Degeneration of unmyelinated axons in the dental root canal induced by 6-OH-dopamine

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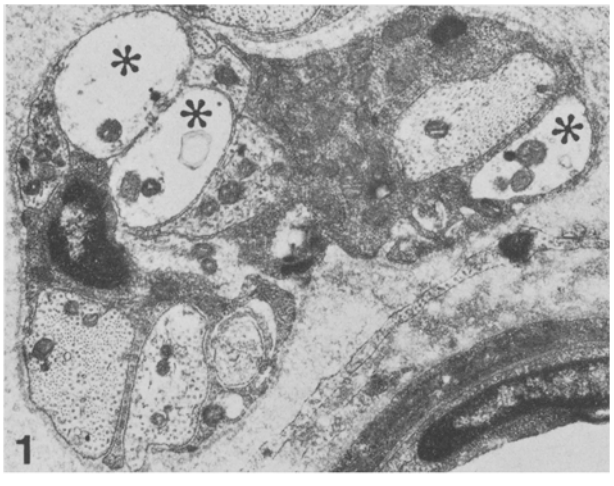
Summary. The pulpal nerve fibres of feline incisors were examined ultrastructurally after i.v. administration of 6-OH-dopamine. The presence of degenerating unmyelinated fibres at this site provides conclusive morphological evidence that sympathetic fibres enter the dental pulp.

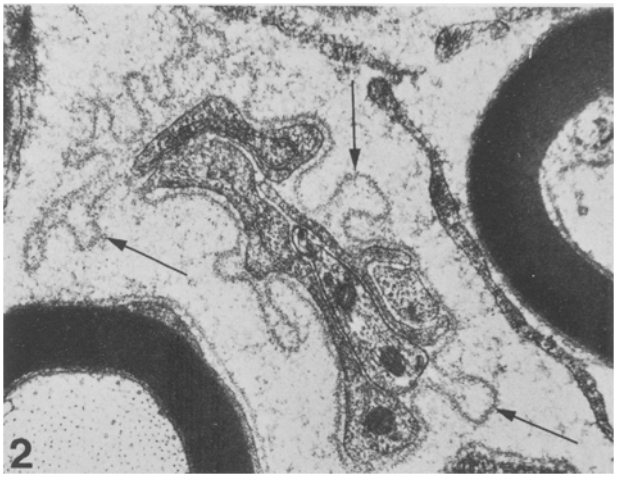
The teeth are supplied with a rich sensory innervation derived from the alveolar branches of the trigeminal nerve. The majority of the pulpal pain fibres appear to be unmyelinated, although some myelinated fibres are present[1]. Efferent adrenergic nerve endings are distributed along the pulpal arterioles of mature teeth, as shown with the Falck-Hillarp method[2]. These pulpal sympathetic efferents mediate vasoconstriction by acting on α-adrenergic receptors[3]. It is generally assumed that most or all of the pulpal sympathetic fibres are unmyelinated[4]. Thus, the dental pulp contains 2 sets of unmyelinated axons - one afferent mediating pain and one efferent composed of vasomotor fibres.

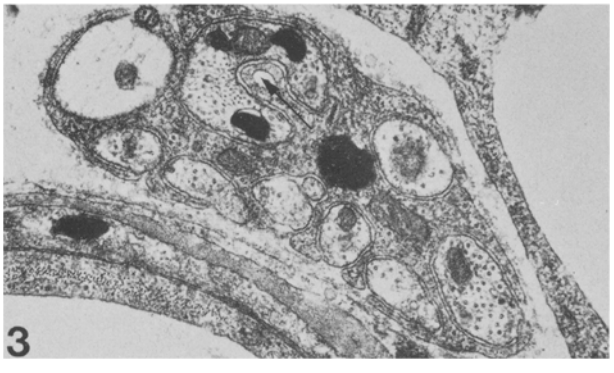
The proportion of pulpal nerve fibres that are sympathetic efferents has long been a matter of controversy. Christensen[4] concluded from light-microscopic degeneration studies on feline dental pulps, that few sympathetic nerve fibres actually enter the dental pulp. Feher et al.[5] observed only a few degenerating unmyelinated fibres ultrastructurally in feline mandibular dental pulps after unilateral exstirpation of the superior cervical ganglion. On the other hand, after transection of the inferior alveolar nerve all myelinated and most unmyelinated fibres disappeared. Both these studies may be questioned. The light-microscopic technique used by Christensen[4] does not allow an exact recognition of unmyelinated fibres. The data of Feher et al.[5] are based upon unilateral lesions which fail to take account of the transmedian innervation of dental pulps[6]. Moreover these authors did not specify either the level in the dental pulps or the type of teeth examined. Myelinated pulpal nerve fibres branch and lose their myelin sheaths during the intrapulpal course[4]. Consequently estimates of the proportion of myelinated and unmyelinated fibres depends upon the pulpal level examined. The nerve fibre population of the dental pulp is probably best examined in the root canal. In this region, very little branching occurs and the nerve fibres are concentrated in a few bundles along the blood vessels[7].

In a wide variety of tissues, degeneration of sympathetic adrenergic neurons has been achieved by the use of 6-OH-dopamine. The specific neurotoxic action of 6-OH-dopamine may provide means conclusively to identify the sympathetic nerve fibres in the dental pulp. 6-OH-dopamine selectively destroys terminal and preterminal sympathetic axons[8], but it remains to be shown whether a degeneration of pulpal sympathetic nerve fibres can also be elicited at the level of the root canal, where the main nerve bundles pass into the pulpal chamber. The present study was performed to test this hypothesis.

Material and methods. 3 adult cats were used as experiment







Figures 1-3. Electron micrographs from the apical region of the root canal in feline incisor pulps 48 h after i.v. administration of 6-OH-dopamine. ×21,000. Fig. 1. 3 degenerating unmyelinated axons lacking neurotubules and neurofilaments (asterisks) are seen together with largely normal axons in the same Schwann cell. Fig. 2. Portion of Schwann cell contained within a basement membrane with several redundant folds (arrows). Unmyelinated axons cannot be identified with certainty. Fig. 3. Protrusion of Schwann cell cytoplasm (arrow) into an unmyelinated nerve fibre, which contains electron-dense inclusions.

animals. Under Nembutal anaesthesia (40 mg/kg b.wt) the right vena saphena magna was exposed at the knee. A freshly prepared sterile solution of 6-OH-dopamine (Hässle, Sweden) dissolved in 0.9% saline containing 0.1 mg/ml ascorbic acid, was slowly injected into the vein. One of the animals received 36 mg/kg b.wt and survived for 24 h. The other 2 animals both received 50 mg/kg b.wt and survived for 24 and 48 h respectively after injection. At sacrifice the animals were perfused with 5% glutaraldehyde under Nembutal anaesthesia as described elsewhere[9]. The mandibular incisors were removed and decalcified for 6 days in a cold solution of 4% EDTA dissolved in a 300 mOsm cacodylate buffer (pH 7.3). The solution was exchanged daily. The decalcified teeth were osmicated, dehydrated in acetone and embedded in Vestopal W. Thin transverse sections, covering the entire root canal, were cut from the apical portion of the roots of 3 incisors from each animal. The thin sections were collected on one hole copper grids coated by carbon-stabilized formvar. After staining with uranyl acetate and lead citrate, the sections were examined in a Philips EM 301 electron microscope.

Results and discussion. In 2 cats sacrified 24 h after injection of 6-OH-dopamine, very subtle alterations were found in the incisor pulps. These were confined to a few (2-4) unmyelinated axons in which degenerative features similar to those seen after surgical sympathectomy[10] were observed. These axons were swollen, presented a loss of microtubules and contained electron-dense inclusions. In the cat surviving for 48 h, marked alterations were found in several unmyelinated fibres. Here, various degenerative features, such as axonal swelling, loss of axonal organelles and an intra-axonal content of a fragmented material, were present (figure 1). Large electron-dense bodies were commonly seen within the axoplasm of the affected fibres. Schwann cells devoid of intact axons were found encluded in a folded basement membrane with several empty pockets. The Schwann cells themselves presented largely a normal picture (figure 2). In addition, protrusions of Schwann cell cytoplasm into unmyelinated axons occurred (figure 3). Degenerating unmyelinated axons were seen together with intact fibres in the same Schwann cell. In no case could any alterations in the myelinated fibres be found. Likewise the non-nervous pulpal tissue had a normal appearance. Examination of pulps of normal permanent incisors in several adult cats failed to reveal unmyelinated fibres with the degenerative features mentioned above.

It seems clear from the present preliminary study that 6-OH-dopamine can be used as a tool for producing a selective degeneration of sympathetic nerve fibres in the root canal of feline mandibular incisors. Axonal degeneration has been achieved at some distance from the site of 6-OH-dopamine uptake at the terminal and preterminal segments. A dose level of 50 mg/kg b.wt. i.v. and a survival time of about 48 h appears to be suitable for this type of study.

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The timing of cyclophosphamide therapy in tumor-bearing rats affects the resistance to tumor challenge in survivors

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Summary. Cyclophosphamide given to rats 2 or 5 days after an injection of Yoshida ascites sarcoma cured approximately the same proportion of animals, but the resistance to a subsequent tumor challenge was found only in rats treated with the drug 5 days after tumor injection.

Cyclophosphamide effectively inhibits the growth of a variety of rodent neoplasms[1] and is widely used in the therapy of human tumors. However, cyclophosphamide is also strongly immunosuppressive[2], and therefore may interfere with the host immune reaction to the tumor. Inasmuch as the drug-induced immunosuppression is more profound in the proliferative phase of the immune response[2,3], the damage to the immune system caused by the drug applied for the purpose of eradication of the tumor may depend upon the stage of the immune response of the host to tumor antigens. The present paper reports on an experiment where the time period between tumor and cyclophosphamide injection significantly influenced the resistance of the treated animals to a subsequent tumor challenge 4 months later.

Materials and methods. 5-month-old female and male rats of WVM strain (derived from Wistar stock) weighing 200-250 g were used as recipients of rat Yoshida ascites sarcoma (YAS) which has been maintained in our laboratory for more than 10 years by weekly passages in WVM rats. Cyclophosphamide (Bosnalijek, Sarajevo) was given i.v. in a dose of 120 mg/kg. Dead animals were checked for the presence of the tumor in the abdominal cavity and other tissues. The 4-month survival rates were compared using the χ-square test with Yates correction.

Results and discussion. Rats were injected i.p. with 10^6 YAS cells/animal and were divided into 3 groups of 21 animals each. The 1st group did not receive any further treatment. Rats in the 2nd group were given cyclophosphamide 2 days after tumor injection, and rats in the 3rd group were injected with the drug 5 days after YAS injection. Within 2 weeks after injection, YAS killed all rats that were not given cyclophosphamide. 14 rats survived in the group that had been treated with cyclophosphamide 2 days after YAS injection, and 10 survived in the group that had been given the drug 5 days after tumor inoculation. The difference in