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Vasoactive intestinal polypeptide (VIP) — immunoreactive nerve fibres in the mammary gland of the pig

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Immunohistochemical studies have been performed to investigate the coexistence of VIP with dopamine-β-hydroxylase (DβH), vesicular acetylcholine transporter (VAChT), somatostatin (SOM) or neuropeptyd Y (NPY) within nerve fibres supplying the immature mammary gland in the pig. Generally, a moderate number of the VIP-immunoreactive (VIP-IR) nerve fibres were located in the nipple and parenchyma of the gland. VIP-IR fibres surrounded smooth muscle cells (SMC), blood vessels (BV) and lactiferous ducts (LD). Double-labelling immunohistochemistry revealed that some of VIP-IR nerve fibres also contained immunoreactivity to DβH. VIP/DβH-IR nerves were associated with BV and SMC and single fibres were observed around the LD in both nipple and parenchyma of the gland. VIP/VAChT-IR nerve fibres were not observed. The majority of VIP-IR fibres associated with SMC were also SOM-IR. Less numerous VIP/SOM-IR fibres supplied the BV and were located around the LD of the gland. A small number of VIP-IR nerves also displayed immunoreactivity to NPY. VIP/NPY-IR nerve fibres supplied the BV of the gland.

key words: mammary gland, pig, innervation, VIP, immunohistochemistry

INTRODUCTION

VIP belongs to the glucagon family of peptides and is present in the central and peripheral nervous system [2, 5]. Nerve fibres containing immunoreactivity to VIP have been observed in many peripheral organs, including the mammary gland [1, 6–8]. VIP-IR nerve fibres which were found in the human [1] or rat [1, 6–8] mammary gland might be involved in the relaxation of SMC, an increase in blood flow and a decrease in ductal tone [1, 8]. However, there are no data dealing with VIP-IR nerve fibres supplying the porcine mammary gland.

Early studies have shown that some neurons which supply the porcine mammary gland originate from sympathetic chain ganglia (SChG) and contain immunoreactivity to VIP [4]. The aim of the present investigation was to disclose the occurrence and co-localisation patterns of VIP and DββH (a marker of catecholaminergic nerve structures) or VAChT (a marker of cholinergic nerve structures) as well as some neuropeptides including SOM and NPY within nerve fibres supplying the immature mammary gland in the pig.

MATERIAL AND METHODS

Three sexually immature female pigs (3 months, 50–55 kg body weight) of the Large White Polish breed were used. The animals were deeply anaesthetised with sodium pentobarbital (Vetbutal, Biowet, Poland; 20 mg/kg, iv.) and transcardially perfused with 4% buffered paraformaldehyde solution (pH 7.4). After perfusion, the whole mammary gland complexes were

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dissected out, postfixed by immersion in the same fixative for 30–45 min and then transferred into 18% buffered sucrose solution (pH 7.4).

The samples were cut into 10 μm thick cryostat sections, mounted on chrome-alum-gelatin-coated glass slides and processed for double-labelling immunofluorescence according to the Wessendorf and Elde method [9]. Antibodies raised in mice (VIP, MaVIP, dilution 1:3000, East Acres), rabbit (DβH, DZ 1020, dilution 1:500, Affiniti, VAChT, H-V006, dilution 1:800, Phoenix Pharm., NPY, RNP1702, dilution 1:5000, Amersham) or rat (SOM, 8330-0009, dilution 1:50, Biogenesis) were used. Omission, replacement and preabsorption tests proved the specificity of the immunostaining.

RESULTS AND DISCUSSION

Generally, the porcine mammary gland was moderately supplied with VIP-IR nerve fibres. VIP-IR nerve fibres were located in the nipple and parenchyma of the porcine gland. SMC located in the connective tissue under the epidermis of the nipple were supplied with numerous VIP-IR nerve fibres running between and in parallel to myocytes. Small arteries of the nipple were richly innervated by these nerves but large arteries and veins were supplied with less numerous VIP-IR fibres. Some VIP-IR nerves were observed around the LD.

In the parenchyma of the gland, VIP-IR nerve fibres were distributed similarly to those located in the SMC of the nipple. A moderate number of VIP-positive nerve fibres were associated with arteries located in the parenchyma of the gland. VIP-IR nerves supplying arteries were mainly circumferentially arranged. Moreover, the veins were supplied with scarce VIP-IR fibres. Most VIP-IR nerves were located around the LD. Single nerve fibres stained for VIP penetrated into the epithelium of the ducts. The distribution of VIP-IR nerve fibres found in the porcine mammary gland was similar to that described in woman [1] and rat [1, 6–8]. However, VIP-IR nerve fibres are more abundant in the human then in the rat mammary gland [1].

Double-labelling immunohistochemistry revealed that some VIP-IR nerve fibres also contained immunoreactivity to DβH. Most VIP/DβH-IR fibres were associated with BV and SMC in both the nipple and parenchyma of the gland, whereas single fibres were observed around the LD (Fig. 1). VIP/VAChT-IR nerve fibres were absent in the porcine mammary gland. These results are comparable to the early findings from observation of the mammary gland SChG-projecting neurons [4]. This study revealed that VIP was found mainly in the population of adrenergic as well as non-adrenergic/non-cholinergic neurons, but not in cholinergic nerve cells [4]. The role of VIP/DβH-IR nerve fibres is still unclear. It is possible that these fibres, which were found in the porcine organs, might induce vasodilatation [2, 3].

Double-labelling study also revealed that the majority of VIP-IR fibres associated with SMC were SOM-IR (Fig. 2). Less numerous VIP/SOM-IR fibres supplied the BV and were located around the LD of the gland. A small number of VIP-IR nerves also displayed immunoreactivity to NPY. VIP/NPY-IR nerve fibres supplied the BV of the gland (Fig. 3). The coexistence of VIP and SOM, or NPY in nerves supply-

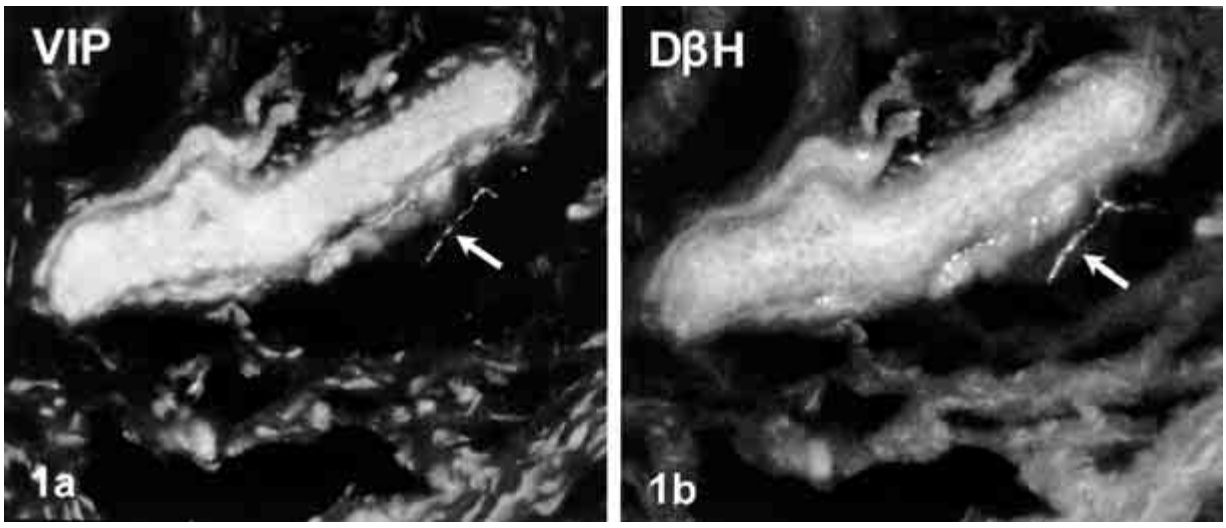

Figure 1. Parenchyma of the porcine mammary gland. Double-immunostaining for VIP (**a** — FITC) and DβH (**b** — Cy3). Nerve fibres (arrow) located around the LD contain both substances (× 400).

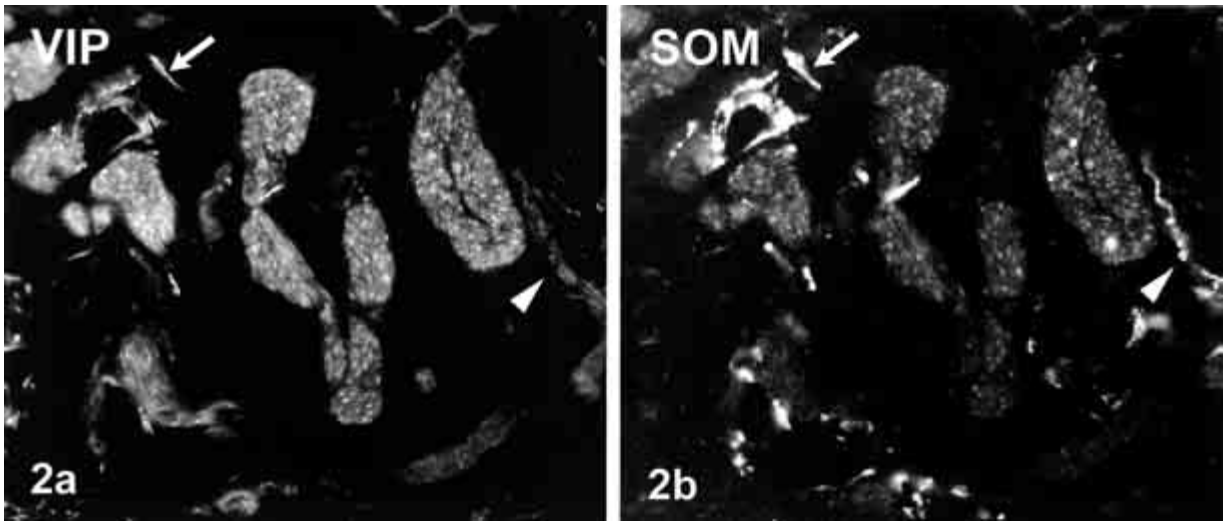

Figure 2. Section of the nipple of the porcine mammary gland. Double-immunostaining for VIP (**a** — FITC) and SOM (**b** — Cy3). VIP-IR nerve fibre associated with SMC is also SOM-positive (arrow), other fibres (arrowhead) located around the SMC contain immunoreactivity to SOM but not to VIP (× 400).

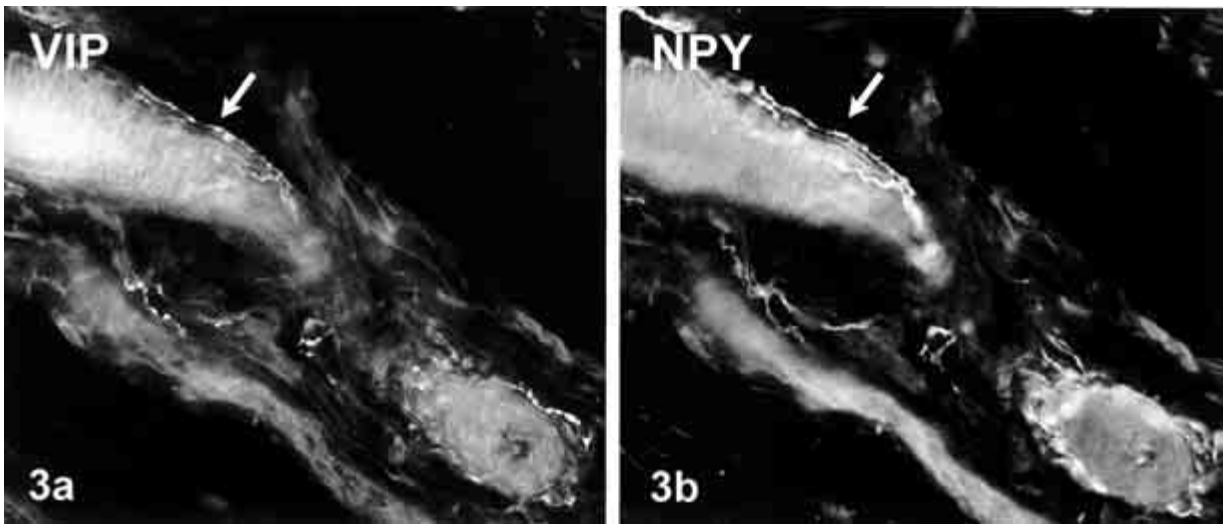

Figure 3. Section of the nipple of the porcine mammary gland. Double-immunostaining for VIP (**a** — FITC) and NPY (**b** — Cy3). Nerve fibres (arrow) surrounding BV display immunoreactivity to both substances (× 400).

ing the mammary gland in other mammals has not yet been studied

Knowledge of the physiological role of VIP in the mammary gland is limited. This peptide, released from nerve fibres supplying the human or rat mammary gland, might play a role in vasodilatation in the gland and may influence the process of milk ejection [8]. Thus, VIP-IR nerve fibres supplying the porcine mammary gland seem to perform a similar function. The physiological consequence of the coexistence of VIP, SOM or NPY in the nerve fibres observed in the porcine mammary gland should be a subject of further investigation.

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