Influence of Occlusal Hypofunction Induced by Opposed Tooth Loss on Periodontal Mechanoreceptors in Rat Molars

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Abstract: It has been critical in clinical dentistry to understand the response properties of periodontal mechanoreceptors (PMRs) when the occlusal condition was changed, because the PMRs are important sensory receptors controlling jaw movement. Therefore, we investigated the effect of opposed tooth loss on the response properties of PMRs in rat molars. All mandibular molars in an in vitro jaw-nerve preparation, made from a rat without opposed teeth, were carefully extracted before electrophysiological recordings. Single-unit discharges evoked by direct mechanical stimuli with von Frey hairs applied to the PMRs were recorded from the inferior alveolar nerve on day 3, and 1, 2, 4, 8, and 12 weeks after opposed tooth extraction. The following results were obtained: (1) in both the 3-day and 1-week post-extraction groups, the von Frey threshold value was significantly lower, although in the 12-week group, it was significantly higher than that in the control group and (2) in the 3-day to 8-week groups, the conduction velocity of nerve fibers innervating PMRs was significantly slower than that in the control group. These results showed that sustained functional changes could be clearly elicited in PMRs by occlusal hypofunction, suggesting that these changes may have critical influences on both jaw reflexes and masticatory movements.
Introduction

The periodontal mechanoreceptor (PMR) is well known as an important sensory receptor excited by mechanical stimuli applied to the teeth. Periodontal inputs are reported to control jaw movements and to influence many reflex systems in the oro-facial region. The physiological properties of the PMR have been studied using several physiological methods in animals and man. In the rat, morphological studies showed that the periodontal ligament was richly supplied by two types of mechanoreceptive sensory receptors, defined as free nerve endings and Ruffini-like endings.

Recently, the precise recording of response properties of PMRs has been realized using an in vitro jaw-nerve preparation. Using this method, Ishii observed two types of response pattern and showed that rat PMRs in incisors and in molars have different physiological properties. Although the responsiveness of PMRs to mechanical stimuli in the normal occlusal condition has been widely investigated, quantitative studies on the response properties of PMRs in abnormal occlusal conditions, such as decreased occlusal contact and traumatic occlusion, have not been attempted. Therefore, this study was undertaken to determine changes in the response properties of PMRs when the opposed teeth were extracted in order to decrease occlusal force.

Materials and Methods

1. Preparation

Thirty-nine male Wistar albino rats (weighing 200—350 g) were used. Under general anesthesia with intraperitoneal injection of thiamylal sodium (60 mg/kg, Isozol®, Yoshitomi Pharmaceutical Co., Ltd., Tokyo, Japan), three right maxillary molars were extracted using a pair of modified surgical pliers to eliminate occlusal force on the mandibular right molars. After extraction of these molars, the experimental animals were divided into six groups: 3-day (n=6) and 1- (n=7), 2- (n=7), 4- (n=7), 8- (n=6) and 12-week (n=6) post-extraction groups. A control group of five male Wistar albino rats (weighing 250—260 g) had intact maxillary molars defined as normal occlusion. After the experimental occlusal hypofunction without opposed tooth was made, an in vitro jaw-nerve preparation was designed as reported elsewhere. In this preparation, three right mandibular molars were carefully extracted by a pair of modified surgical pliers in order to apply mechanical stimuli directly on the periodontal ligaments. The in vitro jaw-nerve preparation was placed on a rubber bed in one of a two-compartment (test pool) plastic chamber, and the lateral side was firmly fixed to the rubber bed with dental cement (Fig. 1).

To test if the periodontium remained intact after tooth extraction, immunohistochemical analysis was conducted. The preparation was fixed in 4% paraform-aldehyde in 0.1 M phosphate buffer (pH 7.4) at 4°C for 12 hours, and decalcified in 5% ethylene diamine tetra-acetic acid (EDTA) -2 Na solution (pH 7.4) at 4°C for 4 weeks. The specimens were immersed in an anti-freezing agent (30% sucrose solution) overnight at 4°C, and processed for freeze-sectioning in a cold microtome (Yamato Kohki, Saitama, Japan). 40-μm thick horizontal sections of the lower jaws were prepared. The rabbit polyclonal anti-human protein gene product (PGP) 9.5 antibody (Ultraclone, Cambridge, UK), which recognizes the cytoplasmic protein distributed in the central and peripheral neurons, was used for immunohistochemical visualization of nerve elements. The rabbit polyclonal anti-human PGP 9.5 antibody used as the primary antibody in this study has been fully characterized previously. The cryostat sections were processed for indirect immunostaining by the avidin-biotin-complex (ABC) method using a commercially available kit (Vector, CA, USA). After inactivation of endogenous peroxidase with 0.3% H2O2 in absolute methanol for 30 minutes, nonspecific immunoreactivity was blocked by pre-incubation in 2% normal goat serum in phosphate-buffered saline. The sections were first reacted with the rabbit polyclonal anti-human PGP 9.5 antibody diluted at 1 : 10,000 with 2% normal goat serum overnight at 37°C followed by two consecutive incubations with biotinylated anti-rabbit IgG (1 : 400) (Chemicon, CA, USA) and ABC complex,
respectively. The immunoreactive sites were visualized with 0.02% 3,3-diaminobenzidine tetrahydrochloride (Dojin Chemical, Kumamoto, Japan) and 0.01% H$_2$O$_2$ in 0.05M tris-HCl buffer (pH 7.6). Sections were then mounted on slide glass using glycerin.

2. Experimental setup

The schematic experimental setup (Fig. 1) illustrates a chamber with two compartments (test and oil pools) separated by a thin vertical plastic plate punctured with a small hole in the center. The nerve trunk containing the inferior alveolar nerve was threaded through this hole and placed in the other compartment of the chamber (oil pool). The two pools were separated by filling the space in the hole surrounding the nerve trunk with vaseline. The oil pool was filled with liquid paraffin to immerse the nerve. The test pool was perfused by a modified Krebs-Henseleit solution (110.9 mM NaCl, 4.8 mM KCl, 2.5 mM CaCl$_2$, 1.2 mM MgSO$_4$, 1.2 mM KH$_2$PO$_4$, 22.4 mM NaHCO$_3$, 20.0 mM glucose), which was saturated with a 95% O$_2$-5% CO$_2$ gas mixture, which was saturated with a 95% O$_2$-5% CO$_2$ gas mixture. Previously warmed fluid was heated by a Peltier-type heater attached to the microtube near the test pool to maintain the temperature at 31°C using a feedback controller. At higher temperatures, the preparation tended to deteriorate in a short time, probably because of increased O$_2$ and glucose consumption. The nerve trunk in the oil pool was slightly pulled to furnish enough tension for easy insertion of the recording electrode.

3. Stimulating and recording procedure

For quantitative mechanical stimulation, the PMRs in the tooth sockets were stimulated by five intensities (1.1, 2.9, 3.3, 7.8, and 11.8 mN) of calibrated von Frey hairs with a tip diameter of 0.2 mm at a position parallel to the tooth axis. Mechanical stimulations by von Frey hairs were attempted three times in each unit, and the lowest value of the three trials was taken as the threshold. In this study, the units were divided into two types according to their response pattern; those that fired continuously for more than five seconds while mechanical stimulation was applied were classified as slowly adapting (SA) type, and the remaining units were all classified as rapidly adapting (RA) type.

To estimate conduction velocity of the innervated fiber, bipolar concentric needle electrodes were employed to deliver electrical stimulation (duration: 0.1 ms, strength: 0.5–1.0 mA, frequency: 1 Hz). The conduction velocity was estimated from the distance and conduction time between the stimulating and recording electrodes, and was corrected to the value at 37°C using the Q$_{10}$ value reported by Paintal.
Fibers with conduction velocity less than 1.2 m/s were regarded as unmyelinated C fibers, those between 1.2 and 10.0 m/s as A\(\delta\) fibers, and those over 10.0 m/s as A\(\beta\) fibers. This criterion has been used by Koltzenburg, et al.\(^{15}\) for an in vitro nerve-skin preparation with mechanosensitive single primary afferents innervating the hairy skin.

Responses were recorded from functional single fibers in the inferior alveolar nerve with a tungsten microelectrode (Type: 25-10-1, tip impedance: 10-12 M\(\Omega\), FHC, Bowdoinham, USA) inserted into the nerve bundle using the conventional microneurographic method\(^{16}\).

ANOVA followed by Fisher's PLSD post hoc test was used to verify statistical significance (\(p<0.05\)).

Results

1. General

In this study, mechanical stimulation was applied directly to the periodontal ligament after extraction of three mandibular molars in an in vitro preparation. Figure 2 A shows the occlusal view of an in vitro jaw-nerve preparation after three molars (M 1, M 2 and M 3) were extracted. Histological study indicated that periodontal ligaments were still present in the preparation from the middle up to the apex of the socket. Figure 2 B shows the immunohistochemical aspect of the periodontium after tooth extraction (a) and with the tooth intact (c). In both cases, some nerve elements can be seen in the periodontal ligaments indicated by oblique arrows (b, d in Fig. 2 B).

The frequencies of the initial spontaneous activity in our preparation were low or none (0—0.3 Hz). This activity was a good indicator that the damage of surgical removal of the jaw and tooth extraction was not so severe for electrophysiological recordings. Stable recordings were made for up to five hours in our preparation.

2. Adaptation properties

Responses to mechanical stimuli were recorded from a total of 120 functional single units and were classified into 110 RA and 10 SA type units. RA types were subdivided into RA on, RA off and RA on-off. RA on unit had a few spikes when mechanical stimulation was applied, and RA off unit had them when mechanical simulation was terminated. RA on-off unit had a few spikes when the stimulation was applied and terminated. Typical examples of each type are shown in Fig. 3 (A : RA on, B : RA off, C : RA on-off, D : SA). Most of the RA type units responded with an on-off pattern (104 units) (Table 1).

3. Threshold

The threshold of the mechanical stimuli was measured in each experimental group. The changes in threshold value with time after opposed tooth extraction were observed. Figure 4 A shows the mean threshold value for each group. Compared with the control group (9.3±0.6 mN : mean±SE, \(n=11\)), significant differences were found in the 3-day (6.2±0.7 mN ; \(n=12\)) and 1-week post-extraction groups (6.3±0.8 mN ; \(n=17\)) with lower thresholds, and in the 12-week post-extraction group (11.1±0.4 mN ; \(n=18\)) with higher threshold values. No significant differences in threshold value between RA and SA type units or between the units innervated by A\(\beta\) and A\(\delta\) fibers were found in any of the experimental groups (Table 2).

4. Conduction velocity

The conduction velocities of 120 mechanoreceptive units were distributed between 2.1 and 34.7 m/s with a mean of 14.6±0.5 m/s. One hundred units were identified as A\(\beta\) fiber units with a mean conduction velocity of 16.2±0.5 m/s and 20 units as A\(\delta\) fiber units with 7.0±0.5 m/s. Although the differences in conduction velocity among each experimental group were not significant, the conduction velocities of five experimental groups (3-day and 1, 2, 4, and 8-week post-extraction groups) were significantly slower than that of the control group (Fig. 4 B).

Discussion

There have been a number of reports on the properties of PMRs in various animal species\(^{5,10,17}\). Since these data were obtained mainly from in vitro preparations, quantitative analyses of the response properties...
were rather difficult. However, using an in vitro jaw-nerve preparation, the precise properties could be investigated electrophysiologically. Our in vitro jaw-nerve preparation has a number of advantages, such as (1) to control quantitatively all environmental variables surrounding the periodontal tissue, and (2) to directly apply von Frey hair stimulation. Although the period of stable recording has so far been limited to five hours, it has proven adequate to record the activities evoked by mechanical stimuli applied to the periodontal tissue. Tissue damage by surgical removal of the jaw and teeth may be a disadvantage. However, the frequencies of the initial spontaneous activity in our preparation were low or none, suggesting that the damage might not be so severe as to interfere with recording.
1. Adaptation properties

There have been many studies on the adaptation properties of PMRs\(^5,9,10,17-19\). Catton\(^{18}\) has suggested that the cause of adaptation may have a mechanical basis, postulating that RA receptors are loosely attached to their surroundings and that slippage occurs during sustained displacement of the tissues.

![Diagram of PMR responses](image)

**Fig. 3** Responses of PMRs.

A: Rapidly adapting (RA) type unit with an on pattern (von Frey stimuli: 7.8 mN).

B: RA type unit with an off pattern (von Frey stimuli: 7.8 mN).

C: RA type unit with an on-off pattern (von Frey stimuli: 11.8 mN).

D: Slowly adapting (SA) type unit (von Frey stimuli: 7.8 mN). Horizontal bars indicate the periods of stimulation.

**Table 1** Adaptation properties of the periodontal units

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<thead>
<tr>
<th>Type</th>
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<td>RA on</td>
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<tr>
<td>RA on-off</td>
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<tr>
<td>RA Total</td>
<td>11</td>
<td>12</td>
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<td>SA</td>
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<td>Total</td>
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All data give number of units.
It is well known that the adaptation properties of periodontal tissue are influenced by the direction and intensity of mechanical stimuli applied to the receptor10). However, the concept that there is only one type of PMR, and that its response characteristics depend on the spatial position of receptors has been suggested by Appenteng, et al. in rabbits19) as well as Linden and Millar in cats17). It is thought that SA type units found in this study might have density changes of PMRs following deformation of the periodontium caused by opposed tooth loss. We observed that most of the RA type units responded with an on-off pattern. Our findings agree with a previous study by Ishii9) , reporting that only RA type units were observed in intact molars and that the majority of the unit types displayed an on-off pattern in the rat molar. Therefore, it seems moot that the loss of occlusal force had significant functional influences on the adaptation properties in rat molars.

2. Threshold

The thresholds of the experimental groups temporarily showed lower values compared with the control group, particularly the first two experimental groups (3-day and 1-week post-extraction groups). In the 12-week post-extraction group, the threshold value was higher than that of the control group. Below, we show four possible explanations for the changes of threshold after opposed tooth extraction.

First, the deformation or displacement of PMR caused by changes in the periodontium could have been responsible for the changes in threshold value. Some histological studies indicated that morphological changes in the periodontal ligaments20) and in ground substances of periodontal ligaments21) occur within a few days after opposed tooth extraction. Moreover, Muramoto, et al.22) showed that occlusal stimuli appeared to be responsible for maintaining the thickness and structure of the periodontal ligament, as well as the function of periodontal Ruffini-like nerve endings. Second, density alterations of each mechanoreceptor followed by narrowing of the periodontium could have been responsible for the changes in threshold value. A decrease in width of the periodontal ligament without masticatory function has been described in a previous report20). Third, degeneration of nerve fibers and receptors may have affected the threshold values. Opposed tooth extraction may be followed by a decrease in blood flow20), which would have induced a shortage of nutrients supplied to the nerve.

Finally, chemical alteration could have been responsible for the changes in threshold value. The causes that influenced the response properties of PMRs would be not only the physical changes in the receptor itself but also some specific substances. A recent study has shown that NFP (neurofilament protein) within nerve fibers increased after experimental tooth movement23).

Therefore, combining the above-mentioned four
possibilities, not only the deformation of PMRs but also the factors involved in the development or regeneration of nerve fibers may have influenced the threshold changes of PMRs.

In this study, no differences in the threshold of PMRs between Aβ and Aδ innervated units were found in any of the experimental groups. This fact suggests that there was little relationship between the threshold value and characteristics of nerve fibers innervating the PMRs. Similarly, no relationship in threshold values of PMRs between RA and SA types was observed in any of the experimental groups. This observation was different from the previous reports as shown by Linden and Millar in cats17), suggesting that these discrepancies may be due to differences in the experimental condition between cats and rats.

3. Conduction velocity

Nerve fibers innervating the PMRs were classified according to the conduction velocities of the units recorded. Our results showed that the conduction velocity in the experimental groups other than the 12-week post-extraction group indicated lower than that in the control group. One possible explanation for these results may be that the physiological properties of PMRs innervated by large-diameter nerves were easy to change after loss of occlusal force. Therefore, it is thought that opposed tooth loss may induce a decrease in the sensory processing rate for mechanosensation evoked by activation of PMRs. This hypoactivity of mechanosensitivity of PMRs may attenuate activities of oro-facial functions.

Conclusion

Sensory information from PMRs plays an important role in jaw movement or mastication. However, influence of occlusal hypofunction induced by opposed tooth loss is still unknown. This study was undertaken to reveal whether occlusal force reduction could induce changes of response properties of the PMRs using an in vitro jaw-nerve preparation.

The von Frey threshold of the mandibular molar was significantly lower on day 3 or 1 week after the extraction of three opposed molars. However, it was higher than the control value after 12 weeks. The conduction velocity of the innervated fibers remained slower than the control during the 8 weeks after opposed tooth extraction. These results indicated that sustained functional changes could be clearly induced in PMRs after opposed tooth loss. It is also suggested that the stomatognathic system, which is dependent on somatosensory inputs, might be severely affected in occlusal hypofunction.

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