

# Immunolocalization of CGRP, NPY and PGP 9.5 in guinea pig skin

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*By means of immunoperoxidase and immune-alkaline phosphatase methods the immunoreactivities to neuropeptides: neuropeptide Y (NPY), calcitonin gene related peptide (CGRP) and a pan-neuronal marker, the protein gene product 9.5 (PGP 9.5), were evaluated in guinea pig deep dorsal skin specimens. CGRP immunoreactive (CGRP-IR) and NPY-immunoreactive (NPY-IR) nerve fibres were dispersed in the papillary dermis and sometimes inside the hair roots and among the sebaceous gland cells. Such localized nerve fibres have not so far been described. In the subcutaneous layer nerve trunks were found composed of CGRP-IR and NPY-IR nerve fibres. Some of these indicated vestigial or negative immunoreactivity to PGP 9.5.*

**Key words:** CGRP, NPY, PGP 9.5, guinea pig, skin

## INTRODUCTION

Very numerous nerves have been observed in the skin composed of afferent, noradrenergic and cholinergic nerve fibres. Neuropeptides can be secreted by all these kinds of nerve fibre at their endings. However, their roles in the physiology and pathology of the skin have not yet been explained. NPY is a 36 amino acid peptide that is widely distributed throughout both the central and peripheral nervous systems [2]. NPY coexists and is co-released with noradrenaline in the sympathetic nerve terminals and is associated with long-term vasoconstriction [1, 9].

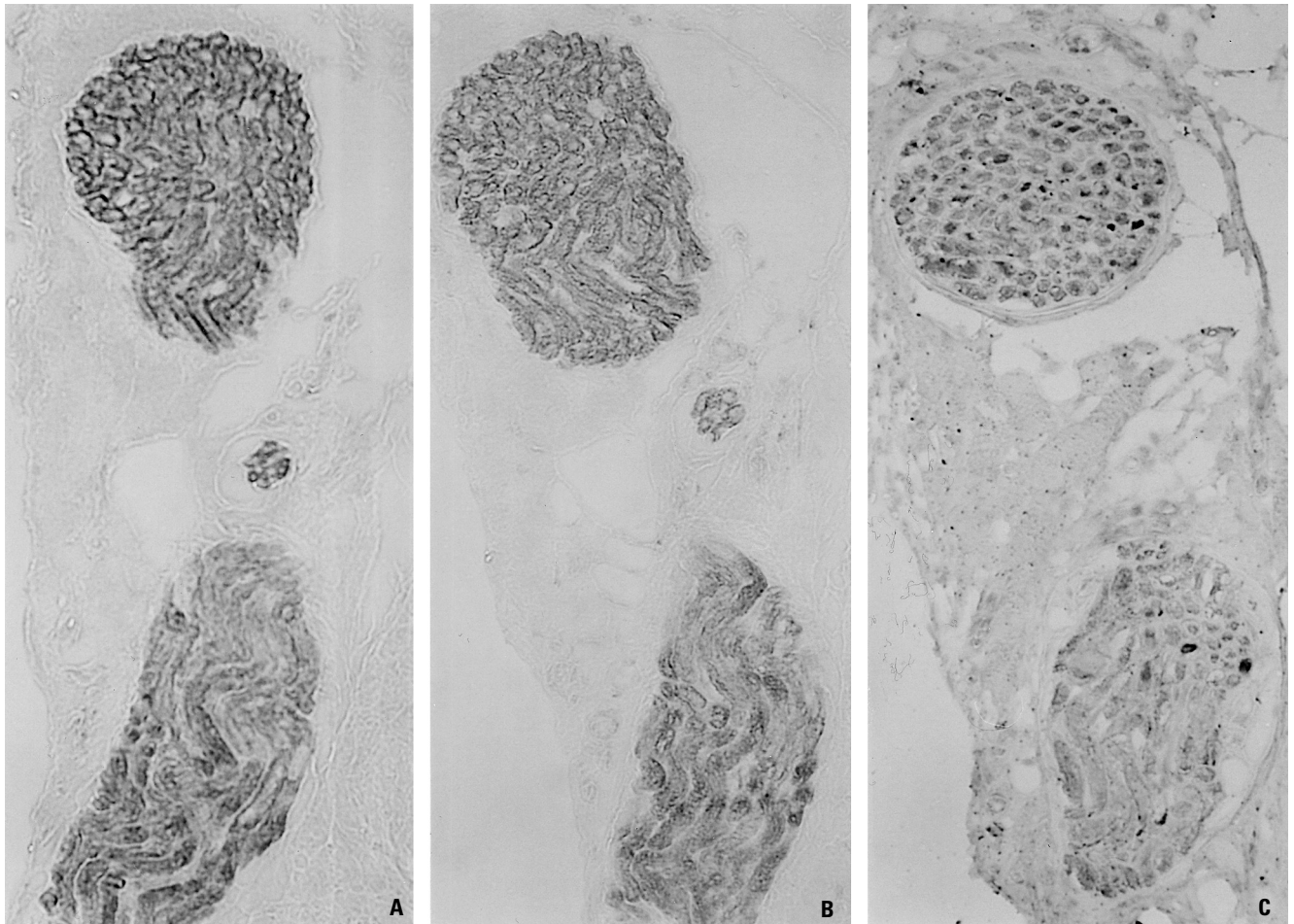
CGRP, a product of the calcitonin/CGRP gene, is localized in and released from primary sensory neurons as a potent and long-acting vasodilator [5]. It is becoming generally accepted that applying antibodies against PGP 9.5 (a general pan-neuronal marker) enables most, if not all, nerve fibres to be detected [4]. In a comparative study of cutaneous nerves in different animals and man it was found that the specific peptides in the nerves innervating various structures were consistent between species [4]. The aim of this study was to compare the distribution

patterns of nerve fibres immunoreactive to NPY, CGRP and PGP 9.5 in guinea pig dorsal skin.

## MATERIAL AND METHODS

Ten male guinea pigs (*Cavia porcellus*) aged 5–6 months and weighing 350–400 g each, were killed by decapitation after being anaesthetised with 35 mg pentobarbital i.p. The procedure was approved by the Local Ethics Committee. Skin specimens containing the deep subcutaneous layer were collected. The material was fixed in Bouin's solution and embedded in paraffin. 6  $\mu$ m slices were used throughout the study.

Immunohistochemical reactions were performed using rabbit polyclonal anti-NPY primary antibody (1:400; Eurodiagnostica), rabbit polyclonal anti-CGRP antibody (1:800; Eurodiagnostica) and sheep polyclonal anti-PGP 9.5 antibody (1:50; The Binding Site, UK). Primary antibodies were incubated for 1 h with tissue sections after blocking endogenous peroxidase with 2% hydrogen peroxide. The slides were washed with PBS and incubated with a proper secondary antibody.



**Figure 1.** In the same subcutaneous nerve immunoreactions to NPY (A), CGRP (B) and PGP 9.5 (C) are shown. Immunoperoxidase method. Magnification 240  $\times$ .

The secondary antibodies used for rabbit primary antibodies were the alkaline phosphatase (AP) or peroxidase (PO) conjugated EnVision antibodies (DAKO, Denmark) and that for the sheep antibody was donkey anti-sheep PO-conjugated antibody (The Binding Site, UK), in dilution 1:50. As chromogens diaminobenzidine (DAB) for PO- and Fast Red TR/Naphthol AS-MX (Sigma) for AP-conjugated antibodies were used.

As negative controls we used slices subjected to immunohistochemical reactions and with the primary antibody omitted or replaced with the non-immune respective serum in the appropriate dilution. The negative control for NPY staining was additionally performed by using the primary antibody pre-absorbed with 20  $\mu$ g of NPY (Sigma)/1 ml of pre-diluted primary antibody. As a positive control, guinea pig jejunum slides were used.

## RESULTS AND DISCUSSION

There were no apparent differences in CGRP-IR or NPY-IR results between the samples treated with the PO-conjugated secondary antibody in comparison with those treated with the AP-conjugated antibody.

NPY-IR and CGRP-IR were found in the dorsal skin in the sparsely scattered (up to 5 in one field of vision using magnification 240  $\times$ ) nerve fibres of the papillary dermis. Sometimes, NPY-IR and CGRP-IR nerve fibres were present inside the hair roots and among the sebaceous gland cells. Nerve fibres so localized have not so far been described, probably because immunofluorescent methods preclude the detection of nerve terminals inside a hair root. However, cells immunopositive to NPY inside the epidermis, described as Langerhans cells in mice [6], were not found by us. The use of the anti-PGP 9.5 antibody allowed detection of numerous nerves and single nerve fibres in the dermis and in

the subcutaneous layer. PGP 9.5-IR nerve fibres were not detected inside hair follicles and sebaceous glands.

In the deep dermis and subcutaneous layer co-expression of NPY-IR and CGRP-IR was not found in the nerve fibres of the blood vessel walls. These nerve fibres were PGP 9.5-IR.

In some large subcutaneous nerves the majority of nerve fibres visible were NPY-IR or CGRP-IR (Fig. 1A, B). Sometimes this occurred on consecutive sections and then NPY-IR and CGRP-IR also appeared to be present in the same nerve fibres. In these nerve trunks the intensity of immunostaining for PGP 9.5 was apparently diversified, ranging from very strong to vestigial or even negative in different nerve fibres (Fig. 1C). The type of nerve fibre that appears to be concomitantly CGRP-IR and NPY-IR seems to be sympathetic, postganglionic and non-adrenergic [7]. The CGRP-IR/NPY-IR nerve fibres found by us are possibly those that innervate the hairs and sebaceous glands in the skin. This kind of fibre is thought to innervate the sweat glands which, in guinea pigs, are practically exclusively present in the glabrous skin of the paws [3].

However, it cannot be ruled out that these are sensory fibres, since in guinea pigs, sensory nerve fibres can transiently express NPY during development [8] and it is possible that in the postnatal period they can express this neuropeptide, too. The roles of the possible NPY-IR/CGRP-IR nerve fibres that we described, as well as the significance of the finding, remain to be elucidated.

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