Multiple functions for NGF receptor in developing, aging and injured rat teeth are suggested by epithelial, mesenchymal and neural immunoreactivity

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Summary

We have used immunocytochemistry to analyse expression of nerve growth factor receptor (NGFR) in developing, aging and injured molar teeth of rats. The patterns of NGFR immunoreactivity (IR) in developing epithelia and mesenchyme matched the location of NGFR mRNA assayed by in situ hybridization with a complementary S35-labeled RNA probe. The following categories of NGFR expression were found. (1) There was NGFR-IR in the dental lamina epithelium and in adjacent mesenchyme during early stages of third molar formation. (2) NGFR-IR nerve fibers were posterior and close to the bud epithelium. (3) During crown morphogenesis NGFR expression was prominent in internal enamel epithelium and predontoblasts; it faded as preameloblasts elongated and as odontoblasts began to make predentin matrix; and it was weak or absent from outer enamel epithelium, the cervical loop, and differentiated ameloblasts and odontoblasts. (4) When NGFR-IR nerve fibers entered the molars late in the bell stage, they innervated the most mature peripheral pulp and dentin in an asymmetric pattern which correlated more with asymmetric enamel synthesis than with mesenchymal NGFR-IR distribution. (5) The mesenchymal pulp cells continued to have intense NGFR expression in adult teeth, especially near coronal tubular dentin. (6) The pulpal NGFR-IR decreased in very old rats or subjacent to reparative dentin (naturally occurring or experimentally induced). (7) During root formation, the preodontoblasts had NGFR-IR but most root mesenchymal cells and Hertwig's epithelial root sheath did not. This work suggests that there are important epithelial and mesenchymal targets of NGF regulation during molar morphogenesis that differ for crown and root development and that do not correlate with neural development. The continuing expression of NGFR-IR by pulpal mesenchymal cells in adult rats was most intense near coronal odontoblasts making tubular dentin; and it was lost during aging, or subjacent to sites of dentin injury that caused a phenotypic change in the odontoblast layer.

Key words: NGF receptor, rat teeth, epithelial, mesenchymal, neural, immunoreactivity, aging, injured.

Introduction

Recent studies have found that some non-neuronal cells express nerve growth factor receptor (NGF) and, therefore, may be capable of responding to NGF. NGF has been demonstrated in developing muscle and connective tissue, and in various adult connective tissues and epithelia using autoradiography to show binding of 125I-NGF or immunocytochemistry with various monoclonal antibodies to NGFR (Ross et al. 1984; Chandler et al. 1984; Raivich et al. 1987; Chesa et al. 1988). The distribution of NGF receptor immunoreactivity in developing tissues suggests that NGF may function as an important regulatory signal between developing epithelia and their associated mesenchyme (Davies et al. 1987; Yan and Johnson, 1988; Chesa et al. 1988). For example, during morphogenesis, skin, lung, testes and ovary have prominent NGF immunoreactivity (IR) in mesenchymal cells associated with epithelia, but not in deeper mesenchyme (Bothwell, unpublished); in most cases, mesenchymal NGFR-IR faded during cytodifferentiation and was undetectable in the adult unless associated with injury or tumors (Chesa et al. 1988; Thompson et al. 1989). Recent work has found evidence for mitogenic effects of NGF during development (Repressa and Bernd, 1989). These results suggest important non-neuronal functions for NGF in addition to its demonstrated effects on sensory and sympathetic nerve fibers (Levi-Montalcini, 1987).

We have found that there is prominent expression of NGF in adult dental tissue of rats (Byers et al. 1988). In addition to NGF expression by some dental nerve fibers and their Schwann cells, pulpal cells in the crown of adult rat molars have NGF immunoreactivity that
is especially intense on cells adjacent to the odontoblasts. This NGFR expression may be related to several unusual properties of coronal odontoblasts: (1) they produce tubular dentin and control its calcification both during tooth development and in the adult; (2) they originate from neural crest mesoderm (Ruch, 1985; Lumsden, 1988); (3) they form a special layer that is linked by numerous gap junctions (Ruch, 1985), and that is the cell barrier between the pulp and the oral cavity (Thomas, 1985); and (4) they receive a dense sensory innervation (Byers, 1984) which includes numerous fibers immunoreactive for calcitonin gene-related peptide (Silverman and Kruger, 1987).

Here we have used immunocytochemistry to study non-neuronal NGFR expression in developing and mature rat teeth compared with neural development. Prominent NGFR-IR in the internal enamel epithelium and dental papilla of rat embryo tooth buds has been reported (Yan and Johnson, 1988). We have analysed the temporal and spatial expression of NGFR during postnatal third molar morphogenesis, cytodifferentiation, maturation and aging, and after injury to adult dentin. The results suggest that there are important non-neuronal functions for NGF and NGFR in developing and mature teeth in addition to neuroregulatory activity.

**Methods**

Normal Sprague–Dawley male rats were studied at the following ages: 1 day (n=4), 4 days (n=3), 6–7 days (n=6), 10 days (n=1); 14 days (n=5), 18 days (n=1), 21 days (n=2), 4–5 weeks (n=2), adult (2.5–4 months old; 275–350 g; w=10) and 12–15 months old (n=3). They were deeply anesthetized by intraperitoneal injection of 2.5% sodium pentothal or Equithesin (1.0% pentobarbitol and 4.25% chloral hydrate; intraperitoneal injection of 2.5% sodium pentothal or Equithesin) in PBS plus 1% bovine serum albumen and 0.5% Triton-X-100. After PBS rinses, sections were incubated in biotinylated anti-rabbit IgG plus 0.5% Triton-X 100 2 hours at 4°C. Following PBS rinses, the sections were incubated in avidin–biotin–peroxidase (ABC reagent; Vector) and then in 0.15% 3,3-diaminobenzidine plus 0.0125 % H2O2 in PBS for 5 min to localize peroxidase activity (Vector). Controls for CGRP specificity used antisera prereacted with CGRP antigen. Some sections from jaws of 4- to 14-day-old rats were reacted with monoclonal anti-neurofilament protein for further analysis of developing innervation, using immunocytochemistry procedures similar to those for NGFR.

Immunoreacted sections were mounted on gelatin-coated slides; some were counterstained with 1% methylene blue plus 1% Azure II in 1.0% sodium borate, as noted. All sections were covered with permount and examined using a Zeiss or Nikon compound light microscope. Camera-lucida tracings were made of representative sections as the basis for the summary diagrams (Figs 1, 10).

In order to compare NGFR-IR with NGFR mRNA location, jaws of two 1-day-old rats were perfusion fixed with 4% p-formaldehyde in 0.1 m phosphate buffer (pH 7.4), post-fixed 1.5 h, dehydrated and embedded in paraffin. In situ hybridization followed the protocol of Angerer et al. (1987). Serial 10 µm sections were mounted on polylysine-coated slides, dehydrated and rinsed in prehybridization solutions. S35-labeled RNA transcripts from either sense or antisense DNA strands of a 260 bp fragment of rat NGFR cDNA in Vector pGEM3Z were used. Probes containing 106 disits min⁻¹ per 50 µl were applied to sections, covered with cover glass, and the slides were submerged in an oil bath for 16 h at 50°C. After removal of the oil and cover glass, sections were incubated in RNase A (20 µg/ml) 30 min at 37°C, rinsed as described by Angerer et al. (1987), dried, coated with Kodak NTB2 emulsion and exposed for 2–4 weeks prior to development.

The technique for injuring rat molars has been described elsewhere (Taylor et al. 1988; Taylor and Byers, 1990). For the present experiments, a cavity was drilled part way through the cervical dentin on the anterior side of one maxillary first molar of each anesthetized adult rat (n=3; 308–408 g) and the cavity was etched 10 s with 37% phosphoric acid to open the dentinal tubules. The contralateral maxillary first molar served as uninjured control. Ten days later the three rats were anesthetized and fixed by perfusion with 4% p-formaldehyde, 0.2% picric acid, and 0.1% glutaraldehyde in phosphate buffer. The maxillary teeth were prepared for NGFR immunocytochemistry as described above.

**Results**

Rat jaws were sagittally sectioned so that all three molars were present in each section of each jaw (Fig. 1). Each of the three molars was at a different developmental stage, and there were gradients of development (anterior–posterior; superior–inferior) within each tooth, so that zones of proliferation of precursor cells, morphogenesis, cytodifferentiation, matrix secretion and maturation were all present within each section from the immature rats. The results are presented in relation to six developmental stages: bud growth; morphogenesis of the crown (cytodifferentiation gradients); innervation development; root growth; tooth maturation; and aging – each with characteristic NGFR-IR patterns. In addition, the effect of dentinal injury...
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Fig. 1. This diagram shows the position, size, and orientation of molar (M1-3) and incisor (INC) teeth of a 10-day-old rat. The developmental stages in subsequent figures primarily concern the third molars which have an entirely postnatal development. TG, trigeminal ganglion; Black, enamel; hatched zone, dentin.

(naturally occurring or experimentally induced) on adult teeth was studied.

(I) Bud growth
The maxillary and mandibular third molar buds formed on the posterior side of the epithelial dental lamina (DL), which connects the oral epithelium to the enamel organ of the second molar (Fig. 2). The buds have a different orientation in the two jaws – with the maxillary third molar buds growing in a superior and slightly anterior direction from a short DL, and the mandibular buds growing in an inferior/posterior direction from a long, V-shaped DL. Both maxillary and mandibular buds had intense NGFR-IR at the growing tip of the epithelium; less NGFR-IR in the DL; NGFR-IR cells of the developing stellate reticulum inside the enamel organ; intense NGFR-IR nerve fibers posterior to the bud; and NGFR-IR mesenchymal cells close to the anterior side of the DL and posterior to the second molar (Fig. 2).

(II) Morphogenesis of the crown (cytodifferentiation gradients)
The first stage of coronal morphogenesis is the cap stage, which quickly grows into the bell stage, followed by the crown stage where the developing enamel organ and dental papilla expand until they achieve the full size of the crown. In the cap stage, the concave inner enamel epithelium (IEE) had intense NGFR-IR, and the outer enamel epithelium (OEE) had less NGFR-IR (Fig. 3A). The stellate reticular cells inside the developing enamel organ had NGFR-IR. At the cervical loop that connected the inner and outer enamel epithelia, NGFR-IR was reduced or absent (Fig. 3A). NGFR-IR mesenchyme located posterior to the second molar separated into three NGFR-IR groups of cells: one encircling the bud would become periodontal tissue; a ball of cells enclosed by the developing enamel organ would become pulpal mesenchyme; and a band of intensely stained cells at the tip of each developing dental papilla would form preodontoblasts (Fig. 3A).

Since NGFR-IR nerve fibers were partially masked by non-neuronal staining during morphogenesis, we immunoreacted alternate sections for CGRP (Fig. 3B) or neurofilament protein (not shown). With both of those antibodies, no labeled fibers were found within the developing dental papilla during cap or early bell stages. Instead they were concentrated near the posterior side of the enamel organ during development and scattered fibers were found elsewhere in the periodontal zone.

In bell and crown stage molars, the NGFR-IR pulpal mesenchyme avoided the regions near NGFR-IR IEE cells and preodontoblasts but approached the pulp periphery near differentiating odontoblasts (Fig. 4). The NGFR-IR of IEE occurred prior to formation of NGFR-IR preodontoblasts. When the latter formed, the IEE cells began to lose NGFR-IR as they elongated and became preameloblasts (Fig. 4). The earliest preodontoblasts had little or no NGFR-IR, but soon had intense NGFR-IR which persisted until predentin formation (Fig. 4); thus, the NGFR-IR of preodontoblast lineage occurred later and extended further into the crown than that of ameloblast lineage cells. The cervical loop in bell- and crown-stage teeth continued to have little or no NGFR-IR (Fig. 4).

Incubation of sections from jaws of two 1-day-old rats with antisense S\textsuperscript{35}-RNA probes complimentary to NGFR mRNA had the same distribution of hybridization as was found for NGFR immunoreactivity (Fig. 5). Label was intense over IEE cells and pulpal mesenchyme near differentiating odontoblasts (those that had begun matrix secretion); it was not found over cervical loop cells, outer enamel epithelium, mesenchyme near inner enamel epithelium or elongated preameloblasts. Hybridization was reduced over matrix-secreting odontoblasts. No hybridization occurred in sections incubated with a control sense-strand RNA probe (not shown).

(III) Innervation development
In order to evaluate developing innervation, we used immunocytochemistry for CGRP and neurofilament protein, since NGFR-IR pulp cells masked NGFR-IR neural staining. Nerve fibers near blood vessels were first seen late in the bell stage in the developing teeth after newly differentiated odontoblasts had made a thin band of dentin. The first pulpal nerves had very weak CGRP-IR compared with older nerve fibers in adjacent teeth or periodontal tissues (Fig. 6A,B). By several weeks after onset of pulp innervation, CGRP-IR neural staining was much more intense in the pulp (Fig. 6C,D). Neurofilament protein immunoreactivity (not shown) was similar to CGRP-IR. The pioneer pulpal nerve fibers did not grow towards all different-
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Fig. 2. NGFR-IR; bud stage. (A,B) No counterstain. In 4-day-old rats, developing maxillary first and second molars are in the bell stage, and the third molar (M3) bud is developing from the dental lamina (DL), which has one branch (DL*) to the oral epithelium (OE) and one to the enamel organ of the second molar (M2). The bud epithelium had intense NGFR-IR. The DL branch to the second molar had less NGFR-IR. Mesenchyme anterior to the DL (m) and posterior to the second molar (mp) had NGFR-IR, but that next to the bud (*) did not. (C,D) Counterstained mandibular M3 of 4-day-old rat. The V-shaped DL had NGFR-IR in the branch to the second molar (M2) but not the branch to the oral epithelium (DL*). Mesenchymal staining was similar to the maxillary molar in Fig. 2A,B. In both jaws, nerve fibers (N) were most numerous posterior to the developing tooth. NGFR-IR stellate reticulum cells (s) inside the DL can be seen in Fig. 2D. Magn: A, 20x; B, 80x; C, 35x; D, 175x. Scales in B and D, 0.1 mm.

tiated odontoblasts; instead they grew towards and first innervated the most differentiated odontoblast layer which was covered by developing dentin and enamel (Fig. 6). This site was usually on the anterior side of maxillary and the posterior side of mandibular crowns. Subsequent increased entry of nerve fibers into dentin also focused on those sites, even though pulpal NGFR-IR was evenly distributed in peripheral coronal pulp of bell- and crown-stage teeth (e.g. Fig. 4C).

(IV) Root growth
After the ameloblasts finish production of enamel and the crown is fully formed, roots begin to grow and the tooth begins to erupt. Once that process begins the enamel organ dies, leaving an epithelial remnant surrounding each growing root base (Hertwig’s root sheath). The preodontoblasts that formed close to the root sheath had strong NGFR-IR until the cells began to make dentin (Fig. 7); however, the epithelial root sheath did not have NGFR-IR. After most of the root elongation had occurred, hard tissue deposition by cementoblasts gave the roots increased girth and length; no NGFR-IR was found in the cells making cementum (Fig. 7C). In the pulp of developing roots, NGFR-IR was prominent on centrally located nerve bundles and adjacent connective tissue, but not in peripheral root zones, and not near the root sheath or root odontoblasts; the root pulp NGFR-IR therefore had a very different appearance compared to the coronal pulp (Fig. 7D). In developing periodontal ligament, NGFR-IR mesenchymal cells were prominent in the ligament cells underlying the developing roots as were NGFR-IR nerve fibers (Fig. 7).

(V) Mature teeth
During juvenile and young adult stages, rat molar odontoblasts slowly make more tubular dentin so that the pulp cavity becomes progressively narrower. Dur-
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M2

3A NGFR B CGRP...

Fig. 3. Cap stage. NGFR-IR without counterstain (A) and CGRP-IR with counterstain (B). Mandibular third molar of 7-day-old rat. In these serial sections, nerve fibers (N) were posterior and inferior to the developing tooth. The inner enamel epithelium (I) was strongly reactive for NGFR-IR as was the developing stellate reticulum (S). The mesenchymal cells have segregated into a group anterior to the DL (m), and three groups posterior to the second molar (M2): preodontoblasts (mo), pulpal (mp) and developing periodontal tissue (m1). The inset in Fig. 3A shows a cervical loop (L) connecting inner (I) and outer (o) enamel epithelia, from a semiadjaacent section reacted for NGFR-IR. Magn: 80×; inset, 200×. Scale: 0.1 mm.

ing this delayed maturation of pulp, the pulpal fibroblasts with strong NGFR-IR became concentrated in the subodontoblastic regions of the crown, including the interradicular zones (Fig. 8A). Nerve fibers immunoreactive for CGRP and NGFR were concentrated in subodontoblast zones, the odontoblast layer, and inner dentinal tubules of the crown, but avoided interradicular zones (not shown). The innervation did not therefore coincide with all areas of pulp NGFR-IR in maturing teeth.

(VI) Aging teeth

In molars from 12- to 15-month-old rats, the NGFR-IR of pulpal fibroblasts underlying tubular dentin decreased or was absent (Fig. 8B). The reduced NGFR-IR in old molars was due to reduced size and number of NGFR-IR pulp cells (Fig. 8C,D).

(VII) Injury reactions

In molars of adult rats, patches of gnarled reparative dentin occur; the pulp subjacent to that altered dentin (and altered odontoblasts) had little or no NGFR-IR, except for nerve fibers (Fig. 9A). In three adult rats (aged 3-5 months), the cervical dentin was injured by drilling and etching a cavity on the anterior side of the first molar. Ten days later, reparative dentin had formed beneath the injury in all three teeth and pulpal NGFR-IR was gone next to the lesion, except for nerve fibers (Figs 9B,C).

Discussion

Tooth development requires differentiation of oral ectoderm into an enamel organ and of oral mesenchyme (including neural-crest-derived cells) into the dentin-producing odontoblasts and associated pulpal and periodontal tissues. Numerous experiments using organ culture and graft recombinations have demonstrated a complex sequence of reciprocal epithelial-mesenchymal interactions that influence the temporal and spatial aspects of dental cytodifferentiation (for review: Kollar and Fisher, 1980; Thesleff and Hurme-rinta, 1981; Kollar, 1983; Slavkin, 1988; Lumsden, 1988). For example, the inner enamel epithelium of the developing enamel organ stimulates odontoblast differentiation; and odontoblasts stimulate conversion of inner enamel epithelium to preameloblasts. It is likely that the various odontogenic inductions depend on diffusible factor(s) – as has been demonstrated for enamel organ induction (Koch, 1967) – and on extracellular matrix molecules and their receptors (Vainio et al. 1989); on paracrine growth factors such as transferrin (Partanen and Thesleff, 1984) and EGF (Partanen and Thesleff, 1987); and on autocrine factors (for review: Kollar, 1983; Slavkin et al. 1988). Our studies of
Fig. 4. (A,B) NGFR-IR: bell stage. Counterstained mandibular third molar of 14-day-old rat. Gradients of development show the transition from NGFR-IR inner enamel epithelium (I) to preameloblasts (PA) and of preodontoblasts (PO) to odontoblasts (*). Dentinogenesis (D) has just begun. Pulpal mesenchyme (mp) next to maturing odontoblasts has NGFR-IR, and appears to interdigitate with the unlabeled odontoblasts (Fig. 4B). The adjacent second molar (M2) was already making roots, with NGFR-IR preodontoblasts at the root tip (open arrow). Magn: A, 50x; B, 170x. Scale in B, 0.1 mm.

(C-E) NGFR-IR: crown stage. Maxillary first and second molars of 4-day-old rat. Pale counterstain. Crown formation was almost complete for M1 and was still in progress for M2; enamel (E) and dentin (D) production were well advanced in M1, but only dentinogenesis was occurring in M2. The pulpal mesenchyme had extensive NGFR-IR except next to the preodontoblast (PO) zone. M2 still had inner enamel epithelium (I), but in M1 those cells had all begun changing to preameloblasts (PA) or had become ameloblasts (A) next to developing enamel. The cervical loop (L) was not NGFR-IR, and the earliest cells of the odontoblast lineage (arrowhead) had weak or absent NGFR-IR. Magn: C, 35x; D,E, 170x. Scale for D and E, 0.1 mm.
NGF immunoreactivity in developing dental tissue are summarized diagramatically in Fig. 10; the results suggest that NGF may be one of the diffusible factors regulating gene expression in mesenchyme and epithelia during tooth development.

The close proximity of nerve fibers to the posterior side of the epithelial bud could indicate an influence of nerve fibers on bud growth. Other work has found that silver-stained nerve fibers were close to oral ectoderm prior to tooth bud initiation in mice (Kollar and Lumsden, 1979), and that the mesenchymal odontogenic capacity was not present until 1-2 days after nerve fibers had arrived (Mina and Kollar, 1987). Presumptive dental laminae and vibrissae are contacted by nerve fibers prior to development (Davies and Lumsden, 1984). However, a lack of neural induction of tooth buds is suggested by the report that mouse brachial arch tissue prior to trigeminal innervation (E9-10) will develop dental crowns when explanted to the nerve-free anterior chamber of the eye at E9-10 (Lumsden and Buchanan, 1986).

Recent experiments on developing otic vesicles suggest that NGF stimulates cellular proliferation but not differentiation (Repressa and Bernd, 1989). The NGFR-IR of developing dental epithelium and mesenchyme did not exactly match zones of proliferation, but rather had specific patterns for each tissue. In bell-stage rat molars, epithelial proliferation occurs throughout the inner enamel epithelium and into the preameloblast zone, as well as in cervical loop and outer enamel epithelial cells (Casasco et al. 1989); the NGFR was, however, confined to the inner enamel epithelium in immunocytochemical (Fig. 4) and in situ hybridization analyses (Fig. 5). Proliferation in the odontoblast lineage of bell-stage rat molars stops well before dentinogenesis begins (Casasco et al. 1989), yet NGFR-IR was weak in the earliest preodontoblasts and continued up to onset of dentin calcification. Finally, pulpal mesenchymal cells were proliferative close to the cervical loop (Casasco et al. 1989), a region devoid of NGFR in the present study. In agreement with Repressa and Bernd (1989) differentiating cells in both the ameloblast and odontoblast lineages lost NGFR, the former early in the preameloblast stage, and the latter once dentinogenesis began.

In developing mouse molars, 125I-EGF binds to proliferating cells of the developing bud epithelium which are adjacent to unlabeled mesenchyme; to outer epithelium and dental mesenchyme during the cap stage, without inner enamel epithelial binding; and to the outer enamel epithelium during the bell stage without binding to the inner epithelium or dental papilla (Partanen and Thesleff, 1987). NGFR-IR was also found in developing rat molars in bud epithelium next to unlabeled mesenchyme (Fig. 2), suggesting that NGF and EGF may both affect proliferating epithelial.
cells at that stage. However, the expression of NGFR in preodontoblasts and inner enamel epithelium, the lack of NGFR-IR in the outer enamel epithelium, and the persistent NGFR-IR of pulpal mesenchyme throughout development and in mature teeth all differed from I125-EGF binding, suggesting that the regulatory functions of NGF in teeth are different from EGF.

When odontoblast elongation and cytodifferentiation began, there were also changes in the adjacent pulpal cytochemistry. The NGFR-IR cells of the dental papilla were numerous near the differentiating odontoblasts but avoided pulp near NGFR-IR preodontoblasts or inner enamel epithelium. As the tooth matured, this NGFR-IR pulpal zone persisted in the crown, but did not extend very far into root pulp, and was missing in pulp covered by reparative dentin. These NGFR depletions suggest that its expression in pulp cells depends on regulatory signals from odontoblasts that are associated with enamel-covered tubular coronal dentin. Supporting this suggestion are the experiments that show that when injury induced replacement of odontoblasts by reparative dentin-producing cells, the adjacent pulp had a focal depletion of NGFR-IR (Fig. 9).

Using electron microscopy, we have recently found that the NGFR-IR of pulpal cells in adult rat teeth was intense for most non-odontoblastic cells in the periphery of the crown, (Byers, 1990). A different pattern was found for class II antigens in rat incisor pulp for which scattered dendritic cells and macrophages were immunoreactive (Jontell et al. 1988). The observed patterns are consistent with the possibility that some of the NGFR-IR pulpal 'fibroblasts' are dendritic antigen-presenting cells as has been suggested for other NGFR-IR dendritic cells (Thompson et al. 1989). If so, their NGFR immunoreactivity may have a purpose different from functions concerned with the slow maturation of tooth pulp, local interactions between pulp and odontoblasts, or pulpal neurobiology.

The asymmetric pattern of nerve development in rat molars correlated with enamel maturation asymmetries rather than with pulpal NGFR-IR patterns. Another suggestion that dentin innervation is controlled by
Fig. 7. NGFR-IR persisted in developing odontoblasts at the tip of the growing roots (open arrow). The adjacent root pulp and Hertwig's root sheath (RS) were not stained (A,B); and mature ameloblasts (a), enamel (e) and dentin (d) were not NGFR-IR. Unstained odontoblasts (o) in the crown (A,D) were associated with NGFR-IR mesenchymal cells (mp), but odontoblasts in the root were not (B-D). When the roots had almost completed their elongation, preodontoblastic NGFR-IR was reduced (C); and the cellular cementum (*) that is deposited around the base of the maturing roots did not have NGFR-IR. The difference between crown (c) and root (R) pulp NGFR-IR can be seen in an erupting molar (D). Periodontal ligament (Lg) was devoid of NGFR-IR except for nerve fibers and for the mesenchyme (ml) underlying the developing roots. Magn: A, 200x; B, 160x; C, 200x; D, 19x. Scale: 0.1mm.

Fig. 8. NGFR-IR, young adult molar, no counterstain. Pulpal mesenchyme had intense NGFR next to interradicular dentin (IR) and in the periphery of the crown. Dotted line indicates crown/root junction. Pulpal cells of upper root regions (arrowhead) also had NGFR-IR in young adult molars. NGFR-IR nerves (arrow) are evident. (B) NGFR-IR, 15-month-old adult rat, no counterstain. Pulpal mesenchyme NGFR-IR was greatly reduced with some remaining along the walls of the crown. NGFR-IR nerve fibers were evident in the pulp (arrows) and entering dentin (open arrows). Pulp of the upper root region (arrowhead) did not have NGFR-IR. (C,D) NGFR-IR of plastic 1μm-thick sections of the comparable pulpal zones of young adult (C) and 15-month-old rat (D) which had been immunoreacted together. The older rat had many fewer NGFR-IR pulp cells (arrows), and those cells had a smaller size than in the young rat. The odontoblast layer (*) had labeled nerves passing through but very little odontoblastic NGFR-IR. Magn: A, 50x; B, 75x; C–D, 550x. Scales: A,B, 0.1 mm; C, 0.025 mm.
Fig. 9. (A) NGFR-IR, adult molar, no counterstain. A patch of reparative dentin (RD) had formed in this unoperated molar. The pulp underlying that abnormal dentin had greatly reduced NGFR-IR, except for nerve fibers. Magn: 50×. (B,C) NGFR-IR with counterstain. The molar in B had been injured 10 days prior to fixation. Underlying the injury, pulp cells have formed reparative dentin (RD). NGFR-IR pulp cells were absent next to the RD even though labeled nerve fibers (arrows) were present. The contralateral control tooth showed normal NGFR-IR of coronal and upper root cells. Magn: 50×.

DENTAL CYTODIFFERENTIATION GRADIENTS

Fig. 10. This diagram depicts the NGFR-IR labeling (stippling) of gradients of cytodifferentiation in developing rat molars. Epithelial NGFR-IR was absent from outer enamel epithelium (o) and cervical loop (L) but was present in the internal enamel epithelium (I); it faded in preameloblast (PA) cells and was absent from ameloblasts (A). The ameloblasts became shrunken and moribund after making enamel (E). The preodontoblasts (PO) had NGFR-IR which faded during predentin (PD) production and was lost during calcification of dentin (D). Pulpal mesenchymal cells (mp) had NGFR-IR during development which became intensely concentrated next to maturing odontoblasts (*). If reparative dentin (RD) formed, the associated pulpal NGFR-IR was lost. Nerve fibers (N) first grew towards the enamel-covered coronal odontoblasts and they innervated that dentin during tooth eruption.

factors other than pulpal NGFR-IR comes from recent studies of rat molars with induced microabscesses; it was found that after 4 days many CGRP-IR nerves innervated dentin throughout the root, a zone that lacks pulpal NGFR-IR (Taylor and Byers, 1990). Thus, the entry of sensory nerve fibers into predentin and dentin appeared to be controlled by tissue chemistry characteristics that differed from the location of mesenchymal NGFR. However, NGFR is also involved in the neurobiology of dental innervation since electron microscopy has found that NGFR-IR is located on unmyelinated fibers in teeth (Byers, 1990), with a pattern similar to that reported for other peripheral nerves (Johnson et al. 1988). There are therefore NGFR functions in dental tissue that concern dental neurobiology in addition to non-neuronal functions during tooth development and maturation. The results suggest that NGF may be the ligand regulating those functions, but further work is
needed to analyze its expression and regulatory activities during development and aging of teeth and following dental injury.

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