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Peripherin- and CGRP-immunoreactive nerve fibers in rat molars have different locations and developmental timing

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Summary Developing rat molars gain mature sensitivity to electric stimulation at 4—5 weeks after eruption, but the related mechanisms are incompletely understood. Preliminary studies showed weak co-localization of calcitonin gene-related peptide (CGRP) immunoreactivity (IR) with peripherin (PER) or neurofilament protein (NF) in rat molar nerve fibers, while the latter two co-localized extensively.
Objective: Our goal was to compare timing and location of PER-IR and CGRP-IR innervation in rat first molars during tooth maturation.
Methods: We used single and double immunocytochemistry to study molars of rats aged 10 days to 1 year. Neural patterns were compared with odontoblast maturation stages, dentinogenesis, formation of cell-free and cell-rich zones, and root closure.
Results: Spatial and temporal patterns showed that most CGRP-IR and PER-IR have different terminal domains in teeth. PER-IR fibers were well established among immature odontoblasts prior to tooth eruption, but CGRP-IR fibers were absent. Two weeks after eruption of first molars, many CGRP-IR beaded fibers entered dentin, the larger PER-IR fibers began shifting away from odontoblasts towards the pulp, and the symmetrical PER-IR pulpal pattern was being established. The CGRP-IR fibers continued to increase their asymmetric dentinal innervation until root growth was completed, during which time odontoblasts matured, the cell-free and cell-rich zones appeared, and roots closed.

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Conclusions: Sensory maturation of rat molars coincides with closed root apices, extensive innervation of dentin by CGRP-IR nerve fibers, and the appearance of the mature avascular odontoblast layer next to cell-free and cell-rich zones in the pulp horns.

Introduction

Human and animal teeth respond poorly to electrical stimulation when they are immature, becoming sensitive to that stimulus once the roots are fully developed with narrow ("closed") apical foramena.[1–3] During tooth eruption and root development, sensory innervation of crown pulp and dentin increases, reaching its maximum at root closure, and then gradually decreasing as teeth age.[4–6] Once sensitivity has matured, the electrophysiological properties appear to be similar for rodents, cats, dogs, monkeys, and humans.[7] Electrical sensitivity then continues so long as the tooth pulp is vital, and the responses are most consistent when the current is applied to the incisal surface of the tooth.[8]

The underlying mechanisms for tooth sensitivity to electrical stimulation are not well understood, though early work in animals found correlations with innervation of dentin. The jaw opening reflex has been shown to be a reliable measure of responses to painful stimuli to tooth pulp in anesthetized animals,[9] and electric pulp stimulation in people also elicits tooth pain.[1,2] Studies in rats showed that the sensitivity of molars (as determined by eliciting a jaw opening reflex) increased rapidly from 4 weeks to when roots closed 2–3 weeks later, during which time the dentinal innervation developed.[10] In another study, rat molars were denervated without affecting gingival innervation, and then re-innervation of dentin beneath the incisal surface occurred simultaneously with return of jaw opening reflex to electrical tooth stimulation.[11] Other correlations of electrical sensitivity with dentinal innervation were found by electrophysiology, in which jaw opening reflexes depended on nerve fibers that innervate dentin (A-fibers), as did the sensation of sharp dental pain.[7]

However, nerve fibers can be present in teeth without being sufficiently sensitive to electrical stimulation to elicit pain or jaw opening reflexes, because the crown pulp and dentin can be fairly well innervated prior to completion of root growth and closure.[6,12–18] Perhaps, some subsets of dental innervation have greater reactivity to electrical stimulation, as suggested by studies of A-beta fibers,[7] and are not sufficiently mature until the roots have formed. Other possible mechanisms are that the architecture of the pulp and root may contribute to intradental current flow and sensitivity, especially at closed root foramina,[2] or that there are co-operations between sensory innervation and other features of teeth, such as odontoblast maturation[19] and function,[20] that are not mature until roots close. Finally, maturation events in trigeminal nerve or CNS would also play a role.

Our goal in the present study was to investigate sensory nerve development in rat molars in relation to maturation of pulp and dentin by comparing teeth before and after onset of mature sensitivity to electrical tooth stimulation, as determined previously.[10] In that earlier study, 4-week-old rats (2 weeks after eruption of first molars), had a mean threshold for the elicited jaw opening reflex of 145 ± 61 μA, whereas in 7-week-old rats it had decreased to 52 ± 18 μA.[10] That difference between 4 and 7 weeks was significant, whereas the 7-week rats had similar thresholds to those of 3–5-month-old rats and to 1-year-old rats, despite the extensive attrition of crown height in older rat molars.

For this study, we decided to compare tooth maturation with development of two populations of dental nerve fibers that our initial work showed either[1] contained calcitonin gene-related peptide (CGRP) immunoreactivity (IR) without much co-localization of the intermediate filament peripherin (PER)-IR or neurofilament protein, or[2] had extensive immunoreactivity for both peripherin and neurofilament protein. Our preliminary studies found an apparent difference in developmental temporal and spatial patterns for those two groups of nerve fibers in relation to initial arrival in the peripheral pulp and/or dentin, mature nerve fiber destination, overall pattern, attraction to or repulsion by dentin, and orientation in relation to immature and mature odontoblasts and to their associated cell-free and cell-rich zones. Here, we analyze a large enough sample to determine which of these dental features is associated with the developing innervation, and the extent to which the PER-IR and CGRP-IR populations differ.

Materials and methods

Animals

The study was performed with the approval and under the supervision of the University of Washington Institutional Animal Care and Use Committee. We analyzed molar teeth from 46 anesthetized,