The early distribution and possible role of nerves during odontogenesis

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ABSTRACT Neural crest cells migrate along specific pathways to reach the mandibular and maxillary arches where they condense under specific areas of the ectoderm which will give rise to the primary and permanent dentition. In the mouse, the trigeminal ganglion becomes evident on E9 and the superior cervical sympathetic ganglion E13. Several studies have suggested that nerves in the vicinity of the developing teeth could influence the surrounding tissues and initiate tooth development, whereas other investigators have suggested that tooth development will proceed without an intact innervation. Innervation of the dental papilla has been reported as early as the cap stage in human teeth using an antibody to PGP 9.5. A large variety of putative neurotransmitters have been localized in the nerves of the dental pulp. Many of the putative neurotransmitters function in vasoregulation while others have unknown functions. A hypothesis is presented describing a possible signal transduction pathway between odontoblasts and nerve terminals.

KEY WORDS: *nerves, odontoblasts, development, immunohistochemistry, signal transduction*

The initial migration of neural crest cells from the mesencephalic region of the developing brain begins prior to the fusion of the neural tube. The neural crest cells migrate as a layer underlying the ectodermal layer along pathways lined with fibronectin and laminin until they reach the mandibular and maxillary arches (Erikson, 1988). At this point, the neural crest cells begin to condense under specific areas of the ectoderm which will eventually give rise to the primary and deciduous dentition. Neural crest cells differentiate into a heterogeneous group of structures in the orofacial region including sensory and autonomic nerves, ganglia, Schwann cells, and cells of the dental pulp including odontoblasts. In the mouse, the trigeminal ganglia become evident on the 9th embryonic day, with emergence of the earliest fibers by day E9.5, and finish leaving the ganglion by E13. The initial fibers reach their terminal fields by E10.5 in the mandibular process and by E11 in the maxillary process. By E15, the majority of the nerves supplying the mandibular and maxillary processes have reached their final target tissues (Davies, 1988). Between E12 and E13 an increased density of nerve terminals have been demonstrated to underlie areas of the oral epithelium where tooth development will occur (Kollar and Lumsden, 1979; Lumsden, 1982). In contrast to the early developing trigeminal nerves, the superior cervical sympathetic ganglion in the mouse does not begin development until E13 (Coughlin *et al.*, 1977, 1978; Lumsden, 1982). Several studies have suggested that the nerves innervating these embryonic tissues could influence the surrounding ectoderm, mesenchyme and ectomesenchyme to initiate tooth development at those specific, densely innervated sites (Kollar, 1981; Mohamed and Atkinson, 1983; Pearson, 1977). This concept seems to be substantiated in a recent paper by Tuisku and Hildebrand (1994) in which they examined the effect of denervation on the formation and eruption of teeth in the teleost cichlid fish, *Tiapia mariae*. One hundred days after resection of the *R. mandibularis trigemini*, a nerve analogous to the inferior alveolar nerve of mammals, they found that the replacement teeth in this polyphyodont species were absent. On the unoperated side of the mandible, the teeth developed normally. The histological results showed the lack of replacement anlage after 100 days. The results of this study clearly demonstrated that denervation inhibited the development and mineralization of the underlying developing teeth in this phylogenetically older species. However, one *in oculo* and several *in vitro* studies have strongly suggested that tooth initiation will proceed in the absence of an innervation in mammalian species (Gerber, *et al.*, 1973; Ruch *et al.*, 1973; Kollar and Lumsden, 1979; Lumsden, 1982; Lumsden and Buchanan, 1986). Other studies have suggested that the density of nerves innervating the tooth primordia and the dental pulp are reminiscent of an older, protective phylogenetic function such as nociception (Halstead-Tarlo, 1968; Lumsden, 1979; Northcutt and Gans, 1983). The above studies suggest that animals of earlier phylogenetic origin have retained the potential to regenerate limb buds and fins (amphibians and fishes) and that the nervous system controls this regenerative process (Geraudie and Singer, 1977). The mammalians appear to have lost this ability or have down-regulated the expression to function in homeostatic maintenance and, perhaps, wound healing.

Other studies have demonstrated the temporal and spatial distribution of nerves entering the developing dental papilla and future pulp using various animal and human models and histologi-

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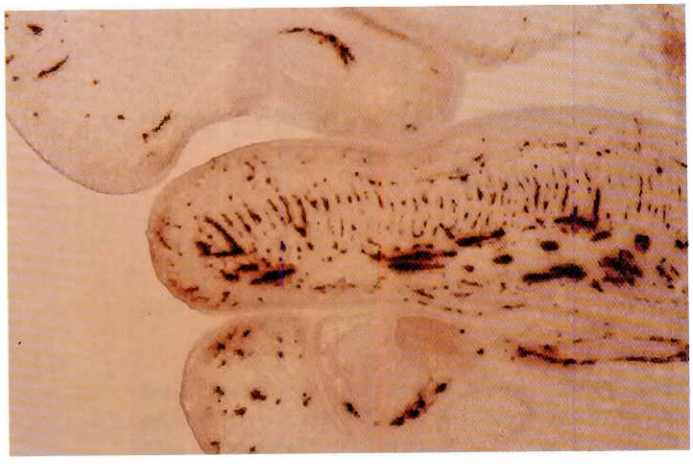

Fig. 1. This low magnification photomicrograph demonstrates the typical immunohistochemical staining pattern for GAP-43 in the anterior portion of an E16 rat fetus. *The dense brown precipitate is concentrated in the neural elements of the maxilla (Mx), the mandible (Md) and the tongue. DAB-IHC with hematoxylin counterstain. Orig. Mag. x5.*

cal methodologies. More recently, immunohistochemical studies have been employed to determine which neurotransmitters are associated with the ingrowing nerves in the dental pulp and developing dental papilla. The functional role of these nerves, however, is still equivocal. Nerves have been suggested to play a role in tooth development as early as the induction of the dental lamina and after development is complete, in repair. A recent study by Christensen *et al.* (1993) using an antibody to protein gene product 9.5 (PGP 9.5) showed that the dental follicle was innervated as early as the cap stage in developing human teeth. Our studies using an antibody to a 43 kDa growth-associated protein (GAP-43) also showed early innervation of the follicle (Figs. 1 and 2). However, the dental papilla was not innervated until after enamel and dentin had been formed. The early nerve fibers were located at the basal portion of the papilla and were usually associated with blood vessels. Similar results have been reported by other investigators. At approximately 7 days postnatal, in the rat, fine nerve fibers can be demonstrated entering the dental papilla/pulp. The majority of these nascent fibers have been reported to be associated with blood vessels although some have also been found lying free in the pulpal extracellular stroma. Kubota *et al.* (1985) have suggested that these early fibers have a sympathetic origin and function in controlling the differentiation of the odontoblasts and the regulation of collagen synthesis and secretion by the pulpal fibroblasts. After the initial secretion of the enamel and mantle dentin, the nerves show an active growth phase. Many more fibers are found in the pulp at 10 days postnatal, which seems to be coordinated with pulpal maturation and mineralization of the dentin and enamel. Although there is a paucity of information on the influence of the nervous system on the regulation of mineralization of the dentin, several recent papers have suggested that nerves can influence the secretion and subsequent mineralization of osseous tissues (Michelangeli *et al.*, 1989).

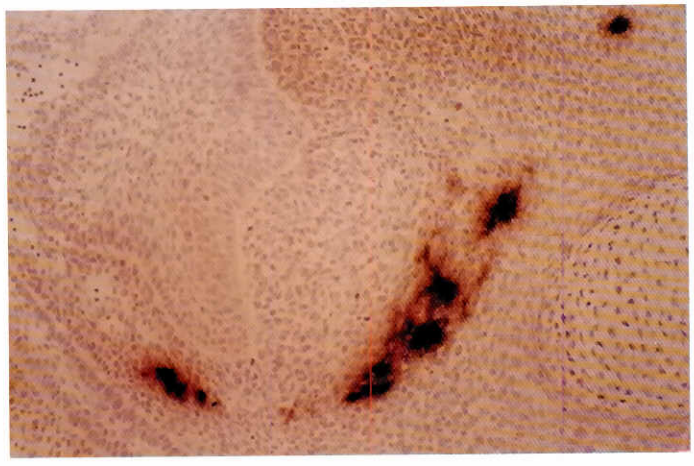

Fig. 2. The mandibular incisor tooth bud of an E16 rat fetus is shown in this medium magnification photomicrograph. *Dense immunoreactivity to GAP-43 is shown in the follicular tissue at the basal and mesial aspects of this tooth bud. The dental pulp was unreactive for GAP-43 in this series of experiments. DAB-IHC with hematoxylin counterstain. Orig. Mag. x20.*

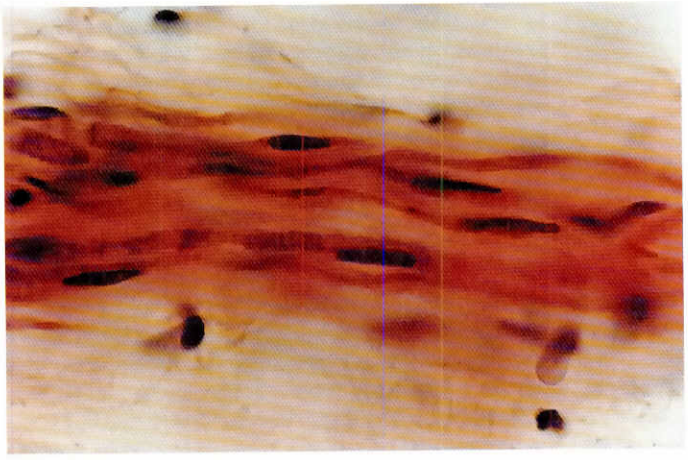

Fig. 3. This high magnification photomicrograph is of a small diameter nerve fiber in a nerve bundle from the pulp proper of a human third molar that demonstrates positive immunoreactivity for Substance K, a putative neurotransmitter associated with vasoregulation. *DAB-IHC. Orig. Mag. x63.*

Several different putative neurotransmitters have been localized in the developing dental pulp using immunohistochemistry. Substance P (SP) immunoreactive nerve fibers have been found in the dental follicle of the mouse incisor 18 days post-conception (Mohamed and Atkinson, 1983). The positively stained SP fibers were found in the developing dental pulp of the incisor as early as 2 days postnatal and in the first molar at 4 days postnatal. In rats, Nagata *et al.* (1994) found SP and calcitonin gene-related peptide (CGRP) in the dental lamina. From birth to the conclusion of the study at postnatal day 15, the number and density of SP and CGRP fibers increased. By day 15 a plexus of nerves containing SP and CGRP immunoreactive fibers could be demonstrated in the oral epithelium overlying the mesiopalatal and mesial cusps of the first molars.

In the mature dental pulp, the majority of the nerves located within the odontogenic zone are unmyelinated A-delta or c-fibers and are thought to be nociceptive fibers or postganglionic sympathetic fibers. These fibers are located below and between the odontoblasts, lying free in the pulp and located in the dentinal tubules of predentin juxtaposed to the odontoblast process. Most of the terminals associated with the odontoblast and the odontoblast

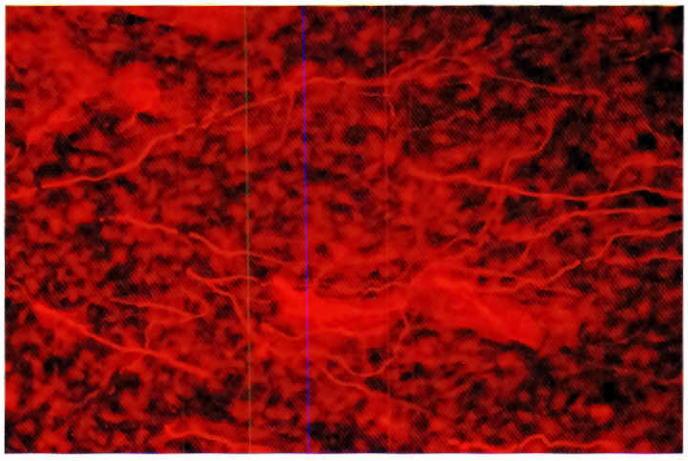

Fig. 4. This photomicrograph demonstrates positive immunofluorescent staining of NF-200 nerve fibers in the pulp proper of a 7-day-old rat pup. *The positively stained fibers exhibit a range of diameters and are branched and unbranched. Strepavidin-Rhodamine-IHC. Orig. Mag. x40.*

process contain a variety of electron dense and/or electron lucent vesicles (Nakano *et al.*, 1970; Panopoulos *et al.*, 1983). These nerve terminals are thought to be postganglionic sympathetic terminals for the regulation of pulpal blood flow (Avery, *et al.*, 1980). Several authors have reported that the dense core containing terminals located in the odontoblastic region have a role in modulating the response of the sensory nociceptors by regulating the vascular supply to these terminals (Ogilvie *et al.*, 1966; Olgart, 1990). Other investigators suggest that these nerve terminals have a role in modifying the response of the odontoblast to insult (Chiego *et al.*, 1981, 1983, 1987) or in recruitment of progenitor cells (Klein *et al.*, 1981; Chiego, *et al.*, 1986; Chiego, 1992) after pulp exposure or deep cavity preparation.

Neurotransmitters such as calcitonin gene-related peptide, enkephalin, neuropeptide Y, VIP, substance P, somatostatin, serotonin, acetylcholine and norepinephrine (Pohto and Antila, 1968; Rapp *et al.*, 1968; Olgart *et al.*, 1977; Schultzberg *et al.*, 1979; Uddman *et al.*, 1980; Lundberg *et al.*, 1982; Lee *et al.*, 1985; Wakisaka *et al.*, 1985), among others, have been reported to be associated with nerves innervating the mature dental pulp. Presumably, these putative neurotransmitters are contained in the vesicles of the nerve terminals adjacent to the odontoblasts. We have recently localized Substance K (SK), a member of the tachykinin family of neuropeptides, to small diameter nerve fibers within the larger nerve bundles traversing the pulp proper of fully developed human third molars (Fig. 3).

Recently, we have used an antibody to a 200 kDa protein found in the intermediate filaments (NF-200) of sensory nerves and an antibody to a 43 kDa growth associated protein (GAP-43) to demonstrate immunoreactive nerves in the first mandibular molar of rat pups from birth to 10 days postnatal using standard avidin-biotin complex immunohistochemistry. Although GAP-43 was a useful marker for nerves in the dental follicle, we could not demonstrate GAP-43 immunoreactive nerves in the dental pulp at any time period in this study. Neurofilament staining was demonstrated in the dental pulp as early as postnatal day 3 and continued to increase in density through the end of this study at day 10. At day 7, positively stained nerves could be seen throughout the pulp and pulp horns and within the odontogenic zone. Neuronal arborizations and varicosities could also be demonstrated at day 7. The distribution of NF immunoreactive nerves at day 10 was similar to day 7. Although some positively stained nerve fibers could be demonstrated running parallel and subjacent to the odontogenic zone, there was little evidence to suggest an intact subodontoblastic nerve plexus (of Rashkow) to the end of study at 10 days postnatal. (Figs. 4 and 5) The results of this study suggest that the patterns of nerves in the dental pulp becomes established as early as 7 day postnatal in the rat first mandibular molar.

Although there are numerous reports in the literature describing nerves and nerve terminals adjacent to odontoblasts, there are not any reports describing synaptic specializations between the two cell types (see review by Byers, 1984). Byers *et al.* (1987) have suggested that the proximity of the nerve terminals and the odontoblast plasma membrane is a synaptic specialization unique to the dental pulp. Functionally, deformation of these nerve terminals could result in depolarization of the nerve and propagation of an action potential. Whether the different conformations of nerve terminals and different neurotransmitters found in the dental pulp function only during the transmission or modulation of nociceptive mechanoreception or whether they have other roles such as modifying the responsiveness of the odontoblasts to iatrogenic or environmental stimuli is currently unknown.

It is possible, however, that the sensory or autonomic nerves that closely approximate the odontoblasts could initiate an odontoblastic response by depletion of specific neurotransmitters released from the vesicles within the nerve terminals. The neurotransmitters would then bind to receptors on the plasma membrane of the adjacent odontoblast and initiate a cascade of events. Then, at some later time small molecules or ions used for intercellular communication, i.e. c-AMP and ionic calcium, would have had time to be synthesized (or mobilized), amplified and transferred to adjacent odontoblasts through gap junctional complexes until the odontoblasts have effected the wound healing.

The following hypothesis proposes a mechanism for signal transduction between odontoblasts and nerve terminals but requires that specific anatomical structures and signaling molecules be present and closely associated with the nerve terminal and the odontoblast.

1. Deformation of the nerve terminal contained within the dentinal

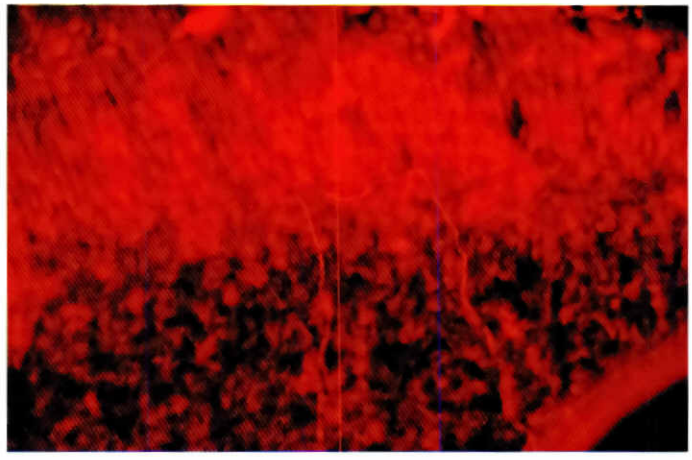

Fig. 5. This photomicrograph demonstrates 2 nerve fibers lying on the odontoblastic layer in the mesial buccal cusp of the first molar of a 7-day-old rat pup. *Strepavidin-Rhodamine-IHC. Orig. Mag. x40.*

tubules or juxtaposed to the odontoblast cell bodies causes the opening of N-type calcium channels with the subsequent release of neurotransmitter(s).

2. The neurotransmitter could then bind to the receptor located on the plasma membrane of the odontoblast (possibly a 7 domain transmembrane protein).
3. Following receptor binding of the neurotransmitter, a signal transduction protein is activated, initiating a cascade of events that increases or decreases intracellular messengers, e.g. c-AMP or Ca^{++} upregulating cellular activity resulting in reparative dentinogenesis. G-proteins have been reported to be efficient transducers of receptor signals into effector responses. Adenyl cyclase, various kinases and hormone and neurotransmitter regulated ion channels (including n-type Ca_2^+ channels) also have been reported to be G-protein regulated (see review by Birnbaumer, 1990).

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