The purpose of this study was to investigate changes in the periodontal masseteric reflex (PMR) after experimentally induced occlusal hypofunction. Wistar rats were divided into control groups (CGs) and hypofunction groups (HGs). Rats in the HGs had their lower incisors cut down every other day for 6 weeks. Electrical stimulation was given to the periodontal ligaments of an upper incisor or the left trigeminal mesencephalic nucleus (MeV) in the CGs and HGs. Recordings of masseter motor unit responses were performed at 0, 1, 2, 4 and 6 weeks after hypofunction. Compared with the CGs, significant longer latencies in the PMR were found in the 4w- and 6w- HGs. After MeV stimulation, no significant difference in latency was found between HGs and CGs. After periodontal stimulation, the threshold value of masseteric motor-unit responses was higher in HGs than in CGs in 4 and 6 weeks respectively. These results suggest that the PMR can be changed by periodontal sensory modification during occlusal hypofunction.

Key words: Occlusal hypofunction; Periodontal masseteric reflex; Mechanoreceptor; Trigeminal mesencephalic nucleus; Rat

Introduction

It has been important in clinical dentistry to understand the characteristic changes in the periodontal masseteric reflex (PMR) under various occlusal conditions, for the PMR is an important reflex controlling jaw movement. In patients with anterior open bite, loss of occlusal contact in the anterior teeth results in decreased activity of the elevator muscles, especially the masseter. The ascendance of the PMR arc consists of the periodontal mechanoreceptor, and the afferent of the PMR, which is a tip branch of trigeminal mesencephalic nucleus (MeV) neuron distributed over the periodontal ligament. The PMR controls the positive feedback regulation of occlusal force; a weak force applied to a tooth during the closure phase of mastication serves as a stimulus that in turn reinforces the occlusal force. In the rat, morphological studies showed that the periodontal ligament is richly supplied by two types of mechanoreceptive sensory receptors, defined as free nerve endings and Ruffini-like endings. The periodontal mechanoreceptors have two types of response pattern, i.e. tonic and phasic, and in the rat, periodontal mechanoreceptors at incisors and molars have different physiological properties. The PMR is similar to the jaw-jerk reflex in that both reflexes have...
almost the same latency. Studies on animals suggest that normal functional occlusion is essential for maintenance of structural integrity of the periodontium, and the morphology and function of the periodontal mechanoreceptors. Especially, the information provided by the periodontal mechanoreceptors of the anterior teeth is important for the motor control of the mandible.

Seki et al. reported changes in the response threshold of the periodontal mechanoreceptors on occlusal hypofunctional teeth stimulated directly, in vitro. Functional changes could be clearly elicited in the periodontal mechanoreceptors by sustained occlusal hypofunction in rat molar. These changes may have critical influences on both jaw reflexes and masticatory movements. However, no available data exist on the changes in physiological properties of the PMR in occlusal hypofunctional conditions. In our experiment, we examined the PMR responses in vivo under occlusal hypofunction, and evoked reflex. We compared these data with normal occlusion. The purpose of the present study was to investigate how occlusal hypofunction would affect the physiological properties of periodontal sensory function in the PMR arc.

Materials and Methods

Animals
Total of sixty female Wistar albino rats, weighing from 250 to 320 g, were used. Animals were randomly divided into control groups (CGs, N=30) and hypofunction groups (HGs, N=30). In the animals of the HGs, lower incisors on both sides were cut at the bottom of the incisor crown under inhalation anesthesia with diethyl ether. After recovery from anesthesia, animals were then returned to their cages. Since the incisors grow continuously, cutting was performed every two days under the same anesthetic condition. After cutting of these incisors, the experimental animals were divided into five groups: 0w- (N=6), 1w- (N=6), 2w- (N=6), 4w- (N=6) and 6week- HGs (N=6). The rats with uncut incisors were the CGs, and were divided into five groups: 0 w- (N=6), 1w- (N=6), 2w- (N=6), 4w- (N=6) and 6week- CGs (N=6). After cutting of these incisors, electrophysiological recordings were obtained at 0, 1, 2, 4, and 6 weeks in both the CGs and HGs. In order to clarify the absence of side effects on the PMR responses which could be caused by the growth deceleration as a consequence of cutting incisors, the body weight of rats in both CGs and HGs was monitored during the whole experimental period. The mean body weights were not significantly different between CGs and HGs.

Stimulation and recording
During the recording, the rats were anesthetized by intraperitoneal injection of thiamylal sodium (Isozol, Yoshitomi Pharmaceutical, Osaka, Japan; 60 mg/kg i.p.). A supplemental injection of 5 mg/kg i.p. was given when necessary. We monitored the level of anesthesia by checking pupil size, flexion and corneal reflexes, and heart rate.

The animals were placed in a prone position with their heads fixed to a stereotaxic frame (models RA-4 and EB-4, Narishige Scientific Instruments, Tokyo, Japan). The frame was then adjusted in the horizontal plane. To avoid mandibular movements, the mandibular incisors were fixed to the stereotaxic frame using a stainless steel bar and dental resin.

Periodontal ligament stimulation
Bipolar stainless steel wires of interpolar distance of about 1.5 mm were used for electrical periodontal stimulation. Stimulating electrodes were inserted into the periodontal ligaments of the left upper incisor (Figure 1A). Electrical stimulation with 0.1 ms in duration was applied at 1.0 Hz. The stimulus intensity was increased gradually until the first spike was evoked in the masseter muscle, just above the threshold value.

MeV stimulation
MeV was stimulated to examine whether the PMR during occlusal hypofunction is affected by the motor collateral. To insert the stimulating electrode into the MeV of the left side, we incised the scalp at the midline and prepared a small aperture about 3 mm diameter in the skull using a dental drill. A monopolar tungsten microelectrode was used (A-M Systems, Inc., Carlsborg, WA, USA; 250- µm diameter shaft with 8° tapered tip, 5 M Ω of AC impedance) for cathodal electrical stimulation in 0.1 ms in duration at 1.0 Hz. The electrode was inserted into the caudal third of the MeV in the left side of the brain following stereotaxic coordinate by Paxinos and Watson (1998) (Figure 1B). The reason why the electrode was inserted into the caudal third was that the periodontal mechanoreceptive neurons are densely situated in the caudal third of MeV.

Recording
Electrodes for single unit recording were placed in
the masseter muscle on left side about 5.0 mm deep from the skin surface. Bipolar stainless steel wires (type E-2, Narishige Scientific Instruments, Tokyo, Japan) of about 2.0 mm interpolar distance, enamel-coated except for the tips, and 100 μm in diameter were used as the recording electrodes (Figure 1A, B). After the recording electrodes were set, liquid paraffin was applied to the exposed masseter to prevent tissue dehydration. Single motor unit responses following stimulation of the periodontal ligament or the MeV were recorded from the masseter on left side. Single unit responses were recognized according to the all-or-none principle, where spikes showed full response after above-threshold stimulus and no response at all from a sub-threshold stimulus. Spike signals were recorded and amplified by a differential amplifier (DAM-80, WPI, Sarasota, FL; x1000 gain, 300 Hz and 3 KHz for low and high filters, respectively).

After recordings, the animals were killed with an overdose of thiamylal sodium. The experimental procedures described here are in agreement with the Animal Care Standard of Tokyo Medical and Dental University and Nagasaki University, and were carried out with the approval from their respective Animal Welfare Committees.

Data and statistical analysis

Data were captured and analyzed in a computer with a CED 1401 interface and the Spike2 software for Windows, version 2.19 (Cambridge Electronic Design, Cambridge, UK). All data were shown as mean ± SD. The Mann-Whitney’s U-test was used for statistical comparison of data from the CGs and HGs; statistical significance was considered as P < 0.05. The software Statview for Windows, version 5.0 (SAS Institute, Cary, NC, USA), aided in statistical analysis.

Results

Latency

Periodontal Ligament Stimulation

In CGs and HGs, the PMR responses to the periodontal stimulation were recorded from 60 units respectively (CGs, N=30; HGs, N=30). Analyses of functional changes of the PMR responses were performed using 2 units from each rat. Typical examples of masseter motor unit responses to periodontal stimulation at 6w were shown in Figure 2. Compared with the CG (2.30±0.20 ms; mean±SD), latency in the HG (2.95±0.25 ms) was clearly delayed at 6w. In the CGs from 0w (2.12±0.21 ms) to 6w (2.30±0.20 ms) CGs, there were no significant differences in latency. However, in the HGs, chronological changes were found in the latency by periodontal stimulation between 0w to 6w. Compared to the latencies in the
CGs, those in the HGs were significantly longer at 4w and 6w, however, no significant difference in latencies at 0, 1, and 2w was found in between the CGs and the HGs as shown in Figure 3.

MeV Stimulation

In CGs and HGs, the PMR responses to the MeV stimulation were recorded from 30 units respectively (CGs, N=30, HGs, N=30). In occlusal hypofunctional conditions, analyses of functional changes of the PMR responses were performed using 1 unit from each rat. Typical examples of masseter motor unit responses to electrical stimulation of MeV at 6w are shown in Figure 4. The latency of the CGs at 6w was 1.38±0.42 ms, the HGs at 6w was 1.40±0.22 ms. No significant differences in latency were found between the CGs and the HGs at every week as shown in Figure 5.

Threshold for periodontal stimulation

Figure 6 shows the changes in threshold from 0w to
The thresholds were significantly higher in the HGs (0.98 ± 0.07 mA) than in the CGs (0.49 ± 0.08 mA) at 4 w and in the HGs (0.99 ± 0.11 mA) than in the CGs (0.48 ± 0.10 mA) at 6 w. However, no significant differences were found between the CGs and the HGs at 0, 1, and 2 weeks.

Discussion

Changes in latency

In this study, after electrical stimulation to the periodontal ligament, the latency of masseteric motor unit responses in 4 and 6w-HGs was increased as compared to the CGs (Figure 3). However, following electrical stimulation to the MeV, no significant differences in the latencies were found between in the CGs and the HGs (Figure 5). These suggested that the conduction velocity of peripheralafferent fibers reduced. One possible explanation for these results is that occlusal hypofunction induced a structural alteration in the peripheral sensory system. We took into consideration that demyelination of the sensory axons might partially be responsible for such alterations and other peripheral neuropathy caused by occlusal hypofunction. When sensory axons are demyelinated, myelin sheaths protecting nerve fibers are damaged and lost and transmit electrical signals at a decreased conduction velocity. Recent studies have indicated that in rats with experimentally induced peripheral neuropathy, conduction velocity of sensory nerve in the reflex obviously decreases, possibly because of demyelination, as compared to that in normal rats. Some studies have reported that rats with demyelination or other types of neuropathy have immature developmental pattern of monosynaptic reflex as compared to normal rats. Clinical studies have also reported that the latencies of masseter reflex responses in patients with severe demyelinating polyneuropathy are strongly delayed as compared to those in the normal patients. These results indicate that demyelination of nerve fibers may influence reflex responses greatly. Although in our experiment, our rats did not have peripheral neuropathy, under occlusal hypofunction changes in the latency were seen implying a similar change such as demyelination. The occlusal hypofunction is associated with alteration of sensory sites and this in turn is likely to induce demyelination of large-diameter nerve fibers. Our study showed that the latencies of PMR responses were delayed during occlusal hypofunction, suggesting that occlusal hypofunction could induce demyelination of peripheral sensory nerves, and thus reduce the conduction velocity of peripheral afferent fibers.

The changes in the latency could be associated with the reconstruction of the central nervous system. In a previous study, after the upper and lower incisors were extracted, the neurons in the MeV disappeared. Another report shows degenerative changes in the MeV neurons after cutting of the
nerves that innervate the masseter. In this experiment we applied electrical stimulation to the MeV, and recordings were performed from the masseter. However, no significant differences in latency were found between the CGs and the HGs at any week. This could be due to the shortness of the experimental period or due to our inability to eliminate all occlusal function, and not causing any degenerative changes in the MeV. The functional changes in the PMR in this experiment were thought to be due to the periodontal sensory function.

Changes in threshold

In this study, after electrical stimulation to periodontal ligaments, the thresholds of the masseteric motor unit responses significantly increased in the 4 and 6w-HGs as compared to the CG (Figure 6). One study has indicated that the thresholds of periodontal mechanoreceptors are higher in rats with occlusal hypofunction than in normal rats. After external tactile stimulation to the maxillary anterior teeth, stimulus threshold in patients with anterior open bite significantly increased as compared to that in patients with normal bite. These results suggest that the sensitivity of periodontal mechanoreceptors may depend on various occlusal condition. Such deterioration in sensitivity may be attributable to the deformation of periodontal mechanoreceptors. Previous histological studies have demonstrated that the periodontal ligament undergoes morphological changes within several days after extraction of the opposed tooth. This phenomenon may be associated with occlusal hypofunction-induced decreases in density of receptor distribution. Moreover, occlusal stimulation may be responsible for the maintaining of not only the periodontal ligament structure but also the function of periodontal Ruffini-like nerve endings.

In this study, occlusal hypofunction-induced changes in threshold of the PMR were seen by the electrical stimulation of the terminal branch of the trigeminal nerve. Although we did not directly stimulate the periodontal mechanoreceptors, per se, since the peripheral input of the PMR arc is the periodontal mechanoreceptor, there must have been some changes in the periodontal mechanoreceptors.

Conclusions

Our results indicated that occlusal hypofunction-induced changes in the threshold and latency of the PMR might greatly affect the coordination of mastication. It was suggested that occlusal hypofunction might cause modification in periodontal sensory function.

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