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Short communication

Distribution and chemical coding of urinary bladder apex-projecting neurons in aorticorenal and testicular ganglia of the male pig

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Abstract

Combined retrograde tracing and double-labelling immunofluorescence were used to investigate the distribution and chemical coding of neurons in aorticoerenal (ARG) and testicular (TG) ganglia supplying the urinary bladder apex (UBA) in the juvenile male pig (n=4, 12 kg. body weight). Retrograde fluorescent tracer Fast Blue (FB) was injected into the wall of the bladder apex under pentobarbital anesthesia. After three weeks all the pigs were deeply anesthetized and transcardially perfused with 4% buffered paraformaldehyde. TG and ARG were collected and processed for double-labelling immunofluorescence. The presence of tyrosine hydroxylase (TH) or dopamine beta-hydroxylase (DBH), neuropeptide Y (NPY), somatostatin (SOM), galanin (GAL), nitric oxide synthase (NOS) and vesicular acetylcholine transporter (VAChT) were investigated. The cryostat sections were examined with a Zeiss LSM 710 confocal microscope equipped with adequate filter blocks.

The TG and ARG were found to contain many FB-positive neurons projecting to the UBA (UBA-PN). The UBA-PN were distributed in both TG and ARG. The majority were found in the left ganglia, mostly in TG. Immunohistochemistry disclosed that the vast majority of UBA-PN were noradrenergic (TH- and/or DBH-positive). Many noradrenergic neurons also contained immunoreactivity to NPY, SOM or GAL. Most of the UBA-PN were supplied with varicose VAChT-, or NOS- IR (immunoreactive) nerve fibres.

This study has revealed a relatively large population of differently coded ARG and TG neurons projecting to the porcine urinary bladder. As judged from their neurochemical organization these nerve cells constitute an important element of the complex neuro-endocrine system involved in the regulation of the porcine urogenital organ function.

Key words: prevertebral ganglia, urinary bladder apex, immunohistochemistry, retrograde tracing, neuropeptides, male pig

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Introduction

Storage and periodic disposal of urine are two main correlated functions of the organs belonging to the lower urinary tract. Micturition is a complex function, which is controlled and regulated by a complex interaction of somatic, sympathetic and parasympathetic innervation, and is modulated by higher nervous centres as the cerebral cortex, the cerebellum and pons (Blok and Holstege 1999). It has been well recognized so far that the innervation of the urinary bladder is supplied by three sets of peripheral nerves: sacral parasympathetic, thoracolumbar sympathetic (hypogastric nerves and prevertebral ganglia-, including testicular (TG) and aorticorenal (ARG) ganglia) and sacral sensory nerves. These pathways are a structural basis for reflexes which either keep the bladder in a relaxed state, enabling urine storage at low intravesical pressure, or initiate bladder emptying by relaxing the outflow region and contracting the detrusor muscle (Pidsudko 2013, 2014, de Groat et al. 2015, Lepiarczyk et al. 2019).

To complete our knowledge of the distribution and chemical coding of the urinary bladder apex-projecting neurons (UBA-PN) in the male pig we combined retrograde tracing and double-immunolabelling to elucidate: 1) the involvement of TG and ARG in this neural pathway and 2) neurochemical features of their UBA-PN.

Materials and Methods

The study was performed on 4 juvenile male pigs of the Large White Polish breed. The animals were housed and treated in accordance with the rules approved by the Local Ethical Commission for Animal Experimentation in Olsztyn. The fluorescent retrograde neuronal tracer Fast Blue (FB; Dr K. Illing KG&Co, Groβ-Umstadt, Germany) was injected into both the left and right side of the urinary bladder apex during laparatomy performed under pentobarbital anesthesia (All the pigs were pre-treated with atropine (Polfa, Poland; 0.004 mg/kg b.w., s.c.) and azaperone (Stresnil, Jansen Pharmaceutica, Belgium; 0.5 mg/kg b.w., i.m.) 30 min before the main anesthetic, sodium thiopental (Sandoz, PLca. 0.5 g per animal, administered according to the effect) was given intravenously in a slow, fractionated infusion). After a survival period of three weeks the pigs were deeply reanaesthetised and transcardially perfused with 4% buffered paraformaldehyde. The collected prevertebral ganglia (i.e., aorticorenal -ARG and testicular -TG) were postfixed by immersion in the same fixative for several hours and finally stored in 18% sucrose until sectioning. The left and right TG and ARG were cut into 12 μm thick cryostat serial sections. FB-labelled cell counts were done prior to the immunohistochemistry. To determine the relative number of UBA-PN, the neurons were counted in every fourth section from both the left and right ganglia in all the animals. Only neurons with a clearly visible nucleus were considered. All the sections containing retrogradely labelled neurons were processed for double-labelling immunofluorescence as described previously (Pidsudko et al. 2001) with antibodies listed in Table 1. Briefly, the sections were mounted on glass slides, air dried, then washed for 3 x 10 min in PB (pH 7.4), incubated for 1 h in blocking solution (10% normal serum of species matching those in which the secondary antibodies were raised (Cappel, Warsaw, Poland) in PBS with addition of 0.25% Triton X-100 (Sigma, St. Louis, MO, USA), and incubated overnight

Table 1. Antisera used in the study.

Antigen	Host	Code	Dilution	Supplier
PRIMARY ANTISERA				
DβH	rabbit	DZ 1020	1:500	Biomol, UK
TH	mouse	1017381	1:40	Boehringer Mannheim, GER
VAChT	rabbit	V5387	1:4000	Sigma
GAL	rabbit	RIN 7153	1:2000	Peninsula, UK
SOM	rat	8330-0009	1:30	Biogenesis, UK
NPY	rabbit	NA 1233	1:400	Biomol, UK
NPY	rat	NZ 1115	1:200	Biomol, UK
NOS	rabbit	11736	1:2000	Cappel
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
Alexa Fluor 555-donkey anti-mouse IγG			1:500	Invitrogen

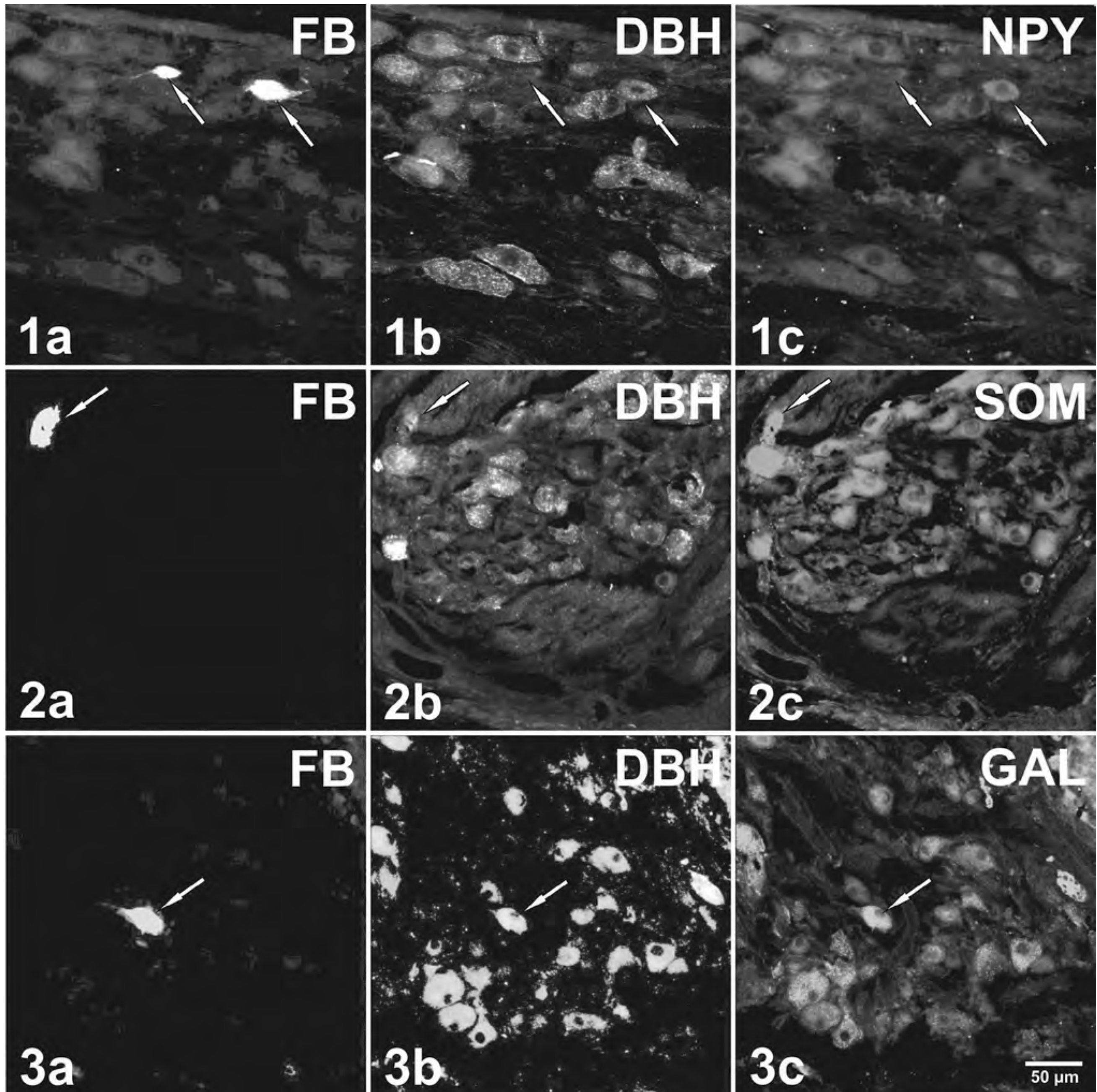

Fig. 1a-c. Urinary bladder apex-projecting neurons in the male pig testicular ganglion - TG; FB-positive neurons (FB+; a); one of them co-expresses DBH- (b) and NPY- immunoreactivity (IR) (c); scale bar = 50 µm.
Fig. 2a-c. UBA-PN in the male pig TG; FB+ neuron (a) co-expresses DBH- (b) and SOM- IR (c); scale bar = 50 µm.
Fig. 3a-c. UBA-PN in the male pig TG; FB+ neuron (a) co-express DBH- (b) and GAL- IR (c); scale bar = 50 µm.

at room temperature (RT) with primary antibodies diluted in blocking solution containing 1% normal swine serum. After incubation with primary antisera, the sections were washed for 3 x 10 min in PBS and further incubated with secondary antisera (Alexa Fluor®, Invitrogen) for 1 h at RT. After incubation, the sections were washed for 3 x 10 min in PBS and coverslipped with buffered glycerol. The labelled sections were studied and photographed with a Zeiss Axiophot fluorescence microscope equipped with epi-ilumination and an appropriate filter set for FITC, Texas Red and FB, and with a confocal microscope (Zeiss LSM 710).

Results and Discussion

The UBA-PN were present only in TG and ARG. The majority were found in the left ganglia, mostly in TG. The whole PRVG complex contained 82 ± 31 neurons (mean± SEM). There were 61 ± 23 and 21 ± 9 neurons in the left and right TG, respectively. Only sin-

gle neurons were found in the left ARG. Immunohistochemistry disclosed that the vast majority of UBA-PN were noradrenergic (i.e. TH- and/or DBH-positive, approx. 86%). A prominent proportion of these neurons contained also immunoreactivity (IR) to NPY - (31.00%; Fig. 1) or SOM- (19.50 %; Fig. 2) and a smaller number was GAL-IR (1.30%; Fig. 3). Most of the UBA-PN were surrounded with varicose nerve fibers exhibiting VAChT- and NOS-IR. The present study has shown that the efferent innervation of the boar urinary bladder apex originates not only from the caudal mesenteric ganglion (CaMG), but also from other prevertebral ganglia. These observations correspond well with findings obtained not only in the female pig but also in laboratory and other domestic animals, in which it has been shown that not only sympathetic chain ganglia, but also prevertebral ganglia, are sources of the efferent nerve supply crucial for the maintenance of the lower urinary tract functions (De Groat and Steers 1988, Pidsudko and Majewski 2004, de Groat and Yoshimura 2015). It should be stressed that sympathetic axons innervating the bladder vasculature most likely originate from neurons in the prevertebral ganglia (sympathetic chain and other prevertebral ganglia), as is the case for sympathetic vasoconstrictor axons in other organs (Keast et al. 2015). Immunohistochemical characteristics of TG and ARG neurons supplying the UBA has shown that the vast majority (86 %) of UBA-PN should be considered as noradrenergic, because they stained for TH/DBH, key enzymes of noradrenaline synthesis. These neurons have been also found to contain NPY, SOM, or GAL. This observation corresponds well with the data obtained in the female pig (Pidsudko and Majewski 2004). In the female pig prevertebral ganglia, including also inferior mesenteric ganglion (IMG) of the rat and cat, the majority of cells contained DBH. In addition to DBH-IR and NPY-IR neurons, numerous SOM-IR nerve cell bodies have been demonstrated (Janig and McLachlan 1987, Pidsudko and Majewski 2004). SOM has been shown to be colocalized with noradrenaline (NA) or DBH in the rat and guinea pig. In the guinea pig 60% of the cell bodies contained both SOM and NA while 16% contained NA and NPY (Janig and McLachlan 1987). In summary, the male pig prevertebral ganglia have been found to contain many neurons projecting to the urinary bladder apex. This study also revealed a different coding of prevertebral UBA-PN, which are probably involved in the neural control of the urinary bladder.

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