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Effect of experimental tooth movement on nerve fibres immunoreactive to calcitonin gene-related peptide, protein gene product 9.5, and blood vessel density and distribution in rats

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SUMMARY The effect of experimental tooth movement on nerve fibres immunoreactive to calcitonin gene-related peptide (CGRP) and to protein gene product (PGP) 9.5 were studied, as well as the coincidence of these responses with changes in blood vessel density and distribution in the periodontal ligament (PDL) and pulp of young Wistar rats. The first right maxillary molar was moved mesially by an orthodontic appliance for 3, 7, 14 and 21 days. Sagittal and horizontal serial sections were incubated alternately with antibodies to CGRP, PGP 9.5 and laminin. Nerve and blood vessel density and distribution between the experimental and control sides were compared in the apical and cervical PDL, and in root and coronal pulp.

The most pronounced changes occurred in the 7 day group. CGRP and PGP 9.5 immunoreactive nerves in the apical PDL showed increased density, being distributed towards the alveolar bone and frequently found in bone resorption lacunae. Numerous nerve fibres were often present adjacent to hyalinized tissue, but were never found near or within root resorption lacunae. Nerve sprouting was also present both in the root and coronal pulp. Increased nerve and blood vessel density generally coincided with each other. At day 14, periodontal nerves and blood vessels were still disorganized compared with the controls. Tissues near cellular cementum and root resorption lacunae were consistently devoid of nerve fibres. After 21 days, PDL nerve and blood vessel density and distribution were nearly at control level. However, nerve fibres were regularly found inside root resorption areas.

In conclusion, experimental tooth movement induces dynamic changes in density and distribution of periodontal and pulpal nerve fibres, indicating their involvement in both early stages of periodontal remodelling and later in the regenerative processes of the PDL, generally occurring in concerted action with modulation of blood vessels.

Introduction

The application of mechanical stress to teeth and their supporting tissues induces a series of responses that are inflammatory in nature (Davidovitch *et al.*, 1988; Cooper and Sims, 1989; Györfi *et al.*, 1994; Vandevska-Radunovic *et al.*, 1994). Experimental evidence clearly indicates that peptidergic sensory nerves are implicated in the modulation of inflammatory reactions by serving not only a sensory role, but also taking part in local effector mechanisms. Stimuli caused by mechanical or chemical irritants, or electrical tooth stimulation, lead to release of neuropeptides in peripheral tissues, upon which direct or indirect effects are exerted (Payan *et al.*, 1984; Holzer, 1988; Mantyh *et al.,* 1989; Fazekas *et al.,* 1990; Laurenzi *et al.*, 1990; Olgart *et al.*, 1993; Heyeraas *et al.*, 1994).

Dental pulp and periodontium are richly supplied by sensory nerve fibres, most of them being immunoreactive to calcitonin gene-related peptide (CGRP) (Silverman and Kruger, 1987; Casasco *et al.*, 1990; Heyeraas *et al.*, 1993). *In vitro* and *in vivo* investigations have shown that CGRP is a multipotent neuropeptide that contributes to the neurogenic component of inflammation by having a vasodilatory effect (Brain *et al.*, 1985; Gazelius *et al.*, 1987), by

stimulating proliferation of endothelial cells (Hægerstrand *et al.*, 1990), by modulation of immune cell function (Payan *et al.*, 1987; Foreman, 1987; Fristad *et al.*, 1995) and by inducing upregulation of endothelial adhesion molecules (Smith *et al.*, 1993). Proliferation of nerves immunoreactive to CGRP has been demonstrated subsequent to dentine and pulpal exposure (Byers *et al.*, 1990; Grutzner *et al.*, 1992), traumatic occlusion (Kvinnsland and Heyeraas, 1992) and short-term orthodontic tooth movement (Kvinnsland and Kvinnsland, 1990; Saito *et al.*, 1991), implying the involvement of these fibres in tissue injury as well as in tissue repair.

In addition to these roles CGRP is shown to have inhibitory potency on bone resorption (Yamamoto *et al.*, 1986; Zaidi *et al.*, 1987) and an osteogenic stimulating effect (Bernard and Shih, 1990). In view of the fact that orthodontic forces induce a variety of tissue reactions such as increased blood flow (Kvinnsland *et al.*, 1989; Vandevska-Radunovic *et al.*, 1994), cellular extravasation, bone resorption and apposition with periodontal fibre reattachment (Rygh, 1973), it is reasonable to expect that nerve fibres may participate in the modulation of these tissue responses incident to orthodontic tooth movement.

Although most of the sensory nerve fibres innervating teeth and their supporting tissues contain CGRP (Kvinnsland *et al.*, 1992; Heyeraas *et al.*, 1993), there are other nerve fibres of sensory and sympathetic origin supplying these tissues. It has been shown that protein gene product (PGP) 9.5—a general cytoplasmic marker of neurons and neuroendocrine cells—visualizes the entire tissue innervation (Day and Thompson, 1987; Wilson *et al.*, 1988; Wang *et al.*, 1990).

The primary goal of this study was to investigate the effect of orthodontic tooth movement on periodontal and pulpal nerve fibres immunoreactive to CGRP as well as on the complete tissue innervation. Knowing that nerve fibres are often intimately associated with blood vessels, it was of additional interest to study the effects of orthodontic tooth movement on blood vessel density and distribution, as well as to evaluate whether neural and vascular responses coincide and co-localize with the known remodelling processes.

Table 1 Distribution of rats used for evaluation at 3, 7, 14 and 21 days after experimental tooth movement.

Experimental period (days)	Number of rats
3	7
7	12
14	11
21	5

Materials and methods

Animals

A total of 35 Wistar male rats, 160–170 g in weight, were used in this study (Table 1). All animals were housed in polycarbonate cages in a conventional animal room. Standard pellet diet (RMI, expanded SDS, U.K.) and tap water were given *ad libitum*.

Experimental procedure

The experiments were carried out with the approval and under the supervision of the Norwegian Experimental Animal Board (NEAB) and were registered by the Board.

The operations were performed under anaesthesia for which purpose subcutaneous injection of fentanyl/fluanison midazolam (Hypnorm Dormicum), 0.2 ml/100 g body weight, was used. An orthodontic appliance, consisting of a coil spring ligated to the first molar connecting it to an orthodontic band cemented onto the incisors, and exerting a force of approximately 50 g, was inserted (Vandevska-Radunovic *et al.*, 1994). The right maxillary molar was moved mesially for 3, 7, 14 and 21 days, while the left, unoperated side served as control. All the rats were weighed before they were killed, and all showed an increase in body weight.

At the end of each experimental period the rats were reanaesthetized with an overdose of Hypnorm Dormicum and transcardiacally perfused with heparinized phosphate buffer, followed by 4 per cent paraformaldehyde and 0.2 per cent picric acid in 0.1 M phosphate buffer,

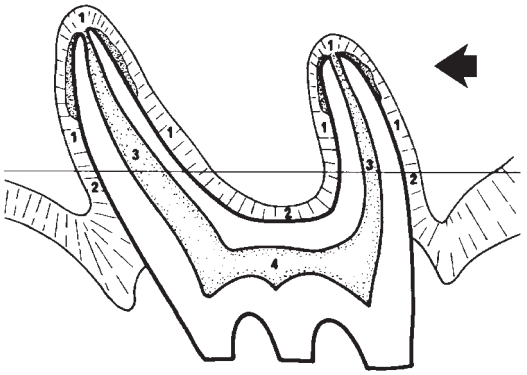

Figure 1 Areas of the first maxillary molar used for evaluation: 1, apical half of the PDL; 2, cervical half of the PDL; 3, root pulp; 4, coronal pulp. Arrow indicates the force direction.

pH 7.4. The jaws were excised and postfixed for 1 day in the same fixative as was used for perfusion, and thereafter demineralized for 4 weeks at 4°C in ethylenediaminetetraacetate (EDTA), containing 7.5 per cent polyvinylpyrrolidone (PVP; Aldrich Chemics, Steinheim, Germany) (Jonsson *et al.,* 1986). After demineralization the jaws were immersed in 30 per cent sucrose in 0.1 M phosphate buffer, pH 7.4, and then serially sectioned, sagittally or horizontally, at 40 μm on a freezing microtome.

Immunohistochemistry

For the purpose of immunohistochemical labelling, free floating sections in tissue-culture wells were used as described by Kvinnsland *et al.* (1991). The sections were alternately incubated for 72 hours in CGRP polyclonal antibody (1:7500 dilution, Cambridge Research Biochemicals, Cambridge, UK), in polyclonal PGP 9.5 antibody (1:8000 dilution, Ultra-Clone Ltd, Cambridge, UK) and in laminin antibody (1:10 000 dilution, Calbiochem Corporation, San Diego, CA, USA).

Antigen–antibody complexes were detected by the avidin–biotin peroxidase (ABC) method, using a commercially available kit (Vectastain ABC kit, Vector Laboratories, Burlinhame, CA, USA) and visualized by 3,3′-diaminobenzidine (DAB) in the presence of 0.2 per cent $(NH_4)_2Ni(SO_4)_26H_2O$ to enhance the immunostaining. Finally, the sections were counterstained with methylene blue/azure II, coverslipped and analysed in a light microscope. The specificity of the immune reaction was tested by omitting the primary antibody, and substituting it with PBS. In these sections, specific immunostaining was not observed.

Table 2 Evaluation of the density of CGRP- and PGP 9.5 immunoreactive nerve fibres in given tissue locations (Figure 1) in experimental maxillary first molars, 3, 7, 14 and 21 days after mesial tooth movement in rats, compared with contralateral controls.

Tissue location	Experimental period (days)			
	3	7	14	21
Apical half of the PDL	0*	+	+	0**
Cervical half of the PDL	0	+	0	0**
Root pulp	0	+	0	0***
Coronal pulp	0	+	0	0

0, equal to the control side; +, increased density compared with the control side.
*Two out of seven animals showed increased nerve fibre density.
**Immunoreactive nerve fibres in root resorption lacunae.
***Local nerve sprouting adjacent to reparative dentine.

Evaluation procedure

Longitudinal and horizontal serial sections of the right and left first maxillary molars were examined under light microscopy. Experimental and control sections showing the most dense innervation in the various tissue locations were used for evaluation. CGRP and PGP 9.5 immunoreactive nerve fibre density, morphology and distribution in experimental and control tissues were compared in the following regions: (1) the apical half of the PDL; (2) the cervical half of the PDL; (3) the root pulp and (4) the coronal pulp (Figure 1). The assessment of immunoreactive nerve density was graded as (0) equal to and (+) increased compared with the controls (Table 2). From serial sections immunolabelled with an antibody to laminin, the corresponding regions were used to study blood vessel density and distribution. The evaluation was undertaken by two independent observers.

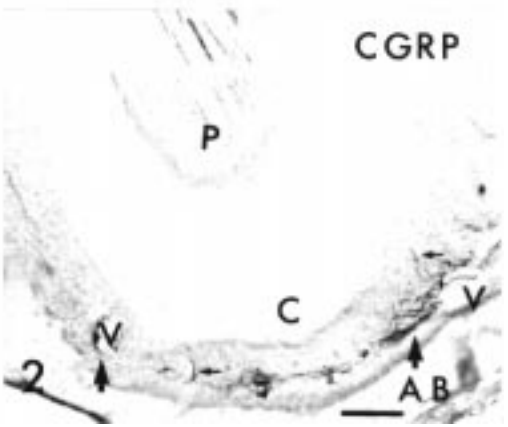

Figure 2 Periapical tissues of a control distal root displaying CGRP immunoreactive nerves (arrows) around blood vessels (V) with some individual fibres (small arrows) extending towards the cellular cementum (C). AB, alveolar bone; P, pulp; bar = 0.1 mm.

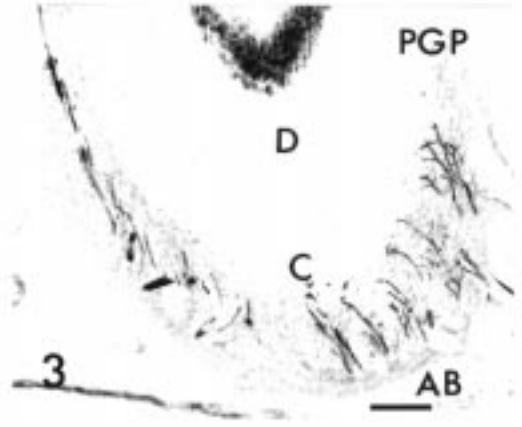

Figure 3 PGP 9.5 immunoreactive nerve fibres in the apical PDL of a control tooth, distal root. A number of nerve fibres are situated proximal to the cellular root cementum (C). AB, alveolar bone; D, dentine; bar = 0.1 mm.

Results

The animals included in this investigation were 6–7 weeks old at the beginning of the experiment. Accordingly, the nerve fibre morphology in all dental tissues was not mature in appearance, having less branching and density than in adult and older rats (Naftel *et al*., 1994; Hildebrand *et al*., 1995).

Nerve fibres

Control side. When compared with the complete dental innervation demonstrated by an antibody to PGP 9.5, the majority of the immunolabelled nerves were CGRP positive and showed similar tissue distribution.

In the apical PDL, CGRP as well as PGP 9.5 immunoreactive nerve fibres mainly followed, and were closely located to blood vessels (Figure 2). Only some fibres extended towards the root surface and the cellular cementum (Figure 3). In the cervical half of the PDL sparse immunoreactive nerves were found, and a few individual fibres were occasionally juxtaposed to the cementum (Figures 3 and 4).

Malassez epithelial cells were regularly distributed in the PDL, close to the root cementum, and occasionally containing cells immunoreactive to PGP 9.5.

In the root pulp, nerve bundles mainly

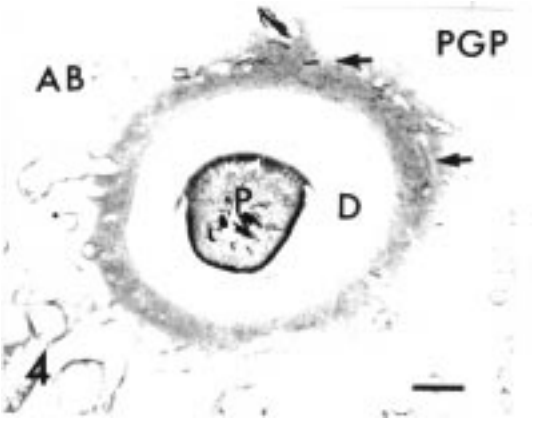

Figure 4 Horizontal section of a control distal root, coronal half of the PDL. Sparse distribution of PGP 9.5 immunoreactive nerve fibres (arrows) which are mainly located in the distal part of the PDL. AB, alveolar bone; P, pulp; D, dentine; bar, 0.1 mm.

followed blood vessels parallel to the long axis of the root, showing almost no branching. After entering the coronal pulp, they ramified, forming a subodontoblastic plexus of fine fibres, some of which terminated in the dentinal tubules (Figures 5 and 6). The odontoblasts regularly displayed immunoreactivity to PGP 9.5 (Figure 6).

Experimental side

Three days. Few changes could be observed in nerve fibre density, morphology and distribution in any of the investigated regions compared with

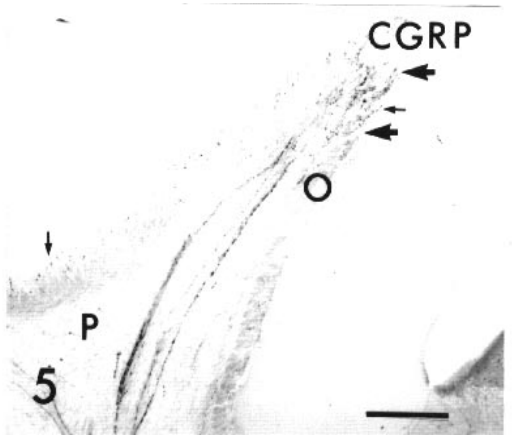

Figure 5 Longitudinal section from the distal pulp horn (P) of a control tooth. CGRP containing nerves (arrows) are distributed in the odontoblastic layer (O) with some fibres (small arrows) in dentinal tubules. Compared with the complete innervation in the serial section shown in Figure 6, most of the pulpal nerve fibres are immunoreactive to CGRP. Bar = 0.1 mm.

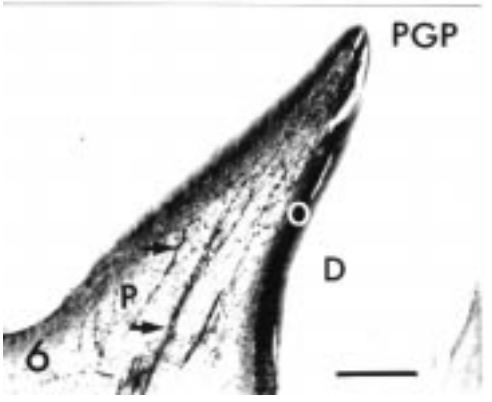

Figure 6 Serial section to Figure 5. Distribution of PGP 9.5 immunoreactive nerve fibres in the distal pulp horn (P) of a control tooth. Nerve fibres (arrows) and odontoblasts (O) are immunoreactive to PGP 9.5. D, dentine; bar = 0.1 mm.

the controls. Only two out of seven animals showed increase in nerve density in the apical periodontal region (Table 2), while cervical PDL, root and coronal pulp nerve fibres demonstrated no changes in distribution and density.

Seven days. After 7 days of tooth movement, nerve fibres on the experimental side showed a marked increase in density (Table 2), as well as changes in distribution and morphology compared with the control side, in all areas investigated.

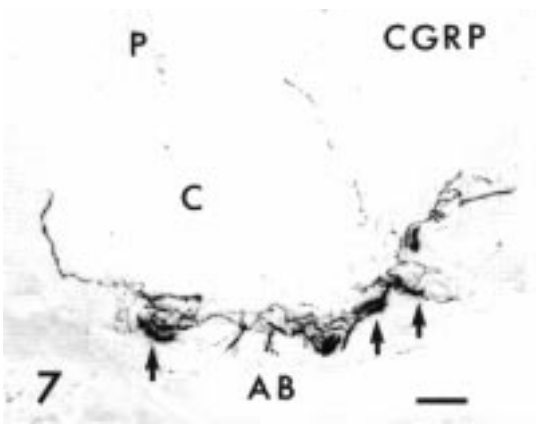

Figure 7 Apical PDL of an experimental tooth, 7 days. Increased density of CGRP immunoreactive nerve fibres (arrows) closely distributed towards the alveolar bone (AB). C, cementum; P, pulp; bar = 0.1 mm.

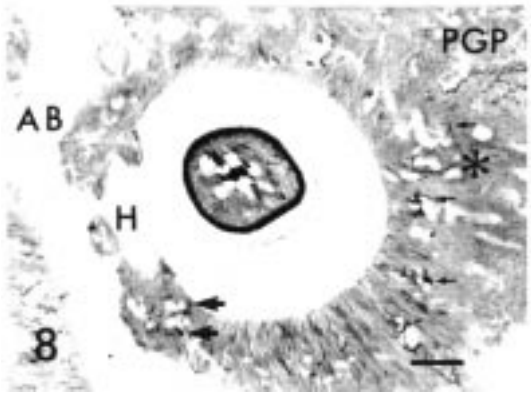

Figure 8 Seven days after experimental tooth movement numerous nerve sprouts (small arrows) are seen in the distal widened tension area (*) of a distal root. A number of nerve fibres (arrows) are present close to the hyalinized tissue (H). AB, alveolar bone; bar = 0.1 mm.

In the apical PDL, thick, beaded nerve fibres, with no regular arrangement, were densely distributed close to the alveolar bone (Figure 7) while branched nerve endings, located against cellular root cementum as seen in the controls (Figure 3), were absent. CGRP and PGP 9.5 immunoreactive nerve fibres were frequently found in the tension area of the distal root, following blood vessels and running proximal to the bone surface (Figure 8). Occasionally, a number of immunolabelled nerve fibres were found in periodontal areas close to hyalinized

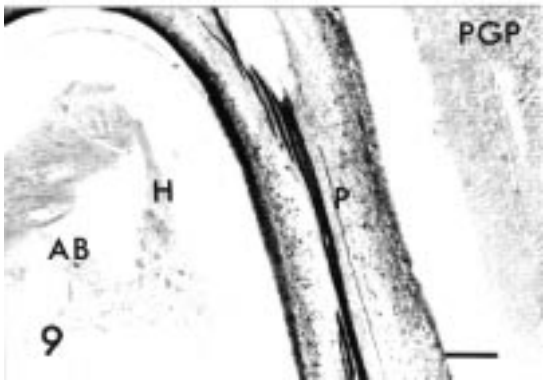

Figure 9 Longitudinal section of experimental tooth, distal root, coronal part of PDL, 7 days after orthodontic tooth movement. Hyalinized tissue (H) is nearly devoid of PGP 9.5 immunoreactive nerves in its periphery. AB, alveolar bone; P, pulp; bar, 0.1 mm.

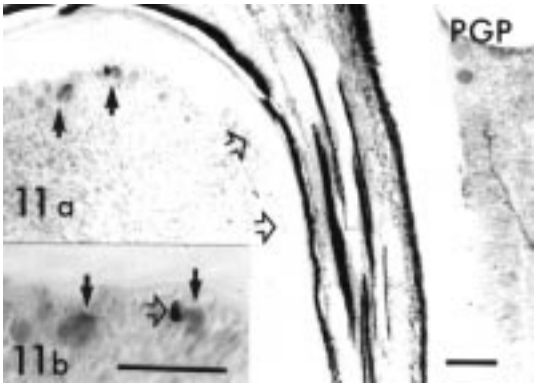

Figure 11 (a) Serial section of Figure 10. The root resorption lacuna (open arrows) lacks PGP 9.5 immunoreactive nerve fibres. Large clusters (arrows) of Malassez epithelium are seen. Bar = 0.1 mm. (b) Enlarged area from (a) Proliferated Malassez epithelium (arrows) containing PGP 9.5 immunoreactive cells (open arrow). Bar = 0.1 mm.

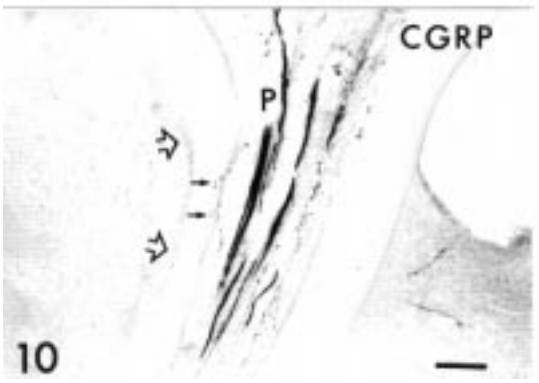

Figure 10 Seven days after experimental tooth movement extensive root resorption lacunae (open arrows) are seen in the coronal PDL of a distal root without CGRP immunoreactive nerve fibres. Pulpal tissue (P) shows sprouting of CGRP containing nerves, with fibres (arrows) in root dentine, adjacent to root resorption. D, dentine; bar = 0.1 mm.

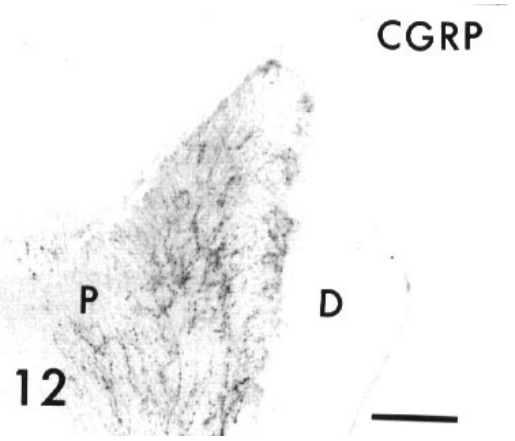

Figure 12 Experimental tooth, distal pulp horn (P), 7 days after experimental tooth movement. Extensive sprouting of pulpal CGRP immunoreactive nerve fibres is seen. D, dentine; bar = 0.1 mm.

tissue (Figure 8), but never near or within root resorption zones (Figures 10 and 11a). In the pulpal tissue opposite either hyalinized tissue or root resorption lacunae, the nerve fibres showed regularly intensified immunoreactivity (Figures 9, 10 and 11a).

In contrast to the control side, the experimental PDL displayed numerous, enlarged clusters of Malassez epithelial cells, occasionally containing cells immunoreactive to PGP 9.5. This was most clearly demonstrated in the bifurcation area, as well as in the tension zone distal to the distal root (Figure 11a,b).

Compared with the controls, increased CGRP and PGP 9.5 nerve fibre density was found both in the root (Figures 9, 10 and 11a) and in the coronal pulp (Table 2). The pulpal horns demonstrated nerve sprouting and intensified immunolabelling (Figures 12 and 13). The pulpal tissues adjacent to the cervical region and opposite to the ligature displayed numerous nerve sprouts (Figure 14).

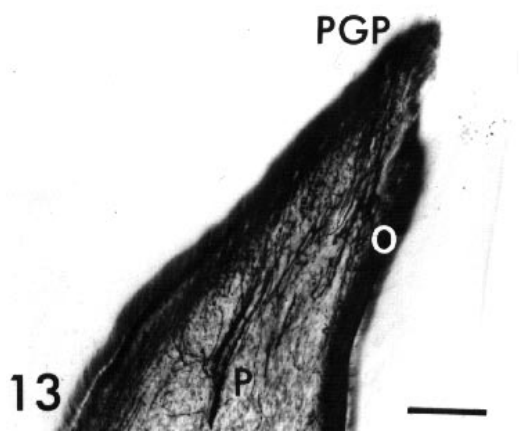

Figure 13 Compared with the contralateral control tooth in Figure 6, a pronounced increase of PGP 9.5 immunoreactive nerve fibre density is found in the distal pulp horn (P) at day 7. O, odontoblastic layer; bar = 0.1 mm.

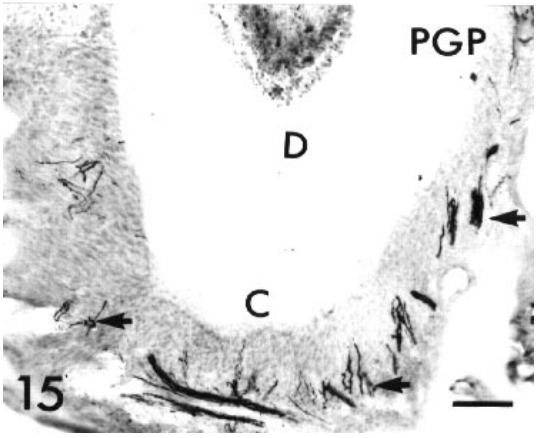

Figure 15 At 14 days PGP 9.5 immunoreactive nerves (arrows) are disorganized compared with the control tooth in Figure 3. Although the fibres show a tendency to return to the control level, they still avoid tissues near the cellular cementum (C). D, dentine; bar, 0.1 mm.

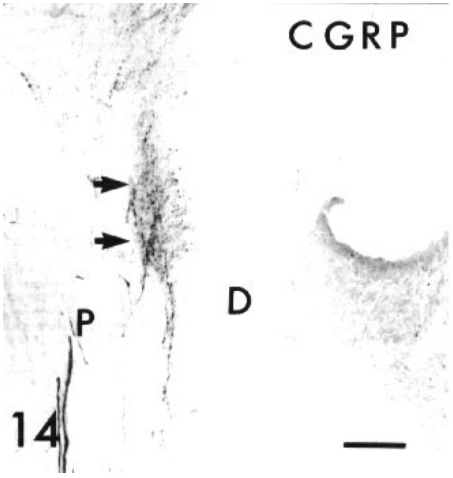

Figure 14 Experimental tooth, 7 days. Extensive sprouting of CGRP containing nerve fibres (arrows) in pulp tissues (P) adjacent to the ligature in the distal gingiva. Cervial PDL is devoid of CGRP immunoreactive nerves. D, dentine; bar = 0.1 mm.

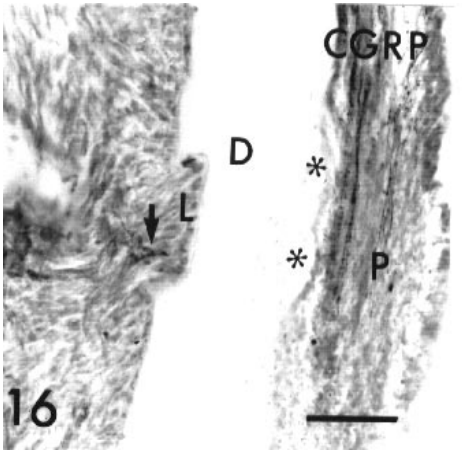

Figure 16 Three weeks after experimental tooth movement CGRP immunoreactive nerve fibres (arrow) are found inside root resorption lacunae (L). Reparative dentine (*) is formed opposite to the resorption cavity in the mid-root area. D, dentine; P, pulp; bar = 0.1 mm.

Fourteen days. Although the periodontal nerve fibres showed a tendency to reorganize at this experimental period, there were still apparent changes in the periapical nerve distribution with an increase in density (Table 2). Compared with the controls (Figure 3), the nerve fibres in the apical PDL (Figure 15) were mainly situated close to the alveolar bone, but were about to approach the cellular cementum. Remnants of hyalinized periodontal tissue were still observed in some sections, but nerve fibres were now very rarely seen in their vicinity. No nerve fibres could be found close to, or within root resorption areas. Enlarged clusters of Malassez epithelial cells were occasionally observed in the distal cervical PDL. Root and coronal nerve fibres showed no differences in density and distribution compared with the controls.

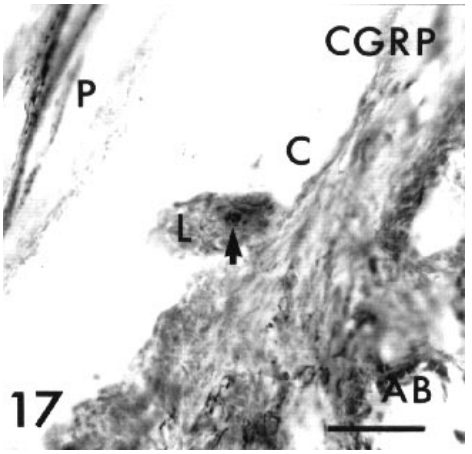

Figure 17 CGRP positive nerve fibres (arrow) located inside root resorption lacuna (L) in the cellular cementum (C) seen three weeks after the beginning of experimental tooth movement. AB, alveolar bone; P, pulp; bar = 0.1 mm.

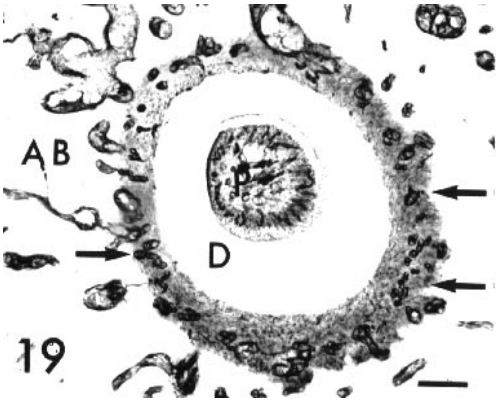

Figure 19 Horizontal section of a distal root of a first maxillary control molar showing the distribution of laminin immunoreactive blood vessels in the coronal PDL. The blood vessels (arrows) are mainly located in the middle part of the PDL and close to the alveolar bone (AB). D, dentine; P, pulp; bar, 0.1 mm.

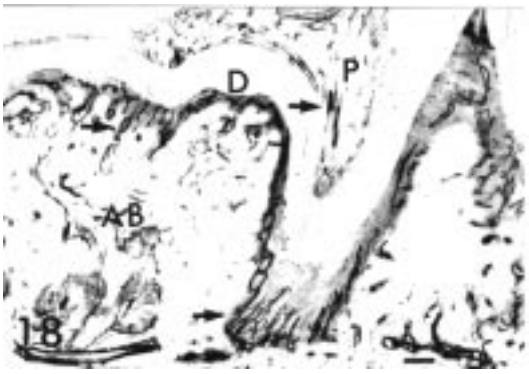

Figure 18 Distribution and density of laminin immunoreactive blood vessels (arrows) in tooth supporting tissues of distal root of a control molar. AB, alveolar bone; D, dentine; P, pulp; bar = 0.1 mm.

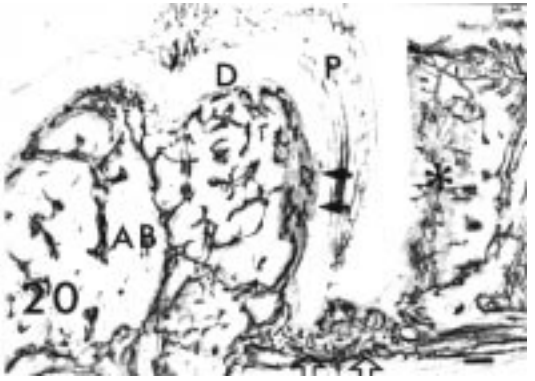

Figure 20. Seven days, experimental tooth, distal root. The PDL shows a widened tension zone (*) and proliferation of blood vessels in the periapical area (open arrows) and in the resorption lacuna (arrows). AB, alveolar bone; D, dentine; P, pulp; bar = 0.1 mm.

Twenty-one days. The periodontal and pulpal nerve supply was now regenerated and reorganized. The hyalinized tissue was completely removed, and the structure of the PDL seemed to be re-established compared with the controls. In three out of five animals, CGRP immunoreactive nerves were regularly found inside root resorption lacunae, proximal to cementoblasts, and at various root levels (Figures 16 and 17). Formation of reparative dentine could be seen opposite the resorption lacunae, with a localized sprouting response in the immunoreactive nerve fibres (Figure 16) (Table 2).

Blood vessels

Control side. The distribution of blood vessels generally followed a similar pattern as CGRP and PGP 9.5 immunoreactive nerve fibres in the investigated tissues. The blood vessels (Figures 18, 19) formed a dense, basket-like pattern in the apical part of the PDL and then ran axially towards the gingiva. In the cervical periodontal area, blood vessels were mainly

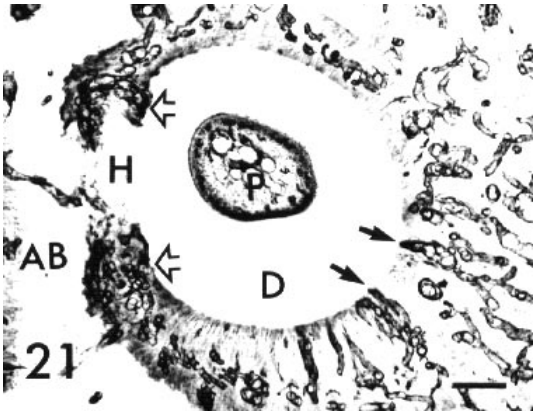

Figure 21 Horizontal section of a distal root, coronal part, at 7 days, showing numerous blood vessels (open arrows) surrounding hyalinized tissue (H) and also located close to the root cementum (arrows). AB, alveolar bone; D, dentine; P, pulp; bar = 0.1 mm.

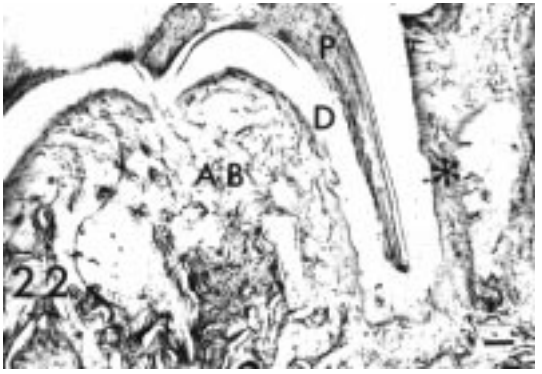

Figure 22 At 14 days, the number of blood vessels is reduced and the distal tension area (*) approaches the width of the control tooth (cf. Figure 20). AB, alveolar bone; D, dentine; P, pulp; bar = 0.1 mm.

located in the middle part of the PDL or closer to the alveolar bone. Generally, very few blood vessels immunoreactive to laminin were situated in the vicinity of the root surface. A few, larger blood vessels entered the root pulp and coursed parallel to the long axis of the root. In the coronal pulp, they branched and formed a network in the pulp periphery encircling the odontoblasts (Figures 18 and 19).

Experimental side. Obvious changes in blood vessel density and distribution were first seen at 7 days. The distribution of blood vessels was changed throughout the whole PDL when compared with the controls. In the widened tension zone of the PDL, blood vessels (Figures 20 and 21) ran not only in the middle and close to the alveolar bone, but also towards the root surface and ended in proximity to the cementum. Blood vessels were also seen in the resorption lacunae (Figure 20), and were densely distributed surrounding hyalinized tissue (Figure 21). Pulpal blood vessels showed almost no changes in density, morphology and distribution either in the root or the coronal region. At 14 days, PDL morphology was about to be re-established (Figure 22). The blood vessel density and distribution showed a tendency to normalize and were about to regain the same position as the control side (Figure 22). At 21 days, a nearly complete restoration of the blood vessel density and distribution was achieved.

Discussion

This study clearly shows that experimental orthodontic tooth movement in rats induces dynamic changes in nerve fibre and blood vessel density, morphology and distribution that correspond to the sequence of connective tissue responses in PDL and bone (Rygh, 1972; Brudvik and Rygh, 1993). At 3 days, only a limited increase in nerve fibre density was noticed, while at 7 days, consistent and marked changes occurred throughout all investigated tissue areas, involving an increase in both nerve and blood vessel density and distribution. Regenerative processes predominated at 14 days, although changes in periapical nerve distribution were still obvious. At 21 days the experimental side achieved nearly complete neural and vascular restoration, apart from the fact that nerve fibres were often found inside root resorption lacunae.

The most prominent changes within the nerve fibre and blood vessel compartments occurred 7 days after the onset of experimental tooth movement. Both the PDL and the pulp displayed a pronounced sprouting response of the nerve fibres containing CGRP and PGP 9.5. This concurs with the most active remodelling

processes of the paradental tissues, which include increase in blood flow (Vandevska-Radunovic *et al*., 1994), formation of new cellular and vascular elements (Rygh, 1972; Okhawa, 1982;) and alveolar bone remodelling with reattachment of the periodontal fibres (Rygh, 1973). The changes in CGRP immunoreactive nerve fibres were most evident in the apical periodontal region and in the tension area of the distal root, where concomitant proliferation of blood vessels occurred (Figures 7 and 8). Nerve sprouting and intensified immunolabelling was also found in the coronal and root pulp, clearly denoting a general pulpal tissue reaction as a response to experimental tooth movement, which agrees well with earlier reports (Kvinnsland and Kvinnsland, 1990; Norevall *et al.,* 1995).

In a previous investigation using the same experimental model (Vandevska-Radunovic *et al.*, 1994) the greatest increase in periodontal and pulpal blood flow was found 7 days after the onset of orthodontic force. The concomitant increase in nerve fibre and blood vessel density in the pulp and tooth supporting tissues as shown in the present study underlines the finding that both neural and vascular reactions may be part of the ongoing inflammatory response. Together, these findings may lead to the suggestion that sensory nerves play an important part in the various stages of tissue remodelling related to orthodontic tooth movement. The involvement of afferent nerves in the regulation of blood flow incident to other clinical and experimental dental procedures in cats has previously been documented (Olgart *et al.,* 1991).

Inflamed dental tissues have been shown to develop an increased density of blood vessels (Nakamura *et al*., 1986; Fristad *et al.,* 1995), which is in agreement with our present results. CGRP is found to have a stimulating effect on proliferation of endothelial cells *in vitro* (Hægerstrand *et al.,* 1990). The simultaneous increase in density of CGRP immunoreactive nerve fibres and blood vessels shown in this study strengthens the suggestion that formation of nerves and blood vessels may influence each other through activation of different cellular mechanisms that occur during inflammation.

Little information is available concerning nerve reaction patterns during orthodontic tooth movement except for responses related to short-term experiments in rats (Kvinnsland and Kvinnsland, 1990; Saito *et al*., 1991; Norevall *et al.,* 1995). Saito *et al*. (1991) found the maximum density and intensity of CGRP immunoreactivity after 3 days, while at 7 days immunoreactive nerves returned to the control level. This disagrees with the results from the present study which is most probably due to the difference in design of the orthodontic appliance used.

Out of the total innervation visualized by PGP 9.5, the majority of the nerve fibres contained CGRP. This finding agrees well with previous results in dental innervation of rat and cat (Kvinnsland *et al.,* 1992; Heyeraas *et al.,* 1993). The wide distribution of CGRP in nerve fibres compared with the total innervation underlines the importance of this neuropeptide in the biology of teeth and their supporting tissues. Extensive evidence indicates that CGRP takes part in multiple regulatory mechanisms (Holzer, 1988) and some of them may also modulate the complex tissue reactions incident to orthodontic tooth movement. In addition to its vasodilatory effect (Gazelius *et al*., 1987), recent findings have shown that immune cells express functional receptors for neuropeptides including CGRP (Umeda and Arizawa,1989; Abello *et al*., 1991; McGillis *et al*., 1991). Furthermore, CGRP is also shown to guide the traffic of immune cells through the blood vessel walls (Foster *et al.,* 1992; Smith *et al.,* 1993) and exert functional effects on macrophages (Nong *et al*., 1989) and leukocytes (Roch-Arveiler *et al*., 1986). Many of the immunocompetent cells that are functionally influenced by CGRP produce various cytokines, some of which are known to have elevated tissue levels in periodontium and bone incident to orthodontic tooth movement (Davidovitch *et al*., 1988). The pronounced sprouting of CGRP containing nerves in the periodontium at 7 days may thus explain, and strongly indicates, their participation in the early inflammatory responses and in the initial remodelling activities within the PDL.

In the vicinity of hyalinized tissue, which was densely surrounded by blood vessels, CGRP and

PGP 9.5 immunoreactive nerves were frequently seen at 7 days. At 14 days, the hyalinized zone was almost completely removed. Evidence presented by Rygh (1974) and Brudvik and Rygh (1994) shows that the removal of the necrotic periodontal tissue is initiated by macrophage- and fibroblast-like cells (Brudvik and Rygh, 1993). Bearing in mind the functional effect that CGRP and other neuropeptides have on macrophages (Peck, 1987; Nong *et al*., 1989), a possible indirect involvement of the immunoreactive nerves on hyalinized tissue removal can not be ruled out.

Seven days after experimental tooth movement most of the nerve fibres were seen close to the alveolar bone and entering bone resorptive lacunae. At the same experimental period, deposition of osteoid tissue on the alveolar bone surface has been found (Rygh *et al*., 1982), indicative of increased osteogenic activity. It has been reported that osteoblastic cell lines *in vitro* express receptors for several neuropeptides among which is CGRP (Bjurholm *et al*., 1992). The nerve sprouting, concomitant with the most active tissue remodelling period could further indicate that peptidergic nerves may act as modulators of bone cell activity and enhance formation of osteoid tissue during orthodontic tooth movement. The osteogenic stimulating effect of CGRP, together with its inhibitory potency of bone resorption, has been well documented both *in vivo* and *in vitro* (Yamamoto *et al*., 1986; Zaidi *et al*., 1987; Bernard and Shih, 1990; Hill *et al*., 1991).

The withdrawal of the nerve fibres from tissues close to the root surface seems to be concurrent with the onset of root resorption. At 7 and 14 days root resorption lacunae were devoid of nerves, but in the 21 day group CGRP immunoreactive nerve fibres were regularly found entering and located within root resorption areas and at various root levels. The distribution of the nerves inside resorption lacunae at this experimental period is coincident with the regenerative activity of the periodontal tissues. Myrick (1988) reported that root resorption in various animal species was causally related to stress situations. Subsequently, bone- or cementum-like substance partly repaired the root resorbed areas. Although he stressed the general, systemic correlation between root resorption and root repair, these findings can also imply local involvement of the sensory and sympathetic peripheral nerves in these events. It is therefore tempting to speculate that under physiological conditions, the nerve fibres might balance the resorptive and reparative activities of the hard tissue forming cells, and thus provide a protective role on the root surface. To be able to reveal more about this hypothesis, well-designed experimental studies are necessary.

It can be concluded that experimental tooth movement induces dynamic changes in the distribution and density of the periodontal and pulpal nerves and blood vessels. The results further indicate their involvement both in the initial stages of periodontal remodelling, as well as in the later regenerative processes of the PDL, all of them occurring in concerted action with the modulation of blood vessels.

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