Neurochemical characteristics of paracervical ganglion in the pig

P. Podlasz, K. Wasowicz

Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Olsztyn-Kortowo, Poland

ABSTRACT: A study on the presence of the selected biologically active substances in nerve structures of the paracervical ganglion in the pig was performed with the use of immunofluorescence and RT-PCR. Immunohistochemical methods revealed that 23% of paracervical ganglion (PCG) neurons contain both tyrosine hydroxylase (TH) and dopamine β-hydroxylase (DβH) and that the remaining 77% contain choline acetyltransferase (ChAT) and vesicular acetylcholine transporter (VAChT). 73% of TH/DβH neurons contained neuropeptide Y (NPY) and 8% contained somatostatin (Som). All ChAT/VAChT positive neurons contained vasoactive intestinal polypeptide (VIP), 87% of them contained Som, 76% contained NPY and 32% contained neuronal nitric oxide synthase (nNOS). Galanin (Gal) was found only in small cells, which were thought to be SIF cells. No pituitary adenylate cyclase-activating polypeptide (PACAP)- or substance P (SP)-positive neurons were found in PCG. Some areas of PCG contained dense plexuses of ChAT- and VAChT-positive nerve fibres. In the ganglion small number of TH-, nNOS-, NPY-, VIP-, Gal-, PACAP-, Som- and SP-positive nerve fibres was also visible. RT-PCR detected the presence of mRNA for TH, ChAT, nNOS, NPY, VIP, Gal and Som, which were visualised as clearly discernible bands on a gel. In cases of PACAP and SP only weak bands were observed.

Keywords: pig; paracervical ganglion; immunohistochemistry; RT-PCR

The paracervical ganglion (PCG) is a unique structure of the autonomic nervous system. It contains components of both the sympathetic and parasympathetic pathways. Postganglionic nerve fibres originating from PCG are responsible for the innervation of the lower urinary and digestive tract as well as reproductive organs.

Previous studies (Mitchell, 1993; Keast et al., 1995) demonstrated the presence of two main types of neuronal somata in PCG: cholinergic [containing, choline acetyltransferase (ChAT), vesicular acetylcholine transporter (VAChT)] and adrenergic [containing noradrenaline (NA), tyrosine hydroxylase (TH), dopamine B-hydroxylase (DβH)], so-called "short" adrenergic neurons. Those two populations also contain other substances acting as neurotransmitters, neurotransmitter synthesizing enzyme, neuro-modulators or trophic factors: neuronal nitric oxide synthase (NOS; Majewski et al., 1995; Papka et al., 1995; Elfvin et al., 1997; Majewski, 1997; Hisasue et al., 2006), vasoactive intestinal polypeptide (VIP; Inyama et al., 1985; Morris and Gibbins, 1987; Keast and De, 1989; Kawatani and de Groat, 1991), neuropeptide Y (NPY; Inyama et al., 1985; Morris and Gibbins, 1987; Papka et al., 1987; Alm and Lundberg, 1988; Mitchell and Stauber, 1990; Majewski, 1997; Kaleczyc et al., 2003), galanin (Gal; Keast, 1991; Kaleczyc, 1997), somatostatin (Som; Morris and Gibbins, 1987; Elfvin et al., 1997; Majewski, 1997; Kaleczyc et al., 2003), substance P (SP; Dail and Dziurzynski, 1985; Dhami and Mitchell, 1991; Kaleczyc, 1997), neurotensin (Alm and Lundberg, 1988), dynorphin (Gibbins and Morris, 1987) and enkephalin (Happola et al., 1990; Keast, 1991; Majewski, 1997; Kaleczyc, 1997).

In order to better understand the roles of the autonomic nerves supplying the genitourinary organs,

it is essential to identify the different populations of neurons involved, based on their content of co-existing neurotransmitters.

Studies on the neurochemical coding of the neurons in the PCG of pigs are scarce (Happola et al., 1990; Czaja et al., 2001). Thus, we decided to study the presence of some biologically active substances in the neurons of the porcine PCG using immunofluorescence and RT-PCR. The substances under study were, TH and DβH – markers of catecholaminergic neurons, ChAT and VAChT – markers of cholinergic neurons, nNOS a marker of nitrergic neurons, as well as peptides: NPY, VIP, pituitary adenylate cyclase-activating peptide (PACAP), galanin, somatostatin and SP.

MATERIAL AND METHODS

The handling of animals proceeded according to the "Principles of laboratory animal care" (NIH publication No. 86-23 rev 1985) and national laws on the treatment of experimental animals.

Seven female, sexually immature (body weight approx. 20 kg) pigs were used. Five animals were heavily anaesthetized with pentobarbital (Vetbutal, Biowet, Poland; 30 mg/kg b.w. *i.v.*) and transcardially perfused with 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4). Uterine cervices and vaginas were removed and postfixed in the same fixative for 30 minutes. They were then placed in 18% sucrose in 0.1M phosphate buffer and stored at +4°C until they sank to the bottom of the container. 10 μm cryostat sections were put on chrome alum-gelatin-coated slides, allowed to dry and stored desiccated at –70°C until processing.

The slides were rehydrated in phosphate buffered saline (PBS, pH 7.4) and processed for double-immunofluorescence as described previously (Kaleczyc et al., 1999). Antibody data are shown in Table 1.

Two animals were heavily anaesthetized as described previously and exsanguinated. The parametrium and adventitia of the utero-vaginal junction were dissected out, snap-frozen in liquid nitrogen and stored at –70°C before RNA extraction. The total RNA was isolated from the ganglia with TRIzol reagent (Gibco BRL, USA). The extracted total RNAs were dissolved in deionized formamide (Sigma, USA), their concentrations were deter-

Table 1. Antisera used in the study

Antigen	Species	Code	Dilution	Supplier
Primary antibodies				
TH	mouse	2/40/15	1:120	Boehringer, Mannheim, GER
DβH	rabbit	DZ1020	1:2 000	Affiniti, Mamhead, UK
ChAT	rabbit	AB143	1:5 000	Chemicon, Temacula, USA
VaChT	rabbit	G4481	1:5 000	Promega, Medison, USA
NOS	rabbit	210-504	1:5 000	Alexis, Lausen, Switzerland
NPY	rabbit	RNP1702	1:5 000	Amersham, Buckinghamshire, UK
VIP	mouse	MaVIP	1:1 500	East Acres, Southbridge,USA
PACAP	rabbit	IHC 8922	1:20 000	Peninsula, SanCarlos, USA
GAL	rabbit	Rin-7153	1:1 600	Peninsula, SanCarlos, USA
SOM	rat	YC7	1:100	Serva, Heidelberg, GER
SP	rat	RPN 1572	1:700	Amersham, Buckinghamshire, UK
Secondary reagents				
FITC-coniug. goat anti-mouse IgG		55493	1:400	Cappel, Durham, USA
FITC-coniug. goat anti-rat IgG		55745	1:400	Cappel, Durham, USA
Biotinyl. goat anti-rabbit IgG		E 0432	1:400	Dako, Glostrup, DK
Streptavidin-CY3		016-160-084	1:4 000	Jackson Immunoresearch Lab. Inc.,USA

Table 2. Design of primers

Substances	PCR primer	Temperature (°C)	Size of PCR product (bp)	Sequence of origin	Nucleotides	Supplier
TH	Sense 5' TGCACCCAGTAYATCCGCCAYGC 3' Antisense 5' TAGYTCCTGAGCTTGTCCTT 3'	60	423	Bovtha	950–1 372	Genset, France
DβH	Sense 5' TGTCCCACTACAGCCCACACTT 3' Antisense 5' GCAGGTGCAGACTTCCTC 3'	60	277	BTDBH	1 284–1 560	Proligo, France
ChAT	Sense 5' TGTCTGAGTACTGGCTGAAC 3' Antisense 5' AGATGCACCGCTCGATCATA 3'	56	576	PIGCHAT	384–959	Genset, France
nNOS	Sense 5' TTCGTGCGTCTCCACACCAA 3' Antisense 5'AGTACTTGAAGGCCTGGAAGAT 3'	60	250	OAU76739	85–334	Proligo, France
NPY	Sense 5' GAGGACTTGGCCAGATACTA 3' Antisense 5' AGAAGGGTCTTCGAGCCTA 3'	52	130	Q9N0M5	71–200	Genset, France
VIP	Sense 5' GAGCAGTGAGGGAGAATCTC 3' Antisense 5' GTTCTGCTCTGTTGAATAG 3'	55	479	Humviph	581–1 059	Genset, France
PACAP	Sense 5'AATACTGCAGACGCTCATGG 3' Antisense 5' CTGAAGTAGCGGAACTGA 3'	52	218	ADCYAP1	1–218	Genset, France
GAL	Sense 5' GGTCACCGGTGAAGGAAAAG 3' Antisense 5' GCTCAAACCTACTCCCAAAG 3'	50	450	M13826	301–750	IDT, Coralville, USA
SOM	Sense 5' GCCAAGTACTTCTTGGCG 3' Antisense 5' TGCAGCCCGCTTTGCGTT 3'	60	172	SSU36385	176–347	IDT, Coralville, USA
SP	Sense 5' AACATGAAAATCATGGAGGC 3' Antisense 5' CATCCCGTTTGCCCATYAAT 3'	57	220	Btta01	141–360	IDT, Coralville, USA
GAPDH	Sense 5' ACATTGTCGCCATCAATG 3' Antisense 5' ATGCCCATCACAAACATG 3'	55	320	AF017079	421–740	Genset, France

mined spectrophotometrically at 260 nm with Ultrospec III spectrophotometer (Pharmacia LKB, Sweden), and were then stored at –20°C.

RT-PCR was performed as a two-tube reaction. 2 μg of total RNA was precipitated with ethanol (Sambrook et al., 1989) and dissolved in 14 μl of nuclease-free water in a 0.5 ml Eppendorf tube. 1 μl of oligo $dT_{(12\text{-}18)}$ primer (Sigma, USA; 0.5 mg/ml) was added, the tube was heated to 65°C for 10 min, then cooled on ice. A 10mM mixture of deoxyribonucleotides (dNTPs; 2.5mM of dATP, dCTP, dGTP and dTTP; Pharmacia LKB, Sweden), Moloney murine leukemia virus-reverse transcriptase (MMLV-RT) buffer (Fermentas, Lithuania), RNase inhibitor (RNasin, Promega, USA), and MMLV-RT reverse transcriptase (Fermentas, Lithuania) were then added. The reverse transcription reaction was carried out at 42°C for 1 h, then heated to 75°C for 10 min and subsequently cooled on ice. Multiplex PCR was performed to semi-quantitatively assess the expression of the studied substances. Primers specific for the studied biologically active substances (except VAChT) were used for PCR together with primers specific for porcine glyceraldehyde phosphate dehydrogenase (GAPDH) as an internal standard. The PCR tube contained 1.25 μl of 10× concentrated Taq polymerase buffer (Fermentas, Lithuania), 100 pm of each primer, 1.25 μl of 10mM dNTPs mixture, 0.75 μl 50mM $MgCl_2$, 0.5μl cDNA preparation, 0.5 IU of Taq thermostable DNA polymerase (Fermentas, Lithuania) and water to a total volume of 12.5 μl. 30 cycles were performed with

the following parameters: denaturation at 95°C for 10 s, annealing for 10 s, and elongation at 72°C for 1 min. The sequences of primers, annealing temperatures and expected sizes of PCR products are listed in Table 2. PCR products were analyzed on a 0.8% agarose gel in TAE buffer with an M1 molecular size marker (DNA Gdansk, Poland). DNA fragments were visualized with ethidium bromide and a 302 nm transluminator (Pharmacia LKB, Sweden). Gels were photographed with a digital camera.

RESULTS

Neurons

Approximately 23% of neurons in the ganglion studied were adrenergic, as proved by the simultaneous presence of TH and DβH (Figure 1). TH/DβH-positive neurons were dispersed irregularly in the ganglion and were usually grouped into smaller or bigger clusters, from a few to few dozens of cells. The number of TH/DβH-immunoreactive neurons decreased moving in the direction from the cranial to the caudal. Those neurons were simultaneously immunoreactive to NPY (73%; Figure 5) and Som (8%; Figure 9). All adrenergical neurons were ChAT- (Figure 2), VAChT- (Figure 3), nNOS- (Figure 4), VIP- (Figure 6), PACAP- (Figure 7) and SP-negative (Figure 10).

The remaining 77% of neurons simultaneously contained both CHAT (Figure 2) and VAChT (Figure 3). Analysis of consecutive sections revealed that it was very likely that all those neurons contained VIP, and they often contained Som (ca. 87%) as well as NPY (ca. 76%) or nNOS (ca. 32%). These cells were TH- (Figure 2), DβH-, Gal-, PACAP- and SP-negative.

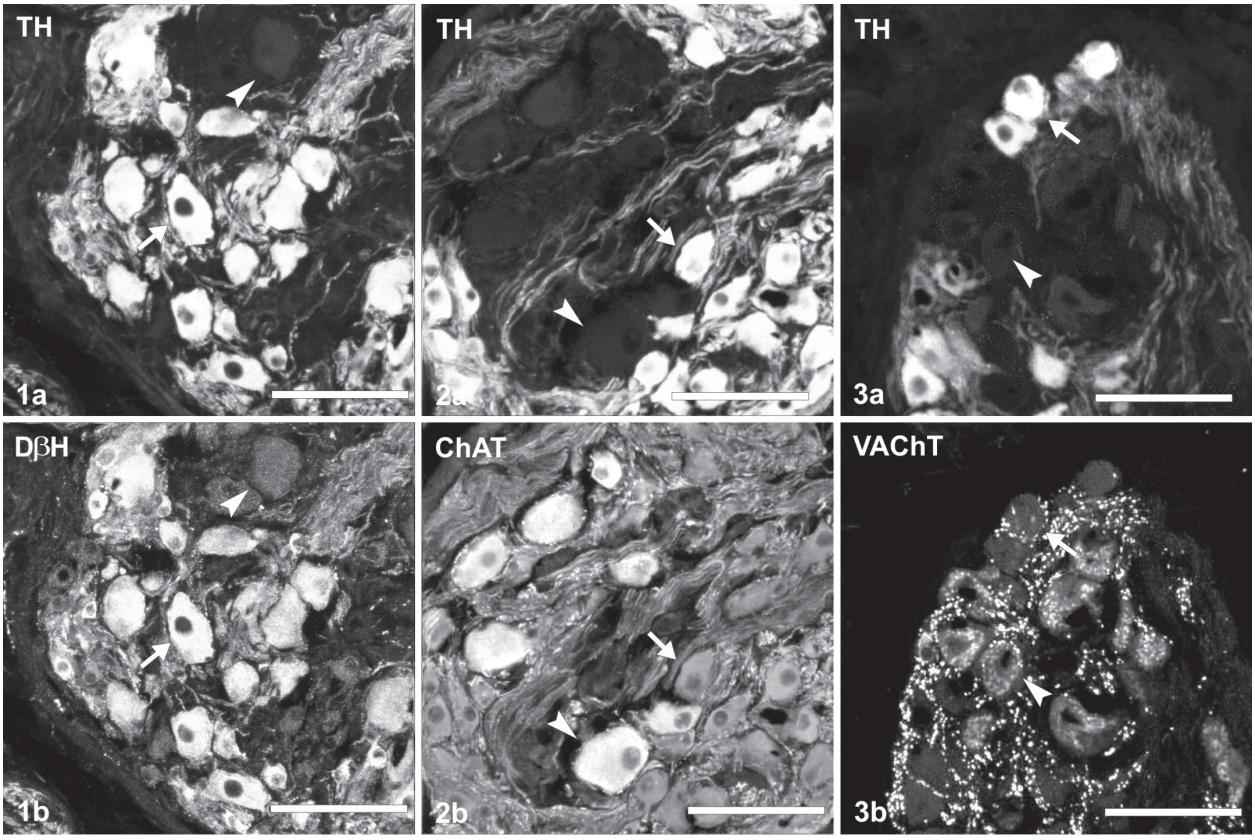


Figure 1. Double-labeling of sections from the porcine PCG for TH (a) and DβH (b). Scale bar 100 μm. Neurons, which stained for TH were simultaneously DβH-positive (arrow). Arrowhead marks TH-, DβH-negative neuron

Figure 2. Double-labeling of sections from the porcine PCG for TH (a) and ChAT (b). Scale bar 100 μm. All neurons, which stained for TH were ChAT-negative (arrow). Arrowhead marks neuron TH-negative/ChAT-positive

Figure 3. Double-labeling of sections from the porcine PCG for TH (a) and VAChT (b). Scale bar 100 μm. All neurons, which stained for TH were VAChT-negative (arrow). Arrowhead marks neuron TH-negative/VAChT-positive

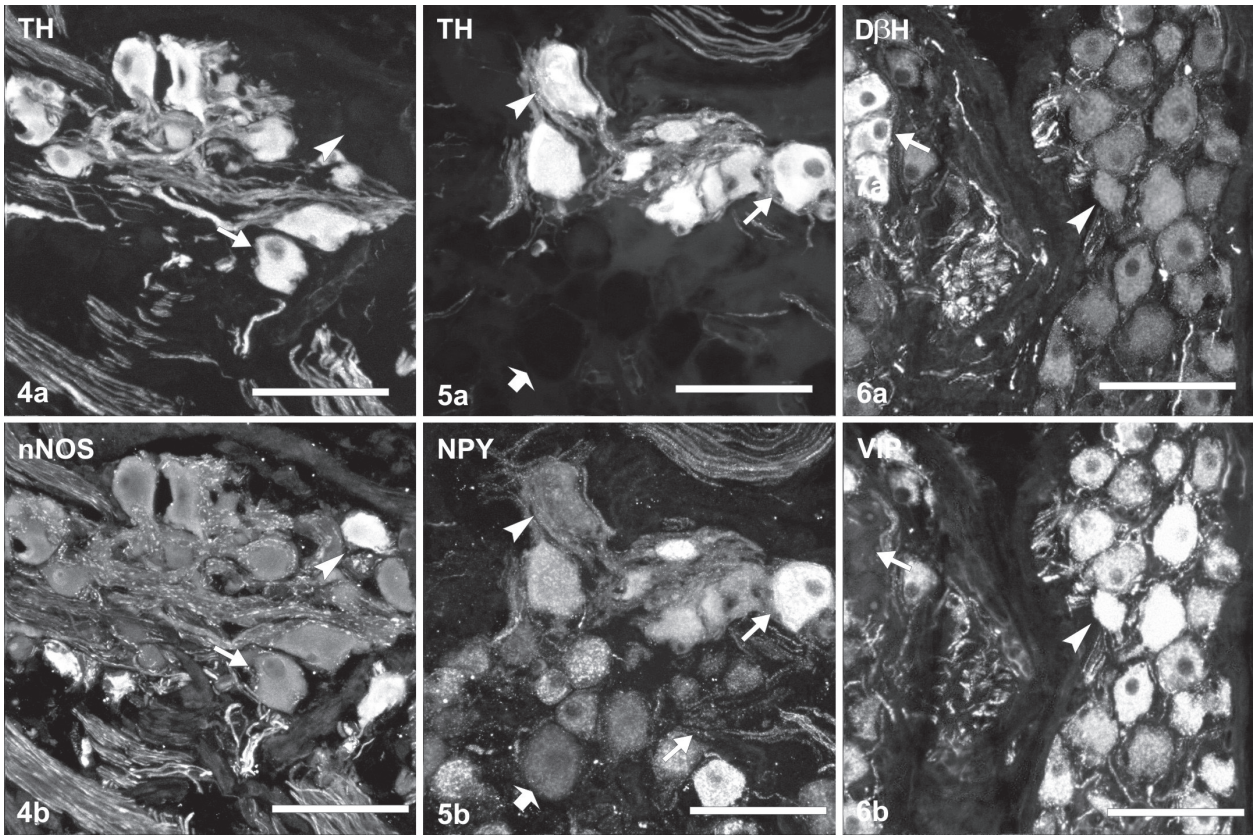


Figure 4. Double-labeling of sections from the porcine PCG for TH (a) and nNOS (b). Scale bar 100 μm. All neurons, which stained for TH were nNOS-negative (arrow). Some of the TH-negative neurons shows immunoreactivity to nNOS (arrowhead)

Figure 5. Double-labeling of sections from the porcine PCG for TH (a) and NPY (b). Scale bar 100 μm. Figure 5b shows different intensity of immunostaining for NPY in nerve cell bodies. Staining revealed three populations of neurons: TH-/NPY-positive (arrow), TH-positive/NPY-negative (arrowhead) and TH-/NPY-negative (thick arrow)

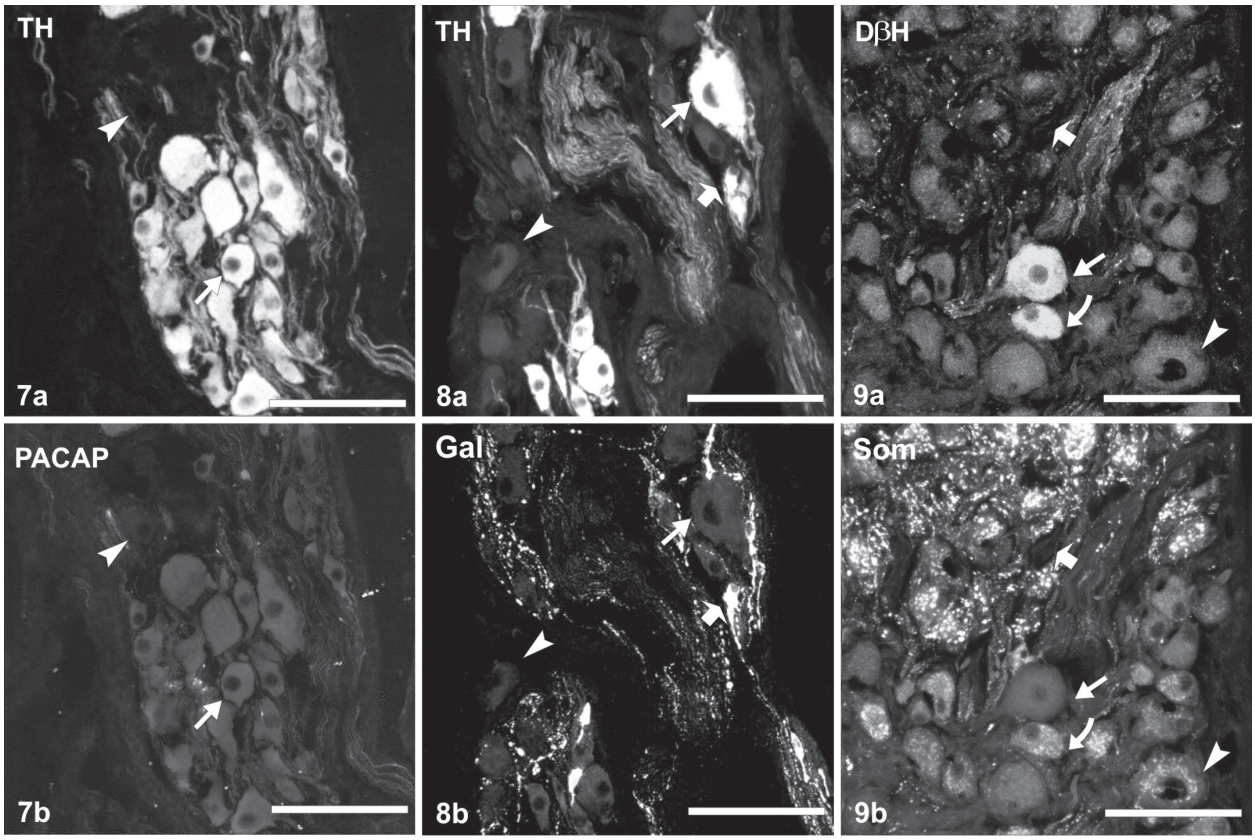
Figure 6. Double-labeling of sections from the porcine PCG for DβH (a) and VIP (b). Scale bar 100 μm. Figure 6b shows different intensity of immunostaining for VIP in nerve cell bodies. All neurons, which stained for DβH were VIP-negative (arrow). Arrowhead marks DβH-negative/VIP-positive neuron

nNOS was detected in 25% of ganglional nerve cells (Figure 4). nNOS-positive cells simultaneously contained ChAT, VAChT and VIP; in addition, some nitrergic neurons contained NPY and Som as well. They were TH- (Figure 4), DβH-, Gal-, PACAP- and SP-negative.

Neurons containing NPY formed a large subpopulation of PCG neurons (75%; Figure 5). This special population can be divided into two subpopulations. Neurons of the first subpopulation (22% of all NPY-positive neurons) contained TH (Figure 5) and DβH. The second one (78% of all NPY-positive neurons) consisted of neurons, in which NPY was colocalized with ChAT, VAChT and VIP (rarely with Som). NPY-positive neurons were Gal-, PACAP- and SP-negative.

Immunoreactivity to VIP was detected in all ChAT-positive neurons of the PCG; however, the intensity of staining was different from neuron to neuron. In some nerve cell bodies strong VIP-positive staining was observed, whereas in other neurons only moderate or weak immunostaining was detected.

No Gal immunoreactivity was found in ganglional neurons. This peptide was found only in small (10–15 μm) cells within the ganglion (Figure 8). These cells contained a small amount of cytoplasm and possessed very numerous and branched processes which seem to be in contact with adrenergic neurons. Immunoreactivity for Gal was very strong, and dispersed evenly in the neuronal cytoplasm. Such cells occurred only individually, but were always in close proximity to



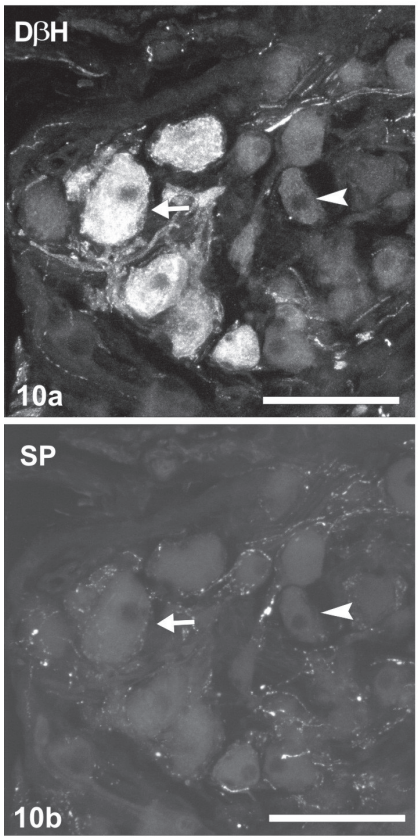


Figure 7. Double-labeling of sections from the porcine PCG for TH (a) and PACAP (b). Scale bar 100 μm. No PACAP-positive neurons were found throughout PCG. Only numerous varicose nerve fibers containing this substance, usually surrounding ganglional neurons, were observed. Arrow marks TH-positive/PACAP-negative neuron and arrowhead marks TH-/PACAP-negative neuron

Figure 8. Double-labeling of sections from the porcine PCG for TH (a) and GAL (b). Scale bar 100 μm. No GAL immunoreactivity was found in ganglional neurons. This peptide was found only in small cells within the ganglion (thick arrow). Arrow marks neuron TH-positive/GAL-negative and arrowhead marks TH-/GAL-negative neuron

Figure 9. Double-labeling of sections from the porcine PCG for DβH (a) and SOM (b). Scale bar 100 μm. Somatostatin was present mainly in cholinergic cells (arrowhead). In the adrenergic neurons the presence of SOM was very rare (curved arrow). Thick arrow shows characteristic group of TH-negative strong SOM-positive neurons, surrounded by varicose SOM-positive fibers, with a high density. Arrow shows TH-positive/SOM-negative neuron

Figure 10. Double-labeling of sections from the porcine PCG for DβH (a) and SP (b). Scale bar 100 μm. No SP-positive cell somata were found throughout PCG. Only numerous varicose SP-positive nerve fibers were observed. Arrow marks TH-positive/SP-negative neuron and arrowhead marks TH-/SP-negative neuron

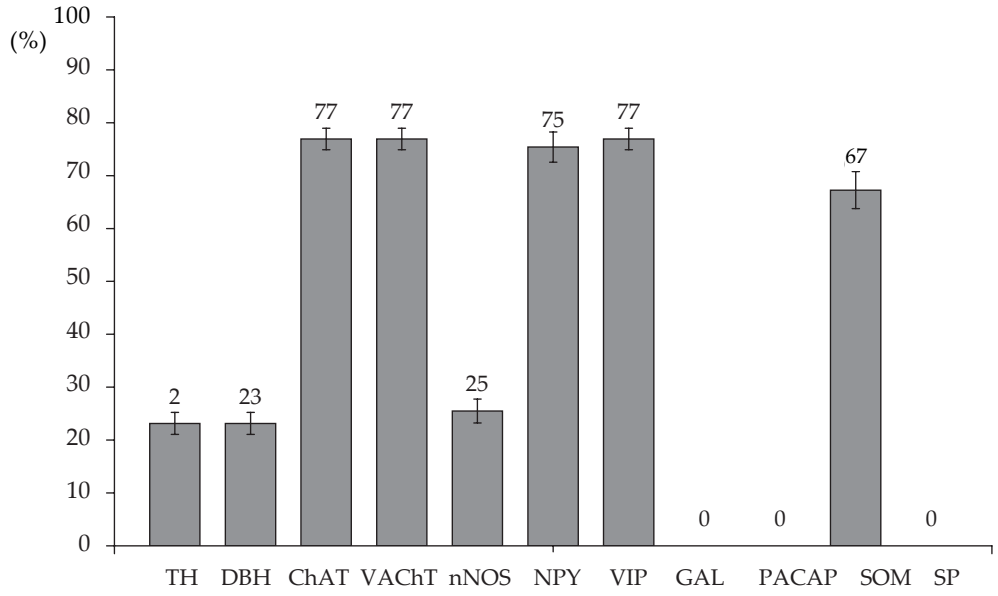


Figure 11. The frequency of population of neurons in PCG

Table 3. The occurrence of studied substances in principal ganglionic neurons of porcine paracervical ganglion. The numbers in brackets show the fraction of neurons positive to the given substances in relation to the total number of the neurons counted

	NPY	Som	nNOS	VIP	PACAP	SP
TH/DBH 23% of total (951/4 124)	73% (2 657/3 625)	8% (298/3 920)	0%	0%	0%	0%
ChAT /VAChT 77% of total (3 652/4 763)	76% (1 025/1 356)	87% (1 120/1 284)	32% (353/1 098)	100%	0%	0%

adrenergic neurons. It is possible that they were SIF cells, and the presence of TH and DβH in them may be proof of this.

No PACAP-positive neurons were found throughout the PCG (Figure 7).

Som was present in 67% of the PCG's nerve cells (Figure 9). Its presence was mainly observed in cholinergic cells. Approximately 97% of these neurons were Som-positive and this peptide was often colocalized with VIP and rarely with nNOS and NPY. In the adrenergic neurons, on the other hand, the presence of Som was very rare (ca. 3%) and colocalized with TH, DβH and NPY. Also, Som-positive neurons contained neither galanin, PACAP nor SP. The intensity of fluorescence in both subpopulations was described in individual neurons as a weak signal (almost invisible grains) compared to the very strong signal (bright and clear granules), distributed evenly throughout the neuronal cytoplasm. Besides the neurons described above Som-positive neurons which showed strong immunoreactivity were also found. The immunoreactivity to Som could be visualised as floccules around the cell nucleus. Most frequently such neurons were grouped into small or larger clusters, containing a few to a few dozen neurons. These were all cholinergic neurons.

No SP-positive perikarya were found throughout the PCG (Figure 10).

Results concerning the presence of neuronal subpopulations in PCG are shown in Figure 11 and Table 3.

Nerve fibres

A large number of TH/DβH-positive nerve fibres was observed in the PCG. They were non-varicose, and were usually grouped into bundles which divided the ganglion into cell clusters.

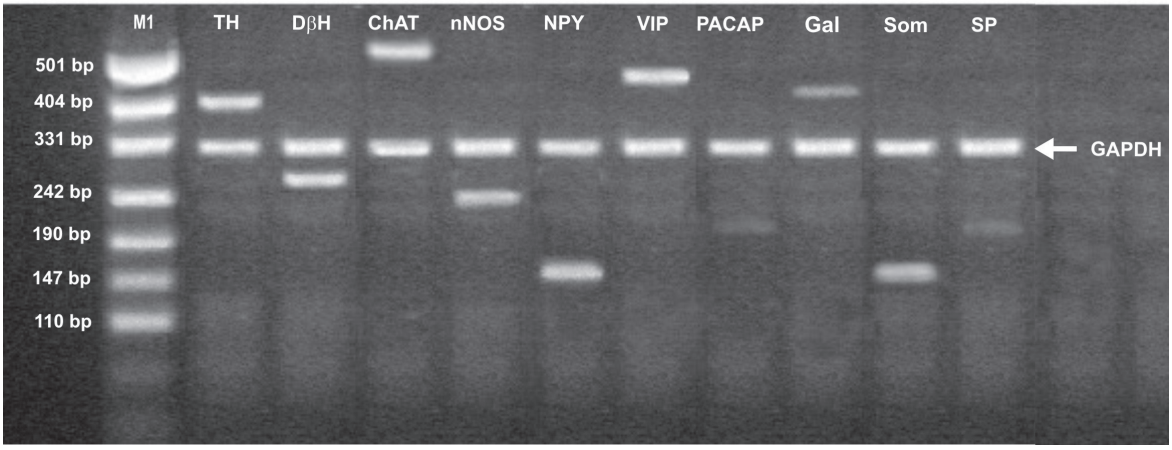


Figure 12. Gel electrophoresis of RT-PCR products corresponding to mRNA species encoding the porcine neuropeptides and neurotransmitter-synthesizing enzymes in the PCG. Strong signal was coming from the transcripts of TH, DβH, ChAT, nNOS, NPY, Gal, VIP and Som. In cases of mRNAs for PACAP and SP only very weak bands of the PCR products were observed. Arrow shows specific product for neuropeptides and neurotransmitter-synthesizing enzymes and arrowhead shows product for GAPDH

DβH-positive nerve fibres were absent in clusters of cholinergic neurons.

Nerve fibres containing ChAT and VAChT were very numerous and unevenly distributed. Some areas of the ganglion contained dense plexuses of such fibres, while in other regions only single fibres were present. ChAT- and VAChT-positive nerve fibres surrounded both TH-positive and TH-negative neurons.

In PCG a significant number of nNOS-positive nerve fibres were present. A number of them (usually non-varicose) were organized into strands which divided the ganglion into parts, the rest of them (varicose nerve fibres) were wound around principal ganglional neurons.

NPY- and VIP-positive nerve fibres in the PCG were numerous. While all of NPY-positive nerve fibres belonged to the smooth fibres subpopulation, in the case of VIP-positive fibres, varicose fibres were also visible in addition to the smooth ones.

Nerve fibres containing Gal were present in a moderate number. These were varicose fibres which mostly supplied TH-positive neurons but some of them were also supplying TH-negative cells.

Varicose nerve fibres containing PACAP were very numerous. They generally formed basket-like structures surrounding ganglionic neurons.

Dense plexuses of Som-positive nerve fibres surrounded clusters of cholinergic neurons where no DβH-positive fibres were observed. Among other ganglionic neurons Som-positive fibres were very rare.

Numerous varicose SP-positive nerve fibres supplied both DβH-positive and DβH-negative cells.

RT-PCR

RT-PCR detected a strong signal from the transcripts of TH, DβH, ChAT, nNOS, NPY, galanin, VIP and Som. In the cases of PACAP and SP the PCR products exhibited only very weak bands on the gel (Figure 12).

DISCUSSION

The majority of data available on the neurochemical coding of neurons in pelvic ganglia was obtained from research on laboratory animals of both genders. The percentage of neurons containing noradrenaline (NA) in pelvic ganglia depends on species and gender and is correlated with a density of adrenergic innervation of genital organs. For example, a rat myometrium exhibits a low number of adrenergic fibres (Adham and Schenk, 1969; Papka et al., 1985), which is correlated with a low number of adrenergic neurons in paracervical ganglia (Papka et al., 1987; Houdeau et al., 1995). The situation is similar in mice (Wanigasekara et al., 2003) and guinea pigs (Morris and Gibbins, 1987; Dhami and Mitchell, 1991; Elfvin et al., 1997).

In humans, about half of the neurons of the pelvic ganglia contain both TH and DβH (Mitchell,

1993; Imai et al., 2006). Such neurons have been detected also in boars (Kaleczyc et al., 2003) and sows (Czaja et al., 2001; Wasowicz et al., 2002). In the present study we found that approximately 23% of neurons in porcine PCG contain TH and DβH as well. Most of these neurons were located in the cranial part of the PCG and their number gradually decreased moving in the direction to the caudal. This observation can most probably be explained by the existence of two regions in the ganglion: the cranial one, where the hypogastric nerve enters, and the caudal one, where the pelvic nerve brings preganglionic parasympathetic fibres. The presence of non-varicose TH/DBH-positive nerve fibres suggests that these fibres are of local origin and are processes of cells located in the PCG.

The PCG is thought to be an essential source of the cholinergic innervation of pelvic cavity organs (Mitchell et al., 1993). Previous studies have revealed that approximately 2/3 of the neurons in the pelvic ganglia of a male rat (Keast et al., 1995) and mouse (Wanigasekara et al., 2003) contain ChAT and a high number of ChAT-positive neurons have also been described in the APG of a male guinea pig (Elfvin et al., 1997). However, in a female rat these neurons constituted the majority of the PCG's neuronal population (Papka et al., 1999). In the present study it was shown that roughly 77% of neurons in the sow PCG were cholinergic nerve cells. The majority of these neurons were located in the caudal region of the ganglion. In all cells ChAT was colocalized with VAChT, and in such neurons neither TH nor DβH were detected. This is in agreement with the results of studies carried out on the pelvic ganglia of a male rat (Keast et al., 1995) and a mouse (Wanigasekara et al., 2003), where all neurons containing ChAT were TH-negative.

This study has revealed that approximately 25% of PCG neurons in the pig contain nNOS. This enzyme was present only in cholinergic neurons. nNOS was always colocalized with VIP, and very often with NPY and Som. Several studies have revealed that nNOS is frequently present in pelvic ganglia neurons of both genders in many species (Majewski et al., 1995; Papka et al., 1995; Elfvin et al., 1997; Majewski, 1997). However, in the rat these cells represent approximately 58% of PCG neurons (Papka et al., 1995). In those neurons nNOS was never colocalized with TH, an observation which is in concord with data described in the present study. The pattern of colocalization of nNOS and other substances described in this paper is very similar to that in guinea pig APG (Elfvin et al., 1997). The two subpopulations of nNOS-positive fibres are probably of different origins. The non-varicose fibres may be of local origin, while the varicose ones most likely originate from an extrinsic source.

In this study NPY presence has been detected in about 75% of ganglionic neurons. This neuropeptide has been observed in approximately 73% of TH-positive and in a slightly higher percentage of TH-negative neurons (ca. 76%). In cholinergic neurons this neuropeptide was frequently colocalized with VIP, Som and nNOS. This is in agreement with data obtained in guinea pig PCG, where NPY was detected in 72–84% of all neurons in the ganglion, and in one half of adrenergic neurons (Morris and Gibbins, 1987). In rat PCG NPY was found in roughly 50% of neurons (Inyama et al., 1985). As far as the domestic pig is concerned, NPY was also found in male pelvic ganglia, where it was present in approximately 64% of APG neurons (Kaleczyc, 1997), and in female pelvic ganglia (Majewski, 1997; Czaja et al., 2001; Wasowicz et al., 2002). The NPY-positive nerve fibres may originate in the PCG since they are non-varicose. This suggests that they do not make any synaptic contact to PCG neurons.

The results presented in this paper suggest that all cholinergic neurons in pig PCG contain VIP. That neuropeptide was very often colocalized with Som, and not so frequently with NPY and nNOS, but it has never been found in adrenergic neurons. The available literature reports similar results to the ones described above (Inyama et al., 1985; Morris and Gibbins, 1987; Papka et al., 1995; Majewski, 1997). In the rat PCG VIP-positive neurons constitute one third to one half of all the PCG's neurons (Inyama et al., 1985; Papka et al., 1987), and in a guinea pig's PCG this number is even higher (62–65%; Gu et al., 1984; Morris and Gibbins, 1987). VIP was also found in numerous neurons in the pelvic ganglia of a boar (Kaleczyc, 1997; Kaleczyc et al., 2003) and sow (Majewski, 1997; Wasowicz et al., 2002). While non-varicose nerve fibres immunoreactive to VIP may be of local origin, the varicose ones are most probably extrinsic nerve fibres. Their exact origin is unknown, however.

As was stated above, Gal was found only in cells with small bodies and branched processess, possibly contacting ganglional neurons. Those cells have shown strong immunoreactivity to galanin, as well as to TH and DβH; they were observed as single cells always in the proximity of adrenergic neurons.

The function of these cells in pig PCG is unknown. They may be interneurons, similarly to SIF cells in rat ganglia (Kanerva and Teravainen, 1972; Baker et al., 1977). Gal inhibits noradrenaline release by hypopolarization of the neuronal cell membrane (Reimann and Schneider, 1993; Sevcik et al., 1993), which can be used to moderate the functions of adrenergic neurons in pig PCG. The presence of galanin in PCG has been detected previously only in a sow (Wasowicz et al., 2002). Additionally, it was found in non-adrenergic neurons in the pelvic ganglia of a boar (Kaleczyc, 1997) and of a male rat (Keast, 1991).

The results presented in our paper showed that Som was present in approximately 67% of all neurons in the studied ganglion. It was usually seen in ChAT-/VAChT-positive neurons, and seldom seen in TH-/DBH-positive neurons. In a female guinea pig about 50% of neurons showed immunoreactivity to somatostatin, and a number of them (1–5% of all neurons in the ganglion) contained TH (Morris and Gibbins, 1987). As far as the domestic pig is concerned, the presence of Som was revealed in the pelvic ganglia of a sow, in both the adrenergic and cholinergic neurons (Majewski, 1997). An interesting subpopulation consists of Som-positive neurons showing strong staining to this neuropeptide. All these cells are cholinergic. Such neurons were also described in the pelvic ganglia of a boar (Kaleczyc, 1997). Until now the function of these neurons is unknown. These neurons are organized into clusters and are surrounded by numerous Som-positive nerve fibres. These fibres form structures suggesting synaptic contacts between fibres and neurons. This suggests also that the fibres are originating outside the ganglion; the site of origin, however, is still unknown.

In this study no SP-positive neuronal somata were found. Numerous varicose SP-positive nerve fibres were observed, supplying both adrenergic and cholinergic neurons. Available data report the presence of SP-positive neurons in pelvic ganglia. However, the subjects of these studies have been mostly males, in which few neurons containing this neuropeptide have been detected: guinea pig (Dhami and Mitchell, 1991), rat (Dail and Dziurzynski, 1985) and pig (Kaleczyc, 1997). However, in pig and rat PCG, only nerve fibres containing SP were observed (Morris and Gibbins, 1987; Majewski, 1997). The origin of these fibers remains unknown; the most probable source are neurons located in the dorsal root ganglia.

RT-PCR used to detect transcripts of the precursors of the substances studied confirmed the results of the immunohistochemical staining. It detected transcripts of TH, DβH, ChAT, nNOS, NPY, VIP and Som, immunoreactivity to which was found in the neurons of the porcine PCG. In case of PACAP and SP, to which immunoreactivity was absent in neuronal cell bodies of the porcine PCG, very weak signals were detected with RT-PCR. This may be due to the presence of the mRNAs for PACAP and SP in other, non-neuronal, cells like leukocytes, but it cannot be excluded that a very low level of transcription of genes for these substances may occur also in neurons of the porcine PCG. No data are available on the expression of the neuropeptides and TH, DβH, ChAT and nNOS at mRNA level in the PCG of other species.

The present results suggest the existence of profound inter-species differences regarding the immunohistochemical characteristics of PCG nerve structures. Further studies are necessary to elucidate the morphological peculiarities of the PCG in different species and their physiological significance.

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Received: 2007–06–14
Accepted after corrections: 2008–02–14

Corresponding Author:

Piotr Podlasz, DVM, PhD, University of Warmia and Mazury, Faculty of Veterinary Medicine, Department of Functional Morphology, Division of Animal Anatomy, 10-957 Olsztyn-Kortowo II, Poland
Tel. +48 89 5233733, fax +48 89 5234986, e-mail: piotr.podlasz@uwm.edu.pl