Postnatal Expression of Calretinin-Immunoreactivity in Periodontal Ruffini Endings in the Rat Incisor: A Comparison with Protein Gene Product 9.5 (PGP 9.5)-Immunoreactivity*

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Received November 26, 1998

Summary. The postnatal expression of immunoreactivity for calretinin, one of the calcium binding proteins, and for protein gene product 9.5 (PGP 9.5), a general neuronal marker, was investigated in mechanoreceptive Ruffini endings in the periodontal ligament of the rat incisor. Age-related changes in the expression of these two proteins in periodontal nerves were further quantified with a computerized image analysis. At 1 day after birth, a few PGP 9.5-immunoreactive nerve fibers and a still smaller number of calretinin-positive fibers were found in the periodontal ligament: they were thin and beaded in appearance and no specialized nerve terminals were recognized. Tree-like terminals, reminiscent of immature Ruffini endings, were recognizable in 4-day-old rats by PGP 9.5-immunohistochemistry, while calretinin-immunostaining failed to reveal these specialized endings. At postnatal 7–11 days when PGP 9.5-immunostaining could demonstrate typical Ruffini endings, calretinin-immunopositive nerve fibers merely tapered off without forming the Ruffini type endings. A small number of Ruffini endings showing calretinin-immunoreactivity began to occur in the periodontal ligament at 24–26 days after birth when the occlusion of the first molars had been established. At the functional occlusion stage (60–80 days after birth), the Ruffini endings showing calretinin-immunoreactivity drastically increased in number and density, but less so than those positive for PGP 9.5-immunoreaction. The delayed expression of calretinin suggests that the function of the periodontal Ruffini endings is established after the completion of terminal formation because Ca**, which binds to calcium binding proteins including calretinin with high affinity, plays an important role in mechano-electric transduction.

The calcium binding proteins include parvalbumin, calbindin, calretinin, S-100 protein, and others. All these proteins are categorized in the “EF-hand family” because of the presence of the common molecular structure called the “EF-hand” which binds Ca** with high affinity. Calretinin, first discovered as a product of a retinal CDNA (ROGERS, 1987), belongs to the category of a ‘buffering-type’ calcium binding protein which serves in passive roles such as the control of intracellular concentrations of Ca** (KRET-SINGER, 1981). Immunohistochemical studies have revealed a wide distribution of calretinin-like immunoreactivity in both central and peripheral nervous systems (cf. ROGERS, 1989; ANDERSEN et al., 1993), indicating that calretinin-like immunoreactivity is a useful marker for neurons.

The mechanism of the transduction in mechanoreceptors has remained obscure, but an important role for Ca** has been suggested (AKOEV et al., 1988). By histochemical technique, TACHIBANA et al. (1992) revealed that mechanical stimuli induced a Ca** influx into axon terminals of cutaneous mechanoreceptors in oral mucosa. Furthermore, they demonstrated the localization of a high affinity Ca** pump and Ca**/Na* exchanger in the axolemmal of mechanoreceptors (TACHIBANA and NAVA, 1992, 1994),

*This study was supported by Grants-in-Aid for Scientific Research (09877344 to S. W.) and Frontier Science (to T. M.) from the Ministry of Education, Science, and Culture, Japan.
suggesting the presence of a calcium channel which permits a change in permeability due to mechanical stimulation. Changes in intracellular Ca^{2+} concentration by mechanical stimuli are precisely controlled in the axon terminals, suggesting the involvement of calcium binding proteins in mechano-electric transduction in mechanoreceptors. In fact, rich contents of calcium binding proteins have been reported in the mechanoreceptors (Duc et al., 1994; Del Valle et al., 1994; Ichikawa et al., 1996, 1997; Ochi et al., 1997a, b).

The periodontal ligament contains many mechanoreceptors which are involved in the neural control of jaw movement (Matthews, 1975; Hannam, 1982). Although various kinds of mechanoreceptors have been reported (cf. Schroeder, 1986), recent morphological studies have revealed that the Ruffini ending, categorized as a low-threshold slowly adapting type II mechanoreceptor (Chambers et al., 1972; Biemesderfer et al., 1978), is the primary sensory receptor in the periodontal ligament (for reviews, Byers and Maeda, 1997; Maeda and Ohshima, 1998; Maeda et al., 1999). The ligual periodontal ligament of the rodent incisor has been favored for the morphological study of Ruffini endings because of the extremely dense distribution of these mechanoreceptors there (Saito et al., 1988, 1989; Kannari, 1990; Kannari et al., 1991).

Although for many decades little information had been available on the postnatal development of the periodontal Ruffini ending (cf. Maeda et al., 1999), our research group succeeded in demonstrating its process by immunohistochemical technique; the periodontal Ruffini ending showed stage-specific configurations which are closely related to tooth eruption during postnatal development, implicating that mechanical stimuli are a prerequisite for the differentiation and maturation of the periodontal Ruffini endings (Nakakura-Ohshima et al., 1993, 1995). On the other hand, a recent experimental study using an antibody to protein gene product 9.5 (PGP 9.5), a general neuronal marker (Doran et al., 1983; Thompson et al., 1983), demonstrated that the terminal morphology of periodontal Ruffini endings returned to normal by 14–21 days following resection of the inferior alveolar nerve (You et al., 1997). By use of the same experimental model, however, we recognized a delayed regeneration for the periodontal Ruffini endings (56 days after nerve resection) by calretinin-immunohistochemistry (Hiroshima et al., 1998). These experimental date lead us to entertain the possibility that the expression of calretinin might reflect the functional recovery of the periodontal Ruffini endings.

In the present immunohistochemical study, the postnatal exhibition of calretinin-like immunoreactivity was investigated in the periodontal Ruffini endings of the rat upper incisor. An antiserum against PGP 9.5 was also applied for demonstrating the postnatal development of the periodontal Ruffini endings. Furthermore, we attempted to quantify the chronological changes in the expression of these two proteins in the periodontal Ruffini endings by use of a computerized image analysis.

**MATERIALS AND METHODS**

All animal experiments were performed under the guidelines by the Institutional Animal Use and Care Committee at the Niigata University School of Dentistry.

**Tissue preparation**

Twenty-six Wistar rats were divided into six groups according to their postnatal days: 1, 4, 7–11, 15–18, 24–26 and 60–80 days after birth, which were defined as P1, P4, P7–11, P15–18, P24–26 and P60–80, respectively (at least 3 rats in each group). At each postnatal stage, the animals were deeply anesthetized by an intraperitoneal injection of chloral hydrate (400 mg/kg) and perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. Following the perfusion fixation, the maxillae including the incisors were removed en bloc and immersed in the same fixative at 4°C overnight. Decalcification was carried out in 10% EDTA-2Na (ethylene diamine tetracetic acid disodium) solution, pH 7.4, for a time period ranging between 4 days and 3 weeks, at 4°C. The decalcified tissue blocks were equilibrated in a 30% sucrose solution overnight for cryoprotection, and rapidly frozen in liquid nitrogen. Serial sagittal sections were cut at a thickness of 40 μm with a freezing microtome (Yamato-Koki, Tokyo, Japan), and collected in 0.01 M phosphate-buffered saline (PBS), pH 7.4.

**Immunohistochemistry**

Free floating sections were processed for the Avidin-Biotin-Complex (ABC) method (Hsu et al., 1981) using a commercially available kit (ABC kits; Vector, Burlingame, CA, USA). After immersion in PBS containing 0.3% Triton-X 100 for 30 min, endogenous peroxidase was inactivated by a treatment with 0.3% H_{2}O_{2} in absolute methanol for 30 min. Non-specific immunoreactivity was inhibited by preincubation in 2.5% normal goat serum (Vector). The sections were primarily incubated overnight
either with a polyclonal anti-calretinin antiserum (1 : 5,000; Chemicon International Inc., Temecula, CA, USA) or with a polyclonal anti-PGP 9.5 antiserum (Ultraclone, Cambridge, UK) at room temperature. Following several rinsings in PBS, they were incubated with a biotinylated anti-rabbit IgG (1 : 1,000; Vector), and subsequently with the ABC complex (Vector) for 90 min each at room temperature. The antigen-antibody reaction sites were made visible by 0.04% diaminobenzidine plus 0.002% H$_2$O$_2$ in 0.05 M Tris-HCl buffer, pH 7.6. Immunostained sections were thaw-mounted onto gelatin-coated glass slides, and were briefly counter-stained with 1% methylene blue plus Azur II.

**Immunospecificity control**

The specificity of the primary antiserum against calretinin was examined by a preabsorption test. The primary antiserum was preabsorbed with an excess recombinant calretinin (SWant, Bellinzona, Switzerland; 1 µg/1 ml of diluted primary antiserum). The origin, characterization, and specificity of anti-PGP 9.5 antiserum have been reported elsewhere (see, Gulbenkian et al., 1987).

**Quantitative analysis**

For quantitative analysis, optimal densities of calretinin-and PGP 9.5-immunoreactivity at the restricted area as shown in Figure 1 were measured from 3 sections per animal (total 9 sections per group). Since thick nerve bundles have been reported to form Ruffini endings immediately after entering the periodontal ligament (Sato et al., 1988, 1989; Nakakura-Oshima et al., 1993, 1995), the site of their entrance was located just below the center of the boxed observation area (1269 µm x 1005 µm; 768 x 576 pixels) under a microscope at a magnification of ×5. Images of the examined area were then directly transferred to a computer equipped with an image analyzer (KS-300; Carl Zeiss, Germany), and the areas of the periodontal ligament (total areas) were extracted on a monitor using an image analysis software. The total areas and areas with positive immunoreactivity were measured using an image analyzer, and the percentages of the latter to the total areas were calculated. The statistical significance of difference was assessed by an unpaired Student's t-test for comparison of PGP 9.5- versus calretinin-positive areas at the same postnatal period, and with a one-way analysis of variance and post-hoc comparison (Fisher's PLSD test) with the previous postnatal stage. A P of value less than 0.05 was considered a significant difference.

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**Fig. 1.** Diagram showing a sagittal section of the rat upper incisor used for image analysis. The periodontal ligament in the boxed area (tooth, alveolar bone and blood vessels excluded) is processed for the computerized image analysis. Thick nerve bundles (NB) are always situated below the center of the defined area. AB alveolar bone, D dentin, DP dental pulp, E enamel.
Fig. 2. Nerve fibers immunoreactive for PGP 9.5 (a, b) and for calretinin (c) in the upper incisor of a 1-day-old rat. a. The incisal edge (arrowheads) is located beneath the oral epithelium. PGP 9.5-positive thick nerve bundles (arrow) enter and ramify in the future lingual periodontal ligament. b. Higher magnification of the boxed area in a. PGP 9.5-positive nerve fibers, thin and beaded in appearance, run parallel to the tooth axis. No specialized ending is found at this stage. c. The calretinin-positive nerve fibers show a distribution pattern and terminal morphology similar to the PGP 9.5-positive fibers. AB alveolar bone, AM ameloblasts, EC epithelial cells, OB odontoblasts, DP dental pulp, PL future periodontal ligament. a: ×49; b, c: ×390
RESULTS

Immunohistochemical observations

The immunostaining with the antisera against PGP 9.5 and calretinin succeeded in demonstrating numerous nerve fibers in the sections examined. In contrast, antiserum against calretinin preabsorbed with the antigen did not show any neural elements in the lingual periodontal ligament or associated tissues. We therefore considered the immunoreactions for calretinin observed in this study to be specific for calretinin.

One day after birth (P1)

In 1-day-old rats, the incisor was located beneath the oral epithelium, preventing the incisal edges from reaching the oral cavity. Thick nerve bundles exhibiting PGP 9.5-immunoreactivity entered the space between the lingual aspect of the tooth germ and the alveolar bone, corresponding to the future lingual periodontal ligament (Fig. 2a). The PGP 9.5-positive nerve fibers, thin and frequently beaded in appearance, ran straight near the alveolar bone along the tooth axis to taper off without forming any specialized structures (Fig. 2a, b). On the other hand, immunohistochemistry for calretinin demonstrated a smaller number of nerve fibers in the lingual periodontal ligament. The distribution pattern and terminal morphology were identical to those reactive for PGP 9.5 (Fig. 2c).

Four days after birth (P4)

At this stage, the lingual periodontal ligament contained more cellular and fibrous elements, and could be divided into two regions: a richly vascularized alveolar half (alveolus-related part as designated by BEERTSEN et al., 1974) and avascular tooth half (tooth-related part) of the periodontal ligament, as have been shown in adult rats (cf. NAKAKURA-OISHIMA et al., 1993). The PGP 9.5-positive nerves increased in number and density in the lingual periodontal ligament in comparison with the earlier stage. The delicate nerve fibers extending from nerve bundles ramified twice or thrice to form tree-like terminals in the periodontal ligament (Fig. 3a). These nerve endings were found to concentrate in the mid-region of the lingual periodontal ligament, and frequently to have axonal swellings in their courses.

Calretinin-immunoreactive nerve fibers also increased in number and density, but appeared less frequently than the PGP 9.5-positive nerves observed at this stage. The calretinin-positive nerve fibers also branched out, but did not display an apparent dendritic ramification (Fig. 3b).
Seven to eleven days after birth (P7-11)

The incisal edges were either located beneath the oral mucosa or exposed to the oral cavity. After entering the lingual periodontal ligament, the PGP 9.5-positive nerve fibers branched out repeatedly to show a tree-like ramification in the mid-region of the lingual periodontal ligament (Fig. 4a). A major population of nerve fibers, beaded in appearance, terminated as a tree-like ramification without expanded terminals in the periodontal ligament. In addition to these endings, some tree-like endings showed different terminal morphology, they were equipped with knob or bell-shaped terminal buttons. At high magnifications, these expanded nerve terminals revealed an irregular outline with fine thread-like projections, reminiscent of Ruffini endings as shown in the periodontal ligament of adult rats (Fig. 4a).

Calretinin-immunoreactive nerve fibers also appeared to increase in number day by day, but they were considerably lower in density than the PGP 9.5-positive nerves. They simply retained their thin and beaded appearance, and did not display dendritic ramifications as seen by PGP 9.5-immunostaining. The calretinin-reactive nerve fibers tapered off in the periodontal collagen fibers without forming any specialized structures (Fig. 4b).

Fifteen to eighteen days after birth (P15-18)

Attrition was recognizable in the incisal edges, suggesting the commencement of occlusion between the upper and lower incisors. Immunohistochemistry for PGP 9.5 demonstrated that a denser distribution of dendritic endings with expanded portions was present

Fig. 5. Immunoreactivities for PGP 9.5 (a, c, d) and for calretinin (b, e, f) in the lingual periodontal ligament at 15 days after birth (a, b) and 25 days after birth (c-f). a. Two types of PGP 9.5-positive nerve endings are recognized: tree-like endings without any expanded portions (arrow), and dendritic endings with expanded portions resembling periodontal Ruffini endings (arrowheads). b. No calretinin-positive expanded and dendritic terminals are found in the lingual periodontal ligament. c. PGP 9.5-positive typical Ruffini endings and tree-like endings composed of thin nerves (arrow) are intermingling in the periodontal ligament of a 25-day-old rat. d. Higher magnification of the boxed area in c. The typical Ruffini endings show irregular outlines due to many microprojections (arrowheads). e. A calretinin-positive dendritic terminal first appears in the mid-region of the periodontal ligament. f. Higher magnification of the boxed area in e. The dendritic nerve ending has fine thread-like projections (arrowheads). BV blood vessels, PL periodontal ligament. a, b, c, e: ×390, d, f: ×980
Fig. 5. Legend on the opposite page.
Fig. 6. Legend on the opposite page.
in the mid-region of the lingual ligament; the terminal formation had progressed more actively than at the previous stage (Fig. 5a). However, no expanded or dendritic terminals immunoreactive for calretinin were found in the lingual periodontal ligament (Fig. 5b).

**Twenty-four to twenty-six days after birth** (P24-26)

By this stage, the occlusion between the upper and lower first molars had started and typical Ruffini endings showing PGP 9.5-immunoreactivity increased in number and density (Fig. 5c). The periodontal Ruffini endings with irregular outlines appeared to have numerous microprojections, giving them a ruffled appearance (Fig. 5d). A large number of thin nerve fibers showing a tree-like ramification were also recognized, occupying the spaces between the typical Ruffini endings (Fig. 5c). At this stage, a small number of dendritic terminals showing calretinin-immunoreactivity were first recognizable in the alveolus-related part at the mid-region of the periodontal ligament (Fig. 5e). Their axonal branches reactive to calretinin were thick and resembled the periodontal Ruffini endings. They had fine thread-like projections, appearing as a ruffled outline (Fig. 5f). Thin and beaded nerve fibers positive for calretinin were still found to terminate in the periodontal ligament.

**Sixty to eighty days after birth** (P60-80)

The functional occlusion had been established between the upper and lower molars by this period. A dense distribution of both PGP 9.5- and calretinin-immunopositive nerve fibers was present along the entire length of the lingual periodontal ligament (Fig. 6a, b). Immunostaining with PGP 9.5 was able to show two types of nerve endings at this stage: Ruffini endings with numerous microprojections, and less-arborized nerve endings with smooth surfaces (Fig. 6c). The latter type of ending showing PGP 9.5 immunoreactivity was located in the middle region of the lingual periodontal ligament in the vicinity of the typical Ruffini endings. This type consisted of thin PGP 9.5-positive fibers, frequently beaded in appearance, and lacking the expanded terminal buttons (Fig. 6a). However, calretinin immunohistochemistry failed to demonstrate any less arborized nerve terminals with a smooth surface (Fig. 6b, d).

**Quantitative analysis**

Since it was hard to obtain three sections per animal at P1 due to the small size of the tooth buds, we analyzed the data from rats older than 4 days. Figure 7 shows the results of the temporal changes in the percentages of immunoreactive areas for PGP 9.5 and calretinin.

The percentage of immunoreactive areas for PGP 9.5 in the restricted area of animals at P4 was 5.7±1.9% (mean±S.E.M.). The percentage for PGP 9.5-immunoreactive areas increased significantly to 14.6±3.7% at P7-10. Thereafter, the percentage increased gradually (17.4±0.7% at P15-18, 19.3±0.6% at P24-26, and 21.8±0.5% at P60-80). There were no significant differences from the previous stage.

The percentage for the calretinin immunoreactive area around P4 was 3.9±1.2%, and increased gradually but with no significant difference from the previous stage (3.6±0.7% at P7-10, 4.6±1.7% at P15-18 and 5.9±0.4% at P24-26). At P60-80, the immunoreactive area increased 10.6±1.6%, which was significantly greater than that of P24-26.

The percentage of the immunoreactive areas for PGP 9.5 was significantly larger than that for calretinin at the same postnatal period, except at P4.

**DISCUSSION**

The present immunohistochemical study demonstrated that periodontal Ruffini endings exhibit intense calretinin-immunoreactivity in the incisor of mature rat, as shown in previous studies (ICHIKAWA et al., 1997; OCHI et al., 1997). Furthermore, this chronological examination revealed a time lag in appearance between PGP 9.5- and calretinin-immunoreactivity in the periodontal Ruffini endings.

The periodontal ligament contains two types of sensory nerve fibers including free nerve endings and...
Ruffini endings, and receives perivascular sympathetic innervation from the surperior cervical ganglion (for reviews, BYERS and MAEDA, 1997; MAEDA and OHISHIMA, 1998; MAEDA et al., 1999). In the present study, we found that numerous PGP 9.5-immunoreactive neural elements were present as compared with calretinin-immunoreactive structures in the mature animal (P60-80); this was confirmed by the quantitative analysis, indicating that calretinin is present in a subpopulation of primary afferents in the periodontal ligament for the following reasons.

BYERS (1985) firstly classified the periodontal Ruffini endings into two types according to their morphological configurations: 1) Type I, enlarged terminals with many axonal fingers called axonal spines or fingers (MÜNGER and IDE, 1988); and 2) Type II, thinner with fewer axonal spines. In the present study, delicate dendritic terminals with smooth outlines demonstrated by PGP 9.5-immunostaining were devoid of calretinin-immunoreactivity, and the Ruffini endings with calretinin-immunoreactivity had ruffled-ended profiles due to the existence of numerous axonal spines (NAKAKURA-OHISHIMA et al., 1995). Immunoelectron microscopic observation showed that calretinin-positive Ruffini endings had typical axonal spines in the lingual periodontal ligament of adult rats (OCHI et al., 1997b). Taken together, the periodontal Ruffini endings immunoreactive for calretinin can be assumed to be Type I Ruffini endings. Secondly, free nerve endings composed of thin nerve fibers contained PGP 9.5, but lacked calretinin-immunoreactivity. This observation was in agreement with previous reports that calretinin-immunoreactivity is not present in small-sized neurons, but in medium-sized to large neurons in rat trigeminal ganglion (ICHIKAWA et al., 1994), which represents the origins of periodontal primary afferents. Finally, no autonomic nerve fibers distributed around the blood vessels showed calretinin-immunoreactivity in the periodontal ligament. We conclude, therefore, that calretinin is present in a subpopulation of the primary afferents, in particular, Type I Ruffini endings in the periodontal ligament of the incisor of the mature rat.

The present results of PGP 9.5-immunostaining in the periodontal ligament during postnatal development are in line with our previous studies (NAKAKURA-OHISHIMA et al., 1993, 1995). Furthermore, the present quantitative analysis clearly showed that PGP 9.5-immunoreactive areas drasti-
cally increased at P7-11 when the incisal edges were exposed to the oral cavity, suggesting that tooth eruption is one of the essential factors for the differentiation and maturation of the periodontal Ruffini endings. As one of the physiological properties, many researchers have pointed out the direction sensitivity of the periodontal mechanoreceptors (for review, Linden, 1990). In the rat incisor, lingual-labial pressure stimulation can more easily evoke the periodontal-masseteric muscle reflex (Goldberg, 1971) than labiobuccal stimulation (Funakoshi and Amano, 1974; Taguchi, 1984). Another physiological study on the periodontal-masseteric muscle reflex in the rat reported that the direction sensitivity was established after 30 postnatal days (Takamiya, 1985), when the morphology and the number of the periodontal Ruffini endings are almost identical to those seen in mature rats as shown in this and our previous studies (Nakakura-Ohshima et al., 1993, 1995). These lines of evidence indicate that the functional maturation of the periodontal mechanoreceptor is followed by morphological maturation.

It is interesting that the appearance of calretinin-immunoreactivity in the periodontal Ruffini endings was delayed in comparison with that of PGP 9.5-immunoreaction; calretinin-immunoreactive areas were almost at the same level by P24-26 when typical Ruffini endings were abundantly found as in PGP 9.5-immunohistochemistry, and increased rapidly after commencement of the occlusion between the upper and lower first molars. Although the function of calretinin has not been understood in detail in the periodontal Ruffini endings, histochemical (Tachibana and Nawa, 1992, 1994; Tachibana et al., 1992) and immunocytochemical (Ichikawa et al., 1997; Ochi et al., 1997b) observations have suggested the possible involvement of this calcium binding protein in Ca²⁺ homeostasis in the axon terminals of the periodontal Ruffini endings against external stimuli such as constant forces and the remodeling of the collagen fibers along with others. Taken together, it is reasonable to consider that the delayed appearance of calretinin-immunoreactivity might be related to the functional maturation of the periodontal Ruffini endings.

This idea is strongly supported by physiological findings on the postnatal development of the jaw closing reflex, where the number of impulses of the masseter muscle reflex activity in mature rats (60 days after birth) was recorded more than two times, in comparison with that in 2-week-old rats (Takamiya, 1985). A similar delayed appearance of calretinin-immunoreactivity was reported during the regeneration process of the periodontal Ruffini endings in our recent experimental study (Hirosima et al., 1998); the number and terminal morphology of regenerated Ruffini endings showing PGP 9.5-immunoreactivity were comparable to those in normal animals around 14-21 days following resection of the inferior alveolar nerve, whereas the regenerated calretinin-immunoreactive Ruffini endings increased in number to return to normal by 56 days after nerve injury. It is likely that the functional completion of the periodontal Ruffini endings occurs after the morphological completion of their terminals, and that calretinin might be one of the markers for the functional maturation of the periodontal Ruffini endings.

The location of the somata of the periodontal Ruffini endings showing calretinin-immunoreactivity in the rat incisor has remained unclear, though the periodontal ligament in the cat has been proved to receive afferent fibers from both trigeminal ganglion neurons and mesencephalic trigeminal neurons (cf. Byers and Maeda, 1997). Previous studies have shown that some neurons both in trigeminal ganglion and mesencephalic trigeminal nucleus are immunopositive for calretinin (Arai et al., 1991; Ichikawa et al., 1992, 1994; Résibois and Rogers, 1999). Physiological experiments have demonstrated that action potentials can be clearly recorded from mesencephalic trigeminal neurons as well as from trigeminal ganglion neurons when mechanical stimuli is applied to the rat incisor (Amano and Iwasaki, 1982). On the other hand, an electron microscopic study combined with an autoradiographic technique has demonstrated that periodontal Ruffini endings in the rat incisor are labeled with the anterograded tracer (³H proline) injected into the trigeminal ganglion, suggesting that the somata of some periodontal Ruffini endings in the incisor are located in the trigeminal ganglion (Byers and Dong, 1989). Our recent immunocytochemical study has demonstrated that S-100 protein β, another calcium binding protein, was present in the axon terminals of Type II Ruffini endings in the periodontal ligament of the rat (Nakakura-Ohshima et al., 1998), and that the origin of S-100 β-immunopositive Ruffini endings seemed to be the trigeminal ganglion since the neurons in the mesencephalic trigeminal nucleus rarely showed S-100 β-immunoreactivity. We therefore suggested that the origin of calretinin immunoreactive Ruffini ending might also be the trigeminal ganglion, but further combined neuronal tracing and immunohistochemical technique will determine whether periodontal Ruffini endings immunopositive for calretinin have their somata in the mesencephalic trigeminal nucleus, which is an important relay site for various oral reflexes.
REFERENCES


