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Possible role of the major pelvic ganglion in the modulation of non-voiding activity in rats

Sara Persyn [a], James Gillespie [b], Jane Eastham [b], Stefan De Wachter [a,*]

[a] Department of Urology, Antwerp University Hospital and University of Antwerp, Faculty of Medicine, Antwerp, Belgium
[b] Uro-physiology Research Group, The Dental and Medical School, Newcastle University, Newcastle upon Tyne NE2 4HH, England

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ABSTRACT

Aims: The existence of a motor-sensory system contributing to bladder sensation is now becoming widely accepted. Although it is clear that the motor component of this system appears to be generated within the bladder wall, recent observations suggest that the mechanisms involved in its modulation may lie outside the wall. The present study was undertaken to gain more insights into the peripheral modulation of non-voiding activity and the role of the major pelvic ganglion.
Methods: Male Sprague-Dawley rats anesthetized with urethane were used. The bladder was filled till 60% of the micturition threshold volume. The baseline pressure and the superimposed non-voiding activity were observed before and after consecutive bilateral transections of the hypogastric and pelvic nerves and bilateral ablation of the major pelvic ganglia.
Results: Hypogastric and pelvic nerve transection didn't significantly change the baseline pressure and superimposed non-voiding activity. Removal of the major pelvic ganglia resulted into an increased baseline pressure when compared with the control and increased amplitude of the non-voiding contractions when compared with both the decentralized condition (both hypogastric and pelvic nerves transected) and the control. The frequency of the non-voiding contractions wasn't affected.
Conclusions: Non-voiding activity during the urine storage phase seems to be modulated at the level of the major pelvic ganglion. This suggests the possibility of local circuits between the bladder and the peripheral ganglia that may be responsible for an inhibitory component influencing non-voiding activity.

1. Introduction

Transient rises in pressure have been recorded during bladder filling, described as non-voiding activity (NVA) (Biallosterski et al., 2011; Gillespie et al., 2012; Streng et al., 2006). This NVA increases as the bladder fills and has been linked to bursts of afferent outflow (Biallosterski et al., 2011; Eastham and Gillespie, 2013; Iijima et al., 2009). These observations have led to the concept that NVA is part of a motor-sensory system that informs the central nervous system (CNS) about bladder volume (Biallosterski et al., 2011; Eastham and Gillespie, 2013). Contractions, similar to NVA, have also been observed in isolated whole bladder preparations suggesting that such activity may be an intrinsic property of the bladder wall (Drake et al., 2003). In addition, this autonomous activity in vitro can be influenced by neural inputs and local environmental factors (Eastham and Gillespie, 2013). However, the fundamental importance of NVA and the mechanisms involved in the generation and modulation of NVA are still not well understood.

Data are accumulating that suggest that the motor-sensory system is augmented by cholinergic (parasympathetic) and inhibited by adrenergic (sympathetic) inputs (Eastham and Gillespie, 2013). The β_3-adrenoceptor agonist, mirabegron, has recently been introduced as a successful treatment for overactive bladder (OAB), since it effectively reduces voiding frequency and urge in these patients (Chapple et al., 2014). Animal models for OAB demonstrate an increased NVA (Lluel et al., 1998) and afferent outflow to the CNS (Iijima et al., 2009) during bladder filling. This phenomenon is therefore thought to be associated with the development of urge sensations (Gillespie, 2004). In these OAB animal models, it has been observed that mirabegron decreases both the amplitude and frequency of NVA suggested via a direct β_3-adrenoceptor mechanism, while voiding activity was not affected (Gillespie et al., 2012). In parallel, it has been shown that anti-cholinergic drugs reduce sensations during bladder filling at plasma

* Corresponding author at: Campus Drie Eiken, D.T.493, Universiteitsplein 1, 2610 Wilrijk, Belgium.
E-mail addresses: sarapersyn@hotmail.com (S. Persyn), james.innes.gillespie@gmail.com (J. Gillespie), j.e.eastham@newcastle.ac.uk (J. Eastham), stefan.dewachter@uantwerpen.be (S. De Wachter).

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concentrations that don't have a major effect on the detrusor neuromuscular junction (Finney et al., 2006, 2007). In addition, isolated whole bladder and detrusor strip experiments in rat and guinea pig have suggested that therapeutic doses of adrenergic and anti-cholinergic drugs may not directly affect the detrusor, but might act on a motor-sensory system controlling NVA and bladder sensation (Finney et al., 2007; Gillespie et al., 2015b).

The pelvic nerve, principally containing parasympathetic fibers, is considered the major pathway for afferent and efferent activity of the bladder. During bladder filling, afferent activity in the pelvic nerve correlates well with bladder pressure (de Groat et al., 2015; de Groat and Ryall, 1969), and is supposed to drive the generation of sensation. During micturition the activity in the pelvic nerve is both afferent, providing feedback for complete emptying, as efferent responsible for detrusor contraction (de Groat et al., 2015). Pelvic nerve transection abolishes the micturition reflex leading to a hyposensitive and acontractile bladder (de Groat and Ryall, 1969). Also, a marked reduction in NVA following bilateral parasympathetic nerve transection has been illustrated (Gjone, 1965).

In contrast, the hypogastric nerves, principally containing sympathetic fibers, don't affect the voiding efficiency but were thought to have a profound regulatory role in determining the timing of voiding (Barrington, 1915). Section of the hypogastric nerves in the cat doubled the voiding frequency and reduced the voided volumes. Also an inhibitory role for the hypogastric nerves on NVA has been suggested (Gjone, 1965).

Furthermore, a modulatory role for the peripheral ganglia in bladder physiology and pathology has been suggested before (De Groat and Booth, 1993). The pelvic plexus consists of autonomic ganglia and axonal pathways that convey afferent and efferent signals between the spinal cord and the peripheral target organs. Synapses in the pelvic plexus have integrative as well as relay functions and are involved in coordinating sympathetic and parasympathetic inputs to the urogenital organs. In the male rat, the pelvic plexus is not a distributed network of ganglia (De Groat and Booth, 1993). Instead, the peripheral neurons are collected into a single large ganglion, the major pelvic ganglion (MPG). Recently, immunoreactivity for the β_3-adrenoceptor and muscarinic (M_3) receptor has been observed in the MPG (Eastham et al., 2015). These observations suggest that the MPG might be a site of action for selective β_3-adrenoceptor and anti-cholinergic drugs on NVA in rats and has been put forward as a possible therapeutic target for OAB treatment in men. Also a sensory input via afferent collaterals has been observed (Eastham et al., 2015), providing a substrate for a complex local synaptic circuitry in the pelvic plexus that might influence NVA.

The present study evaluates the role of peripheral pathways on modulation of NVA to understand the mechanisms underpinning the generation and modulation of bladder sensation. The influence of the sympathetic and parasympathetic nervous system and especially the peripheral ganglia was observed by transecting consecutively the hypogastric nerves and pelvic nerves followed by excision of the MPGs in the rat.

2. Materials and methods

2.1. Animal model

The protocol was approved by the Animal Ethics Committee of the University of Antwerp (EC 2015-22). Male Sprague-Dawley rats (n = 10) weighing 200–300 g were used. The animals were kept under standard laboratory conditions with a cycle of 12 h light and 12 h darkness and free access to food pellets and tap water. They were anesthetized by intraperitoneal injection of urethane (1.5 g/kg).

The rat was positioned supine. A flare tipped PE-50 polyethylene catheter (Clay-Adams, Parsipanny, New Jersey) was inserted in the bladder through the dome via a lower abdominal incision, and externalized via the proximal aspect of the ventral abdominal incision. The ureters were ligated to ensure a constant bladder volume by preventing the flow of urine from the kidneys to the bladder and vice versa. The catheter was connected to a pressure transducer (AD Instruments) and a NE-1000 syringe pump (New Era Pump Systems, Farmingdale, New York) via 3-way stopcocks. All data were monitored using Powerlab 4/35 data-acquisition and LabChart 8 software (AD instruments).

First, a reference micturition threshold volume (MTV) was determined in all animals during three cystometric bladder fillings at a rate of 0.09 ml/min with a 10-minute pause between them. To ensure an empty bladder at the start of each filling, the bladder was emptied and any residual urine evacuated by gravity during the 10 min pause between the different fillings. Average bladder volumes at contraction were calculated from these three measurements and used as a reference.

2.2. Nerve transection protocol

The bladder was filled to 60% of the reference MTV and isovolumetric bladder activity was observed for 30 min. Then, the bladder was drained by gravity and the catheter left open for 5 min. After a control measurement, this protocol was repeated 3 times: after bilateral transection of (1) the hypogastric nerves, (2) the pelvic nerves and (3) excision of the MPGs. The hypogastric and pelvic nerves were transected between the spinal cord and the MPG, near the MPG. After hypogastric and pelvic nerve transection, the bladder is decentralized but still connected with the MPG via peripheral postganglionic sympathetic and parasympathetic fibers and peripheral afferent fibers. After each transection procedure, a 10-minute rest with open catheter was included before starting bladder filling. The protocol is illustrated in Fig. 1.

2.3. Data analysis

The original records were filtered (0.3 Hz) to remove noise. To evaluate the impact of the transections, bladder compliance was calculated for each filling. The compliance was calculated using the following formula: bladder volume filled from start filling until 60% MTV (= stop filling)/bladder pressure increase from start filling until 60% MTV. For the detailed analysis of the superimposed NVA, the filtered original record was smoothed (running smoothing average using 5555 data points, sampling rate 100 Hz). The smoothed record was subtracted from the filtered original record to isolate the NVA. From this isolated NVA, the timed integral of the area under the curve (product of amplitude and frequency, $cmH_2O.s$), amplitude (maximum and mean, cmH_2O) and frequency (events/min) was measured from the last 600 s selections of each condition. The size threshold for each event was set at 0.1 cmH_2O. Smoothed data was used to measure baseline pressure (cmH_2O).

All data are expressed as mean ± standard deviation (SD) and were analyzed using Labchart 8 and Graphpad Prism 6. Normality was tested with a Kolmogorov-Smirnov test. Differences between means were analyzed using repeated measures ANOVA with post hoc Tukey correction. If normal distribution wasn't achieved, the means were analyzed using a Friedman test with Dunn's post hoc test (mentioned in the text). A significance level of $p < 0.05$ was used.

3. Results

Table 1 provides an overview of all parameters measured over the different conditions in 10 rats. The mean values, SDs and the level of significance determined by the p-value are given. Baseline pressure and compliance were not significantly affected after hypogastric nerve transection and the subsequent pelvic nerve transection. A statistical significant increase in baseline and decrease in compliance was found after MPG removal when compared with the control.

The superimposed NVA (Fig. 2A) was analyzed using the integral of activity. This is a measurement of the contractile activity area under the