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

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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, (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.

Keywords

Urothelium; Nerve growth factor; Transient receptor channels; Bradykinin; Neurotoxins; ATP; Primary afferents; Overactive bladder

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,

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2007; Hawtorn et al., 2000; Chess-Williams, 2002; Everaets et al., 2008; Kullmann et al., 2008a, 2008b, 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 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 (1μM), but not the B_1 antagonist (R715, 1μM). 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±1.4pg 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

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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 hours, 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/PBS (26.3±11.2 pg/ml) than in controls (1.40±0.63 pg) ($p = 0.014$). After normalization, the urinary NGF/creatinine levels were significantly greater in IC/BPS (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/PBS patients the serum and urinary NGF levels were not significantly correlated. The clinical characteristics and medical co-morbidities were not significantly difference 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 a., 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). 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

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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/PBS) 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/PBS providing further support for a role of chronic inflammation. However serum and urinary NGF levels were not correlated and only 50% of the IC/PBS patients had increased serum levels. This discrepancy may be related to a heterogeneous pathogenesis of IC/PBS 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 reflex activity. Wild-type (WT) and TRPV1 knockout (KO) mice received daily intraperitoneal injections of NGF (1 μg/10 g) or saline for a period of 4 days, during which time thermal sensitivity was evaluated daily. On the 5th day, mice were anaesthetized and cystometries were performed. The frequency, amplitude and area under the curve (AUC) of bladder reflex contractions were determined. Immunohistochemistry was used to examine c-Fos expression in L6 spinal cord sections of WT and TRPV1 KO mice treated with saline or chronic NGF. Trk-A receptor staining intensity was determined in L6 spinal cord sections and respective dorsal root ganglia of WT and TRPV1 KO mice. Repeated administration of NGF induced thermal hypersensitivity in WT but not in TRPV1 KO mice. The frequency of bladder contractions of saline-treated WT and TRPV1 KO mice was similar, the values, respectively, being 0.45 ± 0.12/min and 0.46 ± 0.16/min. Treatment with NGF enhanced bladder reflex activity in WT mice to 1.23 ± 0.41/min ($P < 0.05$). In NGF-treated KO mice, the frequency of bladder contractions was not changed (0.60 ± 0.05/min). Irrespective of treatment, no differences were observed in the amplitude of bladder contractions of WT and TRPV1 KO mice. The contractile activity measured as AUC was significantly increased in NGF-treated WT-mice, when compared with saline-treated WT-mice. No changes were found in AUC of saline-treated and NGF-treated TRPV1 KO mice. Chronic administration of NGF resulted in a significant increase of spinal c-Fos expression in WT mice ($P < 0.05$ vs KO animals), but not in TRPV1 KO animals. However, Trk-A expression was similar in WT and TRPV1 KO mice. The authors concluded that the NGF-induced bladder overactivity and noxious afferent input to the spinal cord depends on the interaction of NGF with TRPV1 and that the absence of an NGF effect in TRPV1 KO mice is not due to loss of the NGF receptor, Trk-A. Thus, TRPV1 is essential for NGF-driven bladder dysfunction and represents a “bottleneck” target in bladder pathologies associated with NGF up-regulation.

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Commentary

This paper provides evidence that the bladder overactivity and thermal hypersensitivity induced in mice by chronic systemic NGF treatment is dependent on the normal expression of TRPV1. The increased voiding frequency after NGF treatment without a significant change in the amplitude of voiding contractions supports the view that NGF enhances the afferent rather than the efferent limb of the micturition reflex. It is known that exogenous NGF can modulate the expression of TRPV1 as well as other membrane ion channels ($P2X_3$ and Na^+) in afferent neurons that are thought to play a role in inflammation and pain. TRPV1 is important in the generation of bladder dysfunction because desensitization or block of TRPV1 by pretreatment with vanilloids (capsaicin or resiniferatoxin) or receptor antagonists reduces voiding frequency in animal models of bladder overactivity and reduces pain behavior induced by intravesical administration of noxious agents (de Groat and Yoshimura, 2009). These observations coupled with the identification of increased levels of urinary NGF in patients with overactive bladder (Liu et al., 2011; Seth et al., 2013) and increased NGF mRNA levels in overactive bladders after chemically induced cystitis in animals (Vizzard, 2000) raise the possibility that an interaction between NGF released from the urothelium and TRPV1 expressing C-fiber bladder afferents is important for the development of bladder dysfunction in animals and humans. However earlier studies in rats by other investigators (Chuang et al., 2001) showed that the acute bladder excitatory effect of intravesically administered NGF is not reduced by pretreatment with capsaicin that desensitizes TRPV1. This indicates that the early onset excitatory effects of NGF are due to targeting of other ion channels or other types of bladder afferent nerves.

It is noteworthy that TRPV1 knockout mice in the Frias et al., 2012 study did not exhibit a significant change in voiding frequency or amplitude of voiding contractions indicating that activation of TRPV1 in the urothelium or bladder afferent nerves is not essential for normal micturition. On the other hand, TRPV1 knockout mice did exhibit an increase in non-voiding contractions during bladder filling as reported in earlier studies (Birder et al., 2002) suggesting that congential absence of the TRPV1 channels unmasks hyperactivity of the bladder smooth muscle. Frias et al., 2012 speculated that the natural presence of these receptors in sensory nerves or non-neural tissues (urothelial cells or detrusor muscle) may dampen the non-voiding contractions. The urothelium is known to release unidentified substances that inhibit bladder smooth muscle (Levin et al., 1995; Fovaeus et al., 1999; Hawthorn et al., 2000). The release of these agents could be reduced in TRPV1 knock out animals leading to unmasking of non-voiding contractions.

Aizawa, N., Wyndaele, J.J., Homma, Y., Igawa, Y.. 2012. Effects of TRPV4 cation channel activation on the primary bladder afferent activities of the rat. Neurourol. Urodyn. 31, 148–155

Article summary

Transient receptor potential vanilloid 4 (TRPV4) which is expressed in the bladder urothelium and in bladder afferent neurons may play a role in bladder sensory mechanisms. In this paper the authors studied the effects of a TRPV4 agonist (GSK1016790A, GSK), a TRPV4 antagonist (RN1734) and P2X purinoceptor antagonists (TNP-ATP and PPADS) on cystometry (CMG), and the effect of GSK on single afferent fiber activity (SAA) of the rat bladder and its relationship with capsaicin (Cap)-sensitivity. Conscious female Sprague–Dawley rats were used for CMG measurements. In SAA measurements, under urethane anesthesia, SAA was identified by electrical stimulation of the pelvic nerve and by bladder distention. Cystometric parameters were measured before and after intravesical drug instillation. In SAA measurements, the response to saline instillation served as baseline.

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Then, GSK was instilled three times, and finally Cap was instilled to investigate the relationship with Cap sensitivity. Intravesical GSK-instillation transiently decreased bladder capacity and voided volume, which were counteracted by RN1734, TNP-ATP or PPADS. In SAA measurements, Aδ afferent fibers (n= 7) were not affected by either GSK or Cap. Based on the Cap-sensitivity, C-fibers were divided into two subtypes: Cap-insensitive (n= 14) and Cap-sensitive (n = 8). In the Cap-insensitive C-fibers, GSK significantly increased the SAAs during the first instillation, but the increase attenuated with time, whereas GSK did not significantly affect the Cap-sensitive C-fibers. The present results suggest that activation of TRPV4 in the bladder, probably in urothelium, facilitates the micturition reflex by activation of the mechanosensitive, Cap-insensitive C-fiber bladder afferents

Commentary

The effect of a TRPV4 antagonist or P2X purinergic receptor antagonists to block the TRPV4 agonist (GSK) induced decrease in bladder capacity and increase in firing of a subpopulation of bladder afferents indicates that activation of TRPV4 facilitates voiding by enhancing purinergic excitation of bladder afferent nerves. However, neither the TRPV4 antagonist nor the purinergic receptor antagonists affected voiding or afferent activity in untreated bladders suggesting that purinergic/TRPV4 mechanisms are inactive in the normal bladder. The selectivity of GSK for TRPV4 was shown in earlier experiments where intravesical administration induced bladder overactivity in wild type mice but was inactive in $TRPV4^{-/-}$ mice. Because other studies (Birder et al., 2007; Kullmann et al., 2009), demonstrated the expression of TRPV4 in the urothelium and showed that application of a TRPV4 agonist to urothelial cells induced inward currents, increased in intracellular Ca^{2+} and released ATP, Aizawa et al., 2012 logically concluded that GSK must activate a urothelial-afferent purinergic signaling mechanism by triggering the release of ATP from the urothelial cells. Furthermore because GSK did not alter the distension evoked firing of mechanosensitive Aδ or capsaicin-sensitive, C-fiber bladder afferents they proposed that the enhancement of voiding is due to stimulation of mechanosensitive, capsaicin-insensitive, C-fiber afferent nerves. The authors state that this conclusion is consistent with their earlier findings that intravesical administration of ATP mainly excites capsaicin-insensitive C-fiber bladder afferents. However other investigators (Nishiguchi et al., 2005) have reported that the bladder overactivity induced by intravesical administration of ATP is blunted by pretreatment with capsacin indicating that ATP activated capsaicin-sensitive bladder afferents. The latter finding is consistent with patch clamp recording in dissociated bladder sensory neurons which revealed that the large majority of bladder neurons are sensitive to both capsaicin and ATP (de Groat and Yoshimura, 2009). Although the reasons for these discrepancies are unknown, it is clear that activation of urothelial TRPV4 channels which are sensitive to mechanical and osmotic stimuli can indirectly facilitate bladder afferent firing and trigger bladder overactivity. These channels are probably not important in normal bladder function but may play a role in the generation of bladder dysfunction.

Andersson, M., Aronsson, P., Doufish, D., Lampert, A., Tobin, G. 2012. Muscarinic receptor subtypes involved in urothelium-derived relaxatory effects in the inflamed rat urinary bladder. Auton. Neurosci.: Basic Clinical 170, 5–11

Article summary

Functional studies have shown altered cholinergic mechanisms in the inflamed bladder, which partly depend on muscarinic receptor-induced release of nitric oxide (NO). The current study aimed to characterize which muscarinic receptor subtypes are involved in the regulation of the nitrergic effects in the bladder cholinergic response during cystitis. For this

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purpose, in vitro examinations of carbachol-evoked contractions of inflamed and normal bladder preparations were performed. The effects of antagonists with different selectivity for the receptor subtypes were assessed on intact and urothelium-denuded bladder preparations. In preparations from rats pre-treated with cyclophosphamide to induce cystitis, the response to carbachol was about 75% of that in normal preparations. Removal of the urothelium or administration of a nitric oxide synthase inhibitor (L-NNA) re-established the responses in the inflamed preparations. Administration of 4-diphenylacetoxy-N-methylpiperidine (4-DAMP), an M1 and M3 muscarinic receptor antagonist, inhibited the carbachol-induced contractile responses of preparations from cyclophosphamide pre-treated rats less potently than controls. Pirenzepine, an M1 antagonist, or p-fluoro-hexahydro-sila-diphenidol (pFHHSiD), an M3 antagonist, affected the carbachol-induced contractile responses to similar extents in preparations of cyclophosphamide pre-treated and control rats. However, the Schild slopes for the three antagonists were all significantly different from unity in the preparations from cyclophosphamide pre-treated rats. Because L-NNA or removal of the urothelium eliminated differences between preparations from normal and cyclophosphamide treated animals the authors concluded that muscarinic receptor stimulation in the inflamed rat urinary bladder induces urothelial release of NO, which counteracts detrusor contraction.

Commentary

In addition to urothelial factors that affect afferent nerves there is evidence that the urothelium in many species can also release unidentified factors that inhibit bladder smooth muscle activity (Levin et al., 1995; Fovaeus et al., 1999; Hawthorne et a., 2000). A long list of substances including nitric oxide have been evaluated and eliminated as possible factors contributing to the inhibition. However this in vitro study by Andersson et al., confirms and extends previous in vivo observations from their laboratory implicating nitric oxide as an inhibitor released from the urothelium by carbachol in cyclophosphamide inflamed but not in normal rat bladders. Cyclophosphamide treatment shifted to the right the carbachol concentration response curve and this effect was eliminated by pretreatment with a nitric oxide synthase (NOS) inhibitor or removal of the urothelium. The authors note that the urothelium expresses a high level of all five types of muscarinic receptors and releases nitric oxide in response to muscarinic receptor stimulation. In addition cystitis up-regulates certain types of urothelial muscarinic receptors and increases the expression of endothelial NOS in the submucosa/mucosa of the rat. Pharmacological experiments with a range of muscarinic receptor antagonists suggest that the primary mediator of the nitric oxide release is a muscarinic receptor other than M1 or M3. It is clear that the urothelial influence on carbachol induced contractile activity is changed by pathology but the site of the change is uncertain. For example the authors did not establish if the response of bladder smooth muscle to nitric oxide is altered by inflammation. It is known that adult rat bladder smooth muscle is relatively unresponsive to nitric oxide. Thus the proposed urothelial inhibitory mechanism would require an unmasking of the inhibitory response to nitric oxide after cyclophosphamide treatment. In addition nitric oxide or other inhibitory factors might be tonically released from the urothelium rather than evoked by a muscarinic agonist; and therefore the bladder excitatory effects of non-cholinergic agonists might also be enhanced by removal of the urothelium. This should be tested in future experiments. Although urothelium-induced bladder smooth muscle inhibition has been known for many years and has been demonstrated in many species under varying conditions it is fair to state that the mechanism of the inhibition and its physiological significance remain unknown. However the observations of Andersson et al., 2012 are important because they provide another example of pathology-induced plasticity in the intercellular communication between urothelial cells and other cells in the bladder.

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