Neuropeptide Y: occurrence and distribution in dental pulps

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Nerve fibers displaying neuropeptide Y (NPY) immunoreactivity were seen in the dental pulp of several mammals, including man. Generally, the NPY fibers were more numerous in the apical part than in the coronal part and were distributed around small blood vessels and as single fibers in the pulpal stroma. Sequential staining with antibodies against the enzyme dopamine-β-hydroxylase (DBH), a marker for adrenergic neurons, and NPY showed that DBH and NPY were located in the same perivascular nerve fibers. Further, since chemical and surgical sympathectomy caused the disappearance of pulpal NPY fibers, it is conceivable that NPY fibers in the dental pulp are identical with adrenergic ones. There is thus a morphological basis for suggesting that NPY and noradrenaline cooperate in regulating pulpal blood flow. □ *Adrenergic nerves; dental innervation; dopamine-β-hydroxylase (DBH); immunocytochemistry*

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Many regulatory peptides are known to be distributed both in the central and in the peripheral nervous system. Neuropeptide Y (NPY), recently isolated from porcine brain (1), is but one example. NPY is a tyrosine-rich 36-amino acid peptide displaying structural similarities with the candidate hormones pancreatic polypeptide (PP) and peptide YY (PYY). It has therefore been proposed that NPY, PP, and PYY belong to the same peptide family (2).

There is a rich supply of NPY fibers in the upper respiratory tract, in the gastrointestinal tract, and in the genitourinary tract (3–5). Generally, the NPY fibers are located around blood vessels, arteries in particular, and within non-vascular smooth muscle. Sympathetic ganglia, such as the superior cervical ganglion, contain numerous NPY immunoreactive nerve cell bodies (3, 5). Extirpation of this ganglion or treatment with 6-hydroxydopamine (6-OHDA), known to destroy adrenergic nerve fibers, abolishes the NPY immunoreactivity in perivascular fibers.

In the present study, the occurrence and distribution of NPY immunoreactive nerve fibers in the mammalian dental pulp were investigated and compared with those of adrenergic fibers.

Materials and methods

Teeth were collected from adult rats, guinea pigs, cats, sheep, cows, pigs and man. Incisors were extracted from rats and guinea pigs, canines from cats, molars from sheep, cows, and pigs, and premolars and molars from man. Rats and guinea pigs were killed by decapitation under diethyl ether anesthesia, cats by bleeding under sodium pentobarbitone anesthesia. Molars from sheep, pigs, and cows were obtained from a nearby slaughterhouse. Human teeth were extracted under local anesthesia on orthodontic indications. The superior cervical ganglia of three rats were removed bilaterally under chloralose anesthesia 5 days before they were killed. Another three rats were subjected to chemical sympathectomy by intravenous injections of 6-OHDA (100 mg/kg, containing ascorbic acid, 0.2 mg/ml) 72 and 24 h before they were

killed (6). The teeth were split, and the pulps dissected out and immersed for 24 h in a 4% buffered formaldehyde solution. The specimens were then rinsed in Tyrode's solution, containing 10% sucrose, at +4°C for 48 h, frozen on dry ice, and sectioned in a cryostat at 10–20 µm. The sections were processed for the immunocytochemical demonstration of NPY and DBH by the indirect immunofluorescence method (7). The NPY antiserum (code NPYY/2) was a kind gift from Dr. P. Emson, MCR, Cambridge, U.K. The antiserum was raised in a rabbit against porcine NPY and used in a dilution of 1:400. It has been characterized in some detail elsewhere (4). DBH antiserum (a kind gift from Dr. R. Rush, Flinders University, Bedford Park, Australia) was raised in a rabbit against bovine adrenomedullary DBH (8). It was used in a dilution of 1:400. The tissue sections were exposed to the antisera overnight at +4°C and to fluorescein isothiocyanate-labeled goat anti-rabbit IgG diluted 1:20 for 1 h at room temperature. Some specimens from sheep dental pulps were first stained for DBH, examined in a fluorescence microscope, and photographed. They were then briefly treated with acid potassium permanganate for removal of antibodies (9). The completeness of the procedure was tested by the application of fluoresceinated anti-rabbit IgG. Sections devoid of DBH immunofluorescence were then immunostained for NPY and again photographed. Control sections were incubated with NPY antiserum inactivated by the addition of excess amounts of antigen (10–100 µg porcine NPY/ml diluted antiserum). Cross-reactivity with other structurally related peptides and proteins cannot be

Table 1. Relative frequency of NPY nerve fibers in dental pulps from various mammals

Species	No. of teeth examined	Relative frequency of NPY nerve fibers
Rat	8	+
Guinea pig	10	+++
Cat	3	+
Sheep	4	+++
Cow	5	+
Pig	4	+++
Man	4	++

The relative number of nerve fibers was graded arbitrarily: + = few fibers; ++ = moderate number of fibers; +++ = rich supply of NPY fibers.

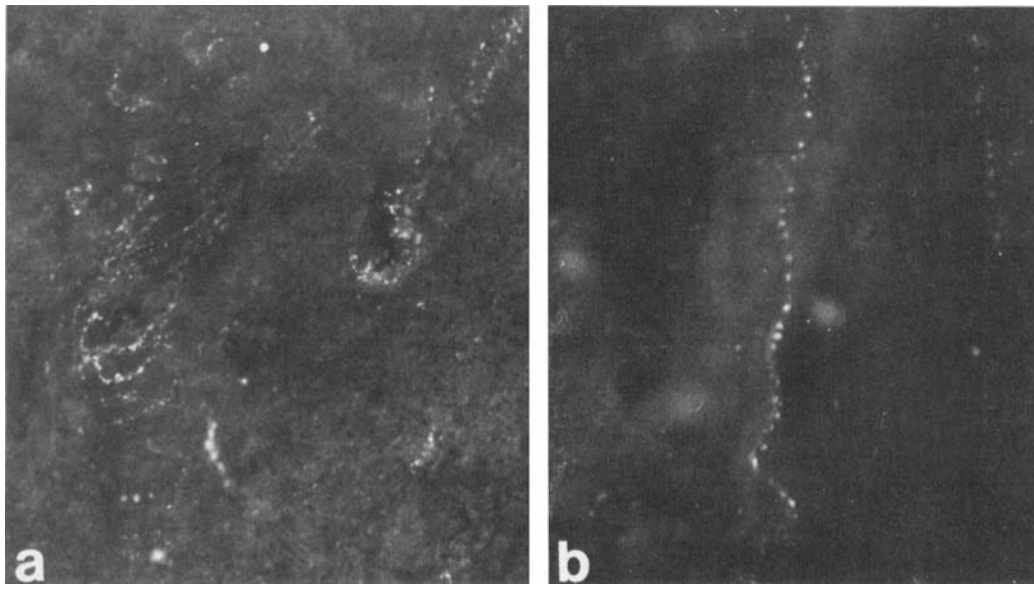

Fig. 1. Human dental pulp. (a) Low-magnification photograph showing distribution of NPY fibers in the apical part mainly around blood vessels (×150). (b) Higher magnification, showing single-beaded NPY fibers without obvious relation to blood vessels (×300).

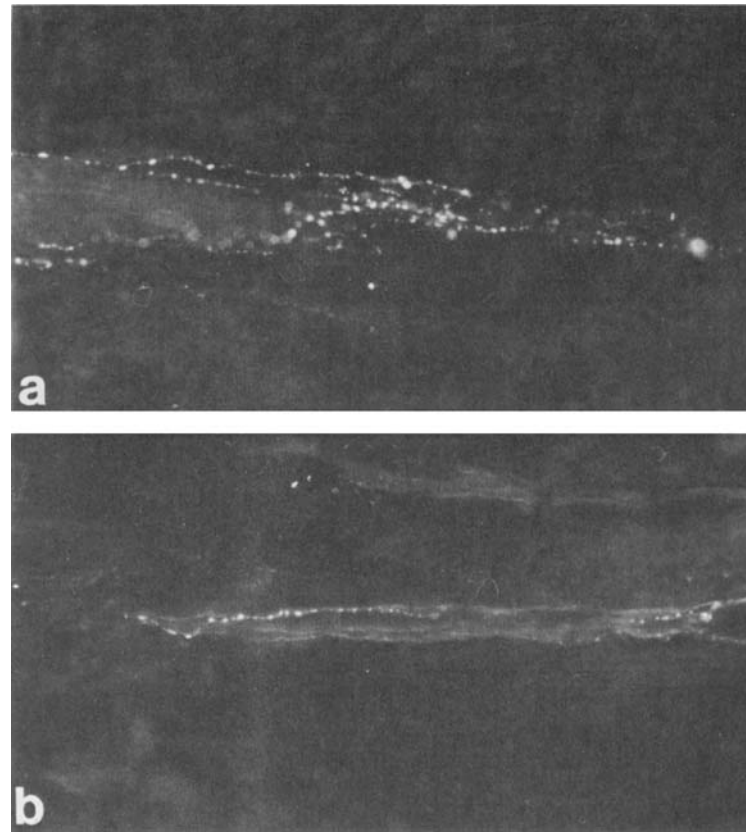

Fig. 2. Dental pulp of cat (a) and pig (b). Obliquely cut blood vessels in the lower part of the pulps innervated by NPY fibers. (×250.)

excluded. It is therefore appropriate to refer to the immunoreactive material as 'NPY-like' or 'DBH-like'. For brevity, however, the immunoreactive nerve fibers are referred to as NPY or DBH fibers.

Results

Nerve fibers displaying NPY immunoreactivity were observed in dental pulp tissue from all species examined. The NPY fibers were numerous in the guinea pig, sheep, and pig and less numerous in the rat, cat, cow, and man. The results are summarized in Table 1.

Fine, varicose nerve fibers were seen both coronally and apically in the pulp of most species studied (Figs. 1–4). Generally, the fibers were more numerous in the apical parts than in coronal parts. Most NPY fibers seemed to be associated with small blood vessels (Figs. 1a and 2). Perivascular NPY fibers were coarse and intensely immunoreactive. However, not all vessels were accompanied by immunoreactive fibers. In addition, single, delicate, moderately immunoreactive NPY nerve fibers ran in the pulpal stroma with no obvious relation to blood vessels (Figs. 1 and 3). Further, single, fine-beaded NPY fibers ran close to the subodontoblastic layer (the zone of Weil) (Fig. 3).

After removal of the superior cervical ganglia or treatment with 6-OHDA no or very few fibers were observed in the rat dental pulps.

In sheep dental pulps, staining with DBH antiserum showed DBH immunoreactive fibers both coronally and apically. The fibers were particularly numerous around small blood vessels in the pulpal stroma. Sequential immunostaining with antisera against DBH and NPY showed that DBH and NPY

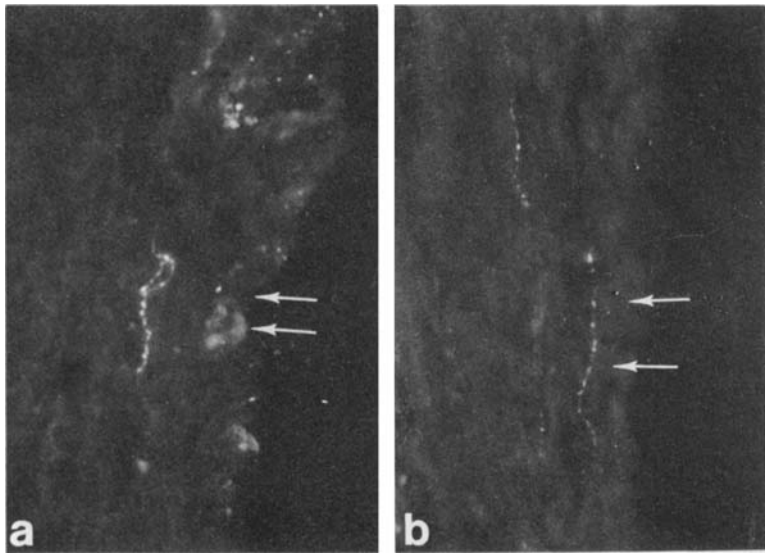

Fig. 3. Dental pulp of cat (a) and pig (b). Single NPY fibers running close to the subodontoblastic layer (arrows). (×200.)

occurred together in nerve fibers around small blood vessels (Fig. 4).

Discussion

In the present study a rich supply of NPY immunoreactive nerve fibers was demonstrated in the dental pulps of several mammals, including man. The NPY fibers were distributed around small blood vessels and as single terminals in the pulpal stroma. Previous studies have shown acetylcholinesterase-positive nerve fibers and noradrenaline-containing nerve fibers in the dental pulps (10, 11). In addition, dental pulps harbor substance P-containing nerve fibers and those storing vasoactive intestinal peptide (VIP) (12, 13).

In the cat stimulation of the inferior alveolar nerve increases the amount of substance P-like immunoreactivity in the pulp perfusates (14). There are numerous substance P

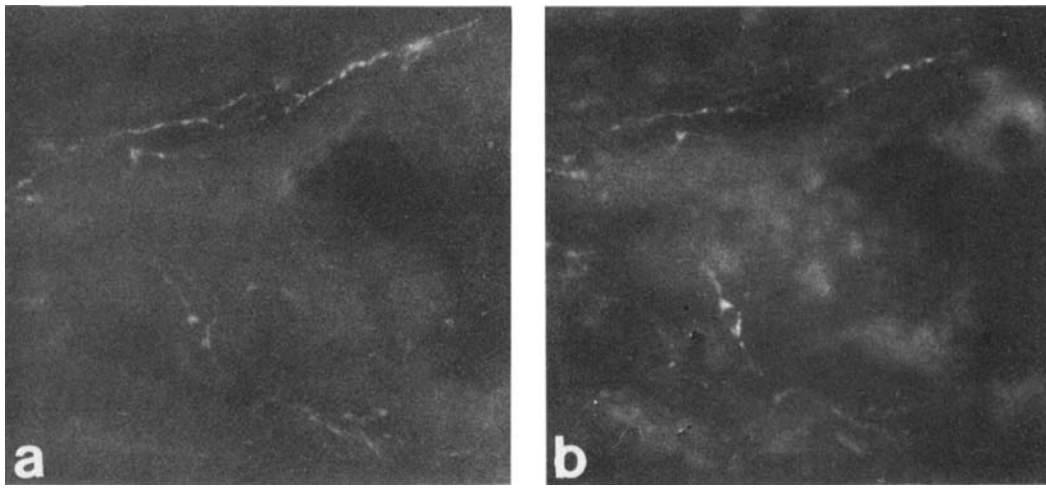

Fig. 4. Sheep dental pulp. Section first immunostained for dopamine-β-hydroxylase, a marker for adrenergic neurons (a). After removal of the antibodies the section was immunostained for NPY (b). Note identity between dopamine-β-hydroxylase- and NPY-storing nerves. (×250.)

nerve cell bodies in the trigeminal ganglia and in the spinal ganglia. Substance P fibers in the pulpal stroma disappear after sectioning of the inferior alveolar nerve but not after sympathectomy (14). These findings point to a sensory nature of pulpal substance P fibers, and it has been proposed that most of the substance P fibers outside the gut represent peripheral ramifications of primary sensory neurons.

VIP fibers are numerous in dental pulps (13), and in several vascular beds VIP gives rise to an atropine-resistant vasodilation (15). In view of the potent vasodilatory action of VIP it is conceivable that pulpal VIP fibers take part in the control of local blood flow.

Electrical stimulation of sympathetic nerve fibers causes constriction of pulpal blood vessels and lowers the tissue fluid pressure in the pulp (16). To date, little is known about the functional role of NPY. Among potential targets for NPY fibers are neurones, blood vessels, non-vascular smooth muscle, and exocrine glands (3). NPY constricts several peripheral blood vessels, and the recent finding that NPY in vitro markedly potentiates noradrenaline-induced vasoconstriction (17) makes it tempting to suggest that NPY in perivascular nerve fibers in dental pulps is involved in the control of pulpal blood flow.

The elimination of perivascular NPY fibers by chemical or surgical sympathectomy and the demonstration that NPY coexists with DBH enable us to conclude that the majority of perivascular NPY fibers are adrenergic. Thus, there is a morphological basis for suggesting that pulpal NPY and noradrenaline cooperate in eliciting vasoconstriction.

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