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Alteration in TRPV1 and Muscarinic (M3) Receptor Expression and Function in Idiopathic Overactive Bladder Urothelial Cells (Running title: Changes in TRPV1/M3 expression and function in human OAB urothelial cells)

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Abstract

Aim—To examine function of both cholinergic (muscarinic) and TRPV1 receptors in human bladder urothelial (HBUC) from non-neurogenic overactive bladder (OAB) patients as compared to control subjects.

Methods—Primary HBUC cultures were derived from cystoscopic biopsies from OAB and control subjects. Muscarinic and TRPV1 function was assessed by acetylcholine (5 μ M) or capsaicin (0.5 μ M) evoked ATP release, measured by luciferase-assay. Overall expression of TRPV1 and muscarinic M3 receptors in bladder urothelial cells was accomplished using western immunoblotting.

Results—Our findings revealed that the response to acetylcholine in OAB HBUC cultures (which was blocked by the nonselective muscarinic antagonist, atropine methylnitrate or AMN) was not significantly different than from controls. The acetylcholine M3 receptor was slightly decreased as compared to control. In contrast, OAB HBUC cultures exhibited a capsaicin-hypersensitivity and augmented release of ATP (3.2 fold higher), which was blocked by the antagonist capsazepine. The increase in capsaicin-sensitivity correlated with increased urothelial TRPV1 expression.

Conclusion—Though characterized in a small number of subjects, augmented release of urothelial-derived transmitters such as ATP could ‘amplify’ signaling between and within urothelial cells and nearby afferent nerves.

Keywords

Bladder urothelium; capsaicin; hypersensitivity; muscarinic; sensor function

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Introduction

Overactive bladder (OAB) syndrome has been defined as a range of symptoms associated with increased urgency, frequency with or without urge incontinence (Wein 2001). Though the pathophysiology has not been fully elucidated, interest has focused on afferent activity generated by different structures in the bladder wall (smooth muscle, urothelium) as playing an important role in the mechanism of this syndrome. Non-neuronal release of transmitters (likely from the urothelium) during the storage phase is thought to play an important role in altering the myogenic activity of detrusor smooth muscle, thereby increasing afferent activity (Kanai & Andersson 2010, Yoshida *et al.* 2006, Yamaguchi 2010). The urothelium is a responsive structure capable of detecting both physical and chemical stimuli and releasing a number of signaling molecules (Birder & de Groat 2007, Apodaca *et al.* 2007). Findings from both human and animal studies have shown that altered production of urothelial-derived factors (nerve growth factor or NGF; ATP; acetylcholine) may influence afferent excitability as well as activity of detrusor smooth muscle (Munoz *et al.* 2011, Birder & de Groat 2007, Apodaca *et al.* 2007, Kanai & Andersson 2010, Schnegelsberg *et al.* 2010). Pharmacologic approaches used to treat symptoms of OAB include antimuscarinic drugs (Abrams *et al.* 2006, Hegde 2006, Yoshida *et al.* 2010, Gulur & Drake 2010) currently the standard treatment for detrusor overactivity though not without side effects and lack of efficacy in a number of patients. The mechanism of action for antimuscarinics remains unclear, but was initially associated with receptor blockade on detrusor smooth muscle, thereby reducing detrusor contractility. However, given evidence that these agents act mainly during the storage phase, it is likely that the efficacy of these antimuscarinics may involve additional sites of action. In this regard, studies have shown that activation of urothelial-muscarinic receptors can result in increased bladder afferent nerve activity (Tyagi 2011), Kullmann *et al.* 2008). A localized effect of these agents on OAB symptoms is supported by evidence that some antimuscarinics are associated with a significant amount of active metabolites excreted into the urine (Kim *et al.* 2006). Thus, urothelial receptors / release mechanisms are likely to contribute to therapeutic efficacy for a number of treatments for lower urinary tract symptoms.

The overall aims of this study were to examine differences in muscarinic as well as TRPV1 signaling in urothelial cells derived from bladder biopsies from OAB and control subjects. Studies have shown that modulation of the capsaicin-sensitive ion channel, TRPV1, has also been proposed as a possible therapeutic approach for treatment of OAB (Fowler 2000), Silva *et al.* 2007, Liu & Kuo 2007). Our findings indicate that human bladder urothelial cells (BUC) are responsive to both capsaicin and acetylcholine, with differences observed in BUC isolated from patients diagnosed with OAB. Taken together these findings will provide a further link to the role of urothelium in bladder disorders including OAB.

Materials and Methods

Subject Selection

The protocol was approved by the University of Maryland Baltimore Institutional Review Board. The study also conforms with good publishing practice in physiology (Persson & Henriksson 2011). Biopsies from 8 OAB and 7 NB subjects were obtained and were all random biopsies in the urinary bladder posterior wall. All inclusion and exclusion criteria have been detailed in a previous study (Li *et al.* 2011). The mean age of the OAB subjects was 53 years old and all were on anti-muscarinics, but with persistent symptoms. The mean age of the control (NB) subjects (no urinary incontinence and no lower urinary tract symptoms) was 40 years.

BUC culture

Human urothelial cultures were performed as previously described (Li, 2011). Briefly, bladder biopsies were obtained using the cold-cup biopsy technique from patients with OAB and NB subjects while they were under general or regional anesthesia. The biopsies were minced manually into 0.5 mm pieces. The samples were placed so that apical urothelium touched the growth surface of the uncoated plastic tissue culture plates. The cell medium used was MEM plus L-glutamine supplemented with 1 U ml⁻¹ insulin, 10% heat-inactivated FBS, 1.25 µg ml⁻¹ amphotericin B, 100 U ml⁻¹ penicillin, and 100 µg ml⁻¹ streptomycin. Samples were immobilized with sterile cover glass slips, and incubated in 95% air 5% CO₂ at 37° C. Once cell growth could be seen and confluent monolayers were present, cells were transferred using 0.25% trypsin. After dissociation, the cell suspension was centrifuged at 2,000g and resuspended in cell medium. Cell counts were obtained and the suspension was titrated with cell medium to give a cell count of 2,000 cells ml⁻¹. Two hundred fifty microliters of suspension was then pipetted onto individual glass cover clips arranged in a sterile petri dish and incubated in 95% air 5% CO₂ at 37° C and used within 2–3 days following plating for all experiments. Specificity of the cultures for epithelial differentiation was confirmed by cytokeratin-17 positive staining.

ATP release

Cultured cells were superfused with HBSS (5mM KCl, 0.3mM KH₂PO₄, 138mM NaCl, 4mM NaHCO₃, 0.3mM Na₂HPO₄, 5.6mM Glucose, 2mM CaCl₂, 1mM MgCl₂, and 10mM HEPES, pH 7.4) at room temperature (flow rate 0.5 ml min⁻¹) until a stable baseline of ATP release was measured (approximately zero); all test agents were bath applied. Perfusate (100µl) was collected at 60-second intervals after agonist (capsaicin or acetylcholine; administered at EC₅₀) application. Antagonists were applied (30 minutes) prior to and also during application of the agonist compounds. ATP levels were quantified using a luciferin-luciferase reagent and concentrations were extrapolated from a standard curve (Adenosine Triphosphate Assay Kit, Sigma-Aldrich, St. Louis MO).

Western Immunoblotting

Protein (whole cell) lysates were prepared and the protein concentrations were determined using the BCA protein assay (Pierce) with BSA as the standard. Proteins were separated on a 10% Criterion Tris-HCl gel (Bio-Rad, Hercules, CA) and then transferred to polyvinylidene fluoride membranes (Bio-Rad). After transfer, the membranes were blocked with 5% Milk TBS-T for 1 hour. After a brief rinse in TBS-T, the membranes were incubated overnight at 4°C with primary antibody (rabbit anti-rat VR1, Neuromics, Edina, MN and rabbit anti M3 AChR, BioTrend, Destin, FL) at 1:5000 in 5% Milk TBS-T. Following extensive washing, the membranes were incubated in secondary antibody (donkey anti-rabbit HRP, Santa Cruz) at 1:20,000 for 1 hr in 5% Milk TBS-T, developed with ECL Prime (GE Healthcare, Piscataway, NJ) and exposed to film. The volume of each band was determined using a Personal Densitometer SI (Molecular Probes). The membranes were stripped (membrane recycling kit from Alpha Diagnostic International, San Antonio, TX) and reprobed overnight with rabbit anti-α-tubulin (52 kDa; Cell Signaling Technology, Danvers MA) as a loading control. A single immunoreactive band, which was blocked by the competing peptide, was observed for TRPV1 (95 kDa). Two immunoreactive bands, which were blocked by the competing peptide, were observed at 120 kDa and 66 kDa (the expected band size) for M3AChR. As is typical of G-protein coupled receptors, M3AChR is known to form dimers, represented here at 120 kDa and the M3AChR antibody used in this study has been fully characterized in studies using null mice (Zarghooni *et al.* 2007).

Materials

All standard chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA) and were either analytic or laboratory grade.

Statistical Analysis

Data were graphed and analyzed using GraphPad Prism 5. ATP responses were expressed as area under the curve (AUC) and all comparisons were made using an unpaired student's t-test. Changes in protein (TRPV1; M3) expression were analyzed using a one-way analysis of variance. Post-hoc significance between pairs of groups was computed using the Newman-Keuls Multiple Comparison Test. Data was considered significantly different when $p < 0.05$.

Results

ATP release from OAB and normal BUC

We first assessed capsaicin (0.5 μ M) evoked ATP release using cultured urothelial cells taken from biopsies from patients with OAB versus asymptomatic controls. OAB HBUC cultures exhibited a capsaicin-hypersensitivity resulting in augmented release of ATP (3.2 fold increase) as compared to that of control (Figure 1A). The responses in both OAB and normal cells were reproducible and typical response reached a peak in (6–10) min after agonist application. The response in both cell types was blocked with pre-incubation with the TRPV1 antagonist, capsazepine (5 μ M, Figure 1A).

Application of acetylcholine (5 μ M) also elicited ATP release from both OAB and control urothelial cells, which could be prevented upon pre-incubation with the non-selective muscarinic receptor antagonist, AMN (25 μ M, figure 1B). This release was reproducible with repeated application and the level of release from OAB urothelial cells was not significantly different from that of control.

OAB versus normal HBUC (TRPV1; M3) receptor expression

There are a number of mechanisms underlying changes in TRPV1 sensitivity including an increased expression of TRPV1 channels. To explore this possibility, we used immunoblotting of TRPV1 to examine whether cultured OAB HBUCs show increased TRPV1 as compared to that of control. Figure 2A reveals a significant increase in urothelial TRPV1 (whole cell lysates) in unstimulated OAB HBUC as compared to asymptomatic controls. All cells were utilized within the same time period (2 days following initial plating). In contrast, though the changes were not significant, there was a trend toward decreased M3 receptor expression in OAB HBUCs as compared to asymptomatic controls (Figure 2B). The expression levels were determined by densitometry and normalized using α -tubulin loading controls.

Discussion

Given the side effect profile of existing anti-muscarinic treatments, it is of considerable interest to develop alternative approaches for the treatment of OAB. The capsaicin-sensitive ion channel, TRPV1, has been linked with normal bladder function in addition to the generation of urgency sensation (Gunthorpe & Szallasi 2008). An increase in TRPV1 expression has been reported in bladder urothelium and suburothelial nerve fibers from patients with sensory urgency and idiopathic detrusor overactivity (Liu & Kuo 2007). The finding of increased TRPV1 expression presented here is consistent with both increased TRPV1 expression (measured with immunofluorescence) and function (measured electrophysiologically) detected in OAB bladder urothelial cells (Li *et al.* 2007). In this

regard, intravesical instillation of vanilloids (capsaicin or resiniferatoxin) has been shown to improve urodynamic parameters in patients with detrusor overactivity (even restoring continence) and to reduce pain in patients with hypersensitivity disorders (Cruz 2004, Fowler 2000, Kissin & Szallasi 2011). This treatment presumably acts via desensitization of C-fiber afferents, though studies in both animals and humans suggest this treatment may target other cell types in the bladder wall including the urothelium (Birder & de Groat 2007, Brady *et al.* 2004). More recently, selective antagonists to TRPV1 are being investigated as a treatment for overactive bladder (Andersson *et al.* 2010, Round *et al.* 2011), which will also be a benefit to patients refractory to anti-muscarinic drugs. However, at this point there are no regulatory agency approved bladder uses of TRPV1 agonists in clinical use today.

The present study has shown that OAB HBUCs exhibit an increased capsaicin sensitivity resulting in augmented ATP release as compared to normal controls. Augmented epithelial-derived release of ATP from 'tubes and sacs' (such as the urinary bladder) has long been regarded to play a prominent role in primary afferent sensitization, likely by affecting P2X3 receptors (Burnstock 2006). Release of ATP from a number of sources including the urothelium can also impact the contractility of smooth muscle. There is substantial evidence supporting a role for alterations in purinergic signaling or transmission (including from the urothelium) in urinary bladder disorders including idiopathic detrusor instability, neurogenic bladder, painful bladder syndrome and outflow obstruction (Rapp *et al.* 2005, Ruggieri 2006, Tan *et al.* 2009). More recently, preclinical findings support the use of small molecule compounds targeting the P2X3 receptors which have the potential to provide relief for patients suffering storage, voiding and sensory symptoms (Gever *et al.* 2010).

The expression and sensitivity of the capsaicin-gated ion channel TRPV1 may be enhanced by proalgesic factors such as NGF. Elevated urinary levels of NGF have been reported in patients with idiopathic and neurogenic detrusor overactivity (Kuo *et al.* 2011). Evidence supports a functional role for NGF in sensitization of mechanosensitive bladder afferents, which are likely to play a role in detrusor overactivity. (Morrison 1991, Dmitrieva & McMahon 1996) Further, chronic administration of NGF in the bladder of rodents results in bladder hyperactivity and blockade of NGF using auto-antibodies prevents plasticity induced changes in bladder hyperactivity in experimental animals (Yoshimura *et al.* 2007). Conditional overexpression of NGF within bladder urothelium in a transgenic animal was shown to have bladder hyperactivity (Schenegelsberg *et al.*, 2010). Evidence also supports increased NGF expression in animal models as well as patients with irritable bowel syndrome or esophagitis (Zhu *et al.* 2011, Shieh *et al.* 2010). Therefore, alterations in NGF are thought to play an important role in modulating the expression and function of TRPV1, which is likely to impact on afferent excitability.

However, human translational studies focused on urothelial expression of NGF (not urinary NGF levels) have not been necessarily congruous with the findings from animal models. When NGF was measured from bladder urothelium in women with varying severities of overactive bladder syndrome, there was no association of NGF with clinical measures of severity (Birder *et al.*, 2007). However, in this particular study, there was no control group of human subjects with no bladder symptoms; therefore, the level of NGF in symptomatic patients were not compared to those without symptoms. We were not able to evaluate the level of NGF in the present study (due to limitations in number and size of samples). In addition, the mechanisms underlying altered TRPV1 sensitivity and expression is likely to be complex, involving additional factors that may be altered in HBUC. Thus, further experiments are needed to fully examine the signaling mechanisms underlying both acute and chronic effects of NGF (or other factors) on urothelial signaling.

In the present study, HBUCs did not exhibit an increased responsiveness to the nonselective cholinergic agonist, acetylcholine. There have been reports of a significant decrease in muscarinic receptor expression in urothelium (and suburothelium) obtained from neurogenic and idiopathic overactive human bladders (Datta *et al.* 2010, Mansfield *et al.* 2007). Though mechanisms underlying this decrease are not known, botulinum toxin treatment in these patients was found to restore muscarinic receptor expression (including M3 within bladder urothelium) to control level. The results in the Datta study used image analysis in order to quantify muscarinic-receptor staining in general tissue regions though did not identify cellular location or distribution. Changes in receptor expression can influence function, which may vary with type of cell as well as in health and disease. Though the M3ACh receptor is thought to play a key role in urinary bladder contraction, there is little information available as to the mechanisms underlying muscarinic receptor subtype mediated signaling involving the urothelium. The M3AChR is sensitive to changes in signaling including agonist-induced intracellular trafficking in a number of cell types including that of epithelial cells (Koenig *et al.* 1996). Changes in muscarinic-receptor expression due to pathology-induced plasticity or with antimuscarinic therapy could impact transmitter release from a number of sources influencing bladder function. For example, we have shown the urothelial release of acetylcholine may participate in a negative feedback mechanism by inhibiting further release of transmitter (Hanna-Mitchell *et al.* 2007). Further, in the presence of a non-selective antagonist of muscarinic receptors (atropine), basal release of acetylcholine could be facilitated or occur in the absence of external stimuli. These and other studies have revealed that release of multiple transmitters is subject to auto regulation (inhibition / facilitation) and can be modulated by receptor blockade.

Taken together, our findings of altered muscarinic receptor expression (which may reflect a shift in muscarinic-receptor traffic to intracellular compartments) may occur as a result of pathology or result of antimuscarinic therapy. These types of changes could impact communication with a number of cells within the bladder wall resulting in alterations in contractility as well as sensation. Thus, a better understanding of muscarinic receptor subtypes and associated intracellular signaling mechanisms in health and disease would be beneficial for the future development of therapies for controlling overactive bladder.

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References

- Abrams P, Andersson KE, Buccafusco JJ, Chapple C, de Groat WC, Fryer AD, Kay G, Laties A, Nathanson NM, Pasricha PJ, Wein AJ. Muscarinic receptors: their distribution and function in body systems, and their implications for treating overactive bladder. *Br J Pharmacol.* 2006; 148:565–578. [PubMed: 16751797]
- Andersson KE, Gratzke C, Hedlund P. The role of the transient receptor potential (TRP) superfamily of cation-selective channels in the management of the overactive bladder. *BJU Int.* 2010; 106:1114–1127. [PubMed: 21156013]
- Apodaca G, Balestreire E, Birder LA. The uroepithelial-associated sensory web. *Kidney International.* 2007; 72:1057–1064. [PubMed: 17667988]
- Birder LA, de Groat WC. Mechanisms of disease: involvement of the urothelium in bladder dysfunction. *Nat Clin Prac Urol.* 2007; 4:46–54.
- Brady CM, Apostolidis AN, Harper M, Yiangou Y, Beckett A, Jacques TS, Freeman A, Scaravilli F, Fowler CJ, Anand P. Parallel changes in bladder suburothelial vanilloid receptor TRPV1 and pan-neuronal marker PGP9.5 immunoreactivity in patients with neurogenic detrusor overactivity after intravesical resiniferatoxin treatment. *BJU Int.* 2004; 93:770–776. [PubMed: 15049988]

- Burnstock G. Pathophysiology and therapeutic potential of purinergic signaling. *Pharmacol Rev.* 2006; 58:58–86. [PubMed: 16507883]
- Cruz F. Mechanisms involved in new therapies for overactive bladder. *Urology.* 2004; 63:65–73. [PubMed: 15013655]
- Datta SN, Roosen A, Pullen A, Popat R, Rosenbaum TP, Elneil S, Dasgupta P, Fowler CJ, Apostolidis A. Immunohistochemical expression of muscarinic receptors in the urothelium and suburothelium of neurogenic and idiopathic overactive human bladders, and changes with botulinum neurotoxin administration. *J Urol.* 2010; 184:2578–2585. [PubMed: 21030043]
- Dmitrieva N, McMahon SB. Sensitisation of visceral afferents by nerve growth factor in the adult rat. *Pain.* 1996; 66:87–97. [PubMed: 8857635]
- Fowler CJ. Intravesical treatment of overactive bladder. *Urology.* 2000; 55:60–64. [PubMed: 10767456]
- Gever JR, Soto R, Henningsen RA, Martin RS, Hackos DH, Panicker S, Rubas W, Oglesby IB, Dillon MP, Milla ME, Burnstock G, Ford AP. AF-353, a novel, potent and orally bioavailable P2X₃/P2X_{2/3} receptor antagonist. *Br J Pharmacol.* 2010; 160:1387–1398. [PubMed: 20590629]
- Gulur DM, Drake MJ. Management of overactive bladder. *Nat Rev Urol.* 2010; 7:572–582. [PubMed: 20930868]
- Gunthorpe MJ, Szallasi A. Peripheral TRPV1 receptors as targets for drug development: new molecules and mechanisms. *Curr Pharm Design.* 2008; 14:32–41.
- Hanna-Mitchell AT, Beckel JM, Barbadora S, Kanai AJ, de Groat WC, Birder LA. Non-neuronal acetylcholine and urinary bladder urothelium. *Life Sciences.* 2007; 80:2298–2302. [PubMed: 17363007]
- Hegde SS. Muscarinic receptors in the bladder: from basic research to therapeutics. *Br J Pharmacol.* 2006; 147:S80–S87. [PubMed: 16465186]
- Kanai A, Andersson K-E. Bladder afferent signaling: recent findings. *J Urol.* 2010; 183:1288–1295. [PubMed: 20171668]
- Kim Y, Yoshimura N, Masuda H, De Miguel F, Chancellor MB. Intravesical instillation of human urine after oral administration of tiropridium, tolterodine and oxybutynin in a rat model of detrusor overactivity. *BJU Int.* 2006; 97:400–403. [PubMed: 16430654]
- Kissin I, Szallasi A. Therapeutic targeting of TRPV1 by resiniferatoxin, from preclinical studies to clinical trials. *Curr Topics Med Chem.* 2011; 11:2159–2170.
- Koenig JA, Edwardson JM. Intracellular trafficking of the muscarinic acetylcholine receptor: importance of subtype and cell type. *J Pharmacol Exp Ther.* 1996; 49:351–359.
- Kullmann FA, Artim DE, Birder LA, de Groat WC. Activation of muscarinic receptors in rat bladder sensory pathways alters reflex bladder activity. *J Neurosci.* 2008; 28:1977–1987. [PubMed: 18287514]
- Kuo HC, Liu HT, Guan Z, Tyagi P, Chancellor MB. Promise of urinary nerve growth factor for assessment of overactive bladder syndrome. *LUTS.* 2011; 3:2–9.
- Li M, Sun Y, Simard JM, Chai TC. Increased transient receptor potential vanilloid type 1 (TRPV1) signaling in idiopathic overactive bladder urothelial cells. *NeuroUrol Urodyn.* 2011; 30:606–611. [PubMed: 21351130]
- Liu HT, Kuo HC. Increased expression of transient receptor potential vanilloid subfamily 1 in the bladder predicts the response to intravesical instillations of resiniferatoxin in patients with refractory idiopathic detrusor overactivity. *BJU Int.* 2007; 100:1086–1090. [PubMed: 17711510]
- Mansfield KJ, Liu L, Moore KH, Vaux KJ, Millard RJ, Burcher E. Molecular characterization of M2 and M3 muscarinic receptor expression in bladder from women with refractory idiopathic detrusor overactivity. *BJU Int.* 2007; 99:1433–8. [PubMed: 17428242]
- Morrison J. The activation of bladder wall afferents. *Exp Physiol.* 1991; 84:131–136. [PubMed: 10081713]
- Munoz A, Somogyi GT, Boone TB, Smith CP. Lumbosacral sensory neuronal activity is enhanced by activation of urothelial purinergic receptors. *Brain Res Bull.* 2011; 86:380–384. [PubMed: 21924327]

- Ohtake A, Sato S, Sasamata M, Miyata K. The forefront for novel therapeutic agents based on the pathophysiology of lower urinary tract dysfunction. *J Pharmacol Sci.* 2010; 112:135–141. [PubMed: 20134114]
- Persson PB, Henriksson J. *J Acta Physiologica.* 2011; 203:403–407.
- Rapp DE, Lyon MB, Bales GT, Cook SP. A role for the P2X receptor in urinary tract physiology and in the pathophysiology of urinary dysfunction. *Eur Urol.* 2005; 48:303–308. [PubMed: 15963632]
- Round P, Priestley A, Robinson J. An investigation of the safety and pharmacokinetics of the novel TRPV1 antagonist XEN-D0501 in healthy subjects. *Br J Pharmacol.* 2011; 72:921–931.
- Ruggieri MR. Mechanisms of disease: role of purinergic signaling in the pathophysiology of bladder dysfunction. *Nat Clin Prac Urol.* 2006; 3:206–216.
- Schnegelsberg B, Sun TT, Cain G, Bhattacharya A, Nunn PA, Ford AP, Vizzard MA, Cockayne DA. Overexpression of NGF in mouse urothelium leads to neuronal hyperinnervation, pelvic sensitivity, and changes in bladder function. *Am J Physiol.* 2010; 298:R534–R547.
- Shieh KK, Yi CH, Tseng HL, Ho HC, Hsieh HT, Chen CL. Evidence for neurotrophic factors associating with TRPV1 gene expression in the inflamed human intestine. *Neurogastro Motil.* 2010; 22:971–978.
- Silva C, Silva J, Castro H, Reis F, Dinis P, Avelino A, Cruz F. Bladder sensory desensitization decreases urinary urgency. *BMC Urol.* 2007; 11:7–9.
- Tan W, Contreras-Sanz A, Kennedy-Lydon T, Alexander SL, Janska S, Bishara S, Lunawat R, Khasriya R, Taylor KM, Crawford C, Malone-Lee J, Wildman SS. Altered urothelial ATP signaling in human overactive (OAB) patients. *Proc Physiol Soc.* 2009; 15:PC183.
- Tyagi P. Pathophysiology of the urothelium and detrusor. *Can Urol Assoc J.* 2011; 5:S128–S130. [PubMed: 21989522]
- Wein AJ. Pharmacological agents for the treatment of urinary incontinence due to overactive bladder. *Exp Opin Inves Drugs.* 2001; 10:65–83.
- Yamaguchi O. Antimuscarinics and overactive bladder: other mechanism of action. *Neurourol Urodyn.* 2010; 29:112–115. [PubMed: 19693952]
- Yoshida M, Inadome A, Maeda Y. Non-neuronal cholinergic system in human bladder urothelium. *Urology.* 2006; 67:425–430. [PubMed: 16461116]
- Yoshida M, Masunaga K, Nagata T, Yono M, Homma Y. The forefront for novel therapeutic agents based on the pathophysiology of lower urinary tract dysfunction: pathophysiology and pharacotherapy of overactive bladder. *J Pharmacol Sci.* 2010; 112:128–134. [PubMed: 20134115]
- Yoshimura N, Kaiho Y, Miyazato M, Yunoki T, Tai C, Chancellor MB, Tyagi P. Therapeutic receptor targets for lower urinary tract dysfunction. *Naun Schmiedeberg's Arch Pharmacol.* 2007; 377:437–448.
- Zarghooni S, Wunsch J, Bodenbenner M, Bruggmann D, Grando SA, Schwantes U, Wess J, Kummer W, Lips KS. Expression of muscarinic and nicotinic acetylcholine receptors in the mouse urothelium. *Life Sciences.* 2007; 80:2308–2313. [PubMed: 17337281]
- Zhu Y, Colak T, Shenoy M, Liu L, Pai R, Li C, Mehta K, Pasricha PJ. Nerve growth factor modulates TRPV1 expression and function and mediates pain in chronic pancreatitis. *Gastroenterol.* 2011; 141:370–377.

Figure 1.

Capsaicin as well as acetylcholine evokes ATP release from HBUC cultures. In A, graph depicts mean release of ATP from cultured normal (N) HBUC (open bar) or OAB HBUC (solid bar) following stimulation with the TRPV1 agonist, capsaicin (CAP, 0.5 μ M). In B, graph depicts mean release of ATP from cultured normal (N) HBUC (open bar) or OAB HBUC (solid bar) with the non-selective cholinergic agonist, acetylcholine (ACh, 5 μ M). The responses in both OAB and normal cells were blocked by the respective antagonists (in A, capsazepine (CPZ, 5 μ M) or in B, atropine methyl nitrate (AMN, 25 μ M). Values are means $\pm$ SE from recordings in a minimum of 5 independent experiments. *P 0.05, significantly different from control.

Figure 2.

Alterations in TRPV1 and M3 receptor expression (whole cell lysates) in OAB HBUC. In A, the graph depicts increased TRPV1 expression using western immunoblotting in normal (N) HBUC (open bars) versus OAB HBUCs (solid bar) expressed as a percentage of α -tubulin loading control. In B, we also show western blot analysis of the cholinergic muscarinic receptor subtype 3 (M3) in normal (N) HBUC, open bars) versus OAB HBUCs (solid bar) expressed as a percentage of α -tubulin loading control. TRPV1 or M3AChR expression in human urothelial cells was eliminated with the antibody preabsorbed with antigenic peptide (not shown). Values are means $\pm$ SE; * $P < 0.05$, significantly different from control.