Pulpal neuropeptidergic fibers

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SUMMARY

A study was carried out on Met- and Leu-enkephalin, Gastrin / CCK-, SP-, CGRP-, NPY- immunoreactive fibers using paraffin sections of dental pulp taken from 8 apparently normal teeth (wisdom teeth or teeth extracted for orthodontic reasons).
Within the limitations of the samples studied, dental pulp is characterized by the presence of sensory (Enkephalin-, Gastrin / CCK- immunoreactive) and pain fibers (SP-immunoreactive) and of fibers with a potent vasodilatory action (CGRP-immunoreactive) and by the absence of fibers with a vasoconstrictor action (NPY-immunoreactive).

KEY WORDS:

Immunohistochemistry, pulpal nerve fibers, neuropeptides.

RÉSUMÉ

Notre étude a porté sur la mise en évidence sur des coupes après inclusion à la paraffine des fibres Met- et Leu-enképhaline, Gastrine / CCK-, SP-, CGRP- et NPY-immunoréactives, dans la pulpe de huit dents normales (dents de sagesse ou dents extraites pour des raisons orthodontiques).
Dans la limite des échantillons utilisés, la pulpe dentaire est caractérisée par la présence de fibres sensitives (Enképhalin-, Gastrin / CCK-immunoréactives), des fibres de la douleur (SP-immunoréactives) et de fibre à action vasodilatatrice (CGRP-immunoréactives) et par l'absence de fibres à action vasoconstrictive (NPY-immunoréactives).

MOTS CLÉS:

Immunohistochimie, fibres nerveuses pulpaires, neuropeptides.

INTRODUCTION

Until fairly recently (see Ogilvie and Ingle, 1965), the traditional methods of staining nerve fibers (e.g. Palmgren) had supported the belief that the innervation of dental pulp consisted of:

- myelin fibers accompanying the vascular ramifications;
- free nerve endings deriving from the central nervous system (CNS);
- sensory fibers in apparent contact with the odontoblasts and predentin.

In the last ten years or so, the refinement of the methods used in immunohistochemistry has led (i) to the ever-growing availability of monoclonal antibodies and (ii) to the possibility of performing immunohistochemical reactions on ordinary paraffin sections and of developing the reactions in a completely satisfactory manner (see Barbolini et al., 1988).

The availability of antibodies against the S100 proteins has facilitated the refinement of a method for

the morphological study of nerve structures that is both easy to perform and highly reproducible.

In particular, the so-called rabbit anti-cow S100 refers to an antibody (class IgG) which was originally known for its activity against a cerebral antigen (isolated by Moore, 1965, in the form of soluble cow-brain protein) but then shown to be capable of reacting with a whole series of cells and structures: gliocytes and ependymal cells; melanocytes and Langherans cells; Shawm cells and peripheral nerve endings; serous cells of the peribronchial glands and their nerve endings. The sharp morphological image produced by the reaction does not, however, admit of a direct functional determination of the nerve structures shown.

On the other hand, immunohistochemistry has evolved into an extremely useful method for demonstrating different types of compounds, among them neuropeptides, both in the central and peripheral nervous systems and in other tissues (e.g. endocrines). Important information about such compounds has generally been obtained by immunofluorescence techniques, for example in the case of the airways (Uddman and Sundler, 1987).

The aim of this paper is to establish whether it might be possible to apply immunohistochemical techniques for neuropeptides to pulpal sections embedded in paraffin and so ascertain the function of the nerve fibers.

MATERIALS AND METHODS

The present study was carried out on the dental pulp of eight apparently normal teeth (wisdom teeth or teeth extracted for orthodontic reasons) from subjects of both sexes aged between 16 and 28. Immediately after extraction, a series of six holes was drilled in each tooth with a tungsten bur so as to allow the fixative (10% neutral formalin) to permeate the pulp. Then, with the aid of a powerful magnifying glass, the tooth was cut in a longitudinal (coronoapical) direction with a water-cooled diamond disk mounted on a straight hand-piece so as to obtain two symmetrical halves. All the pulp fixed *in situ* was then carefully removed and embedded in paraffin in the usual way.

Indirect immunostaining method was performed on 5 μm paraffin sections and the antigen-antibody reaction shown by the ABC method, as reported elsewhere (Barbolini et al., 1988).

Each series of preparates had its positive and negative control sections.

The antiserums (host: rabbit; class IgG) were supplied by DAKO (S100) and Amersham International (neuropeptides). The latter were prepared in rabbits using synthetic neuropeptides conjugated to bovine serum albumin using glutaraldehyde as coupling agent.

Plate 1 – Immunohistochemistry of pulpal nerve fibers. Predentin and odontoblasts are at the top or at the right.
Planche 1 – Immunohistochimie des fibres nerveuses de la pulpe. La prédentine et les odontoblastes sont en haut ou à droite de l'image.

Fig. 1 – S100: a strong reaction of the nerve fiber bundles is noticeable (×320).
Fig. 1 – Protéine S100: on note une importante réaction au niveau des fibres nerveuses (×320).

Fig. 2 – Met-enkephalin immunoreactive fibers are perivascular with free nerve endings and diffuse network of fine interlacing fibrils (×540).
Fig. 2 – Les fibres Met-enképhaline-immunoréactives sont périvasculaires avec des terminaisons nerveuses libres et un réseau diffus de fines fibrilles entrelacées (×540).

Fig. 3 – Leu-enkephalin immunoreactive fibers are perivascular and penetrate the odontoblast/predentin layer (×320).
Fig. 3 – Les fibres Leu-enképhaline-immunoréactives sont périvasculaires et pénètrent dans la couche odontoblastes/prédentine (×320).

Fig. 4 – Isolated bundles of gastrin/CCK-immunoreactive fibers are visible (×125).
Fig. 4 – On aperçoit des faisceaux isolés de fibres gastrine/CCK-immunoréactives (×125).

Fig. 5 – The SP-immunoreactive fibers are perivascular, running below and parallel to the odontoblast/predentin layer and penetrating it; they are also in the form of a diffuse network of fine interlacing fibrils (×540).
Fig. 5 – Les fibres SP-immunoréactives sont périvasculaires, courant en dessous et parallèlement à la couche d'odontoblastes/prédentine, et la pénétrant; elles se présentent également sous la forme d'un réseau diffus de fines fibrilles entrelacées (×540).

Fig. 6 – A picture similar to that of fig. 5, if less intense, presented by the CGRP-immunoreactive fibers (×540).
Fig. 6 – Une image similaire à la figure 5, quoique moins intense, est présentée par les fibres CGRP-immunoréactives (×540).

Plate 1 – Planche 1

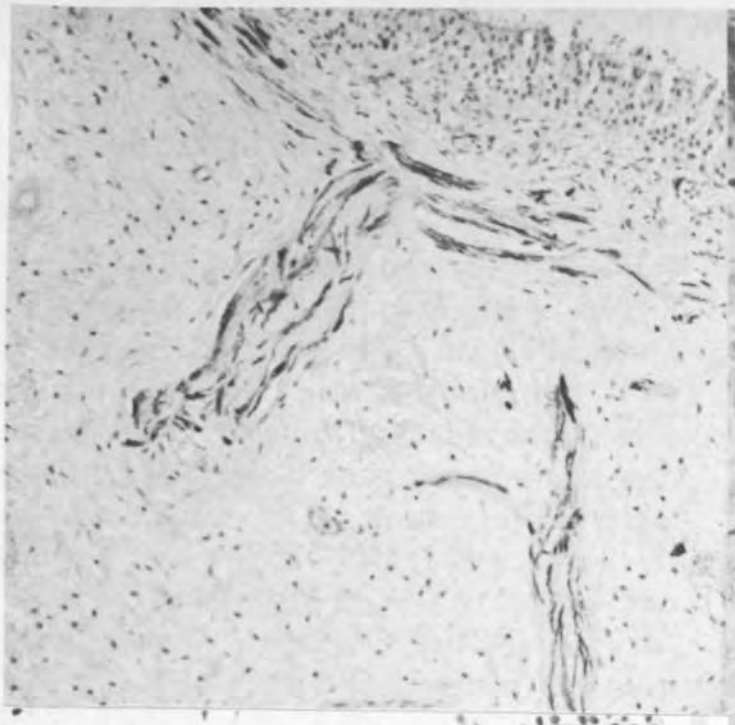

Fig. 1

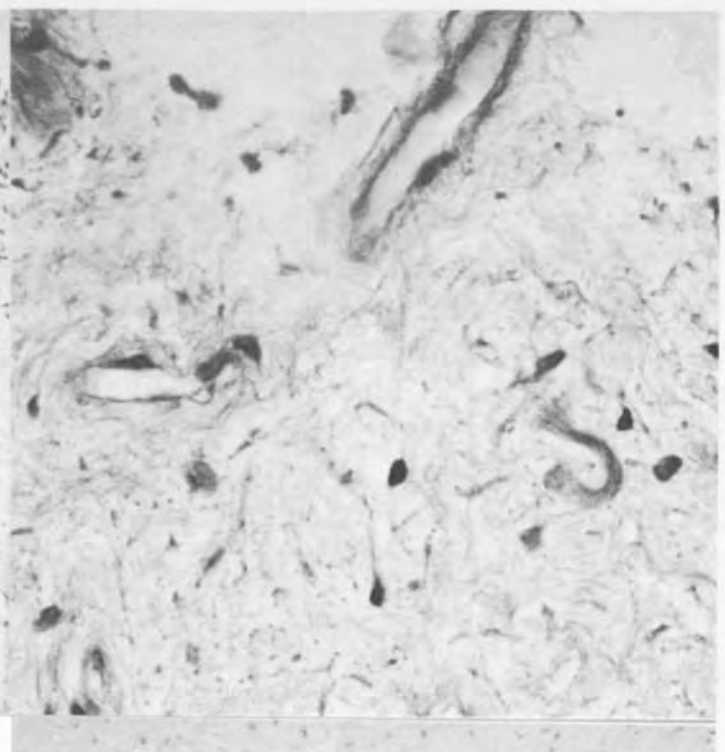

Fig. 2

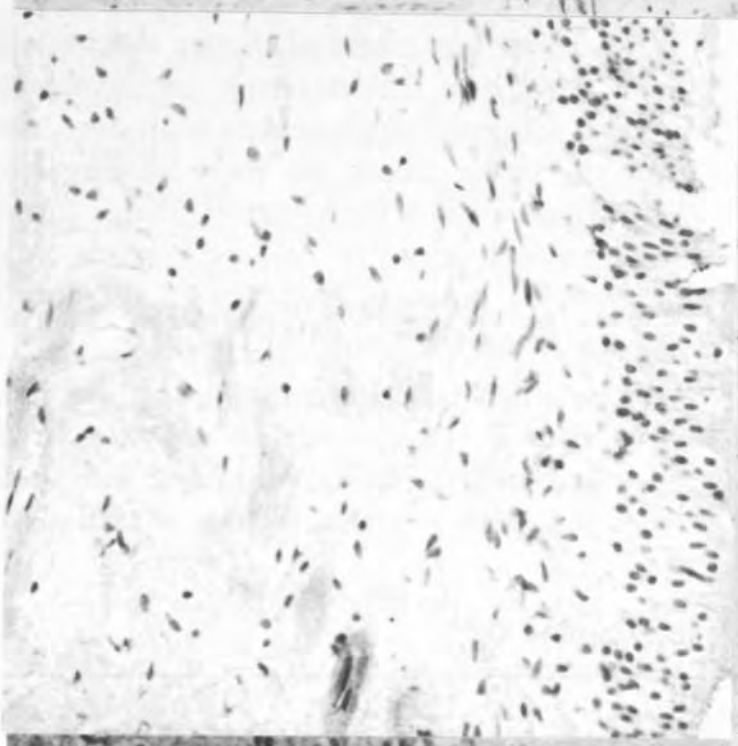

Fig. 3

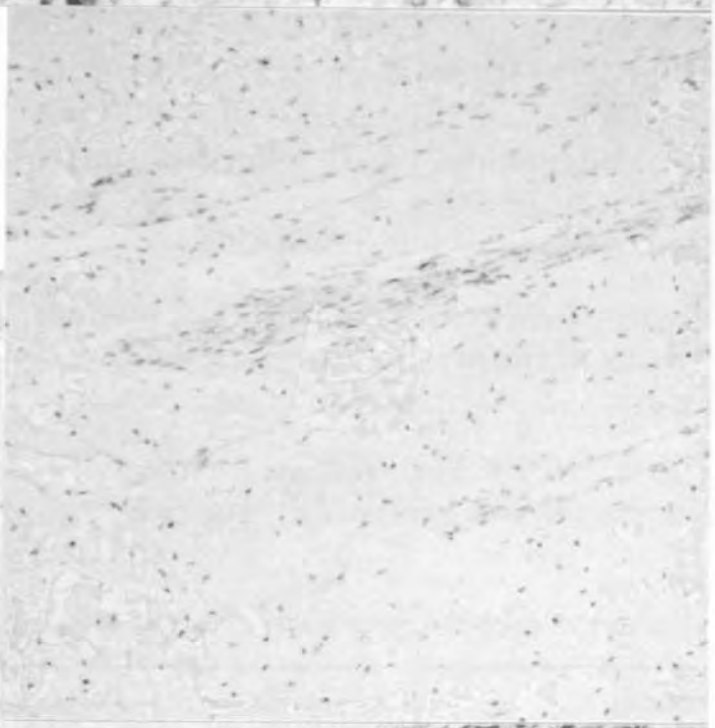

Fig. 4

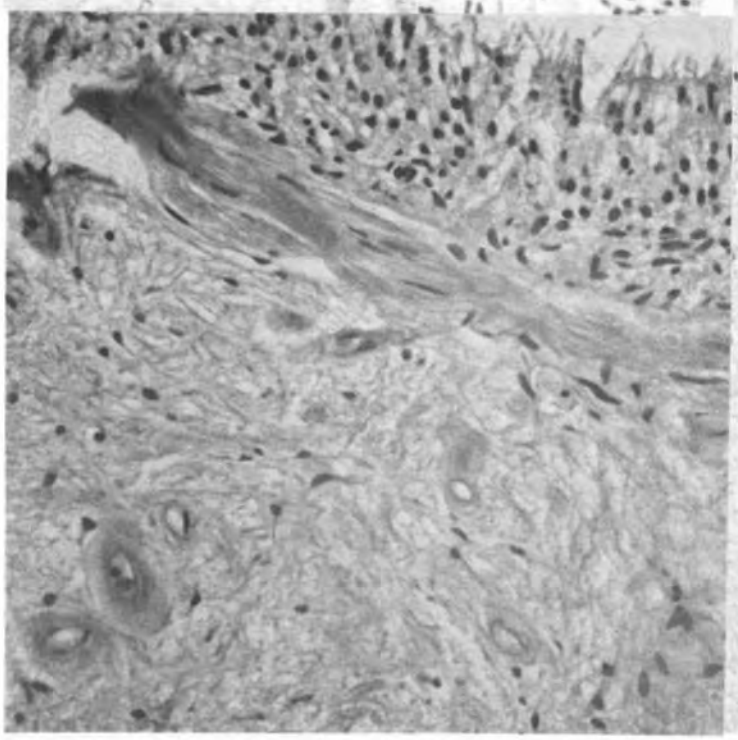

Fig. 5

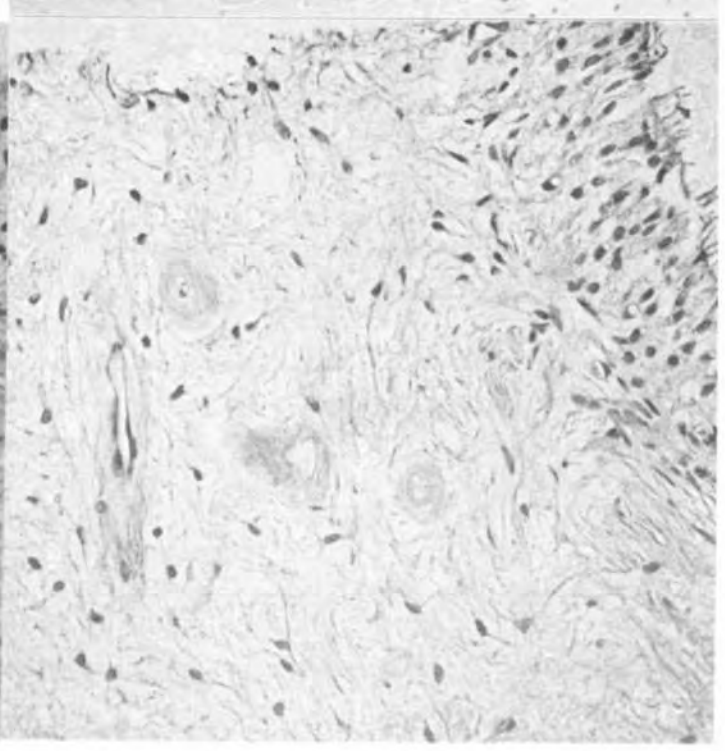

Fig. 6

RESULTS

S-100 proteins (fig. 1). The isolated myelin sheaths are readily recognized, as are the nerve fibers, particularly if they are in discrete bundles perpendicular or parallel to the predentin and penetrating it.

Met (5-L-methionine)- and Leu (5-L-leucine) enkephalin (figs. 2 and 3). The overall immunohistochemical pattern is finer than the previous one and similar for the two neuropeptides with perivascular fibers that penetrate the predentin. The Met-enkephalin-immunoreactive fibers stain with consistently greater intensity than the Leu-immunoreactive fibers; also, they show free nerve endings and a diffuse network of fine, interlacing fibrils (fig. 2).

Gastrin-CCK (fig. 4). The reaction is largely restricted to isolated bundles of nerve fibers in the pulp.

Substance P (SP). The SP immunoreactive fibers react in a homogenous, diffuse fashion and follow a clearly perivascular configuration (fig. 5). Also, they can be seen close to or penetrating the predentin together with a diffuse network of fine, interlacing fibrils.

Calcitonin gene-related peptide (CGRP). The reaction is similar to that of the previous peptide, though less intense (fig. 6).

Neuropeptide Y (NPY). The reaction for the NPY-immunoreactive fibers is consistently negative.

DISCUSSION

The immunohistochemistry of pulpal fibers is a little-known but extremely promising future field of research that can yield insight into the delicate biological, physiopathological and pathological mechanisms of dental pulp; from a methodological point of view it can be performed now following standardized, reproducible procedures that are relatively simple to carry out using paraffin sections. However, from a theoretical and operative point of view, the main limiting factors for the comprehension of the role and function of pulpal neuropeptidergic-immunoreactive fibers are: (i) the paucity of specific information on the subject; (ii) the possible co-existence of peptides and «classic» neurotransmitters and/or of several peptides in the same neurone; (iii) the absence of specific, potent antagonists to most of the neuropeptides.

Table 1 sets out essential, hence theoretically pertinent, data from the literature on the neuropeptides studied. Comparing these data with ours, the result is that the only datum we were able to find in the literature with which our findings agree is that substance P can be released from peripheral, presumably sensory, branches in the tooth pulp of the cat (Olgarth et al., 1977).

On the other hand, according to Hökfelt et al., 1980, substance P is involved in sensory processes of various types, including pain, and our findings may offer a morphological basis for the explanation of the

TABLE 1. Overview of the better known neuropeptides investigated in this study

Classical transmetter (MW ~ 200)	Neuropeptides (MW ~ 3000)	Located area (in addition to the PNS and endocrine system)	Remarks	Function
Dopamine	Enkephalin (5-L-Met.)	CNS (limbic system)	Preganglionic neurones	Sensory nature
	Enkephalin (5-L-Leu.)	CNS	Preganglionic neurones	Sensory nature
	Gastrin/CCK	CNS (limbic system, cortex)	Gastrointestinal in origin	Sensory nature
Serotonin	Substance P	CNS (limbic system)	Tachykinin coexistence with CGRP	Sensory nature (pain)
	CGRP	Trigeminal and other ganglia	Association with sensory neurones	Potent vasodilator
Norepinephrine	Neuropeptide Y	CNS (extrapyramidal system)	Coexistence with VIP (vasoactive intestinal peptide)	Vasoconstrictor

pain classically associated with pulpitis. Furthermore, Lundberg et al. (1984*a* and *b*) have reported that substance P mediates protein extravasation and vasodilatation in the airways, and its coexistence with CGRP may explain the hyperemia which is constantly associated with acute pulpitis. On the contrary the negative reaction for NPY suggests that dental pulp does not possess effective defence mechanisms of the vasoconstrictor type and is therefore a highly-vulnerable tissue. This first series of problems can best be elucitated in the course of further research devoted to pathological pulpal tissue.

The different immunohistochemical pattern emerging for enkephalin-immunoreactive fibers agrees with the notion put forward by Larsson et al. (1979), namely, that Leu- and Met-enkephalin neurones constitute separate systems. More generally, the wealth and the structural complexity of peptidergic fibers presented by dental pulp may offer a morphological basis for an explanation of the awareness – and the fear connected with it – that the patient displays when afflicted by dental disease. In order to evaluate the possibility of a functional link between the CNS and dental pulp, we propose to study the neuropeptidergic pattern of the dental pulp of patients known to be affected with various cerebral and/or mental disease.

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