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Highlights in basic autonomic neuroscience: Contribution of the urothelium to sensory mechanisms in the urinary bladder

Prepared by: William C. de Groat

Department of Pharmacology and Chemical Biology, University of Pittsburgh Medical School, Pittsburgh 15261, USA

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ABSTRACT

Urothelial cells in the urinary bladder express neural properties including: (1) release of neurotransmitters and neurotrophic factors, (2) expression of neurotransmitter receptors and ion channels, and (3) sensitivity to mechanical and chemical stimuli. These properties have focused attention on the possible contribution of the urothelium to the storage and emptying functions of the bladder. In addition chemicals released from urothelial cells can affect the excitability of adjacent afferent nerves and this interaction can be affected by pathological conditions. This raises the possibility that abnormal urothelial–afferent interactions may contribute to bladder dysfunctions and therefore be a target for drug therapy.

Introduction

The afferent innervation of the urinary bladder which consists of small myelinated (Aδ) and unmyelinated (C-fiber) axons expresses various types of receptors and ion channels that respond to mechanical stimuli as well as chemicals present in urine or released in the bladder wall by neural and non-neural cells (de Groat and Yoshimura, 2009). Distension of the bladder activates non-nociceptive Aδ afferents and triggers the normal sensation of bladder filling; while pathological conditions activate nociceptive C-fiber afferents leading to urinary urgency, increased voiding frequency, nocturia, urinary incontinence and pain. The urothelium which lines the luminal surface of the bladder and which forms a barrier between the urine and the bladder wall also exhibits neural properties (Ferguson et al., 1997; Birder et al., 1998) and participates in bladder sensory mechanisms (Cockayne et al., 2000; Vlaskovska et al., 2001; Birder et al., 2002). Urothelial cells release neurotransmitters (ATP, nitric oxide, acetylcholine, substance P) and neurotrophic factors (nerve growth factor) that can target adjacent afferent nerves. In addition urothelial cells express neurotransmitter receptors (purinergic, muscarinic, nicotinic, adrenergic) and transient receptor potential channels (TRPV1, TRPV2, TRPV4, TRPM8) (Birder et al., 1998, 2001, 2007; Hawthorn et al., 2000; Chess-Williams, 2002; Everaerts et al., 2008; Kullmann et al., 2008a,b, 2009) that allow the urothelium to respond to chemical and mechanical stimuli. Thus the urothelium is believed to have both sensory and transducer functions that complement those of the sensory nerves (Birder and de Groat, 2007). Pathological changes in the urothelium or in urothelial–afferent signaling may play a role in various types of lower urinary tract dysfunction and therefore are the focus of considerable research. Various drugs used to treat disorders of the lower urinary tract, such as antimuscarinics, β_3 adrenergic agonists, phosphodiesterase-5 inhibitors as well as neurotoxins (capsaicin, resiniferatoxin and botulinum neurotoxin-A) may act in part by modulating urothelial–afferent signaling as well as directly affecting the afferent nerves.

Ochodnický, P., Michel, M.B., Butter, J.J., Seth, J., Panicker, J.N., Michel, M.C.. 2013. Bradykinin modulates spontaneous nerve growth factor production and stretch-induced ATP release in human urothelium. Pharmacol. Res. 70, 147–154.

Article summary

The urothelium responds to mechanical stress and chemical stimulation by producing several diffusible mediators, including ATP and, possibly, nerve growth factor (NGF); and therefore may play an important role in integrating urinary bladder sensory outputs. By activating underlying afferents urothelial mediators may contribute to normal bladder sensation and possibly to the development of bladder overactivity. The muscle-contracting and pain-inducing peptide bradykinin is produced in various inflammatory and non-inflammatory pathologies associated with bladder overactivity. This study examined the effect of bradykinin on a human urothelial cell line, UROtsa, that was shown by real-time-PCR to express mRNA for both B_1 and B_2 subtypes of bradykinin receptors. Bradykinin concentration-dependently (pEC_{50} = 8.3, E_{max} 4434 ± 277 nM) increased urothelial intracellular

E-mail address: wcd2@pitt.edu.

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calcium levels and induced phosphorylation of the mitogen-activated protein kinase, ERK1/2. Activation of both signaling pathways by bradykinin was completely abolished by the B_2 antagonist, icatibant (1 μM), but not the B_1 antagonist (R715, 1 μM). Activation of B_2 receptors by bradykinin (100 nM) markedly increased (192 ± 13% of control levels) stretch-induced ATP release from UROtsa cells in hypotonic medium, the effect being dependent on intracellular calcium elevations. UROtsa cells also expressed mRNA and protein for NGF and spontaneously released NGF to the medium (11.5 ± 1.4 pg NGF/mg protein/h). Bradykinin increased NGF mRNA expression and accelerated urothelial NGF release to 127 ± 5% in a protein kinase C- and ERK1/2-dependent manner. Bradykinin also up-regulated mRNA for the transient-receptor potential vanilloid 1 (TRPV1) sensory ion channel in UROtsa cells. In conclusion, this paper showed that bradykinin represents a versatile modulator of human urothelial phenotype, accelerating stretch-induced ATP release, spontaneous release of NGF, as well as expression of the sensory ion channel TRPV1. The authors concluded that bradykinin-induced changes in urothelial sensory function might contribute to the development of bladder dysfunction.

Commentary

This study in a human urothelial cell line (UROtsa) extended earlier experiments in rat primary urothelial cell cultures that showed that stimulation of B_2 bradykinin receptors elevated intracellular Ca^{2+} and evoked ATP release (Chopra et al., 2005). In UROtsa cells B_1 receptor expression was detected by RT-PCR but these receptors were not functional. In the rat urothelium, B_1 receptors were not detected by immunohistochemistry or functional assays in normal tissue but were up-regulated 1 and 8 days after cyclophosphamide-induced cystitis indicating that urothelial expression of bradykinin receptors is plastic and altered by pathology. In vivo cystometry performed by Chopra et al., 2005 on control anesthetized rats revealed that intravesical instillation of bradykinin activated the micturition pathway and that this effect was reduced by the P2 purinergic receptor antagonist, PPADS. On the other hand in rats with cystitis induced by pretreatment with cyclophospamide for 24 h, the bladder hyperactivity was significantly reduced by intravesical administration of either B_1 or B_2 receptor antagonists. Because bradykinin not only increased ATP release from UROtsa cells but also enhanced TRPV1 and NGF mRNA expression and increased urothelial NGF release in a protein kinase C- and ERK1/2-dependent manner it is clear that bradykinin can induce multiple changes in urothelial sensory mechanisms via distinct intracellular signaling pathways and thereby may contribute to the development of acute as well as chronic types of bladder dysfunction.

Liu, H.T. & Kuo, H.C.. 2012. Increased urine and serum nerve growth factor levels in interstitial cystitis suggest chronic inflammation is involved in the pathogenesis of disease. PLoS One.7, e44687.

Article summary

This study investigated the nerve growth factor (NGF) levels in serum and urine of patients with interstitial cystitis/bladder pain syndrome (IC/BPS), a bladder disorder that may be induced by localized chronic inflammation. Thirty patients with IC/BPS and 28 normal subjects without lower urinary tract symptoms were recruited from an outpatient clinic. IC/BPS was diagnosed by frequency, bladder pain, and the presence of glomerulations during cystoscopic bladder hydrodistention. Serum and urine were collected prior to treatment. Serum NGF and urinary NGF/creatinine levels were compared in IC/BPS patients and controls. Urinary NGF levels were significantly higher in patients with IC/BPS (26.3 ± 11.2 pg/mL) than in controls (1.40 ± 0.63 pg/mL) ($p = 0.014$). After normalization, the urinary NGF/creatinine levels were significantly greater in IC/BPS patients (0.69 ± 0.38 pg/mg) than in controls (0.20 ± 0.01, $p = 0.011$). Relative to the levels in control subjects (1.90 ± 0.38 pg/mL), the mean serum NGF levels were higher in IC/BPS patients (3.48 ± 0.55 pg/mL) ($p = 0.015$). However in IC/BPS patients the serum and urinary NGF levels were not significantly correlated. The clinical characteristics and medical co-morbidities were not significantly different between IC/BPS patients with higher and lower serum NGF levels. Increased urinary NGF levels in IC/BPS patients suggest that chronic inflammation is involved in this bladder disorder. Increased circulating serum NGF levels were noted in over half of patients with IC/BPS; however, the urinary and serum NGF levels were not inter-correlated and elevated serum NGF did not relate with clinical features.

Commentary

Various studies have revealed increased levels of NGF in the urine of patients with idiopathic overactive bladder dysfunction (Liu et al., 2011) and in the bladders of patients with neurogenic lower urinary tract dysfunction (Giannantoni et al., 2006). NGF is produced by the urothelium as well as bladder smooth muscle and can be released by chemical or mechanical stimuli (Seth et al., 2013). Exogenous NGF can induce bladder nociceptive responses and bladder overactivity in rats when applied acutely in the bladder lumen (Chuang et al., 2001) or chronically to the bladder wall or intrathecally to the lumbosacral spinal cord (Yoshimura et al., 2006). Bladder overactivity induced by chronic spinal cord injury or cyclophosphamide induced cystitis is associated with increased NGF mRNA levels in the bladder (Vizzard, 2000). Overexpression of NGF in the urothelium in transgenic mice also induces bladder hyperinnervation and bladder overactivity (Schnegelsberg et al., 2010; Girard et al., 2012). Endogenous NGF seems to contribute to lower urinary tract dysfunction after spinal cord injury in rats because intrathecal administration of NGF antibodies which neutralize NGF in the spinal cord suppresses detrusor hyperreflexia and detrusor sphincter dyssynergia in spinal cord injured animals (Seki et al., 2002). Urinary NGF levels are reduced in patients by treatments (antimuscarinic drugs or botulinum neurotoxin-A) that reduce bladder overactivity (Giannantoni et al., 2006; Seth et al., 2013). Thus urinary NGF levels have been considered as a useful biomarker for certain types of bladder dysfunction. NGF is thought to act in part on bladder afferent nerves by increasing expression of certain neurotransmitters, modulating ion channels and increasing excitability. This study by Liu and Kuo, 2012 which showed that patients with interstitial cystitis/bladder pain syndrome (IC/BPS) have an almost 20 fold increase in urinary NGF and 2 fold increase in serum levels of NGF suggests that chronic bladder inflammation is involved in this disorder. Previous studies by these authors also revealed that serum C-reactive protein levels are increased in patients with IC/BPS providing further support for a role of chronic inflammation. However serum and urinary NGF levels were not correlated and only 50% of the IC/BPS patients had increased serum levels. This discrepancy may be related to a heterogeneous pathogenesis of IC/BPS or co-morbidity with other non-urologic disorders such as irritable bowel syndrome or vulvodynia that might contribute to the increased serum NGF levels.

Frias, B., Charrua, A., Avelino, A., Michel, M.C., Cruz, F., Cruz, C.D.. 2012. Transient receptor potential vanilloid 1 mediates nerve growth factor-induced bladder hyperactivity and noxious input. B.J.U. International. 110, E422–428.

Article summary

This study examined the role of transient receptor potential vanilloid 1 (TRPV1) in the excitatory effects of chronic administration of nerve growth factor (NGF) on bladder-generated sensory input and