

Parotid Saliva Secretion by Mechanical Stimulation is Site-Specific in the Oral Cavity in Human Subjects

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Abstract

For oral health, it is an important issue to keep and, if deteriorated, to improve salivary secretion. Although mechanical stimulation in chewing and masticating bolus induces the salivary secretion, it is not well-known which sites in oral cavity are effectively stimulated. The present study was designed to investigate the effective sites in the cavity to the mechanical brushing stimulation to induce parotid saliva in human subjects. Parotid saliva was collected using a modified Lashley cup. Saliva secretion was induced by brushing, with normal toothbrush, of the palatal gingival margin of molars, ipsilateral and contralateral to the cup. The occlusal surface of the molars, the gingiva, the palatal rugae, and the tongue were also stimulated by the brushing. Salivary secretion from gingival margins of ipsilateral maxillary and mandibular molars showed comparatively higher rates among them. This indicates that mechanical brushing stimulation of the gingival margin of molars is effective to secrete parotid saliva, possibly activating mechanoreceptors in the mucosa and the periodontal ligament. These may provide beneficial information to improve salivary secretion in oral rehabilitation.

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Introduction

Saliva has several specific and essential functions, including protection of the oral mucosa and teeth, antibacterial action, facilitation of alimentation, cleaning, and buffering. Saliva is reflexively secreted in response to several stimulants, for example, taste and odorous substances and other chemicals, temperature, and mechanical stimulation. In mastication, saliva is primarily secreted on the ipsilateral side, via the masticatory-salivary reflex [1]. Evidence suggests that afferent information from mechanoreceptors in the mucosa and periodontal ligament contributes to this reflex [2].

Hypofunction of salivary gland is induced by systemic disorders such as Sjögren's syndrome, medications and irradiation therapy for head and neck cancer [3, 4]. In addition, aging [5] and diet [6] may elicit a decrease of salivary secretion. In the field of oral rehabilitation, it is important to improve such salivary gland hypofunction. In order to improve the hypofunction, mechanical stimulants such as sugarless gum and gustatory stimulants such as lemon drops are used in the clinical field [7].

Toothbrushing as a mechanical stimulant induces salivary secretion [8]. A study shows an improvement in salivary secretion in patients with salivary hypofunction by using a powered toothbrush [9]. However, there is no evidence suggesting which sites in the oral cavity are effectively

stimulated to induce salivary secretion by mechanical stimulation. The aim of the present study is to determine which sites in the oral cavity contribute to effective parotid salivary secretion by mechanical stimulation with brushing in human.

Materials and Methods

Subjects

The subjects were 16 healthy young adults (mean age: 22.0 ± 0.7 , male 12; female 4), who were in the 3rd year of undergraduate studies at Kyushu Dental University. No subjects were taking medication or had previous diseases that might affect salivary secretion. The procedures were explained in full to all subjects and signed consent forms were obtained prior to the experiments. The study design was approved by the Institutional Review Board of Kyushu Dental University.

Collection of Parotid Saliva

Collection of parotid saliva was performed at 15:00-17:00. Subjects were restricted in eating, drinking, smoking or brushing their teeth 1 h before saliva collection. In order to allow to collecting parotid saliva in brushing the oral cavity, a modified Lashley cup, which was made from polyethylene (Fukae MFG. Co., Kitakyushu, Japan), was used in the present study. The outer diameter and height were 16 and 4.5 mm, respectively. The inner circular chamber is connected to a polyethylene tube (outer diameter 1.6 mm, No. 5, Hibiki Co., Tokyo, Japan) for collection of parotid saliva. The outer chamber is connected to a polyethylene tube (outer diameter 2.0 mm, No. 6, Hibiki Co., Tokyo, Japan) and a rubber bulb which is squeezed to create a negative pressure that maintains the cup in place without movement. The inner chamber was positioned over the orifice of the parotid salivary duct on one side of the mouth. The saliva was collected into glassware and measured with an electric balance (GF-400, A & D, Tokyo, Japan). Flow rates of the parotid saliva were estimated assuming the specific gravity to be 1.0.

The subjects were seated in a chair in a quiet laboratory. Following placement of the cup and confirming saliva drops from the end of polyethylene tube, unstimulated saliva was

measured for 5 min. Then the subjects were asked to brush around regions illustrated in Figure 1 with toothbrush, with the same pressure in brushstroke as when they normally brushed teeth. Each region was brushed for 1 min at intervals of 5 min. The gingival margins of one of the following regions were brushed according to the Bass method [8, 10]: the ipsilateral/contralateral and palatal/lingual/buccal sides of the maxillary/mandibular and molars/incisors. According to the method, toothbrush was placed at the gingival margin at 45 degree to long axis of tooth. The brushing was always started from the ipsilateral palatal side of the maxillary molars. After brushing these regions, they were asked to brush the occlusal surface of the ipsilateral maxillary molars, palatal gingiva beside the molars, the palatal rugae and the tongue. The subjects were also asked to chew gum base (0.25g each) supplied by Lotte (Tokyo, Japan), in either ipsilateral or contralateral sides to the cup for 2 min. Brushing of the buccal side of ipsilateral maxillary molars was not performed because the modified Lashley cup was too near to the brushing site and was sometimes accidentally detached during the brushing in trials done before data collection. In the preliminary experiments by KI and TI, it was confirmed that the flow rates obtained under the present protocol were repeatable when the order of brushed regions was randomized.

Figure: 1

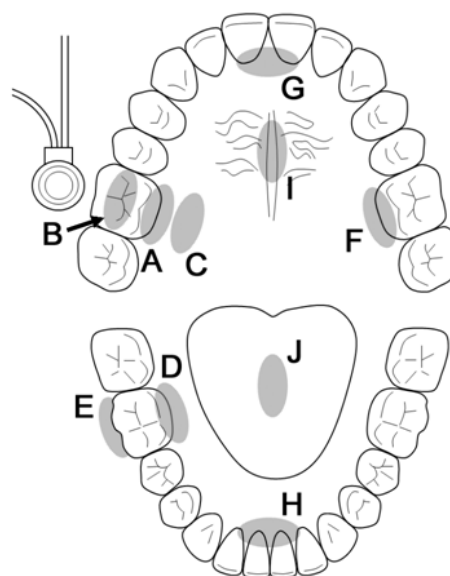


Figure 1: Schema of brushing sites with a toothbrush. A-E show brushing sites ipsilateral to a modified Lashley cup. Brushing of the A, D, E, F, G and H regions was performed according to the Bass method, in which gingival margins are brushed. A: palatal gingival margin of ipsilateral maxillary molars, B: occlusal surface of ipsilateral maxillary molars, C: palatal gingiva beside ipsilateral maxillary molars, D: lingual gingival margin of ipsilateral mandibular molars, E: buccal gingival margin of ipsilateral mandibular molars, F: palatal gingival margin of contralateral maxillary molars, G: palatal gingival margin of maxillary incisors, H: lingual gingival margin of maxillary incisors, I: palatal rugae, J: tongue.

Statistical Analyses

Differences in flow rates were assessed using a paired Student's t-test. Relationships yielding P values < 0.05 were considered to be significant. All values are expressed as the mean $\pm$ SEM.

Results

Brushing of all oral regions tested increased salivary secretion (Figure 2). Compared to the flow rate of unstimulated parotid saliva, flow rates were significantly increased by brushing of the palatal gingival margin (A), occlusal surface (B) of ipsilateral maxillary molars and palatal gingiva beside the molars (C). There was no significant difference between (A) and (B) while the salivary flow rate in (B) is quite smaller than that in (A). Brushing of the palatal gingival margin of the ipsilateral maxillary molars (A) was the most effective among the three regions. The flow rates generated by brushing of lingual (D) and buccal (E) gingival margins of the ipsilateral mandibular molars, but not the palatal gingival margin of the contralateral maxillary molars (F), were significantly increased compared to the flow rate of the unstimulated parotid saliva. There was no significant difference among regions (A), (D), and (E). The flow rate generated by brushing of the palatal gingival margin of the maxillary incisors (G) was significantly, although small, increased, but not that of the mandibular incisors (H). The flow rates by brushing of the palatal rugae (I) and the tongue (J) were also significantly increased over the unstimulated parotid saliva.

The mean flow rates from ipsilateral (0.37 ± 0.06 ml/min, $p < 0.001$) and contralateral sides (0.08 ± 0.02 ml/min, $p < 0.001$) to the cups of parotid saliva stimulated by gum chewing were significantly greater than that of the unstimulated parotid (0.04 ± 0.01 ml/min). The contralateral flow rate was significantly smaller than the ipsilateral ($P < 0.001$).

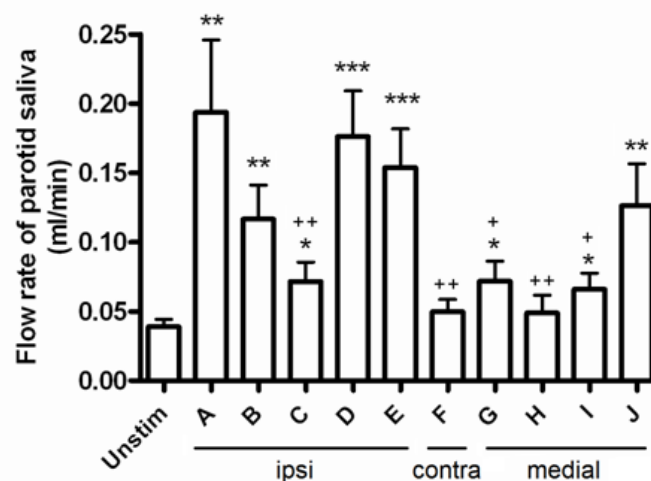


Figure 2: Effects of brushing gingival margin of the teeth, occlusal surface of the molars, the gingiva, the palatal rugae and the tongue on flow rate of parotid saliva. Unstim: the flow rate of unstimulated parotid saliva. A-J shows the flow rates induced by brushing. A-E was obtained from the ipsilateral (ipsi) side to the modified Lashley cup, F from the contralateral (contra) side, and G-J from the medial (medial) side. The brushing sites are shown in Fig. 1. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, vs. the unstimulated parotid saliva. +: $P < 0.05$, ++: $P < 0.01$, vs. A.

Discussion

This is the first report to show that flow rate of parotid saliva by mechanical stimulation is site-specific in the oral cavity in humans. Parotid salivary secretion was increased by brushing with toothbrush. This is consistent with results in previous studies showing increase of whole saliva secretion during toothbrushing [8, 11]. Greater parotid salivary secretion was obtained by brushing the gingival margin of the molars, which were ipsilateral to the modified Lashley cup. The volume of secretion was approximately half of saliva secreted during

chewing gum in the ipsilateral side. Salivary secretion generated in brushing the contralateral side showed no significant change over unstimulated saliva secretion. This is in accord with results of previous research into the flow rates of parotid saliva in rabbits and humans, showing increased production of saliva on the chewing side versus the non-chewing side [2]. We obtained also the same results in this study, as for the parotid salivary secretion in chewing. These point to involvement of mechanoreceptors in the periodontal ligaments and tactile mechanoreceptors in the mucosa around the gingival margin of the molars in parotid saliva secretion, and support the occurrence of the salivary reflex primarily on the ipsilateral side [1, 2]. In the brushing of ipsilateral lingual or buccal sides of mandibular molars, a similar (but rather smaller) volume of parotid salivary secretion was observed to that seen in the brushing of the ipsilateral palatal side of maxillary molars. This indicates that afferent inputs from the gingival margins of both ipsilateral mandibular and maxillary molars to the center of salivary reflex cooperate and secrete parotid saliva in masticating bolus.

Brushing palatal incisors increased salivary secretion by a significant but small amount, compared to that of the unstimulated parotid saliva and that of the lingual gingival margin of the mandibular incisors showed no significant change in parotid salivary secretion. It is known that parotid saliva is secreted more selectively for mastication, bolus formation and swallowing than submandibular saliva [12, 13]. Since bolus is present in the posterior boundary of oral cavity at the time points, regions in the anterior boundary may be less affected by mechanical stimulation with bolus. Considering this, it is a reasonable physiological phenomenon that parotid saliva is more selectively secreted by mechanical stimulation around the molars during mastication, bolus formation and swallowing because the orifice of the parotid salivary duct is opened near the maxillary molars.

During empty clenching and tooth grinding, research has found that parotid saliva is not secreted and may even be suppressed [14]. In the present study, increased salivary secretion was observed in brushing of the occlusal surfaces of the molars. It

may be that the movement of a toothbrush over the occlusal surfaces activates mechanoreceptors in periodontal ligaments and induces salivary secretion. According to this line of thought, activation of mechanoreceptors in periodontal ligaments in clenching and tooth grinding must increase parotid salivary secretion. But it actually did not occur. That may be caused by the following reasons. In the clenching and tooth grinding, masseter muscles are also activated. A previous report has suggested that afferent information from the masseter muscles influences and suppresses salivary secretion [14]. Although results in the present study may support this hypothesis indirectly, the suppression mechanism of salivary secretion in clenching and tooth grinding is still unclear.

The salivary secretion is influenced by diet [6] and food consistency [15, 16]. Fasting significantly decreases salivary secretion in human [6]. Animal studies of rats have shown that feeding of a liquid diet elicits structural and functional changes of the parotid gland and decreases the salivary secretion by less than two weeks [17, 18], suggesting that stimulation of mechanoreceptors during mastication is important to keep salivary functions. The studies have also demonstrated that the decreased salivary function is recovered less than two weeks after returning to the normal meals. This indicates that salivary glands have a highly plastic ability, implying that the deteriorated salivary functions may be improved by suitable mechanical stimulation.

A clinical report shows that repeated use of a powered toothbrush increases salivary flow rates in xerostomic patients [9]. The present study demonstrates that the tooth-lingual margin of molars is an effective site to induce parotid saliva production. Although it may be hard to apply the present results directly to hypo-salivation patients, the information may provide beneficial guidance as to techniques for improving salivary secretion with mechanical stimulation in oral rehabilitation.

In conclusion, salivary secretion generated by mechanical brushing stimulation with toothbrush is site-specific within oral regions, and brushing the tooth-lingual margins of the molars is comparatively effective to induce parotid salivary secretion.

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