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Original article

Immunohistochemical characteristics and distribution of neurons in the intramural ganglia supplying the urinary bladder in the male pig

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Abstract

This study investigated the distribution and chemical coding of neurons in intramural ganglia of the urinary bladder trigone (UBT-IG) and cervix (UBC-IG) in the male pig using combined retrograde tracing and double-labelling immunohistochemistry. Additionally, immunoblotting was used to confirm the presence of marker enzymes for main populations of autonomic neurons. Retrograde fluorescent tracer Fast Blue (FB) was injected into the wall of both the left and right side of the bladder trigone, cervix and apex during laparotomy performed under thiopental anaesthesia. Twelve μm-thick cryostat sections were processed for double-labelling immunofluorescence with antibodies against tyrosine hydroxylase (TH), dopamine β-hydroxylase (DBH), neuropeptide Y (NPY), somatostatin (SOM), galanin (GAL), vasoactive intestinal polypeptide (VIP), nitric oxide synthase (NOS), calcitonin gene-related peptide (CGRP), substance P (SP) and vesicular acetylcholine transporter (VAChT). UBT-IG and UBC-IG neurons in both parts of the organ formed characteristic clusters (from few to tens of neuronal cells) found under visceral peritoneum or in the outer muscular layer. Immunohistochemistry revealed several subpopulations in UBT-IG and UBC-IG neurons, namely noradrenergic (ca. 76% and 76%), cholinergic (ca. 22% and 20%), non-adrenergic/non-cholinergic nerve cells (ca. 1.5% and 3.8%), NPY- (ca. 66% and 58%), SOM- (ca. 39% and 39 %), VIP- (ca. 5% and 0%) and NOS- immunoreactive (IR) (ca. 1.5% and 3.8%), respectively. Immunoblotting using antibodies to TH and VAChT showed the presence of studied proteins as revealed by the presence of protein bands of the correct molecular weight. This study has revealed a relatively large population of differently coded UBT- and UBC- IG neurons, which constitute an important element of the complex neuroendocrine system involved in the regulation of the male urogenital organs function.

Key words: intramural ganglia, urinary bladder trigone and cervix, retrograde tracing, neuropeptides, neurotransmitter synthesising enzymes, male pig

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Introduction

The urinary bladder has two main functions: storage of urine and its periodical and voluntary voidance. These mentioned functions require a complex neural control system that coordinates the activities of a variety of effector structures including the smooth muscle of the urinary bladder and the smooth and striated muscle of the urethral sphincters (Andersson and Arner 2004, Fowler et al. 2008). It has been well recognized so far that the innervation of the urinary bladder is supplied by three sets of peripheral nerves: sacral parasympathetic (pelvic nerves consisting of mainly preganglionic fibres supplying the intramural ganglia as well as of postganglionic fibres (Crowe and Burnstock 1989, Gabella 1990, Birder et al. 2009), thoracolumbar sympathetic (hypogastric nerves – their fibres are mainly postganglionic and a few preganglionic supply so called short adrenergic neurons' found within ganglia located very close to pelvic organs (Downie 1981, Feher and Vajda 1981) and sacral sensory (pudendal nerves (De Groat and Booth 1993). These pathways are a structural basis for constitution of reflexes, which either keep the bladder in a relaxed state, enabling urine storage at low intravesical pressure, or which initiate bladder emptying by relaxing the outflow region and contracting detrusor muscle. Integration of the autonomic and somatic efferents results in the contraction of the detrusor muscle preceded by a relaxation of the outlet region, thereby facilitating bladder emptying. On the contrary, during the storage phase, the detrusor muscle is relaxed and the outlet region is contracted to maintain continence.

It has long been known that ganglion cells are present within the wall of the bladder. Intramural ganglia are surrounded by a capsule and lie along nerve trunks being interconnected to form a plexus. They can occur in every region of the bladder but are more common in the trigone region where the ureters enter the organ (Gosling 1986, Gabella 1990). The trigone is a small region of the dorsal wall of the bladder located in the vicinity of the bladder cervix which has a different embryological origin from the detrusor muscle and represents an extension of the longitudinal muscle of the ureters (De Groat and Booth 1984). Also the bladder neck (cervix) is not a homogenous anatomical structure but consist of extensions of the lower detrusor and trigonal musculature. Thus, the urinary bladder intramural nervous system might play an important functional role, at least in the species where it is widely distributed, as in humans (Gilpin et al. 1983), female pigs (Lakomy et al. 1990, Pidsudko 2004), dogs (Arrighi et al. 2008), cats (Feher et al. 1979) and guinea-pigs (Crowe et al. 1986, Gabella 1990).

Furthermore, pigs, as opposed to smaller animals, such as cats and rats, present the advantage that the anatomy and functionality of their urinary system resembles the human one (Swindle et al. 1992, Dalmose et al. 2000).

Since our knowledge on the distribution and chemical coding of the intramural ganglia neurons of the urinary bladder (UB-IG) in the male pig is very limited, combined retrograde tracing with a fluorescent tracer Fast Blue (FB) and double-immunolabelling were used to elucidate the exact localisation and neurochemical features of UB-IG neurons involved in this neural pathway. Additionally, immunoblotting was used to confirm the presence of the proteins of marker enzymes for major subpopulations of autonomic neurons – tyrosine hydroxylase and vesicular acetylcholine transporter.

Materials and Methods

The study was performed on 15 juvenile male pigs of the Large White Polish breed. The animals originated from a commercial fattening farm. They were kept under standard conditions with free access to water. The pigs were divided into four groups: group T (n=4; 12 kg. of body weight; injection of FB solution into the trigone of the urinary bladder), group C (n=4; 12 kg. of body weight; injection of FB solution into the cervix of the urinary bladder), group A (n=4; 12 kg. of body weight; injection of FB solution into the apex of the urinary bladder) and group I (n=3; 12 kg. of body weight; for immunoblotting analysis of the expression of TH and VAChT, biologically active substances specific for adrenergic and cholinergic neurons present in the ganglia regulating the functions of the urinary bladder). All the animals were housed and treated in accordance with the rules approved by the local Ethics Commission (conforming to the "Principles of Laboratory Animal Care", NIH publication No. 86-23, revised 1985).

Experiments for retrograde tracing

At the beginning of the experiment the animals from group T, C and A were treated in the same way: all were pre-treated with atropine (Polfa, Poland; 0.04 mg/kg b.w., s.c.) and azaperone (Stresnil, Jansen Pharmaceutica, Belgium; 0.5 mg/kg b.w., i.m.) thirty minutes before the main anesthetic, sodium thiopental (Sandoz, PL, ca. 0.5 g per animal, administered according to the effect) was given intravenously in a slow, fractionated infusion. During a mid-line laparotomy the dorsal wall of the urinary bladder was

Table 1. Antisera used in the study.
1

Antigen	Host	Code	Dilution	Supplier
Primary Antisera				
DβH	Rabbit	DZ 1020	1:500	Biomol, UK
TH	Mouse	1017381	1:40	Boehringer Mannheim, GER
ChAT	Goat	AB144P	1:50	Milipore, USA
VAChT	Rabbit	V5387	1:4000	Sigma, USA
CGRP	rabbit	11535	1:2000	Cappel, UK
SP	rat	8450-0505	1:250	ABD Serotec, UK
GAL	rabbit	RIN 7153	1:2000	Peninsula, UK
SOM	rat	8330-0009	1:30	Biogenesis, UK
VIP	rabbit	Ab22736	1:2000	Abcam, USA
NPY	rabbit	NA 1233	1:400	Biomol, UK
NPY	rat	NZ 1115	1:200	Biomol, UK
NOS	rabbit	11736	1:2000	Cappel, UK
Secondary Reagents				
Alexa Fluor 488-donkey anti-rabbit IγG			1:500	Invitrogen, USA
Alexa Fluor 488-donkey anti-mouse IγG			1:500	Invitrogen, USA
Alexa Fluor 488-donkey anti-rat IγG			1:500	Invitrogen, USA
Alexa Fluor 555-donkey anti-rabbit IγG			1:500	Invitrogen, USA
Alexa Fluor 555-donkey anti-mouse IγG			1:500	Invitrogen, USA
Alexa Fluor 555-donkey anti-goat IγG			1:500	Invitrogen, USA

gently exposed and the aqueous suspension of FB (Dr K. Illing KG & Co, Gros-Umstadt, Germany) was injected into every mentioned structure – to the left and right side of the bladder trigone, cervix and apex – in a total volume of 40 µl 5% of FB solution per each animal and structure. Each side was injected 20 times (1 µl of the dye solution per 1 injection, under the serosa, with a Hamilton syringe equipped with a 26G needle) along the whole extension of the urinary bladder trigone, cervix or apex keeping a similar distance between the places of the injections. To avoid leakage the needle was left in place for one minute. The wall of the injected organ was then rinsed with physiological saline and gently wiped with gauze. After a survival period of four weeks the animals were sacrificed. Shortly, the pigs were deeply anaesthetised (following the same procedure as describe above) and transcardially perfused with 4% buffered paraformaldehyde (pH 7.4). The animals were then dissected and the whole urinary bladders were cut out and the fragments of the apex, cervix and bladder trigone were postfixed by immersion in the same fixative for 2 h. Then, they were washed with 0.1 M phosphate buffer (pH 7.4), and finally transferred to and stored in 18% buffered (pH 7.4) sucrose solution until further processing.

Samples of the apex, cervix and trigone (with intramural ganglia) of the urinary bladder were cut into transwerse 12 µm-thick cryostat serial sections. Sections were put on chrome alum-gelatine-coated slides, air dried and examined under the fluorescent microscope equipped with a filter set specific for FB. FB-positive neurons were counted in every fifth section and the results were pooled for every experimental animal. The results were statistically analysed and mean number of FB-positive cells was calculated. Results were expressed as mean ± SEM. The statistical analysis was performed with GraphPad Prism 5 software (GraphPad Software, La Jolla, Calif., USA). All the sections containing retrogradely FB-labelled nerve cells were processed for double-labelling immunofluorescence with antibodies listed in Table 1 and labelling techniques was applied as described previously (Pidsudko et al. 2001). The sections labelled were studied and photographed with a Zeiss Axiophot fluorescence microscope equipped with epi-illumination and an appropriate filter set for FITC, Texas Red and FB, and with confocal microscope (Zeiss LSM 710). Relationships between immunohistochemical staining and FB distribution were examined directly by interchanging filters.

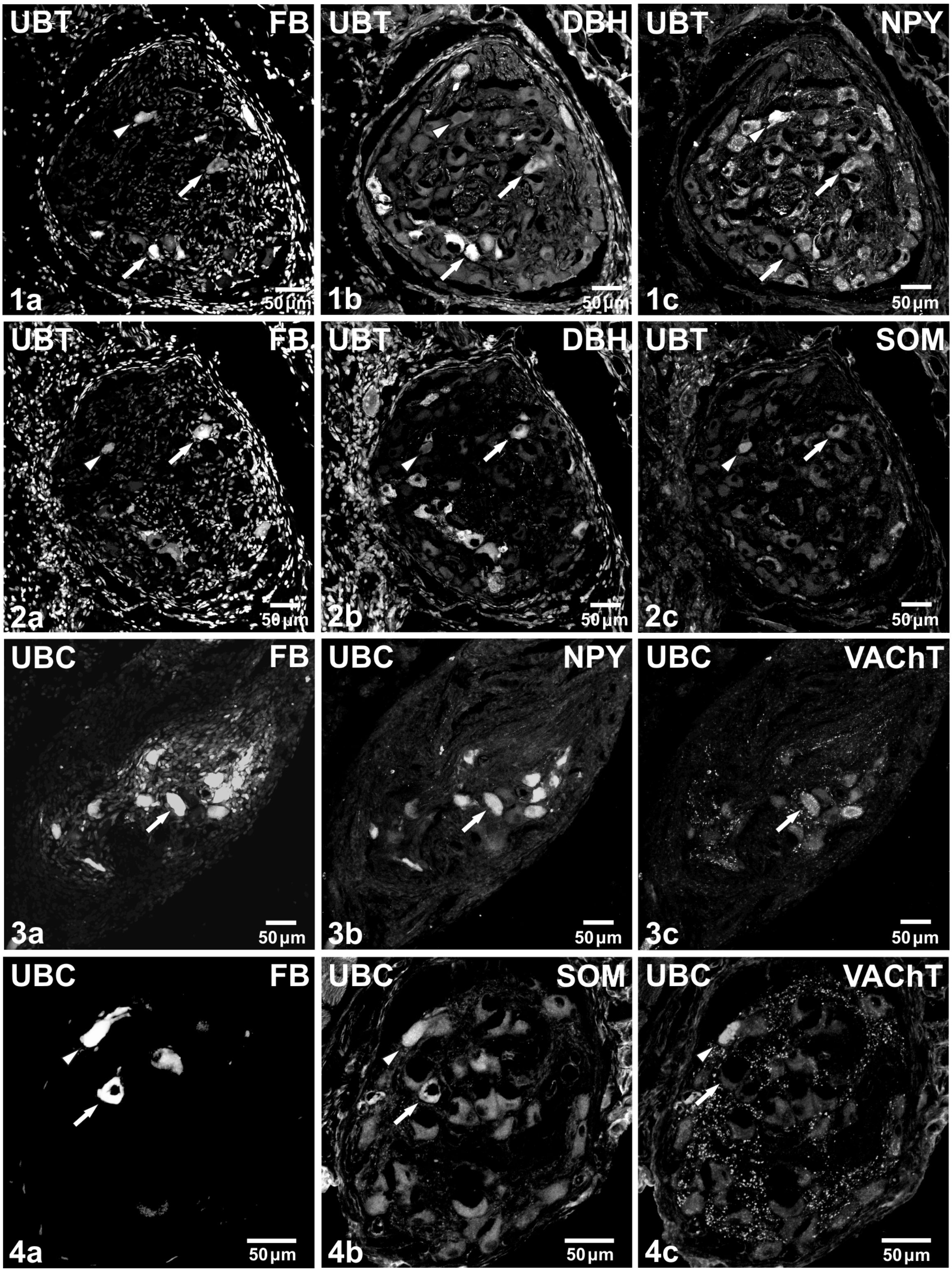


Fig. 1a-c. Intramural ganglia of the urinary bladder trigone (UBT-IG). Some FB-positive neurons (a) co-exhibited DBH (b; FITC visualization, applies to all the figures; arrows) and NPY-immunoreactivity (c, arrows; TXR visualization, applies to all figures; arrowhead shows NPY-positive neuron only. Scale bar = 50 µm; Fig. 2a-c. UBT-IG. A loose cluster of FB-positive neurons (a), one of them was DBH-(b; arrow) and SOM-positive (c, arrow; small arrowhead shows a neuron which was SOM-positive only). Scale bar = 50 µm; Fig. 3a-c. Intramural ganglia of the urinary bladder cervix (UBC-IG). Many FB-positive neurons (a) contained NPY-immunoreactivity (b; arrow), whereas only small numbers of them were simultaneously VAChT-positive (c; arrow). Scale bar = 50 µm; Fig. 4a-c. UBC-IG. A loose cluster of FB-positive neurons (a), one of them (small arrowhead) is VAChT- (b; small arrowhead) and SOM-positive (c, small arrowhead; arrow shows a neuron which was SOM-positive only). Scale bar = 50 µm.

Controls

Standard tests (preabsorption of the neuropeptide antisera, omission and replacement by non-immune sera of all the primary antisera used) were applied to control the specificity of immunofluorescence.

Counting of neurons

Sections from three, evenly distributed, regions of the trigone and cervix samples containing FB^+ neurons were chosen for immunohistochemical stainings. To determine the percentage of particular neuronal populations, at least 300 FB^+ (FB-labelled) neuronal profiles were investigated for each combination of antisera. All traced cells found in particular sections were counted. To avoid double counting of the same neurons, the neuronal cells were counted in every fifth section. The number of immunolabelled profiles was calculated as a percentage of neurons immunoreactive to particular antigen related to all FB^+ perikarya counted. Finally, data were pooled from all animals in particular groups and expressed as means ± SEM and then analysed by using GraphPad Prism 5 software.

Immunoblotting

All the animals from I group were deeply anaesthetised as described previously and exsanguinated. Then, fragments of urinary bladder where FB-positive neurons were previously localized were precisely dissected out and used for molecular biology. They have been analysed for the presence of main enzymes/markers of particular classes of ganglionic neurons – TH and VAChT by Western Blot method. Immunoblotting was performed as described elsewhere (Wasowicz 2003).

Results

The intramural ganglia of the male urinary bladder were found only in such anatomical structures as trigone and cervix. No ganglia were found in the body and apex of the organ.

In the animals from group T, intramural ganglia of the urinary bladder (UB-IG) were found under visceral peritoneum or in the outer muscular layer of the trigone (T). The UBT-IG neurons formed characteristic clusters (containing from a few to tens of neuronal cells). Ganglia were surrounded by a capsule. In the ganglia under study, the mean number of FB^+ neurons was $1661 \pm 136{,}7$ per animal.

In the animals from group C, UB-IG were also found under visceral peritoneum or in the outer muscular layer of the cervix (C). The UBC-IG neurons formed also characteristic clusters (containing from a few to tens of neuronal cells). Ganglia were surrounded by a capsule and they were a little more aggregated than in the trigone area. In the ganglia under study the mean number of FB^+ neurons was $1146 \pm 48{,}7$ per animal.

Immunohistochemistry revealed that both the UBT- and UBC-IG FB^+ neurons could be divided into three populations of neuronal cells. It was found that the vast majority of UBT- and UBC-IG, $76.38 \pm 1.54\%$ and $76.11 \pm 1.84\%$, respectively, belonged to the subset of noradrenergic cells (they contained colocalized TH and DBH; Fig. 1, 2, 7). $22.37 \pm 1.17\%$ and $20.07 \pm 1.39\%$ of UBT- and UBC-IG, respectively, were cholinergic (as may be judged from the existence of CHAT or VAChT in their somata; Fig. 3, 4, 5, 6). $1.42 \pm 0.2\%$ and $3.67 \pm 0.53\%$ of UBT- and UBC-IG, respectively, were considered as nonadrenergic/noncholinergic (NANC; on the basis of the comparison of consecutive sections; Fig. 6).

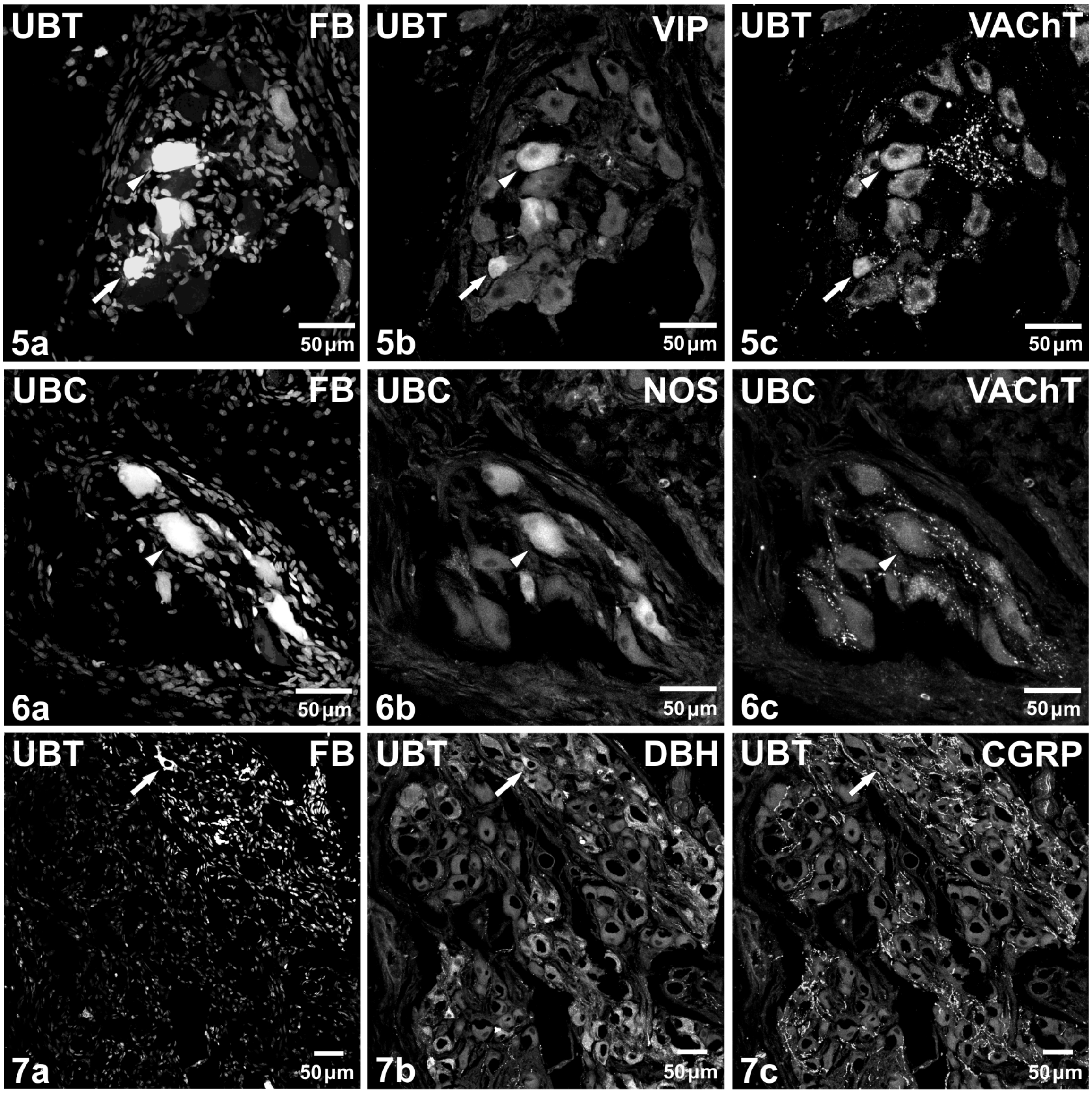
Immunohistochemical stainings with antisera for the studied nuropeptides and NOS revealed that NPY-immunoreactivity (IR) was present in $59.29 \pm 2.1\%$ and $51.87 \pm 1.94\%$ of FB^+ perikarya (Fig. 1, 3) of UBT-IG- and UBC-IG, respectively. Respective numbers for SOM-IR were $39.88 \pm 1.52\%$ and $39.55 \pm 1.36\%$ (Fig. 2, 4), for VIP-IR were 5.06 ± 0.14 and 0% (Fig. 5) and for NOS-IR were $1.42 \pm 0.2\%$ and $3.67 \pm 0.53\%$ (Fig. 6).

$66.2 \pm 1{,}8\%$ and $58.52 \pm 2.58\%$ of DBH-IR FB^+ neurons in UBT-IG and UBC-IG, respectively, contained also immunoreactivity to NPY (Fig. 1), while $44.46 \pm 1.77\%$ and $40.28 \pm 1.76\%$ of such neurons in UBT-IG and UBC-IG, respectively, contained SOM (Fig. 2).

$14.43 \pm 1.01\%$ and $21.43 \pm 1.84\%$ of the VAChT-IR FB^+ neurons in UBT-IG and UBC-IG, respectively, were also stained for NPY (Fig. 3). $4.66 \pm 0.54\%$ and $10.51 \pm 1.21\%$ of such neurons (Fig. 4) in UBT-IG and UBC-IG, respectively, contained SOM, while $5.65 \pm 0.53\%$ and 0% of such neurons (Fig. 5) in UBT-IG and UBC-IG, respectively, contained VIP.

Among NANC FB^+ nerve cells, all the neuronal profiles were NOS-positive (Fig. 6). GAL-, SP- and/or CGRP-IR FB^+ neurons were not found.

The UBT-IG and UBC-IG received a moderate CHAT/VAChT-, TH/DBH-, SP- and/or CGRP-IR nerve supply (Fig. 1-7). These nerve terminals formed dense network, evenly distributed within the ganglia investigated. It should be mentioned that nerve fibres


UBT
FB
5a
50 μm
UBT
VIP
5b
50 μm
UBT
VAChT
5c
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UBC
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UBC
NOS
6b
50 μm
UBC
VAChT
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UBT
FB
7a
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UBT
DBH
7b
50 μm
UBT
CGRP
7c
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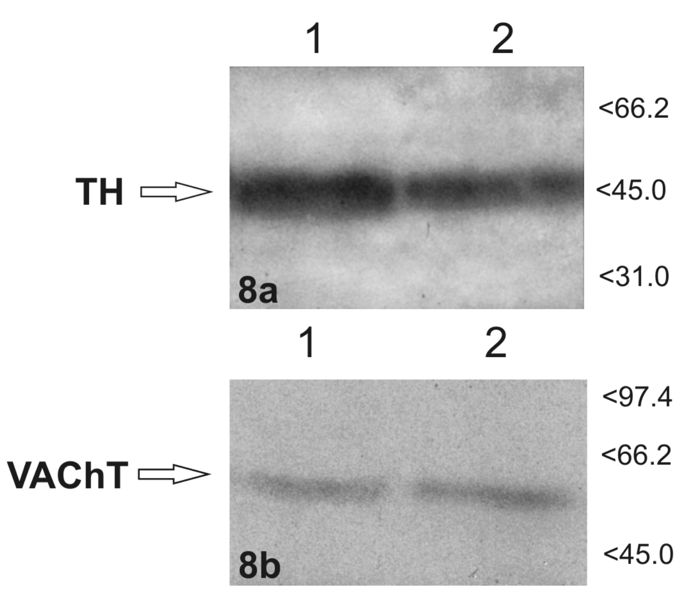
1
2
TH
<66.2
<45.0
<31.0
8a
1
2
VAChT
<97.4
<66.2
<45.0
8b

immunoreactive to CGRP formed basket-like structures surrounding TH/DBH-IR neurons (Fig. 7), whereas nerve terminals exhibiting other neuropeptides were less numerous. The TH/DBH-IR nerve terminals surrounded very often NPY-positive nerve cells.

Only very few nerve terminals were found to be immunoreactive to SOM, GAL, NOS and VIP.

Immunoblotting

Western Blot done on protein extracts from the samples of the urinary bladder trigone and cervix in male pig with TH (arrow) antibody detected specific TH (molecular weight ca. 45 kDa; Fig. 8a) bands in samples from the trigone and cervix. No differences in TH band intensity were detected. Western Blot performed with VAChT-antibody detected specific VAChT (arrow) protein band (molecular weight ca. 56 kDa; Fig. 8b) in samples from the trigone and cervix. No differences in VAChT band intensity were detected.

Discussion

The present study indicates that the male pig urinary tract has a complex neuronal supply. It is well known that the innervation of the urinary bladder originates not only from the prevertebral ganglia and pelvic plexus ganglia, but also from intramural ganglia distributed within the wall of the urinary bladder. These intramural ganglia consist of both noradrenergic and cholinergic neurons. It is known that between mammals there are some differences in the distribution of the IG-UB. They are numerous in the guinea-pig (Gabella 1990) and less numerous in the cat (Feher et al. 1979) ferret, rabbit (Gabella 1990), horse (Prieto et al. 1989) and female pig (Pidsudko 2004). No, or very few, UB-IG have been found in the mouse (Grozdanovic et al. 1992) and rat (Gabella and Uvelius 1990).

In the present work, the intramural neurons were observed in two areas of the bladder – the trigone and bladder neck. In the literature on this topic several studies have reported that intramural neurons are dispersed throughout the urinary bladder wall, while in many animal species they have been mainly concentrated around the entry points of the ureters – the trigone, and in the bladder neck (Feher et al. 1980, Gabella 1990, Dixon et al. 1997). Also, earlier experiments performed by the author (Pidsudko 2004) conducted in the female pig confirmed this observation, but only the intramural ganglia of the trigone were investigated.

Immunohistochemical investigations performed in the present study have revealed two major populations of UB-IG neurons, noradrenergic (those immunoreactive to DBH; approximately 76%) and cholinergic (those immunoreactive to VAChT; approx. 22%). These observations are in agreement with previous findings performed in the female pig (Pidsudko 2004) although the population of noradrenergic neurons was less numerous (approximately 52%). Also Dixon et al. (Dixon et al. 1997, Dixon et al. 1998, Dixon et al. 1999) observed that up to 50% of UB-IG ganglia in humans contain TH/DBH, but they also were VAChT -IR, so they contained both markers of noradrenergic and cholinergic traits. However, this was not the case in the pig. It may be attributed most probably to interspecies differences or to different stage of the development of this part of the peripheral nerve system, that is known to be able to make so-called “transmitter switch” in the presence of particular trophic molecules *vide* the plasticity of the sweat gland innervation (Landis and Fredieu 1986). The finding of such big proportion of noradrenergic neurons in the bladder wall is probably due to the fact that similarly to human all the ganglia presently examined came from the bladder trigone and neck where noradrenergic nerves have been shown to be concentrated (Schulman et al. 1972), especially in males (Dixon et al. 1983). In this region noradrenergic nerves have been shown to cause smooth muscle contraction and thus closure of the bladder neck (Lincoln and Burnstock 1993) and in the male such nerves are activated at the time of seminal emission and prevent the reflux of semen into the bladder (so-called “retrograde ejaculation”) (Krane and Olsson 1973). As in

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Fig. 5a-c. UBT-IG. A loose cluster of FB-positive neurons (a), one of them (small arrowhead) was VIP-(b; small arrowhead) and VAChT-positive (c, small arrowhead). Scale bar = 50 µm; Fig. 6a-c. UBC-IG, a small group of FB-positive neurons (a) all of them exhibited immunoreactivity to NOS (b) and were VAChT-negative (c; arrowhead). Scale bar = 50 µm; Fig. 7a-c. UBT-IG; many FB-positive neurons (a) contained DBH-immunoreactivity (b; arrow), but were CGRP-negative (c; arrow). Scale bar = 50 µm; Fig. 8a-b. Immunobloting. Expression of TH and VAChT in intramural ganglia of the urinary bladder trigone and cervix in male pig. a – Protein bands of TH (arrow). The intensities of protein bands are uniform in all samples. b – Protein bands of VAChT (arrow). The intensities of protein bands are uniform in all samples. Lane 1 – urinary bladder trigone; lane 2 – urinary bladder cervix. Marks on the right show positions of bands of molecular weight marker (Broad Range, Bio Rad, USA). Numbers indicate molecular weights of standard bands in kilodaltons (kDa).

the female pig (Pidsudko 2004) the present finding that subpopulation of DBH- and VAChT-IR neurons contain NPY is consistent with previous studies which have shown that NPY is present in a proportion of both noradrenergic and cholinergic nerves in man (Lundberg and Hokfelt 1986). Furthermore, NPY-IR has been shown to be widely distributed throughout the human detrusor muscle (Gu et al. 1984, Jen et al. 1995) and ureters (Edyvane et al. 1994) and many of these nerves presumably originated from intramural ganglion cells. NPY has also been detected in intramural ganglia of the guinea-pig (James and Burnstock 1988) and horse (Prieto et al. 1989) urinary bladder as well as of the human urethra (Crowe et al. 1988).

Similarly to the results obtained in the female pig (Pidsudko 2004) the present study has revealed that subpopulation of DBH- and VAChT-IR neurons contain SOM-IR. These results correspond well with data obtained in other mammals and dealing with immunohistochemical characteristic of UB-IG neurons (for references, see Pidsudko 2004). Since the relaxing effect in the detrusor muscle is very low (Callahan and Creed 1986, Crowe et al. 1986) it seems possible that neurons containing SOM could act in the local intraganglionic transmission or have a modulatory or inhibitory effect on the action of other transmitters (e.g. ATP; Callahan and Creed 1986). SOM also causes a concentration-dependent rise in the basal tone but not phasic contraction in the urinary bladder of guinea-pig, rabbit and rat (Sjogren et al. 1982) suggesting that it is not the transmitter for the non-adrenergic, non-cholinergic nerves.

The present study has also shown VIP-IR in the studied neurons. These results do not correspond with data obtained in female pig (Pidsudko 2004). The precise functions of these peptide-containing nerves in the pig are unknown. VIP has a concentration-related relaxant effect on the detrusor muscle of the human urinary bladder and is a candidate for the transmitter of non-adrenergic, non-cholinergic *inhibitory* nerves that supply the neck of the bladder in the human and pig (Klarskov et al. 1984). The distribution of VIP-containing nerves in the smooth muscle, around blood vessels and beneath the epithelium in the pig urinary tract suggests that these may participate in the regulation of smooth muscle activity, perhaps acting as a local modulators of neuromuscular transmission, blood flow and epithelial activity.

The present investigation has shown a small populations of NANC neurons in the ganglia studied. All of this nerve cells contain immunoreactivity to NOS. This observation agrees with the results of earlier studies on the pig urinary bladder (for references see Pidsudko 2004). It is known that NO may act as a non-adrenergic, non-cholinergic inhibitory transmitter in the urogenital tract (Rand 1992). There is accumulating evidence that neurogenic relaxation of both the bladder neck and the urethra is reduced by NOS inhibitors (Thornbury et al. 1992, Persson and Andersson 1992). This suggests that NO may play a significant role in non-adrenergic, non-cholinergic inhibitory transmission and thus internal sphincter function.

The neurons in the male pig UB-IG are moderately supplied with VAChT-, DBH-, SP- and/or CGRP-IR nerve fibres and poorly supplied with SOM-, GAL-, NOS- and VIP-IR nerve terminals. Similar intraganglionic distribution pattern has been described in the pig (Crowe and Burnstock 1989, Pidsudko 2004) as well as in other mammals (Hoyle 1994, Zhou and Ling 1998) but so far it is not known which ganglia contribute to the nerve supply and the source of the observed nerve terminals remains to be discovered. Correlating the present results and results of previous publications it can be assumed that the nerve fibres containing such peptides as SP, CGRP and GAL are collaterals of sensory neurons, the nerve terminals containing immunoreactivity to ChAT-, NOS-, and VIP-IR probably represent preganglionic axons (Kondo et al. 1985, Gibson et al. 1986), whereas at least some DBH-, NPY- and/or SOM-IR nerve fibres are processess of CAMG neurons (Pidsudko 2000). However, this hypothesis should be verified using anterograde tracing method (e.g. anterograde tracer DiI injected directly to into CaMG).

In summary, the porcine IG-UB have been found to contain many neurons projecting to the urinary bladder trigone and neck. This study has revealed a relatively large population of differently coded IG-UBT and UBC neurons, which constitute an important element of the complex neuroendocrine system involved in the regulation of the male urogenital organ function. In addition to noradrenaline and acetylocholine, numerous substances have been localized in neuronal cell bodies and terminal varicosities which have the potential for transmitters and/or neuromodulator actions. In many cases the precise mechanism of action of these substances has yet to be defined. However, it is likely that modulation and integration of the nervous control of the bladder occurs not only by the anatomical overlap of the nerve pathways but also by the functional interactions of the variety of transmitters or neuromodulators within the separate nerve population. Furthermore these intramural neurons do not simply act as relay stations in the bladder trigone and neck but are capable of complex interaction, presumably serving to coordinate and regulate smooth muscle activity during both the filing and emptying phases of bladder function.

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