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Distribution and sub-types of afferent fibre in the mouse urinary bladder

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ABSTRACT

Aim: Increased afferent fibre activity contributes to pathological conditions such as the overactive bladder syndrome. Nerve fibres running near the urothelium are considered to be afferent as no efferent system has yet been described. The aim of this study was to identify sub-types of afferent nerve fibres in the mouse bladder wall based on morphological criteria and analyse regional differences.
Materials and methods: 27 bladders of six month old C57BL/6 mice were removed and tissues were processed for immunohistochemistry. Cryostat sections were cut and stained for Protein Gene Product 9.5 (PGP), calcitonin gene related polypeptide (CGRP), neurofilament (NF), vesicular acetylcholine transporter (VAChT) and neuronal nitric oxide synthase (nNOS).
Results: In the sub-urothelium, different types of afferent nerve fibre were found, i.e. immunoreactive (IR) to; CGRP, NF, VAChT, and/or nNOS. At the bladder base, the sub-urothelium was more densely innervated by CGRP-IR and VAChT-IR nerve fibres, then at the lateral wall. NF- and nNOS nerves were sparsely distributed in the sub-urothelium throughout the bladder. At the lateral wall the inner muscle is densely innervated by CGRP-IR nerve fibres. NF, VAChT and nNOS nerves were evenly distributed in the different muscle layers throughout the bladder. Nerve fibre terminals expressing CGRP and NF were found within the extra-mural ganglia at the bladder base.
Conclusions: Different types of afferent nerve fibres were identified in the sub-urothelium of the mouse bladder. At the bladder base the sub-urothelium is more densely innervated than the lateral wall by CGRP-IR and VAChT-IR afferent nerve fibres. CGRP and NF afferent nerve fibres in the muscle layer probably relay afferent input to external ganglia located near the bladder base. The identification of different afferent nerves in the sub-urothelium suggests a functional heterogeneity of the afferent nerve fibres in the urinary bladder.

1. Introduction

There is accumulating evidence that aberrant afferent sensory nerve activity plays an important role in the overactive bladder syndrome (OAB) (Fowler et al., 2008). During bladder filling, sensory information is relayed to the central nervous system by the pudendal, pelvic and hypogastric nerves. Each of these nerves contains different types of afferent fibres, which can be identified and differentiated functionally and structurally. They consist of small myelinated Aδ fibres, mainly located in detrusor muscle, and unmyelinated C nerve fibres innervating all layers of the bladder wall. These types can functionally be distinguished based on the conduction velocity, or their response to mechanosensitive or chemosensitive stimuli. However, no functional relationship between conduction velocity and receptor type has been described (Shea et al., 2000; Janssen et al., 2016). Histological studies have shown that bladder afferent nerve fibres originate from the dorsal root ganglia (DRG) and contain multiple neuropeptides, e.g., Substance P (SP), Neurofilament protein (NF) and neuronal Nitric Oxide Synthase (nNOS) and Calcitonin Gene Related Peptide (CGRP) (Fowler et al., 2008). The exact distribution of terminal endings of these peptide-containing afferents is still largely

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unknown but they appear to be more densely present in the sub-urothelium just beneath the urothelium (Gosling and Dixon, 1974). Nerve fibres in the sub-urothelium near the urothelium are considered to be afferent fibres as no efferent system has yet been identified (Gosling and Dixon, 1974). Furthermore, the afferent innervation of the bladder neck and trigone is higher in density than the lateral wall and dome (Andersson, 2002). Regional variation in the sub-urothelial cholinergic innervation has been observed in the guinea pig urinary bladder, suggesting functional differentiation between the lateral wall and bladder base (Grol et al., 2008). Moreover, co-localization of different neuropeptides has been shown also suggesting a functional heterogeneity (Gillespie et al., 2006). This may explain why some fibres respond to a wide range of mechanical and chemical stimuli, such as stretch, pain, chemical modulation and motor/sensory elements (Gillespie et al., 2009). In the mouse bladder, using electrophysiological techniques, at least four functionally different types of afferent nerve fibre have been identified (Xu and Gebhart, 2008). Based on their responses to mechanical stimuli (perpendicular von Frey probing, urethral stroking, stretch), urothelial, muscular/urothelial, muscular, and serosal afferents were found. Lagou et al. have already described the presence of CGRP and nNOS nerve fibres in the sub-urothelium but no specific attention was paid to regional differences (Lagou et al., 2006). In this study, we attempt to identify afferent nerves in the mouse bladder and describe differences in the bladder base and lateral wall with particular attention to the sub-urothelium and urothelium.

2. Materials and methods

All experimental procedures were approved by the local animal ethical committee of Maastricht University, and were conducted in accordance with Dutch governmental guidelines. In total 27 six months old, C57BL/6 male mice, were used. The animals were housed in a temperature and light (12 h light/dark cycle) controlled room and allowed free access to food and water. Mice were killed by cervical dislocation. The urinary bladder was removed and divided into two pieces, dome/lateral wall and base and fixed in an ice-cold solution of 4% freshly prepared depolymerised paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 2 h at 4 °C. Afterwards the tissue was washed at 4–8 °C in 0.1 M phosphate buffer containing 10% sucrose (24 h), 20% sucrose (24 h) and 30% sucrose (24 h). Tissue was snap frozen with isopentane cooled in liquid nitrogen and placed in Tissue-Tek O.C.T. compound (Bayer Corporation, Pittsburgh, PA, USA) to form a single block. Cryostat sections (10 μm) were cut in a manner so that each section was perpendicular to the urothelial surface. Sections were then thawed onto chrome–alum–gelatine-coated slides and processed for immunohistochemistry.

Immunohistochemistry Sections were dried for 30 min at room temperature followed by three washes with Tris-buffered saline (TBS; pH 7.6) for 5 min and thereafter incubated overnight with primary antibodies at 4 °C. Primary antibodies were diluted in TBS containing 0.3% (v/v) Triton X-100 (TBS-T). We used several different antibodies to stain for afferent nerves and counterstained

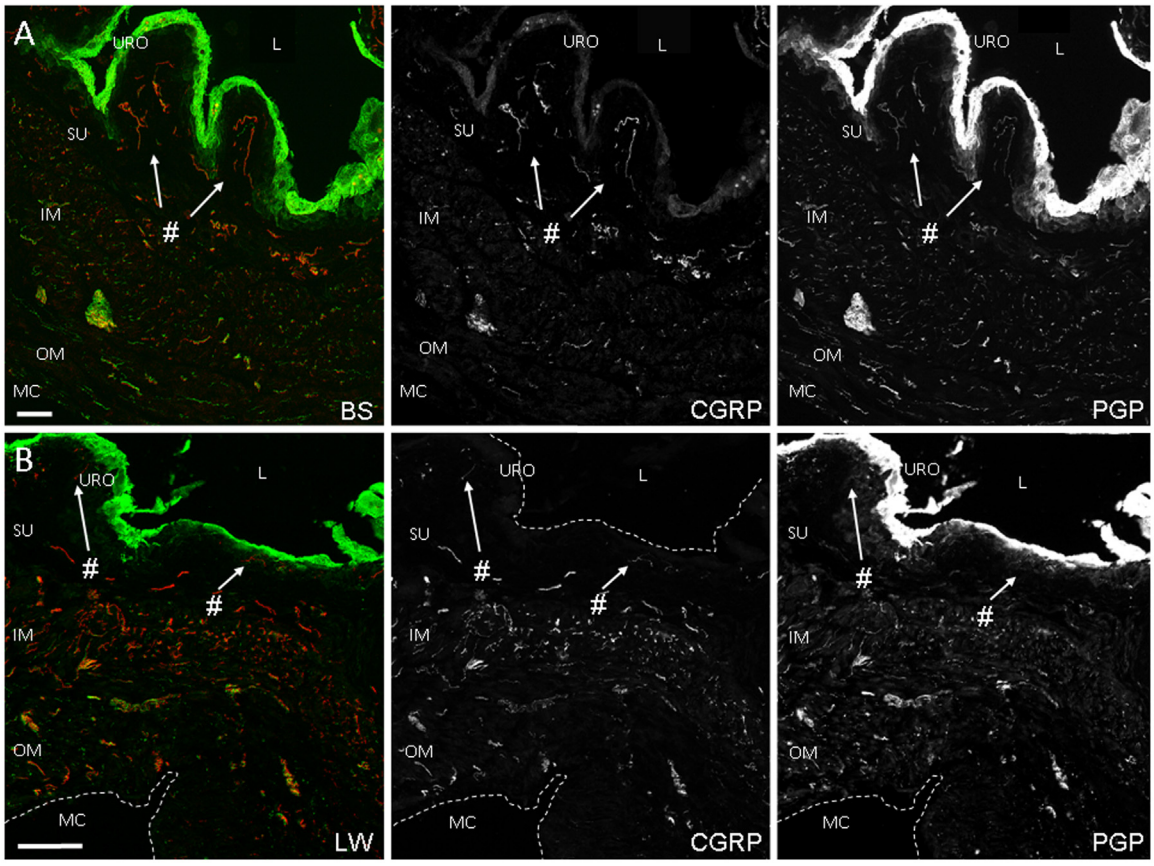

Fig. 1. Photomicrographs taken from sections of the base (A) and lateral wall (B) of a C57BL/6 mouse bladder. The sections are double stained for PGP (green) and CGRP (red). In both panels A and B the double staining is shown with the black and white images of CGRP and PGP next to it. The nerves in the sub-urothelium (# arrows) are more densely innervated in the base (A) compared to the lateral wall (B). In the lateral wall (B) a clear difference between CGRP nerve distribution in the inner and outer muscle layer can be seen. More CGRP is visible in the inner muscle layer compared to the outer. BS = bladder base; LW = lateral wall; URO = urothelium; L = lumen; SU = sub-urothelium; IM = inner muscle layer; OM = outer muscle layer; MC = muscle coat. Calibration bars: 25 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)