



Title	Effects of occlusal force on the development and maintenance of periodontal mechanoreceptors of the rat
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論文内容の要旨

Aim:

The periodontal ligament serves as a supporting and buffering apparatus between the tooth and alveolar bone, and as a sensory receptor to mechanical stimuli to make mastication smooth, and to protect the jaw and tooth. Recent morphological studies have shown that there are at least two types of sensory nerve endings in the ligament; free nerve nociceptive endings and specialized mechanoreceptive endings. Among many types of mechanoreceptors, the Ruffini ending, a low-threshold slowly adapting type II mechanoreceptor, is the primary mechanoreceptor. Developmental studies have demonstrated that periodontal Ruffini endings have age-specific morphological changes related to the changes in the pattern of mastication, indicating that the occlusion is one of the important factors for morphological maturation of the periodontal Ruffini endings. There are few studies, however, on the effect of occlusal forces on the development and maintenance of periodontal Ruffini endings. The present study was designed to examine the effect of occlusal forces on the morphological development and maintenance of the axon elements of the periodontal Ruffini endings of the rat incisor by immunohistochemistry of protein gene product 9.5 (PGP 9.5), a general neuronal marker.

Materials and methods:*Animals, treatment and tissue preparation*

Three groups of different ages of Sprague-Dawley rats (adults, weighing 150-170 g, n=30), postnatal day 28 (PN28d) (n=39) and PN14d (n=30) were used in the present study. Under anesthesia with chloral hydrate (400 mg/kg, i.p., supplemented as necessary), the incisal edges of right upper and lower incisors were ground at least 2 mm every two days. The body weight and length of eruption were measured. In each age group, animals without any treatment served normal control. Animals with anesthesia but no grinding served as anesthetized control.

After appropriate survival times (3, 5, 7, 14, 28, 42 and 56 days after initial treatment for adult group ; 3, 7, 14 and 28 days for PN28d group ; 7, 14 and 28 days for PN14d group), animals were deeply anesthetized with chloral hydrate (600 mg/kg, i.p.) and perfused transcardially with a mixture of 4% paraformaldehyde and 0.05% glutaraldehyde in 0.1M phosphate buffer (pH 7.2). The upper jaws including incisors were removed and further fixed in 4% paraformaldehyde for 2-3 days and decalcified with Plank-Rychlo solution. Following completion of decalcification, specimens were equilibrated in 20% sucrose overnight, cut at a thickness of 20 μ m with cryostat, and treated as free floating sections.

Immunohistochemistry

Following inactivation of endogenous peroxidase activity, sections were incubated with polyclonal anti-PGP 9.5 (1 : 5000 ; Chemicon) for 16-18 hours at room temperature. After several rinses, they were incubated with biotinylated anti-rabbit IgG (1 : 500 ; Dako) and subsequently with ABC complex (Vector). The antigen-antibody binding sites were visualized by incubation with 0.04% 3-3' diaminobenzidine and 0.003% H₂O₂ in 0.05 M Tris-HCl buffer (pH 7.5) with nickel ammonium sulfate (0.08-0.1%) enhancement.

Quantitative analysis

For quantitative analysis, optimal densities of PGP 9.5-immunoreactivity at the restricted area were measured from 5 sections per animal. Since thick nerve bundles form Ruffini endings immediately after entering the periodontal ligament, the site of their entrance was located just below the center of the boxed observation area. The total areas of periodontal ligament and immunoreactive areas were measured using the Scion Image software package, and the percentages of the latter to the area of periodontal ligament were calculated. The statistical significance of difference was assessed by paired Student's t-test for comparison between immunoreactive areas of ground side and those of non-ground side of the same animal, and with one way analysis of variance (ANOVA) for comparison between immunoreactive areas of control animals and those of experimental animals. A P value less than 0.05 was considered a significant difference.

Results

General observations

The body weight was increased gradually in all groups. The eruption speed at the ground side was significantly faster than that at the non-ground side and control animals. No significant difference in eruption length between non-ground side and control animals.

Distribution and morphology of Ruffini endings

In the normal adult, PGP 9.5-immunoreactive structures were restricted to the alveolar half of the ligament (alveolus-related part ; ARP), and never in the tooth-related part (TRP). Ruffini endings were easily identified as the extensive arborization of their expanded terminal portions. The expanded terminal portion had the finger-like projections, showing the ruffle-ended outlines. The morphology of Ruffini endings of normal animals at PN28d was almost identical to that of normal adults. In PN14d, the terminal portions of the Ruffini endings were less expanded, but the ruffled-ended outlines were recognized.

Following grinding the cutting edge of incisors unilaterally, the density of Ruffini endings at the ground side was not different from that at the non-ground side and that of normal animals. However, most of Ruffini endings at the ground side showed smooth appearance, and they were occasionally found in the TRP. In contrast, Ruffini endings at the non-ground side showed clear ruffle-ended outlines. In all experimental animals, percentages of PGP 9.5-immunoreactive areas at the ground side were significantly smaller than those at the non-ground side and those of normal animals throughout the experimental period.

Discussion and Conclusion

The present observation clearly demonstrated that the grinding of the cutting edges of incisors affect the distribution and morphology of Ruffini endings as well as the eruption speed. Although the precise occlusal forces were not measured, we believe that occlusal force of the ground side was smaller than that at the non-ground side and normal animals. At the ground side, outlines of terminal portions of the Ruffini endings became smooth-surfaced appearance, while those at the ground non-grinded side showed extensively ruffled-appearance. It is reported that ruffled-outlines reflect the presence of microspines. Therefore, changes in the occlusal forces also cause the changes in number of microspines in the axon terminals.

In conclusion, the present results clearly suggest that occlusal force (or mechanical stimulation to the periodontal ligament) is essential to the development and maintenance of periodontal Ruffini endings.

論文審査の結果の要旨

本研究は、咬合力が歯根膜機械受容器の形成および維持にどのような影響を及ぼすかをラット切歯歯根膜を用いて免疫組織化学的に検索したものである。

その結果咬合力を軽減させると、歯根膜機械受容器の数の減少や歯根膜感覚受容に重要な役割を果たす歯根膜機械受容器の軸索小突起の消失が認められ、これらの影響は歯根膜機械受容器の形成過程でより顕著であった。以上の知見は適切な咬合力が歯根膜機械受容器の形成、維持に必要不可欠であることを示し、博士（歯学）の学位に値するものであると認める。