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Short communication

The distribution and chemical coding of urinary bladder trigone-projecting neurons in testicular and aorticorenal ganglia in male pigs

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Abstract

Combined retrograde tracing and double-labelling immunofluorescence were used to investigate the distribution and chemical coding of neurons in testicular (TG) and aorticoerenal (ARG) ganglia supplying the urinary bladder trigone (UBT) in juvenile male pigs (n=4, 12 kg. of body weight). Retrograde fluorescent tracer Fast Blue (FB) was injected into the wall of the bladder trigone 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 expression 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 UBT (UBT-PN). The UBT-PN were distributed in both TG and ARG. The majority of them were found in the right ganglia, mostly in TG. Immunohistochemistry disclosed that the vast majority of UBT-PN were noradrenergic (TH- and/or DBH-positive). Many noradrenergic neurons contained also immunoreactivity to NPY, SOM or GAL. Most of the UBT-PN were supplied with VAChT-, or NOS- IR (immunoreactive) varicose nerve fibres.

This study has revealed a relatively large population of differently coded prevertebral 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 trigone, immunohistochemistry, retrograde tracing, neuropeptides, male pig

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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

Introduction

Micturition can be described as a process in which neural circuits in the brain and spinal cord coordinate the activity of smooth muscle in the bladder and urethra. These circuits act as on-off switches to alternate the state of the lower urinary tract between two modes of operation: storage and elimination (Yoshimura and Chancellor 2003). Micturition is under voluntary control and depends on learned behavior that develops during maturation of the nervous system, whereas many other visceral functions are regulated involuntarily. Micturition also requires the integration of autonomic and somatic efferent mechanisms to coordinate the activity of visceral organs (i.e., bladder and urethra) with that of urethral striated muscles (de Groat 1995). 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 ones. 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 enrich our knowledge on the distribution and chemical coding of the urinary bladder trigone-projecting neurons (UBT-PN) in the male pig we have combined retrograde tracing and double-immunolabelling to elucidate: 1) the involvement of TG and ARG in this neural pathway, and 2) neurochemical features of these neurons.

Materials and Methods

The study was performed in 4 juvenile male pigs of the Large White Polish breed. The animals were housed and treated in accordance with the rules of the local Ethics Commission (affiliated to the National Ethics Commission for Animal Experimentation, Polish Ministry of Science and Higher Education).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 trigone during laparotomy 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, PL;ca. 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 animals were deeply reanaesthetised and transcardially perfused with 4% buffered paraformaldehyde. The collected prevertebral ganglia (i.e., TG and ARG) were postfixed by immer-

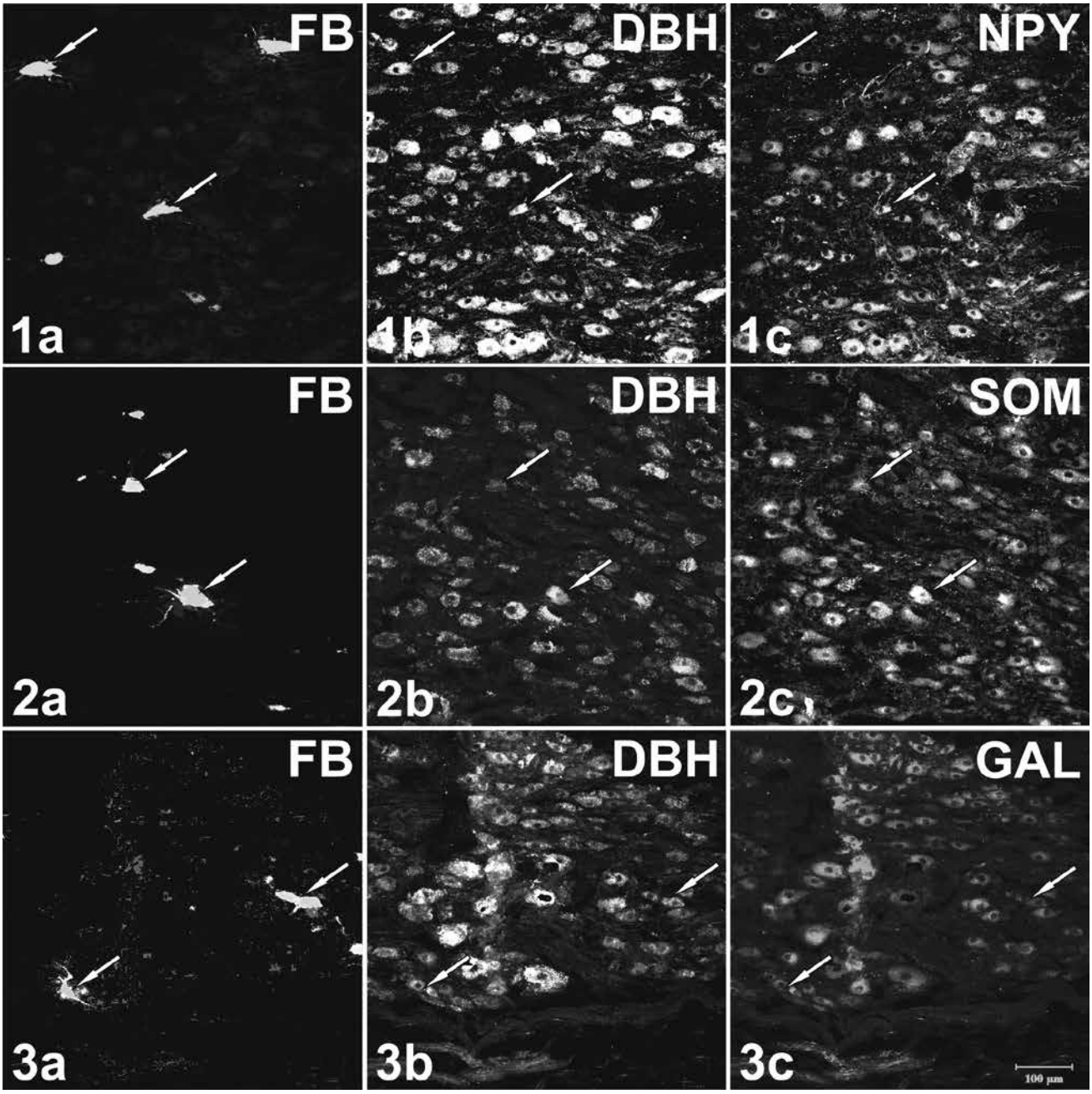

Fig. 1a-c. Urinary bladder trigone-projecting neurons (UBT-PN) in the male pig testicular ganglion (TG). FB-positive neurons (FB+; a); some of them co-express DBH- (b) and NPY- immunoreactivity (IR) (c) . Scale bar 100 µm

Fig. 2a-c. UBT-PN in the male pig TG. FB+ neurons (a) co-express DBH- (b) and SOM- IR (c) . Scale bar = 100 µm

Fig. 3a-c. UBT-PN in the male pig TG. FB+ neurons (a) co-express DBH- (b) and GAL- IR (c). Scale bar = 100 µm

sion 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 the UBT-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. 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 confocal microscope (Zeiss LSM 710).

Results and Discussion

The UBT-PN were present in TG and ARG. The majority of them were found in the right ganglia, mostly in TG and a small number in ARG. The whole prevertebral ganglia contained 186±31 neurons (mean± SEM). There were 155±23 and 31±9 neurons in the right and left TG, respectively. Only single neurons were found in the right ARG. Immunohistochemistry revealed that the vast majority of UBT-PN were noradrenergic (i.e. TH- and/or DBH-positive; approx. 87%). A prominent proportion of these neurons contained also immunoreactivity to NPY (33%; Fig. 1) or SOM- (17.5 %; Fig. 2) and a smaller number was GAL-IR (1.50%; Fig. 3). Most of the UBT-PN were surrounded with varicose nerve fibers exhibiting VAChT- or NOS-immunoreactivity. The present study has shown that the efferent innervation of the boar urinary bladder trigone originate not only from the caudal mesenteric ganglion (CaMG), but also from other prevertebral ganglia. This observation corresponds well with findings obtained not only in female and male pigs innervation of the apex of the urinary bladder) 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 (Pidsudko and Majewski 2004, de Groat and Yoshimura 2015, de Groat et al. 2015, Pidsudko et al. 2019). It should be mentioned that in female pigs the same structures of urinary bladder are supplied by the homologous ganglia, but the neurons are much more numerous (Pidsudko and Majewski 2004).

Immunohistochemical investigations performed in this study revealed that the vast majority (87%) of UBT-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 findings obtained in previous study performed in female and male pigs (Pidsudko and Majewski 2004, Pidsudko et al. 2019). In the pig, prevertebral ganglia, including also inferior mesenteric ganglion (IMG) of the rat and cat, the majority of cells contained DBH. In addition to the DBH-IR and NPY-IR, numerous SOM-IR nerve cell bodies have been demonstrated.

It should be stressed that the adrenergic UBT-projecting neurons have also a visceromotor function. The role of the visceromotor adrenergic neurons on the UBT smooth muscle appears complex. According to Yamanishi et al. (Yamanishi et al. 2002), the functional role of the bladder base, including the bladder trigone, is to open and close the bladder neck during filling and emptying, respectively. The superficial trigonal layer, composed of smooth muscle of mesodermal origin, is thought to be sensitive to noradrenaline, with responses being predominantly mediated by α-adrenoceptors (Russo et al. 2013). During bladder filling, the adrenergic stimulation causes the contraction of the superficial layer to keep the bladder neck closed. The deep trigonal layer, which is instead of endodermal origin, like the detrusor muscle, responds to the same noradrenergic stimuli by β-adrenoceptors, relaxing and not opposing the closure of the bladder neck, but favoring flattening and elongation of the bladder base. During emptying, the detrusor and the deep layer of the trigone respond to acetylcholine via muscarinic receptors by contracting and facilitating the opening of the bladder neck (Russo et al. 2013).

In summary, the male pig prevertebral ganglia have been found to contain many neurons projecting to the urinary bladder trigone. This study has also revealed differently coded prevertebral UBT-PN, which are probably involved in the neural control of the urinary bladder.

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