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Research Report

Bladder outlet obstruction causes up-regulation of nicotinic acetylcholine receptors in bladder-projecting pelvic ganglion neurons

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ABSTRACT

Pelvic ganglion (PG) neurons relay sympathetic and parasympathetic signals to the lower urinary tract, comprising the urinary bladder and bladder outlet, and are thus essential for both storage and voiding reflexes. Autonomic transmission is mediated by activation of the nicotinic acetylcholine receptor (nAChR) in PG neurons. Previously, bladder outlet obstruction (BOO), secondary to benign prostatic hyperplasia, was found to increase soma sizes of bladder-projecting PG neurons. To date, however, it remains unknown whether these morphological changes are accompanied by functional plasticity in PG neurons. In the present study, we investigated whether BOO alters acetylcholine receptor (nAChR) transcript expression and current density in bladder PG neurons. Partial ligation of the rat urethra for six weeks induced detrusor overactivity (DO), as observed during cystometrical measurement. In rats exhibiting DO, membrane capacitance of parasympathetic bladder PG neurons was selectively increased. Real-time PCR analysis revealed that BOO enhanced the expression of the transcripts encoding the nAChR α3 and β4 subunits in PG neurons. Notably, BOO significantly increased ACh-evoked current density in parasympathetic bladder PG neurons, whereas no changes were observed in sympathetic bladder and parasympathetic penile PG neurons. In addition, other ligand-gated ionic currents were immune to BOO in bladder PG neurons. Taken together, these data suggest that BOO causes upregulation of nAChR in parasympathetic bladder PG neurons, which in turn may potentiate ganglionic transmission and contribute to the development of DO.

Abbreviations: ACh, acetylcholine; BOO, bladder outlet obstruction; BPH, benign prostatic hyperplasia; DiI, 1,1′-didodecyl-3,3,3,3′-tetramethylindocarbo-cyanineperchlorate; DO, detrusor overactivity; nAChR, nicotinic acetylcholine receptor; PG, pelvic ganglion

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1. Introduction

The lower urinary tract (LUT) comprises the urinary bladder and bladder outlet. For normal storage and periodic voiding of urine, coordination of the two functional units of the LUT is achieved by the neural circuit containing autonomic and somatic pathways under the command of the micturition center located at a supraspinal level (for review, see de Groat, (2006), Fowler et al., (2008)). Part of the autonomic pathway, the pelvic ganglion (PG) contains the motor neurons that project to the LUT and mediate storage and voiding reflexes (de Groat et al., 1993; Lincoln and Burnstock, 1993). Unlike other autonomic ganglia, the PG contains sympathetic and parasympathetic neurons receiving preganglionic inputs from the lumbar and sacral segments of the spinal cord, respectively. Throughout the storage phase, sympathetic activation maintains closure of the bladder outlet and relaxes the detrusor muscles of the bladder. During voiding, parasympathetic activation contracts the detrusor and opens the bladder outlet. Both types of ganglionic transmission are primarily mediated by the activation of nicotinic acetylcholine receptor (nAChR) in PG neurons (Janig and McLachlan, 1987). The main combination of nAChR subtypes in rat PG neurons is found to be α3β4* (K.S. Park et al., 2006), the expression of which is susceptible to certain pathological conditions (Girard et al., 2013; Huang et al., 2011).

Benign prostatic hyperplasia (BPH), a common problem affecting middle-aged and elderly men, is characterized by the enlargement of the prostate glands surrounding the urethra which leads to bladder outlet obstruction (BOO) (Irwin et al., 2009). In early stages of BPH, the urinary bladder undergoes compensatory hypertrophy to generate higher voiding pressure for overcoming the increased outlet resistance. However, BOO gradually develops involuntary detrusor overactivity (DO) which may lead to overactive bladder (OAB) syndrome with symptoms including urinary urgency, often with frequency, incontinence, and nocturia (Abrams et al., 2002; Ouslander, 2004). DO is diagnosed by spontaneous, non-voiding contraction (NVC) of the detrusor muscles during the filling phase of a cystometrogram (Abrams et al., 2002) and simulated by partial urethral ligation in rat (Chai et al., 1999; Levin et al., 1993; Lluel et al., 1998; Malmgren et al., 1987; Uvelius et al., 1984). To date, neural mechanisms involved in BOO-induced DO remain incompletely understood. The voiding reflex is normally mediated by a supraspinal pathway (Mallory et al., 1989). In a rat model of BOO, however, the spinal pathway was found to be facilitated, suggesting that BOO affects components of the spinal reflex arc (Steers and De Groat, 1988). BOO has been found to cause hypertrophy (Steers et al., 1991a) and sensitization (Andersson, 2004) of bladder afferent neurons. The latter is suggested to be a primary neural mechanism underlying DO and OAB syndrome (Andersson, 2004; Steers and De Groat, 1988). Similar to the afferent neurons, the bladder-projecting PG neurons also undergo morphological changes in rats with BOO (Gabella et al., 1992; Gabella and Uvelius, 1993). Until now, however, studies on BOO-induced functional plasticity of PG neurons have been lacking. We show here for the first time that BOO, induced via partial urethral ligation, upregulates nAChR transcripts and increases nAChR currents in parasympathetic bladder PG neurons.

2. Results

2.1. BOO induces hypertrophy of the bladder

We first evaluated morphological changes in the bladders of the BOO group. Six weeks after surgery, there was no difference in body weight between sham-operated control ($n=20$) and BOO ($n=15$) groups (data not shown). Gross anatomic examination of the BOO group consistently revealed an enlarged bladder without any fibrotic or inflammatory changes. On average, the bladder weight was increased 2.3-fold in the BOO group from 181 ± 4 mg ($n=20$) to 420 ± 6 mg ($n=15$). Consistent with the increase in bladder weight, conventional histological examination with light microscopy revealed that BOO caused hypertrophic changes in the bladder detrusor muscles, which increased the bladder wall thickness (data not shown).

2.2. Urodynamic analysis of BOO-induced DO

Fig. 1 shows typical cystometrical recordings acquired from conscious and free-moving rats. Distension of the bladder by continuous infusion of saline increased intravesicular pressure (IVP), which eventually triggered the voiding of urine. When compared with the control group, the voiding pattern was significantly altered in the BOO group. The values of urodynamic parameters in control and BOO groups are summarized in Table 1. Notably, frequent non-voiding contraction (NVC) was observed during the urine storage phase in the BOO group. On average, the frequency of NVC was increased from 6.7 ± 3.4 Hz ($n=5$) to 51.3 ± 7.6 Hz ($n=5$) in the BOO group. Consistent with these results, there was a significant increase in the threshold pressure (TP) for voiding in the BOO group. BOO-induced DO was not coupled with voiding for a significant period of time, which prolonged the intervoiding interval and thereby increased voiding volume (VV). Voiding in the BOO group was not complete, thus leaving a large residual volume (RV) in the bladder. Bladder capacity (BC) and voiding pressure (VP) were also increased in the BOO group, reflecting enlargement and hypertrophy of the bladder.

2.3. BOO increases membrane capacitance of parasympathetic bladder PG neurons

BOO has been shown to increase the soma size of bladder neurons in the PG (Gabella et al., 1992; Steers et al., 1990). Because the PG contains both sympathetic and parasympathetic neurons and innervates different target tissues, we tested whether the BOO-induced augmentation of soma size is cell type- and target tissue-specific. Both the bladder and the corpus cavernosa-specific PG neurons were identified by retrograde DiI labeling. As shown in Fig. 2A, the membrane capacitance, indicative of soma size of sympathetic and parasympathetic bladder neurons was 62 ± 8 pF ($n=12$) and 25 ± 2 pF ($n=21$), respectively. BOO significantly increased capacitances of parasympathetic bladder neurons with little effect on sympathetic bladder neurons (Fig. 2A). In addition,