232

Persyn S[1], De Wachter S[1], Eastham J[2], Gillespie J[2]
1. *Department of Urology, University of Antwerp, Antwerp, Belgium,* ***2.*** *Uro-physiology Research Group, The Dental and Medical School, Newcastle University, Newcastle upon Tyne, England*

POSSIBLE LOCAL CIRCUITS BETWEEN THE BLADDER AND THE MAJOR PELVIC GANGLION IN THE MODULATION OF NON-VOIDING ACTIVITY IN THE RAT.

Hypothesis / aims of study

Bladder afferent outflow, linked to sensation, plays a critical role in bladder pathology: abnormal outflow results in altered sensation, leading to increased voiding frequency, urge and often incontinence. β3-adrenoceptor agonists have been suggested to be beneficial in treating these symptoms by mimicking sympathetic nerve activity and directly relaxing the detrusor (1). However, we hypothesize that the primary site of action may not be directly at the detrusor muscle. For many years, it has been suggested that the peripheral ganglia might play a modulatory role in bladder physiology (2). Therefore, in the present study, immuno-histochemical techniques were employed to identify structures in the major pelvic ganglion (MPG) of the rat that might be involved in such neural circuitry. To further explore the role of the pelvic plexus in the control of bladder activity, we evaluated isovolumetric non-voiding activity (NVA) in a rat model in which the MPG's were cut bilaterally.

Study design, materials and methods

Seventeen male Wistar rats (250-270 g) were killed by stunning and cervical dislocation. The MPG's were surgically removed and fixed with 4% paraformaldehyde, frozen and sections (7-8 µm) were prepared for immunohistochemistry. Adrenergic signalling elements were identified with antibodies to detect immunoreactivity (IR) against vesicular mono-amine transporter (VMAT), tyrosine hydroxylase (TH) and β3-adrenoceptors. Antibodies against vesicular acetylcholine transporter (vacht) and calcitonin gene related peptide (cgrp) were also used. For the functional studies, 4 male Sprague-Dawley rats (200-250 g) were anesthetized with urethane (1.5 g/kg, intraperitoneally). The bladder was catheterized through the dome and filled to a volume equal to 60% of the control micturition threshold volume. The control NVA (= pressure measurements) was observed for 30 minutes and compared with the NVA at the same volume after a bilateral transection of the MPG's (30 minute observation).

Results

Approximately half of the neurones in the MPG are adrenergic, as demonstrated by VMAT-IR (Figure 1A). In addition, there are also SIF cells showing VMAT-IR. The remainder are large cholinergic neurones that control the detrusor = motor-activity. These are vacht-IR and also receive vacht-IR cholinergic inputs (Figure 1B). All three types of neurones are surrounded by cgrp-IR sensory nerve fibers (Figures 1A-B). However, only the SIF cells demonstrate β3 AR-IR in the MPG (Figure 1C). No large neurones have β3 AR-IR.

Experiments in vivo were then done to explore the functional role of the MPG in possible local reflexes. Figure 2 shows an increase in the amplitude and a decrease in frequency of the NVA after cutting the MPG's.

Interpretation of results

The three neuron types: large adrenergic, cholinergic and SIF cells observed in the MPG appear to receive sensory inputs. Therefore, the ganglion has all of the components to form reflexes. These may involve afferent cgrp fibers and the large cholinergic and adrenergic neurones. Although the function of SIF cells is not known, it has been suggested that they play an integrated role within the ganglia. The fact that many of them receive input from cgrp fibers and they express β3-adrenoceptors supports a modulatory role (Figure 1D).

The role of this circuitry is unknown. However, removing the ganglia alters NVA. NVA forms the motor component of a motor-sensory system, that sends information related to bladder volume to the central nervous system. Mirabegron, a β3-selective adrenergic agonist, was reported to reduce the NVA in rats (3). Therefore, the mechanisms underlying NVA have been suggested to be an alternative therapeutic target for β3-adrenoceptor agonists. The current study has shown a major increase in the amplitude of the NVA after bilateral transection of the MPG's. This increase in the amplitude of intrinsic spontaneous bladder contractions could increase their duration and result in their decrease in frequency. These results are indicative for the release of an inhibitory local reflex residing in the MPG.

Concluding message

There is evidence that a local circuit may function to modulate NVA during the filling phase and that β3-adrenoceptors may be an integral part of this system.

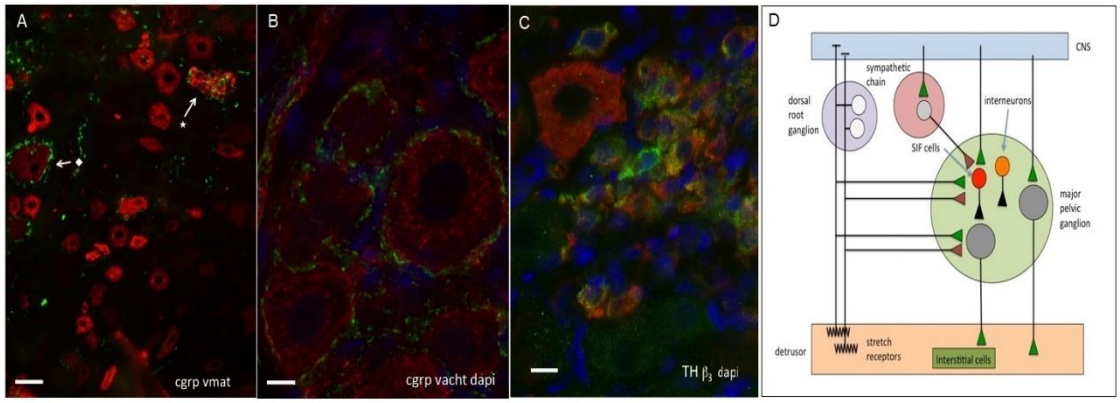

Figure 1. Illustration of the complex interactions between sensory, cholinergic and adrenergic systems in the major pelvic ganglion (MPG). A demonstrates large adrenergic (red: VMAT-IR) neurones (♦) and adrenergic SIF cells (*) in the MPG. Both cell types are sometimes surrounded by cgrp-fibers (green). B illustrates that the large cholinergic neurones (red: vacht) receive cgrp-IR inputs (green) and vacht-IR inputs (red). C demonstrates that only the SIF cells are β3-adrenergic receptor immunoreactive (β3 AR-IR: green). These cells are also TH-IR (red). The section in B and C are stained with the nuclear stain dapi (blue). D illustrates the possible reflex pathways and intra-ganglionic elements involved in the motor-sensory regulation of the bladder. Calibration bars: A 30 µm; B 15 µm; C 10 µm.

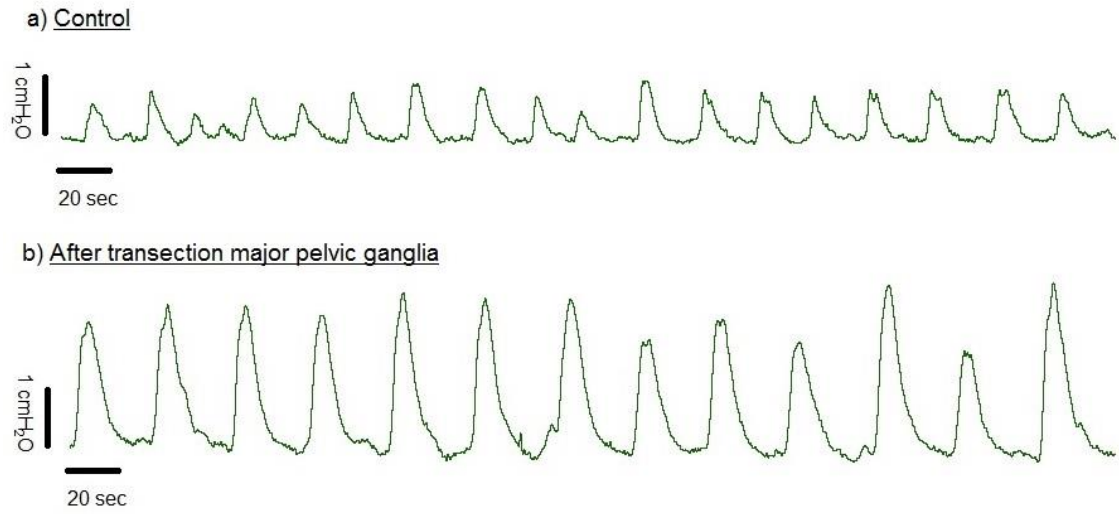

Figure 2. Pressure recording of the non-voiding activity in a rat bladder before (a) and after the transection of the major pelvic ganglia (b).

References

1. Sacco E, Bientinesi R. Mirabegron: A review of recent data and its prospects in the management of overactive bladder. Therapeutic Advances in Urology 4: 315-324.
2. de Groat WC, Booth AM. Synaptic transmission in pelvic ganglia. In: Maggi CA (ed) Nervous control of the urogenital system. Harwood academic publishers
3. Gillespie JI, Palea S, Guilloteau V, Guerard M, Lluel P, Korstanje C. Modulation of non-voiding activity by the muscarinergic antagonist tolterodine and the beta(3)-adrenoceptor agonist mirabegron in conscious rats. BJU Int 110: E132-142.

Disclosures

Funding: None **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Rat **Ethics Committee:** Animal Ethics Committee of the University of Antwerp (EC 2015-22)