VIA MEDICA

ORIGINAL ARTICLE

Vol. 60, No. 3, pp. 205–211

ISSN 0015–5659
www.fm.viamedica.pl

Distribution and immunohistochemical characterisation of paracervical neurons innervating the oviduct in the pig

Krzysztof Czaja, Krzysztof Wąsowicz, Magdalena Klimczuk, Piotr Podlasz, Mirosław Łakomy

Department of Animal Anatomy, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Olsztyn-Kortowo II, Poland

[Received 1 June 2001; Accepted 9 July 2001]

The present study was aimed at disclosing the distribution of paracervical neurons projecting to the ampulla and isthmus of the porcine oviduct and the pattern(s) of co-existence of tyrosine hydroxylase (TH), dopamine β-hydroxylase (DβH), neuropeptide Y (NPY), substance P (SP), calcitonin gene-related peptide (CGRP) and nitric oxide synthase (NOS) within these nerve cell bodies. The fluorescent retrograde tracer Fast Blue (FB) was injected into the wall of the ampullar (n = 3) and isthmal (n = 3) part of the organ in six sexually immature female pigs. After a survival period of three weeks paracervical ganglia (PCG) were collected. 10 µm-thick cryostat sections of the ganglia were examined for the presence of FB-positive (FB^+) nerve cells under the fluorescent microscope. Tracered neurons were counted in every third section and processed for double-labelling immunofluorescence according to the method of Wessendorf and Elde. 78.6% of FB^+ neurons were projecting to the isthmus while 21.4% of the studied population innervated the ampulla of the oviduct. Double-labelling immunofluorescence revealed the existence of the following different chemically coded subpopulations of the studied perikarya: $TH^+/D\beta H^+$, TH^+/NPY^+, TH^+/NOS^+, TH^+/NOS^-, SP^-/NOS^+, $SP^+/CGRP^+$.

key words: tracing, immunohistochemistry, innervation, oviduct, pig

INTRODUCTION

The afferent and efferent innervation of genital organs in small laboratory animals have been relatively well studied. However, the origin of nerve fibres supplying reproductive organs in breeding animals has not been studied thoroughly [5–9, 32–34]. The investigations performed in breeding animals involved extirpations of fragments of the reproductive organs, which allowed for the detection of nerve centres including those localised in pelvic ganglia contributing to the innervation of the removed segments of the organs. Although previous studies have revealed that some nerve fibres supplying the porcine oviduct may be of PCG origin [32, 33], the neurochemical nature of their perikarya is still obscure. Application of the retrograde tracing method is commonly considered to be one of the most advanced and precise approaches in localising specific neuronal populations supplying any particular organ under study. However, the information dealing with sources of nerve fibres supplying the oviduct based upon the tracing method is very limited. On the other hand, comprehensive retrograde tracing investigations were performed in other female genital organs in-

Address for correspondence: Dr Krzysztof Czaja, Department of Animal Anatomy, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Bldg. 105 J., ul. Oczapowski 13, 10–917 Olsztyn-Kortowo II, Poland, fax: +48 89 52 34 986, e-mail: czajak@moskit.uwm.edu.pl

cluding guinea pig [1], pig [31], rat [12, 25] and cat [14] uterus as well as on the rat [12, 21] and pig [19, 20] ovary. It has been found that PCG neurons contain different combinations of biologically active substances and belong to the noradrenergic [18, 23, 26, 30], cholinergic [17, 26–28, 30] or non-adrenergic non-cholinergic (NANC) [10, 15, 23, 29] neurochemical populations. However, there is a paucity of data dealing with the origin and immunohistochemical nature of PCG neurons supplying the porcine oviduct.

Therefore, by means of combined retrograde tracing and double-labelling immunofluorescence [35], the present study was aimed at disclosing the distribution of "oviductal" PCG neurons and the pattern of putative co-existence of TH, DβH, NPY, SP, CGRP and NOS within these nerve cell bodies.

MATERIALS AND METHODS

The experiment was performed on six sexually immature female pigs of the Great Polish breed, about 15 kg of body weight (b.w.), obtained from a commercial fattening farm. The animals were kept under standard laboratory conditions. Thirty minutes before the main anaesthetic was given, all the animals were pre-treated with atropine (Polfa, Poland; 0.04 mg/kg b.w., s.c.) and propionyl-promasine (Combelen, Bayer, Germany; 0.4 mg/kg b.w., i.m.). The main anaesthetic, sodium pentobarbital (Vetbutal, Biovet, Poland; 30 mg/kg b.w.), was given intravenously. During laparotomy the right oviduct was gently removed from surrounding tissues and the fluorescent retrograde tracer Fast Blue (FB; Dr. K. Illing GmbH, Grob-Umstadt, Germany) was injected into the wall of the ampullar (n = 3) and the isthmal (n = 3) part of the organ. A total volume of 10 μl of FB was injected into each part of the oviduct using a Hamilton syringe. Particular care was taken to minimise the contamination with the dye of adjacent tissues by washing the pelvic organs thoroughly with isotonic saline after each injection. No leakage from injection sites was visible, either immediately after the injection or just prior to closing the abdomen. After a survival period of three weeks the animals were deeply reanaesthetised (following the same procedure as applied before the laparotomies) and perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Both oviducts and PCG were collected. All the tissue specimens were postfixed overnight by immersion in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) and then stored at 4°C in 0.1 M phosphate buffer containing 18% sucrose and 0.01% NaN_3 until sectioning. 10 μm-thick cryostat sections of the organs were processed for double-labelling immunofluorescence according to the method of Wessendorf and Elde [35]. Primary and secondary antisera used are listed in Table 1. The following combinations of the primary antisera were applied: TH/DβH, TH/NPY, TH/NOS, SP/NOS, SP/CGRP. The specificity of the primary antisera was tested by the preabsorption control. 1 μM concentration of the respective peptide completely abolished the fluorescence. There was also no immunostaining when primary antisera were omitted or replaced by a normal rabbit, rat or mouse serum. FB-positive (FB^+) neurons were counted in every third section. This strategy eliminated the likelihood of counting the same neuron twice. The labelled sections were studied and photographed with a Zeiss-Axiophot microscope, equipped with epi-illumination fluorescence and an appropriate filter set for Texas Red and fluoresceine izothiocyanate (FITC).

In the experiments, the principles of laboratory care as well as the specific national laws on the protection of animals were followed.

RESULTS

Results of the present study show that 78.6% of FB^+ neurons were projecting to the isthmus while 21.4% of the studied population innervated the ampulla of the oviduct. Those perikarya were located mainly in the cranial and medial parts of the studied ganglia.

Table 1. Antisera used in the study

Antigen	Species	Code	Dilution	Supplier
Primary antisera				
TH	mouse	1017381	1:40	Boehringer
DβH	rabbit	TE103	1:400	ETI
NPY	rabbit	RPN1702	1:500	Amersham
NOS	rabbit		1:1000	B. Mayer
SP	rat	1021	1:200	Medicorp
CGRP	rabbit	RPN1842	1:800	Amersham
Secondary antisera and fluorochromes				
FITC-conjugated mouse anti-rabbit IgG			1:400	Cappel
FITC-conjugated rabbit anti-mouse IgG			1:400	Cappel
FITC-conjugated rabbit anti-rat IgG			1:400	Cappel
Biotynylated mouse anti-rabbit IgG			1:100	Cappel
Biotynylated rabbit anti-mouse IgG			1:100	Cappel
Biotynylated rabbit anti-rat IgG			1:100	Cappel
Texas Red-conjugated streptavidin			1:100	Amersham

In the cranial part of PCG, FB^+ neurons formed groups containing 3–5 cells while in the medial part of the ganglion tracered neurons were irregularly dispersed. FB^+ neurons represented two different size populations. The first one was formed by small cells with a diameter (in the case of oval perikarya the long axis) between 20–33 μm. As regards the shape of the neurons, the most numerous were oval neurons (about 70%), round cells composed about 20% of this population, and approximately 10% of cells were fusiform in shape. The second population was formed by large perikarya with a diameter of 40–50 μm, which were oval (about 60%) or round (about 40%) in shape. Double-labelling immunofluorescence revealed the existence of the following different chemically coded subpopulations of studied perikarya: $FB^+/TH^+/NOS^-$ (Fig. 1a–c), $FB^+/SP^-/NOS^+$ (Fig. 2a–c), $FB^+/TH^+/D\beta H^+$ (Fig. 3a–c), $FB^+/TH^+/NPY^+$ (Fig. 4a–c), $FB^+/TH^+/NOS^+$ (Fig. 5a–c), $FB^+/SP^+/CGRP^+$ (Fig. 6a–c).

After analysing the content of TH and DβH within the studied neurons, it was found that about 70% of FB^+ cells represented noradrenergic population while 30% of the studied neurons had non-noradrenergic characteristics. Noradrenergic neurons were located mainly in the cranial part of the ganglion while the non-noradrenergic were concentrated in the medial part of PCG.

DISCUSSION

Based on the preparation studies made in man [4, 16] and rat [2, 3], it can be postulated that the pelvic neurons participate in the innervation of the reproductive organs. The above hypothesis was confirmed by extirpation method [7, 32, 33] as well as by retrograde tracing [20, 31]. Results of the present study also showed that the porcine oviduct is innervated by the pelvic neurons. FB^+ neurons innervating both ampulla and isthmus of the oviduct were located in the PCG. Distribution of the studied neurons did not show somatotopic organisation. PCG have been found to be the place of origin for so called "short adrenergic neurons" [for review see 24], due to their close localisation to effector organ structures. Furthermore, PCG have been recognized as providing most of the autonomic nerve supply to the pelvic urogenital and distal bowel organs [11, 13]. The porcine PCG has appeared to be a "mixed" autonomic ganglion consisting of both adrenergic neurons, containing catecholamine-synthesing enzymes (TH and DβH) and non-adrenergic nerve cells. The vast majority of FB^+ neurons belonged to the population of adrenergic neurons, which may sug-

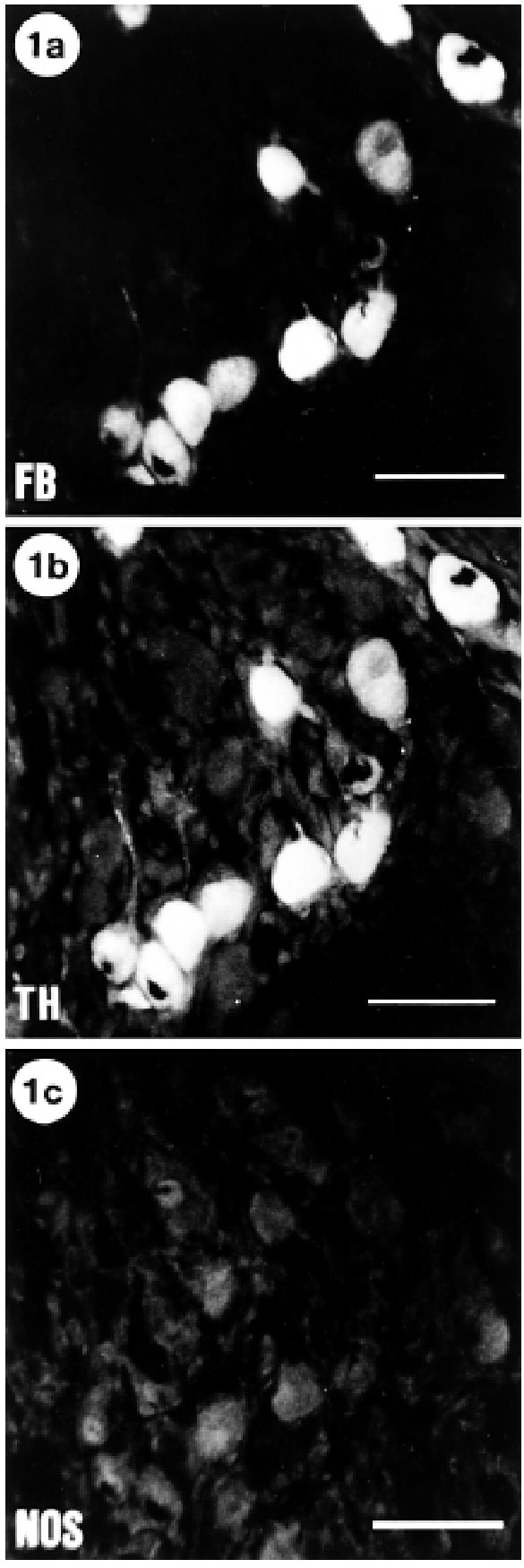

Figure 1a–c. Group of small and round or oval in shape $FB^+/TH^+/NOS^-$ neurons located in cranial part of PCG. Scale bar — 50 μm.

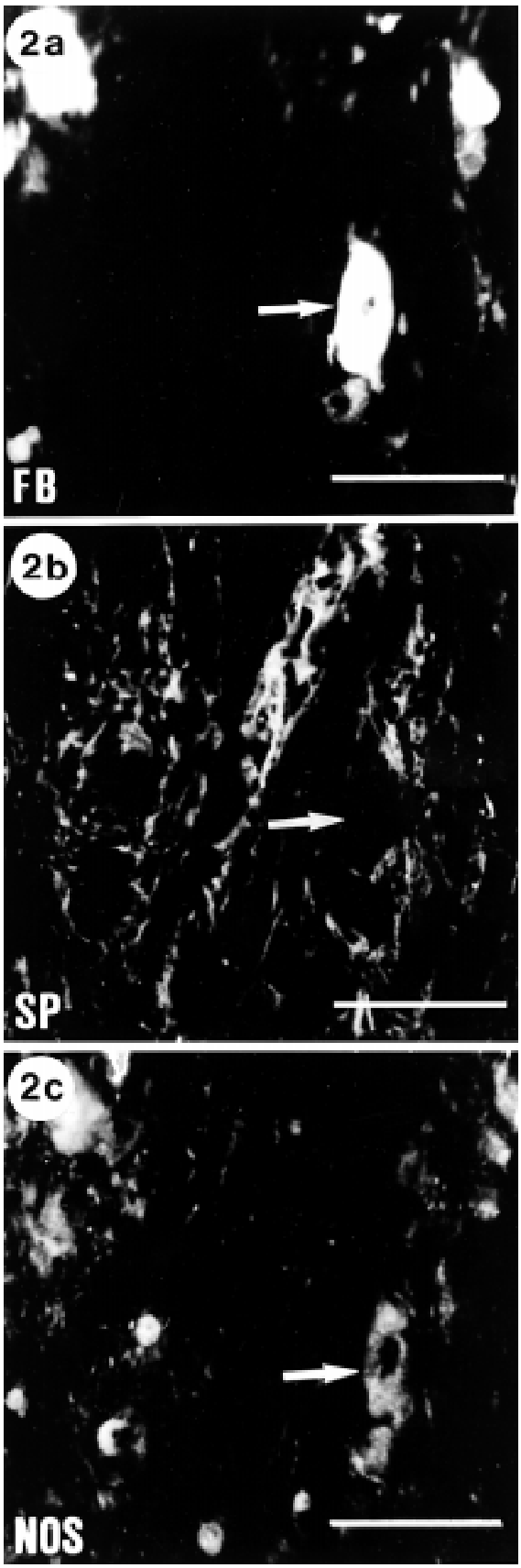

Figure 2a–c. The large and oval $FB^+/SP^-/NOS^+$ neuron (arrow) located in cranial part of PCG. Scale bar — 50 μm.

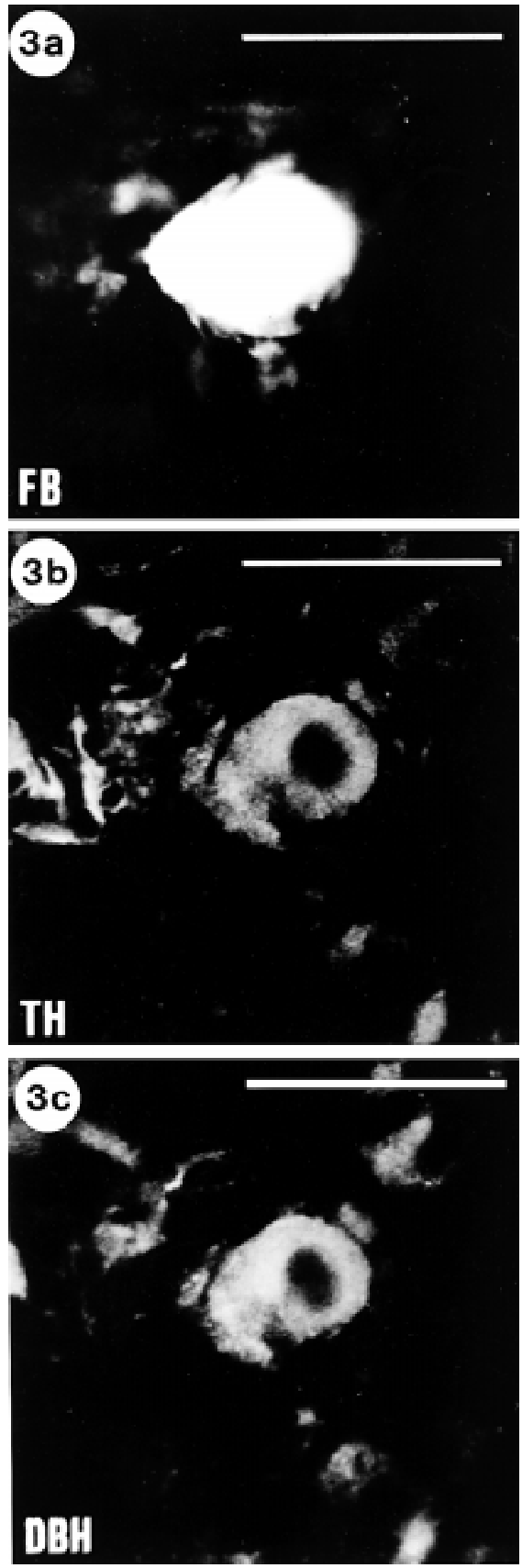

Figure 3a–c. The large and round $FB^+/TH^+/D\beta H^+$ neuron located in cranial part of PCG. Scale bar — 50 μm.

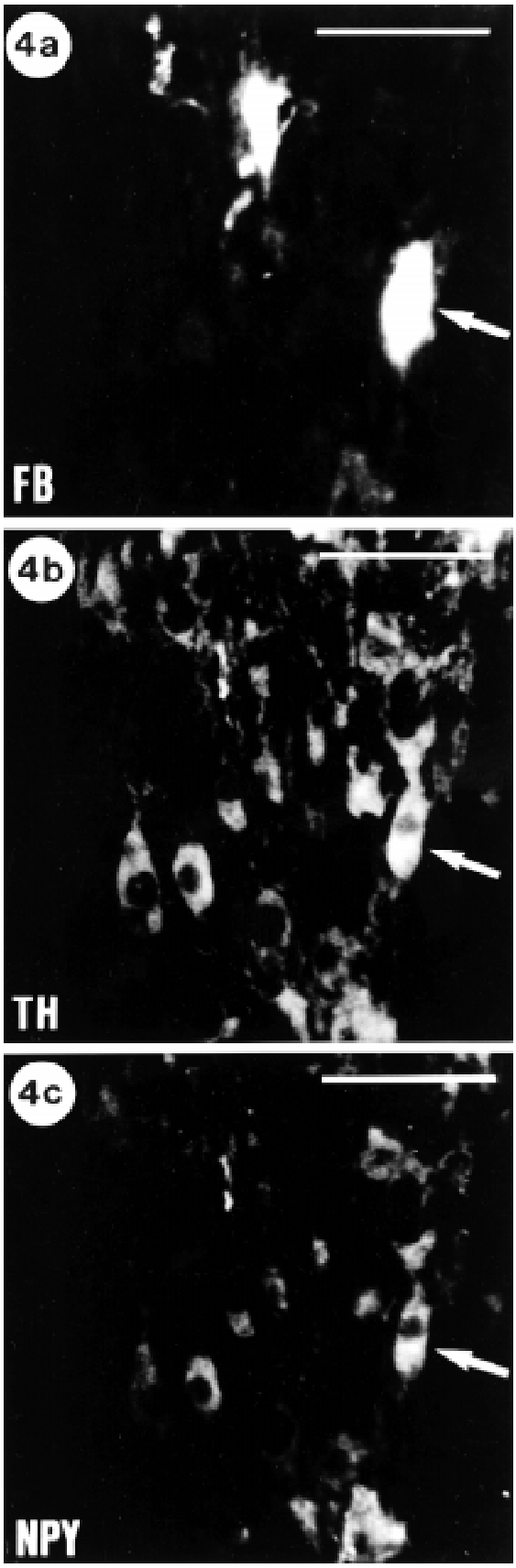

Figure 4a–c. The medium sized and oval in shape FB$^+$/TH$^+$/NPY$^+$ neuron (arrow) located in median part of PCG. Scale bar — 50 μm.

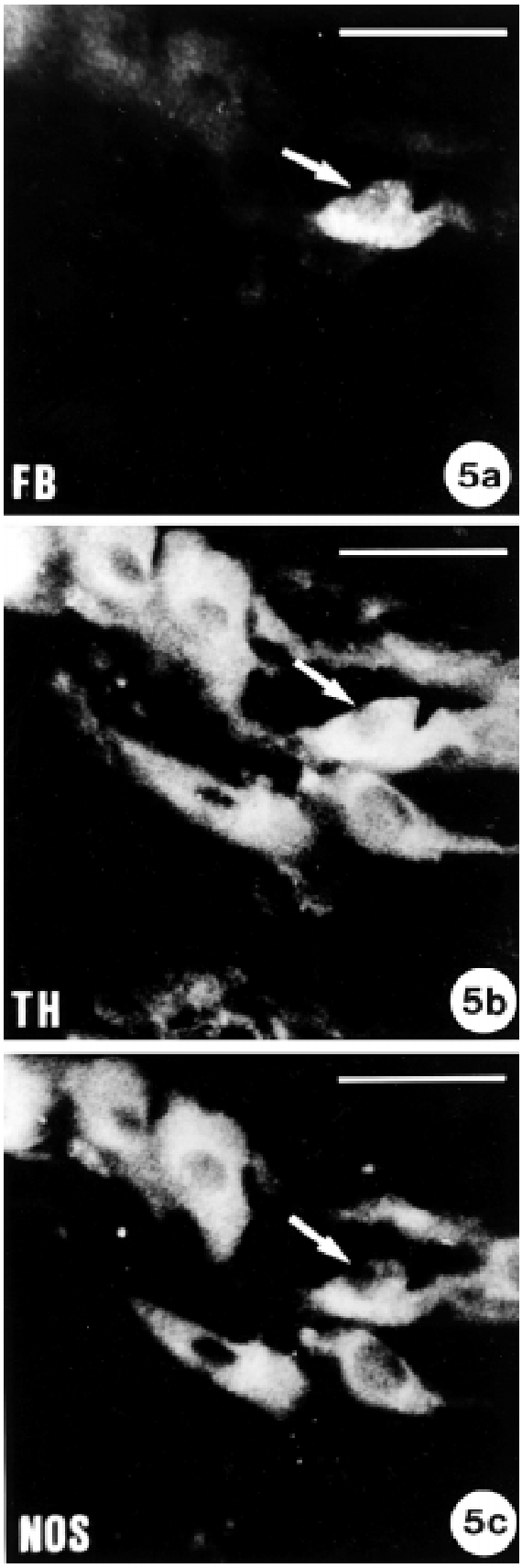

Figure 5a–c. The large FB$^+$/TH$^+$/NOS$^+$ (arrow) located in median part of PCG. Scale bar — 50 μm.

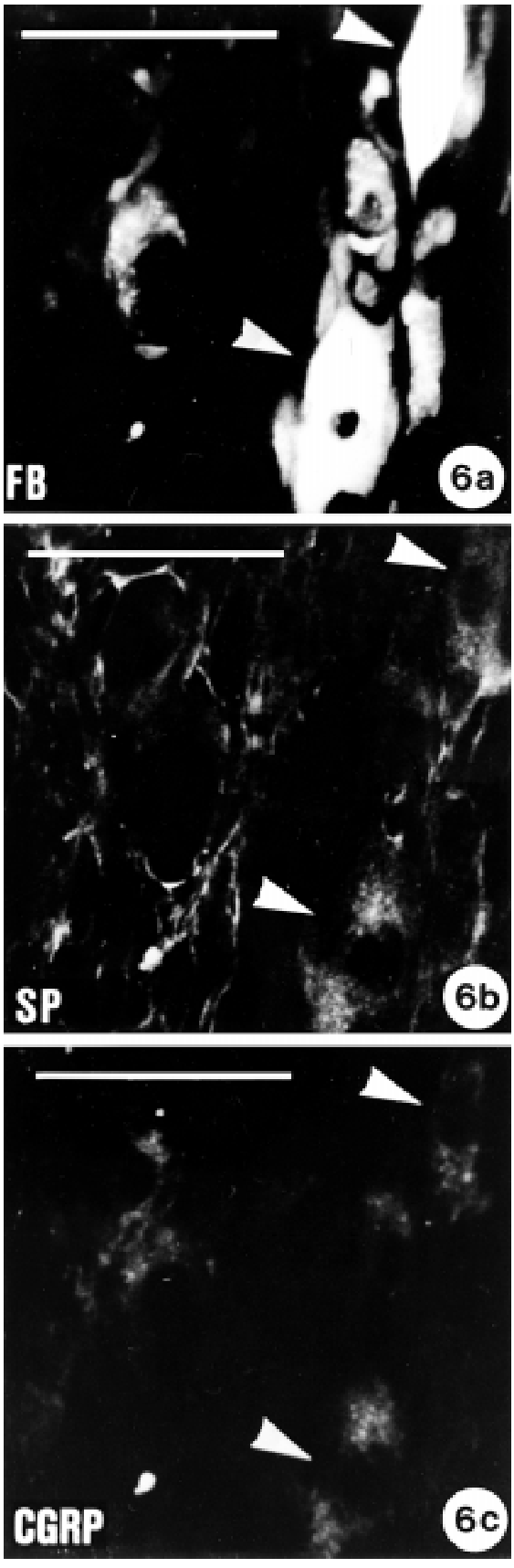

Figure 6a–c. Two large and fusiform in shape FB$^+$/SP$^+$/CGRP$^+$ neurons (arrowheads) located in caudal part of PCG. Scale bar — 50 μm.

gest the presence of "short adrenergic neurons" innervating the porcine oviduct. Based on the fact that chemical coding of FB$^+$ localised in PCG (TH$^+$/DβH$^+$, TH$^+$/NPY$^+$, TH$^+$/NOS$^+$) and perivascular nerve fibres in the oviduct are very similar, it is possible that "short adrenergic neurons" of this ganglion control the vasoactive activity of the oviduct. We have also found that non-noradrenergic FB$^+$ neurons localised in PCG contain CGRP and/or SP, therefore this ganglion should also be considered as a prominent source of cholinergic innervation to the oviduct in the pig. Because similar coded pelvic neurons in the guinea pig have been described as a source of perivascular nerve fibres inhibiting contraction of blood vessels [22], the non-noradrenergic axons in the pig (putative cholinergic) may be "agonists" for the perivascular noradrenergic terminals originating from the sympathetic ganglia or PCG. This population of noradrenergic neurons was located mainly in the cranial part of the ganglion, while the non-noradrenergic perikarya innervating the oviduct were concentrated in the medial part of PCG. Similar models of distribution of the above-mentioned neuronal populations localised in PCG were observed in other mammalian species [15]. This fact proclaims the morphological organisation of PCG neurons with respect to their functions.

ACKNOWLEDGEMENTS

The skilful technical assistance of Ms Maria Marczak, Ms Grażyna Greniuk and Mr Aleksander Penkowski is greatly acknowledged.

REFERENCES

1. Alm P, Lundberg LM (1988) Co-existence and origin of peptidergic and adrenergic nerves in the guinea pig uterus. Retrograde tracing and immunocytochemistry, effects of chemical sympathectomy, capsaicin treatment and pregnancy. Cell Tissue Res, 254: 517–530.
2. Baljet B, Drukker J (1979) The extrinsic innervation of the abdominal organs in the female rat. Acta Anat, 104: 243–267.
3. Baljet B, Drukker J (1980) The extrinsic innervation of the pelvic organs in the female rat. Acta Anat, 107: 241–267.
4. Baljet B, Drukker J (1982) Some aspects of the innervation of the abdominal and pelvic organs in the human female fetus. Acta Anat, 111: 222–230.
5. Boratyński Z, Flieger S, Welento J, Krzyżanowski J, Sławomirski J (1988) Sources of autonomic and afferent fibers to the uterine cervix in pig. Folia Morphol (Warszawa), 47: 71–76.
6. Flieger S (1977) Eksperymentalne badania nad lokalizacją ośrodków nerwowych narządów płciowych u owcy. Pol Arch Wet, 20: 89–119.

7. Flieger S, Boratyński Z, Welento J., Eustachiewicz R, Szalak M, Krzyżanowski J, Sławomirski J (1984) Eksperymentalne badania nad lokalizacją ośrodków nerwowych jajnika i jajowodu u krowy. Pol Arch Wet, 24: 261–273.
8. Flieger S, Boratyński Z, Welento J, Krzyżanowski J, Sławomirski J (1988a) Sources of the autonomic and afferent fibers to the horns and body of the uterus in pig. Folia Morphol (Warszawa), 47: 77–82.
9. Flieger S, Welento J, Boratyński Z, Krzyżanowski J, Sławomirski J (1988b) Sources of the autonomic and afferent fibers to the clitoris in pig. Folia Morphol (Warszawa), 47: 83–88.
10. Gibbins IL (1995) Chemical neuroanatomy of sympathetic ganglia. In: McLachlan EM (ed.). Autonomic ganglia, Harwood Academic Publishers, Luxembourg, Luxembourg, pp. 73–121.
11. Hondeau E, Prud'homme MJ, Rousseau A, Rousseau JP (1995) Distribution of noradrenergic neurons in the female rat pelvic plexus and involvement in the genital tract innervation. J Auton Nerv Syst, 54: 113–125.
12. Inyama CO, Wharton J, Su HC, Polak JM (1986) CGRP--immunoreactive nerves in the genitalia of the female rat originate from dorsal root ganglia T11-L3 and L6--S1: a combined immunocytochemical and retrograde tracing study. Neurosci Lett, 69: 13–18.
13. Kaleczyc J (1998) Origin and neurochemical characteristics of nerve fibres supplying the mammalian vas deferens. Micros Res Tech, 42: 409–422.
14. Kawatani M, de Groat WC (1991) A large proportion of afferent neurons innervating the uterine cervix of the cat contain VIP and other neuropeptides. Cell Tiss Res 266: 191–196.
15. Keast JR (1995) Pelvic ganglia. In: McLachlan EM (ed.). Autonomic ganglia, Harwood Academic Publishers, Luxembourg, Luxembourg, pp. 445–479.
16. Koppen K (1950) Histologische Untersuchungsergebnisse über die Nervenversorgung des Ovars beim Menschen. Zentralbl Gynäkol, 72: 915–921.
17. Lundberg JM, Hökfelt T, Schultzberg M, Uvnäs-Wallensten K, Köhler C, Said SI (1979) Occurrence of vasoactive intestinal polypeptide (VIP)-like immunoreactivity in certain cholinergic neurons of the cat: evidence from combined immunohistochemistry and acetylcholinesterase staining. Neuroscience, 4: 1539–1559.
18. Lundberg JM, Terenius L, Hökfelt T, Goldstein M (1983) High levels of neuropeptide Y in peripheral noradrenergic neurons in various mammals including man. Neurosci Lett, 42: 167–172.
19. Majewski M, Kummer W, Kaleczyc J, Heym Ch (1991a) Peptidergic pathways in the inferior mesentric ganglion of pig. J Auton Nerv Syst Suppl, 109–110.
20. Majewski M, Wasowicz K, Heym Ch (1991b) Peptiderge Projection vom Ganglion paracervicale uteri zum Eierstock des Schweins. Verh Anat Ges, 86: 176.
21. McNeill DL, Burden HW (1986) Neuropeptide Y and somatostatin immunoreactive perikarya in preaortic ganglia projecting to the rat ovary. J Reprod Fertil 78: 727–732.
22. Morris JL (1993) Co-transmission from autonomic vasodilator neurons supplying the guinea pig uterine artery. J Auton Nerv Syst, 42: 11–21.
23. Morris JL, Gibbins IL (1987) Neuronal colocalization of peptides, catecholamines, and catecholamine-synthesizing enzymes in guinea pig paracervical ganglia. J Neurosci, 7: 3117–3130.
24. Owman C, Stjernquist M (1988) Origin, distribution, and functional aspects of aminergic and peptidergic nerves in the male and female reproductive tracts. In: Björklund A, Hökfelt T, Owman C (eds.). Handbook of chemical neuroanatomy: The peripheral nervous system, Elsevier, Amsterdam, pp. 445–544.
25. Papka RE (1990) Some nerve endings in the rat pelvic paracervical autonomic ganglia and varicosities in the uterus contain calcitonin gene-related peptide and originate from dorsal root ganglia. Neuroscience, 39: 459–470.
26. Papka RE, Cotton JP, Traurig HH (1985) Comparative distribution of neuropeptide tyrosine-, vasoactive intestinal polypeptide-, substance P-immunoreactive, acetylcholinesterase-positive and noradrenergic nerves in the reproductive tract of the female rat. Cell Tiss Res, 242: 475–490.
27. Papka RE, McCurdy JR, Williams SJ, Mayer B, Marson L, Platt KB (1995a) Parasympathetic preganglionic neurons in the spinal cord involved in uterine innervation are cholinergic and nitric oxide-containing. Anat Rec, 241: 554–562.
28. Papka RE, McNeill DL, Thompson D, Schmidt HHHW (1995b) Nitric oxide nerves in the uterus are parasympathetic, sensory, and contain neuropeptides. Cell Tiss Res 279: 339–349.
29. Papka RE, Traurig HH (1993) Autonomic efferent and visceral sensory innervation of the female reproductive system: special reference to neurochemical markers in nerves and ganglionic connections. In: Maggi CA (ed.). Nervous control of the urogenital system, Harwood Academic Publ Chur, Switzerland, pp. 423–466.
30. Papka RE, Traurig HH, Klenn P (1987) Paracervical ganglia of the female rat: histochemistry and immunohistochemistry of neurons, SIF cells, and nerve terminals. Am J Anat, 179: 243–257.
31. Wąsowicz K, Majewski M., Łakomy M. (1998) Distribution of neurons innervating the uterus of the pig. J Auton Nerv Syst, 74: 13–22.
32. Welento J, Flieger S, Boratyński Z, Krzyżanowski J, Sławomirski J (1987b) Experimental examinations on the localization of the nerve centres of the reproductive organs in the pig. Zesz Probl Post N Roln, 339: 73–88.
33. Welento J, Flieger S, Boratyński Z, Krzyżanowski J, Strzałka B, Sławomirski J, Szalak M, Eustachiewicz R (1984) Experimental investigations on localization of the nerve centers concerned in the innervation of the ovary and the uterine tube in pig. Folia Morphol (Warszawa), 43: 85–90.
34. Welento J, Szalak M, Flieger S, Eustachiewicz R, Boratyński Z, Krzyżanowski J, Slawomirski J (1987a) Experimental studies on localization of the nervous centers in the uterus of the cow. Pol Arch Wet, 25: 297–305.
35. Wessendorf MW, Elde RP (1985) Characterization of an immunofluorescence technique for the demonstration of coexisting neurotransmitters within nerve fibers and terminals. J Histochem Cytochem, 33: 984–994.