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Effects of denervation on ^{3}H-fucose incorporation by odontoblasts in the mouse incisor[1]

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Summary. The present study was designed to determine the effects of denervation on glycoprotein synthesis in the predentinal matrix of the mouse incisor. The inferior alveolar nerve (IAN), superior cervical ganglion (SCG) or both (IAN+SCG) were unilaterally resected in adult mice with the contralateral side remaining intact as a control. Fourteen days after surgery and 4 h prior to killing, 0.2 mCi of ^{3}H-fucose was injected intravenously and mandibles were processed for standard histological and autoradiographic techniques. Silver halide grains were counted over the predentin matrix for 2000 μm per tooth. The results showed that the IAN and SCG resection affected ^{3}H-fucose incorporation into the predentinal matrix; however, the highest absolute mean grain counts occurred after IAN+SCG resection. SCG resection increased the amount of ^{3}H-fucose incorporated into the predentinal matrix by 48%, that of IAN by 24% and that of IAN+SCG by 14% as compared to contralateral controls. These data indicate a regulatory role for the nervous system and a possible interaction of neural components in the control of glycoprotein synthesis by odontoblasts in the mouse incisor.

Key words: Odontoblasts – Denervation – Glycoproteins – Predentin – ^{3}H-Fucose

Numerous studies have attempted to define the role of innervation in tooth growth and maintenance. The inferior alveolar nerve (IAN) contains primarily sensory fibers innervating pulps of the teeth while the superior cervical ganglion (SCG) supplies sympathetic adrenergic fibers. The eruption rate of the rat incisor is increased after IAN denervation (Taylor and Butcher 1951; Brown et al. 1961; Devoto et al. 1966), but SCG appears to have

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no effect on this parameter (Taylor and Butcher 1951). IAN resection produces an altered pattern of cellular proliferation in the progenitive compartments of the continuously erupting mouse incisor (Chiego et al. 1981) while SCG has only a minor influence on cellular proliferation (Klein et al. 1981).

Analysis of denervation effects on secretory activity by the incisor has demonstrated irregular calcification patterns within the dentin (Weatherred 1965). Odontoblasts are entrapped within a widened predentin zone and globular dentin is found after IAN resection (Rehak 1963). Cholinesterase and succinic dehydrogenase levels in the pulp are altered by surgical denervation in the rabbit (Avery et al. 1971). Cholinesterase levels are decreased primarily by IAN or SCG + IAN resection while histochemical staining for the oxidative enzyme succinic dehydrogenase increased after IAN, SCG or IAN + SCG denervation (Avery et al. 1971). Odontoblastic cells beneath the predentin showed the most dramatic increase in succinic dehydrogenase and a decrease in cholinesterase activity. The rate of deposition of reparative dentin after a cavity preparation is increased by IAN or IAN + SCG denervation and appears to be influenced by sympathetic innervation (Avery et al. 1974).

Autoradiographic analysis of dentinogenesis has demonstrated that odontoblasts show large fluctuations in ^{3}H-proline incorporation after IAN resection in the mouse (Chiego and Singh 1974). Therefore, collagen synthesis in the odontoblastic layer is apparently influenced by neural factors. ^{3}H-fucose has been used by Weinstock et al. (1972) to investigate the formation of glycoproteins by odontoblasts. ^{3}H-fucose is added to glycoprotein synthesized in the Golgi apparatus. The glycoproteins subsequently migrate to the odontoblastic processes, ultimately being discharged into the dentinal matrix (Warshawsky and Josephsen 1981).

The purpose of the present study was to determine the effects of IAN, SCG or IAN + SCG on ^{3}H-fucose incorporation into the predentinal matrix of the mouse incisor. It was anticipated that this study would provide data on the role of innervation in the synthesis of glycoproteins in the continuously erupting mouse incisor.

Materials and methods

Seventeen female Swiss-Webster mice (Carworth, Wilmington, MA) 1 month old were used. Mice were anesthetized with sodium pentobarbitol (60 mg/kg) body wt. and the following surgical procedures were performed: IAN resection (5 mice), SC ganglionectomy (4 mice), IAN resection + SC ganglionectomy, (4 mice) or Sham-surgery (4 mice). All surgery was performed on the left side with the right side as the unoperated control. Sham control mice were treated in the same way as resected mice except for the actual resection process. IAN resection was performed by the method of Chiego and Singh (1974) while the SCG and 3–4 mm of the sympathetic trunk were removed by the method of Avery et al. (1971).

Fourteen days after resection, 0.20 mCi ^{3}H-fucose with a specific activity of 56.0 Ci/mmol, in a 9:1 ethanol:water solution (New England Nuclear Co., Boston, MA), was slowly injected into the dorsal tail vein. The mice were then sacrificed 4 h after the injection so that the maximal amount of label would be incorporated on the dentin side of the predentin-dentin

junction (Weinstock et al. 1976). The mandibles were immediately removed and placed into Karnovsky's fixative for 1–2 h.

Following fixation, the mandibles were demineralized in fixative/EDTA for 34 days. Demineralization was determined to be completed by observing radiographs of the mandibles. Mandibles were embedded in paraffin and sectioned at 5 μm. Glass slides with mounted sections were deparaffinized and hydrated, coated with Kodak NTB-2 emulsion, placed in light tight boxes with dessicant and stored at 4° C for a 2-week exposure period. The slides were developed in Kodak D-19b for 6 min, dipped in H_2O for 0.5 min, fixed for 4 min in Kodak Rapid Fix and washed in H_2O. The slides were then lightly stained with Harris' hematoxylin and eosin.

By means of a 10×10 net micrometer, the reduced silver halide grains were counted for a distance of 10 micrometers from the odontoblasts into the predentin at 400 X. The secretory zone of the incisor was counted for a length of 2000 μm per tooth. The resultant mean grain counts were analyzed by use of a 't' test for paired observations.

Results

Comparison of the experimental and control structures showed an increase in label on the experimental side in all three experimental groups: IAN resection, SCG resection and SCG + IAN resection (Table 1). A comparison of experimentals and sham controls showed no differences between values from the IAN resected side and sham controls; while values from sympathectomized and combination resected incisors were significantly increased over sham control values.

IAN resection increased the amount of ^{3}H-fucose incorporation by 24%, SCG by 48%, and resection of both neural components by 14% over contralateral controls. However, absolute incorporation (mean grain counts) increased from 10.78 ± 0.80 in the IAN resected group to 13.48 ± 0.11 in the sympathectomized group and 14.79 ± 0.53 in the combined resection group. Comparison of values from resected incisors and sham controls indicates that IAN resection and sympathectomy have both ipsilateral and contralateral effects on ^{3}H-fucose incorporation. Contralateral effects of IAN denervation have previously been demonstrated as to eruption rate (Devoto et al.

Table 1. Mean ± S.E.M. of autoradiographic grain counts for denervated (D) versus contralateral controls (C) 14 days after denervation and 4 h after injection of ^{3}H-fucose. Sham control values are also indicated.

Group	N	$\bar{x} \pm$ S.E.M.
D. IAN	5	10.78 ± 0.80[a]
C. IAN	5	8.24 ± 0.56[b]
D. SCG	4	13.48 ± 0.11[a]
C. SCG	4	7.04 ± 0.15[b]
D. IAN + SCG	4	14.79 ± 0.53[b]
C. IAN + SCG	4	12.67 ± 0.26[b]
Sham controls	4	10.66 ± 0.70

[a] Denervated side differs from contralateral control ($p < 0.05$)
[b] Grain count differs from that of sham controls ($p < 0.05$)

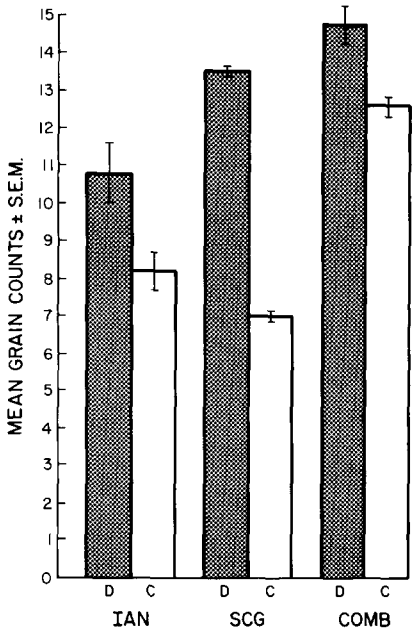

Fig. 1. Mean grain counts of odontoblasts after ^{3}H-fucose administration for control (C) and denervated (D) mouse incisors. Stippled bars indicate IAN (Inferior Alveolar Nerve), SCG (Superior Cervical Ganglion) or COMB (IAN + SCG) resected side vs. control

1966), mineralization (Skopakoff and Stiefel 1972) and ^{3}H-thymidine labeling (Chiego et al. 1981).

In the present study 4 h after the injection of ^{3}H-fucose, silver grains were observed on the predentin side of the predentin-dentin junction in all groups and not on the dentin side as reported previously in rats (Weinstock et al. 1972; Warshawsky and Josephsen 1981). This slight variance may be due to the neural effects or differences in animal models. However, ^{3}H-fucose incorporation may be impaired due to the effects of ethanol on glycoprotein synthesis and secretion (Mookerjea and Chow 1969; Tuma and Sorrel 1981) or ethanol-induced disturbances of calcification and dentin formation (Iida and Mimura, 1967).

Discussion

The resection of the inferior alveolar nerve and/or superior cervical ganglion increased the incorporation of ^{3}H-fucose into the predentin matrix of the mouse incisor on the resected side versus the contralateral control. Since ^{3}H-fucose is specifically incorporated into glycoproteins of the non-collagenous dentinal matrix (Weinstock et al. 1972) denervation appears to increase glycoprotein synthesis by the odontoblasts.

Various dystrophic changes have previously been reported in the rodent mandibular incisor after IAN resection. The loss of sensory innervation results in shorter, thinner, and less pigmented incisors (Taylor and Butcher 1951; Ronning and Isotupa 1973) at the macroscopic level. Sensory denervation results in an accelerated eruption rate (Taylor and Butcher 1951) and

altered cell proliferative indices at various intervals after surgery (Chiego et al. 1981).

Microscopically, qualitative changes in the secretion of predentin and enamel have been demonstrated in the weanling and adult rat after IAN resection (Rehak 1963; Weatherred 1965). The increase in ^{3}H-fucose incorporation into glycoprotein in the present study may reflect irregular calcification patterns, widened predentin and accelerated eruption rate previously seen in denervated incisors.

The increased uptake of ^{3}H-fucose after superior cervical (SC) ganglionectomy may be due to the increased blood flow and increased nutrient supply resulting from decreased vascular tone and increased vascular permeability. However, sympathectomy is ineffective in altering incisor eruption rate (Taylor and Butcher 1951) and cell proliferation except in the stellate reticulum and stratum intermedium (Klein et al. 1981). These zones have been hypothesized to play a role in the regulation of absorption and secretion of enamel matrix (Kallenbach 1978). Adrenergic endings in mouse molars are found primarily around blood vessels in the pulp and juxtaposed to odontoblasts in the pulp horns (Avery et al. 1980). If the pattern of adrenergic endings is similar in the incisor then the increase in ^{3}H-fucose as seen in this study after ganglionectomy may be due to modulation of odontoblastic activity and vascular tone by the sympathetic nervous system.

The response of odontoblasts to IAN and SCG resection appears to differ from the response of osteoblasts. Following interruption of sensory (IAN resection) or sympathetic (surgical removal of the SCG or guanethidine treatment) denervation there is a decrease in ^{3}H-proline incorporation into mandibular osteoblasts located mesial to the first molar (Chiego and Singh 1981; Singh et al. 1982). The response of osteoblasts is attenuated with time possibly as part of a compensatory mechanism and at least in the case of sympathectomy there is a decrease in matrix secretion as well as incorporation into osteoblasts (Singh et al. 1982). The odontoblastic response to IAN resection (as measured by ^{3}H-fucose incorporation) also differs from the pattern of ^{3}H-proline incorporation into odontoblasts. IAN resection increased ^{3}H-proline incorporation into ameloblasts, but increased the variability in ^{3}H-proline incorporation into odontoblasts as compared to controls (Chiego and Singh 1974).

In the present study, combination resection results in the highest absolute mean grain counts of all the experimental groups. This increase suggests a possible interaction involving first order sensory neurons in the inferior alveolar nerve with postganglionic sympathetic fibers from the superior cervical ganglion in the secretion of glycoprotein. Comparison of denervated and control incisors indicates that the greatest percentage increase in ^{3}H-fucose incorporation occurs after SC ganglionectomy. The IAN is intact in these mice indicating that loss of sympathetic inhibition may remove the modulating influence of the sympathetics on IAN regulation of odontoblastic activity. Neurotrophic influences on odontoblasts, ameloblasts, and osteoblasts have been hypothesized previously (Chiego et al. 1981; Singh et al. 1982). Neurotrophic dependence has been demonstrated in limb regen-

eration in newts (Dresden 1969; Mailman and Dresden 1979). Resection of the spinal nerves reduces DNA and protein synthesis in limb regenerates. However, there is a greater effect of denervation on collagen synthesis than noncollagenous protein synthesis (Mailman and Dresden 1979) although, only about 40% of protein synthesis is nerve dependent (Lebowitz and Singer 1970). Neural factors also appear to be involved in the regulation of collagen and non-collagen protein synthesis by odontoblasts in the mouse incisor. Further studies with various denervation procedures are now in progress to analyze the neural and temporal sequence of denervation effects on odontoblastic activity.

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