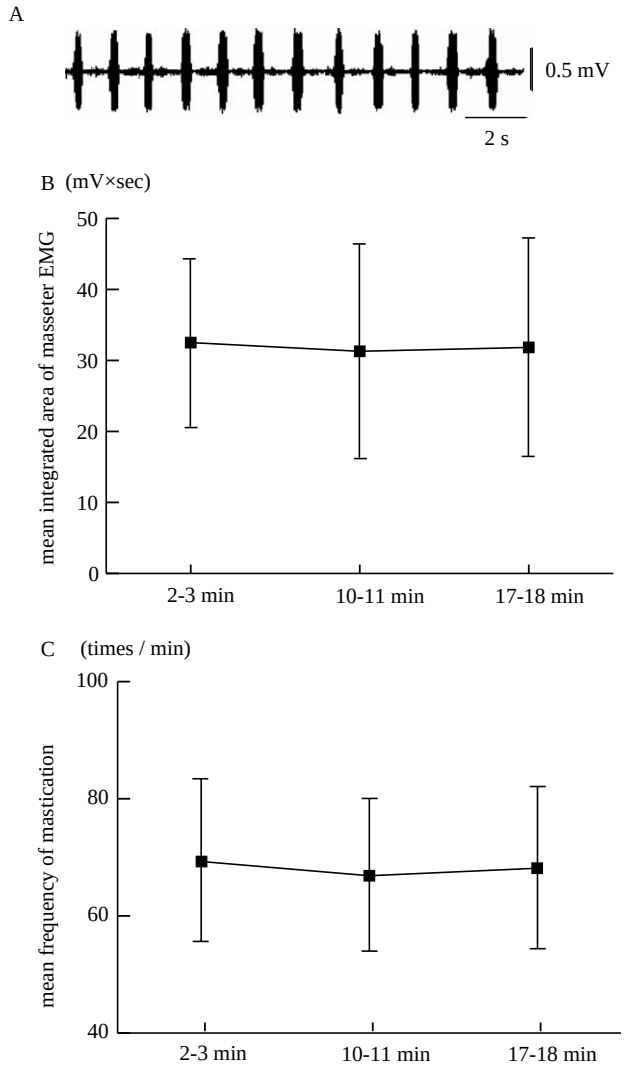

Fig. 2. (A) A typical example of masseter EMG recorded during gum chewing. (B) Mean integrated area of masseter EMG during a masticatory cycle at 2–3, 10–11, and 17–18 min after the onset of gum chewing. There were no significant changes in the mean integrated area throughout the task of gum chewing ($n=9$). (C) The mean frequency of mastication at 2–3, 10–11, and 17–18 min after the onset of gum chewing. There were no significant changes in the mean frequency during the task of gum chewing.

mean NFR area persisted until 30 min after cessation of gum chewing and returned to a level corresponding to that obtained during the first analysis period at 60 min after the end of gum chewing.

The time course of the mean NFR area while resting without mastication is shown in Fig. 4 ($n=6$), with the data expressed as percentages of the basal level. Resting without mastication for 20 min consisted of 12 analysis periods in this control experiment, as was the case in the task experiment. There were no significant changes in the mean NFR area.

3.3. Changes in VAS

The time course of VAS during gum chewing is shown in Fig. 5(A). VAS decreased significantly during gum chewing ($F=9.182$, $P=0.0001$). There was a significant post hoc difference between VAS at the beginning and VAS values obtained at 5- and 15-min after the onset of gum chewing. A significant decrease in VAS was also observed just after stopping gum chewing ($n=8$, $P<0.01$). VAS at 30 min after cessation of gum chewing returned to the score obtained at the beginning of gum chewing.

Fig. 5(B) shows the time course of VAS while resting without mastication for 20 min. There was no significant change in VAS during the control experiment, i.e. at rest without mastication or clenching for 20 min.

Since both mean NFR area and VAS showed significant decreases during gum chewing, we evaluated the relationships between these two parameters. VAS scores (abscissa) were plotted against mean NFR areas (ordinate) in Fig. 6. Linear regression analysis showed a high correlation between mean NFR area and VAS during gum chewing. Reductions in mean NFR areas correlated significantly with VAS reductions ($R=0.65$, $P<0.0001$).

3.4. PPP 5-HT level

Fig. 7(A) shows changes in the PPP 5-HT level before, and also just after and 30 min after cessation of gum chewing, with the data expressed as percentages of the basal level. The PPP 5-HT level just after gum chewing increased by $33.6\pm22.3\%$ from the basal level. ANOVA showed a significant main effect of gum chewing on the PPP 5-HT level ($F=6.126$, $P<0.01$). There was a significant post hoc difference between before gum chewing and just after the end of gum chewing ($n=8$, $P<0.01$). The PPP 5-HT level had returned to $110.2\pm29.9\%$ of the basal level at 30 min after cessation of gum chewing.

There were no significant changes in the PPP 5-HT level while at rest for 20 min ($n=8$).

3.5. WB 5-HT level

Fig. 7(B) shows changes in the WB 5-HT level before, just after and 30 min after the end of gum chewing, with the data expressed as percentages of the basal level. There was a tendency for WB 5-HT level to increase just after the end of GC. The WB 5-HT level at 30 min after the end of gum chewing increased by $11.7\pm8.7\%$ from the basal level. ANOVA showed a significant main effect of gum chewing on the WB 5-HT level ($F=6.206$, $P<0.05$). There was a significant post hoc difference between before gum chewing and 30 min after cessation of gum chewing ($n=8$, $P<0.01$).

There was no significant change in the WB 5-HT level while resting for 20 min (n = 8).

3.6. Urinary 5-HT level

Fig. 7(C) shows changes in the urinary 5-HT level before, just after, at 30 min and at 60 min after the end of gum chewing, with the data expressed as percentages of

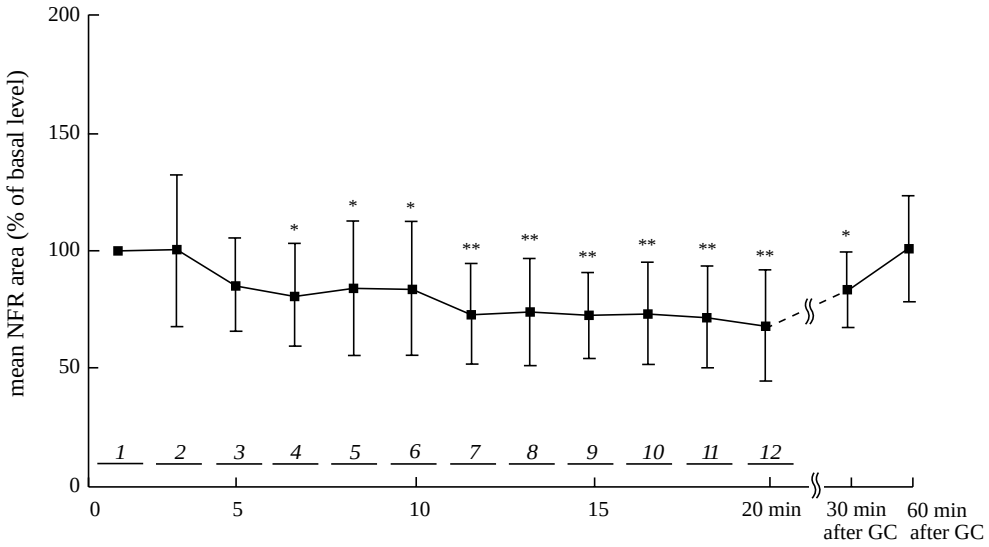

Fig. 3. Time course showing changes in the mean NFR area during and after gum chewing (GC, $n=9$), with the data expressed as percentages of the basal level. Each point is expressed as the mean of 10 successive NFR areas during an analysis period marked by a numbered line. Since 10 successive NFR stimuli were delivered randomly every 5–15 s, each analysis period was approximately 1.6 min. Gum chewing for 20 min consisted of 12 analysis periods. There were significant decreases in the mean NFR area during gum chewing. The significant decrease in the mean NFR area persisted until 30 min after cessation of gum chewing, and had returned to the level seen during the first analysis period by 60 min after cessation of gum chewing. Vertical lines show means ± SD. $*P<0.05$ and $**P<0.01$ as compared to the first analysis period of gum chewing (see text for details).

the basal level. Urinary 5-HT increased but not significantly, by 13.1 ± 28.9%, from the basal level at the end of gum chewing. Neither an increase nor a decrease was observed after the cessation of gum chewing.

There was no significant change in the urinary 5-HT level while resting for 20 min ($n=8$).

The 5-HT levels determined by HPLC are shown in Table 1.

4. Discussion

Two main findings are derived from the present study. First, prolonged chewing suppressed nociceptive responses, as evidenced by the significant NFR suppression at 5–7 min after the onset of chewing; such significant suppression persisted until 30 min after cessation of chewing. Second, the blood 5-HT levels rose significantly in response to chewing, suggesting that 5-HT neurons may be involved in antinociception during gum chewing.

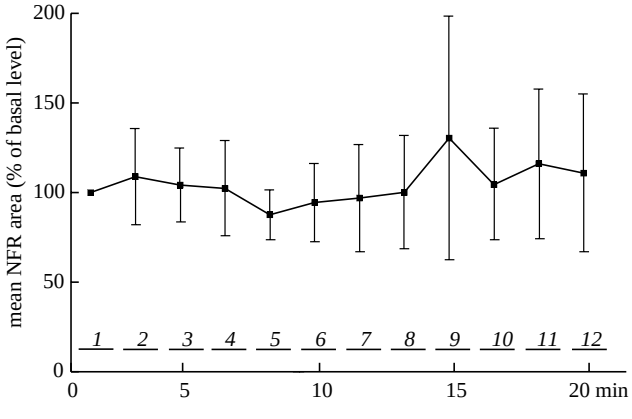

Fig. 4. Time course of the mean NFR area while resting without gum chewing ($n=6$), with the data expressed as percentages of the basal level. Each point is expressed as the mean of 10 successive NFR areas during an analysis period marked by a numbered line, which corresponds to approximately 1.6 min. Vertical lines show means ± SD. Note that there were no significant changes in the mean NFR area during resting.

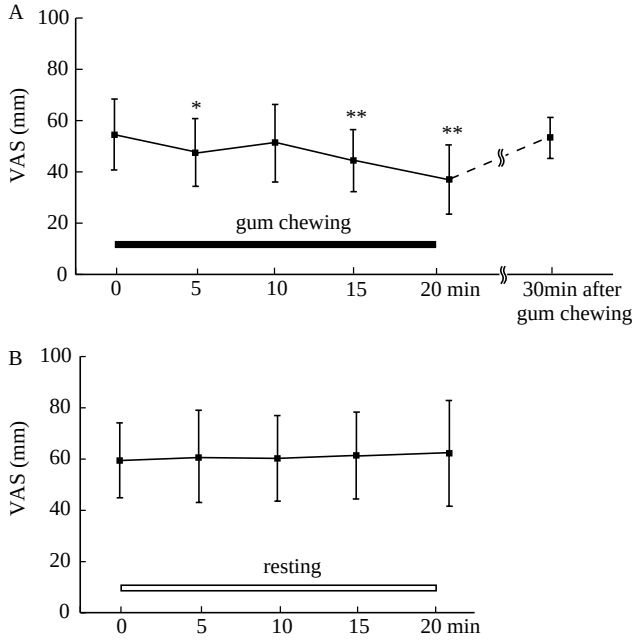

Fig. 5. (A) Time course showing decreases in VAS (visual analogue scale) during gum chewing ($n=8$). $*P<0.05$ and $**P<0.01$ as compared to VAS just after the onset of gum chewing. (B) Time course showing VAS during rest ($n=6$). There were no significant changes in VAS while resting. Vertical lines show means ± SD.

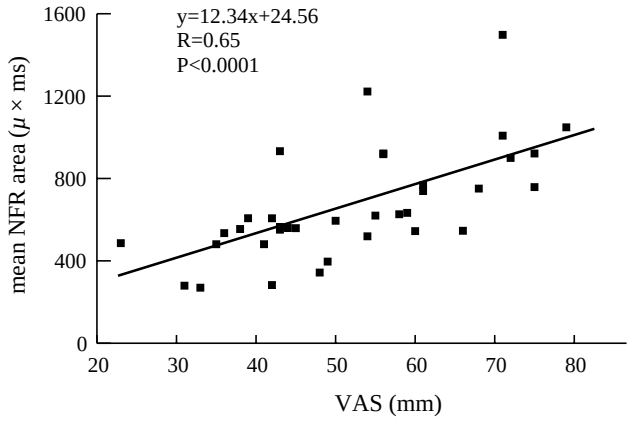

Fig. 6. Linear regression analysis shows a high correlation between the mean NFR area and VAS. Reduced mean NFR area correlated significantly with VAS reduction. Each point represents the mean of the ten NFRs obtained in each subject at about the time of the VAS determination.

Evidence of the value of NFR as an objective tool in pain assessment (Skljarevski and Ramadan, 2002) has accumulated since the first report on NFR by Hugon (1973). It has been well established that the latency of NFR is consistent with the conduction velocity of A-delta afferents and that the NFR area is related to the subjective intensity of pain (Willer, 1977). We demonstrated the NFR area to have decreased significantly by the 4th analysis period (5–7 min) and this significant NFR area reduction persisted until 30 min after the end of chewing. Note that there was not the significant suppression of the NFR during the first three analysis periods (~5 min) of chewing. Taken together, the chewing-induced antinociception is characterized by a delayed onset and a prolonged action of analgesia.

The time course of the change in NFR corresponded to that of VAS during chewing. Linear regression analysis revealed the NFR suppression to correlate significantly with a reduction in VAS. Since NFR and VAS showed no changes while resting without chewing, we conclude that prolonged chewing causes the delayed antinociceptive action.

When conducting pain-related studies, one must be aware of the placebo effect of expectation. Since the subjects were not informed about the chewing-induced analgesia, they were not expecting gum chewing to reduce pain. Although we did not made a double-blind placebo-controlled experiment in this study, we indicate that the placebo effect of expectation might not occur in the chewing experiment, based on the following unique results. The antinociceptive effect was observed at 30 min after the end of chewing, when the subjects were not performing a chewing exercise. On the contrary, a significant inhibition of the nociceptive responses did not occur during the first three analysis periods (~5 min), when the subjects were making a chewing exercise. These results indicate that chewing-induced antinociceptive effect is not primarily linked with the ongoing behavior of chewing. Thus, it is unlikely that

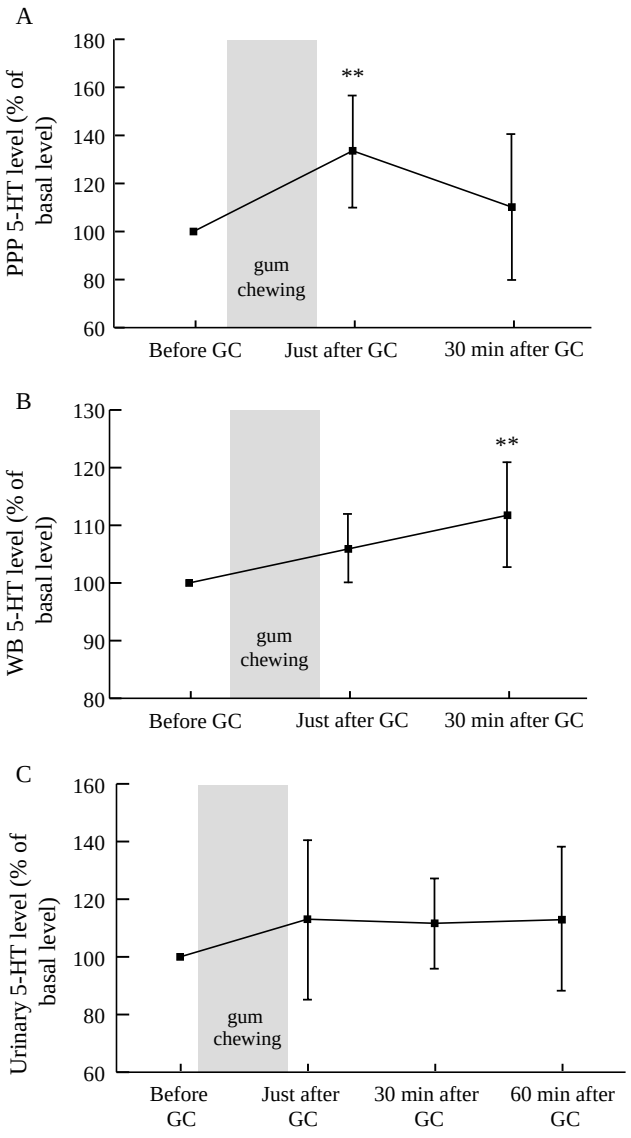

Fig. 7. (A) Changes in PPP (platelet poor plasma) 5-HT level after cessation of gum chewing (GC), with the data expressed as percentages of the basal level. Vertical lines show means ± SD ($n=8$). PPP 5-HT level increased significantly just after the end of GC and returned to the pre-chewing level at 30 min after cessation of GC. $^{**}P<0.01$ as compared to 100% of the basal level. (B) Changes in the whole blood (WB) 5-HT level after gum chewing (GC), with the data expressed as percentages of the basal level. Vertical lines show means ± SD ($n=8$). The WB 5-HT level obtained at 30 min after cessation of GC was significantly greater than that before GC. There was a tendency for WB 5-HT level to increase just after the end of GC. $^{**}P<0.01$ as compared to 100% of the basal level. (C) Urinary 5-HT level after gum chewing (GC), with the data expressed as percentages of the basal level. Vertical lines show means ± SD ($n=8$). Urinary 5-HT increased, but not to a significant extent, after cessation of gum chewing.

the significant inhibition of nociceptive responses is caused by the placebo effect of expectation. We conclude that prolonged chewing exercise is necessary for the induction of antinociceptive action obtained herein.

It is generally known that distracting attention from a painful stimulus reduces pain perception, while directing

Table 1
Numerical of 5-HT levels before and after gum chewing (GC), with the data expressed as means ± SD ($n = 8$)

5-HT level (ng/ml)	Before GC	Just after GC	30 min after GC	60 min after GC
PPP	5.81 ± 4.43	7.54 ± 5.46	5.40 ± 3.32	–
WB	85.6 ± 40.9	91.2 ± 45.3	93.5 ± 38.4	–
Urine	82.5 ± 36.2	89.7 ± 32.1	87.8 ± 29.8	88.6 ± 33.2

attention to a pain stimulus increases its perceived intensity (Bantick et al., 2002). Maintaining gum chewing is considered as a cognitive task, which distracts attention from the nociceptive stimulation. Therefore, the attention-related modulation of pain may be responsible in part for the chewing-induced analgesia. The anterior cingulate cortex seems involved in the mechanisms underlying this attention-related modulation, but our understanding of the precise neuronal basis remains poor.

On the other hand, athletes can continue strenuous exercise in the face of severe injuries, indicating a notion that exercise alters pain perception. The exercise-induced analgesia has been examined most often in humans with running or cycling (Koltyn, 2000), which is defined as a prolonged rhythmic movement of leg muscles. Since chewing is also defined as prolonged rhythmic movement of jaw muscles, chewing-induced analgesia may be explained by the same mechanisms underlying the exercise-induced analgesia.

Regarding the mechanisms for exercise-induced analgesia, some investigators have found that naloxone administration attenuated the post-exercise analgesia, while others have not observed the involvement of the endogenous opioid system (Koltyn, 2000). Thus, it is suggested that opioid and non-opioid systems would be involved in exercise-induced analgesia. Regarding the non-opioid systems, several neurotransmitters such as 5-HT and noradrenalin have been implicated.

Hoffman et al. (1990) examined the role of 5-HT system in increased pain threshold (an analgesic effect) following 60 min of rhythmic muscle stimulation of the hind leg in rats. They revealed that the analgesic effect was abolished by a 5-HT synthesis blocker (*p*-chlorophenylalanine), suggesting that 5-HT system was responsible for the analgesia induced by prolonged rhythmic stimulation of hind muscle. This result indicates that 5-HT descending inhibitory pathway may play an important role in exercise-induced analgesia. If the chewing-induced analgesia is linked with the exercise-induced analgesia as discussed above, it is reasonable to speculate that the chewing-induced analgesia is also mediated by 5-HT descending pathway.

The present study was based on the hypothesis that activation of 5-HT neurons by the rhythmic behavior of chewing might enhance the 5-HT descending inhibitory pathway and could suppress nociceptive responses in humans. The hypothesis was based on the animal experiments performed by Jacobs and Fornal, (1993). They have demonstrated that voluntary rhythmic movements, such as mastication, locomotion and breathing, produce an increase in 5-HT neuronal activity in the raphe nuclei. Since locomotion in animals is considered to correspond to running or cycling in humans, we suggest that our hypothesis can be applied to the mechanism for the exercise-induced analgesia, i.e. running-induced analgesia or cycling-induced analgesia.

There are a few previous reports concerning chewing-induced antinociception in humans. Lewkowski et al. (2003) have examined the effects of gum chewing (for 1 minute) on routine painful procedures, which involved needles in children. They measured pain intensity and unpleasantness, as scored by the children. The result was negative, showing that chewing did not have an analgesic effect. Kemppainen et al. (2001) have demonstrated that repeated masticatory-like jaw movements (jaw opening and closing) produce a reduction of pulpal sensitivity, whereas modulation of the pulpal pain threshold is considerably weaker. Based on the present results, we suggest that relatively short period of chewing might result in no or weaker antinociceptive effect in those studies. As demonstrated in this study, the antinociceptive effect manifested in the 4th analysis period (5–7 min) after the onset of chewing. It is important to note that more than 5 min of chewing is necessary to induce the antinociceptive action.

To assess indirectly the activation of 5-HT neuronal activity in humans, we measured blood (PPP and WB) 5-HT levels in this study. We found significant increases in the blood 5-HT levels after chewing, although there was no significant change during resting without chewing. Therefore, it is suggested that the rise in blood 5-HT levels could be derived from augmentation of 5-HT neuronal activity induced by the chewing exercise.

To assess such increases in the peripheral blood level as an index of augmentation of 5-HT neuronal activity in the brain, one must consider a possible mechanism for 5-HT transport across the blood–brain-barrier (BBB). In this connection, Wakayama et al. (2002) recently have discovered a 5-HT transporter (SERT) at the BBB: SERT is located on the luminal and abluminal sides. This is a structural evidence for 5-HT transport across the BBB.

We have recently examined the possibility that whether the increased 5-HT activity in the brain can be estimated by measuring the peripheral blood 5-HT levels in rats with gastrointestinal and kidney resections and liver inactivation. Besides the brain, these organs are established to be important 5-HT depots (Kema et al., 2000). We found that blood 5-HT levels showed a significant augmentation whenever brain 5-HT level was elevated by administration of 5-hydroxytryptophan (Nakatani et al., 2004). This physiological evidence strongly indicates that an increase in 5-HT within the brain can be estimated by measuring the increase in the peripheral blood 5-HT levels.

Since voluntary rhythmic movements enhance the activity of 5-HT neurons (Jacobs and Fornal, 1993), other

prolonged repetitive movements such as locomotion or breathing are considered to produce the same effect. We have recently confirmed an elevation of the urinary 5-HT levels accompanied by changes in EEG after prolonged voluntary abdominal breathing in humans (Fumoto et al., 2004).

There are other possible mechanisms underlying the chewing-induced antinociception. As for opioid system, Ogawa et al. (2003) have reported the experiment demonstrating that masticating hard-food suppresses chronic pain in rats. The antinociception is reversed after application of naloxone, transection of the inferior alveolar nerve, or ablation of the somatosensory cortex. Thus, they suggest that opioid system combined with thalamocortical pathway would be involved in the antinociception induced by masticating hard-food. However, they could not observe a 100% reversal of antinociception after removing those three structures, indicating an involvement of other neuronal pathways contributing to the antinociception induced by mastication. Based on the discussion described above, we suggest that 5-HT descending inhibitory pathway is one of the important candidates for the non-opioid systems of antinociception. In this connection, it is important to note that 5-HT neurons in the RVM do not directly mediate the antinociceptive effects of supraspinal opioids in rats (Potrebic et al., 1994).

In summary, the present study revealed that prolonged chewing exercise suppressed the nociceptive responses and that the blood 5-HT levels rose significantly in response to chewing. These results support our hypothesis that the rhythmic behavior of chewing suppresses nociceptive responses via the 5-HT descending inhibitory pathway.

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