Published in final edited form as:

Neurol Urodyn. 2016 February ; 35(2): 299–303. doi:10.1002/nau.22809.

Do We Need to Know More About the Effects of Hormones on Lower Urinary Tract Dysfunction? ICI-RS 2014

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Abstract

This review article reflects the presentations and subsequent discussions during a think tank at the 5th International Consultation on Incontinence Research Society's annual meeting, held in Bristol, UK (September 22–24, 2014). It reviews the current state of knowledge on the role of hormones in lower urinary tract dysfunction (LUTD) and overactive bladder (OAB) and in particular: highlights some specific basic research findings from discussion participants; reviews future research topics; and discusses potential new therapeutic opportunities for LUTD and OAB. The role of the large conductance voltage- and Ca^{2+} -activated K^{+} (BK) channels, as novel therapeutic targets for OAB was discussed, in particular as recent studies on human detrusor smooth muscle suggest that estradiol exerts a direct non-genomic activation of the BK channels. Recent developments on the roles of sex hormones on diuresis, as well as the roles of melatonin and vitamin D on LUTD were also discussed. It was concluded that further basic science and translational studies are needed to better understand hormonal regulatory mechanisms of the lower urinary tract and the implications for novel treatment options for LUTD and OAB.

Keywords

BK channel; detrusor overactivity; estrogen; lower urinary tract dysfunction; melatonin; overactive bladder; vasopressin; vitamin D3

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Dr. Mickey Karram led the peer-review process as the Associate Editor responsible for the paper.

Conflicts of interest: Nothing to disclose.

INTRODUCTION

Overactive bladder (OAB) is the most common form of lower urinary tract dysfunction (LUTD). OAB, often associated with detrusor overactivity (DO), affects ~17% of the population and its prevalence increases with age.¹ The primary OAB pharmacotherapy is based primarily on antimuscarinics, which are associated with dose-related side effects.² The selective β 3-adrenoceptor agonist mirabegron has been recently approved to treat OAB; however, its long-term effectiveness remains uncertain. Other newer therapies such as intravesical botulinum toxin are not only invasive and expensive, but also raise some safety concerns.³ A critical step for the development of more effective therapies for LUTD/OAB is a better understanding of the age and sex-specific molecular and cellular hormonal mechanisms that regulate detrusor smooth muscle (DSM) function under normal conditions and in LUTD. Further, sex and age-specific differences are important determinants in LUTD and should be considered in developing personalized LUTD/OAB treatments. Hormonal replacement therapy, both for men and women, has emerged as a treatment for age-related diseases, including LUTD.⁴ Indeed, the urinary bladder shares a common embryonic origin with the genital system, and its function is, in part, regulated by sex hormones.⁴ Substantial sex-related differences in the severity of LUTD, such as OAB, have been well documented,^{1,4} and therefore sex differences should be important considerations in the treatment of LUTD. Specifically, women with OAB are more likely to have associated urge urinary incontinence (UI), whereas benign prostatic obstruction, the prevalence of which increase with age, is a major contributing factor for LUTD in men.¹ Therefore, to effectively treat sex-specific LUTD, the role of sex hormones in bladder physiology and pathophysiology should be clearly understood.

Do We Need to Know More About Estrogen Replacement Therapy and LUTD?

Estrogen has an important role in the regulation of lower urinary tract (LUT) function and estrogen and progesterone receptors have been demonstrated throughout the pelvic floor and the LUT.^{5,6}

For many years, systemic estrogen therapy was felt to be beneficial for LUTD but this evidence has been challenged by large epidemiological studies investigating the use of systemic hormone replacement therapy in the prevention of cardiovascular disease and osteoporosis.⁷⁻⁹ In these trials systemic estrogen replacement therapy was found to increase the risk of developing UI, and in those women who complained of UI at baseline, the symptoms deteriorated.

These conclusions have recently been reappraised with regard to the effect of estrogen on the LUT. This most recent analysis, in addition to findings from further meta-analyses and systematic reviews, support the use of local estrogen therapy, but not systemic, in the treatment of urge UI and OAB.¹⁰

The effect of estrogen on LUTD may partly be explained by the effect that exogenous estrogen therapy has been shown to have on collagen remodeling. Estrogen therapy has been shown to lead to a reduction in total collagen concentration,¹¹ decrease in collagen cross linking,¹² and an increase in the levels of collagen turnover markers.¹³ Additionally, women

with stress UI have been shown to have a reduction in total collagen and an increase in the level of collagen degradation products after taking oral estrogen for six months.¹⁴ This change in the structure and composition of connective tissue may affect the peri-urethral tissue and increase urethral mobility, thereby reducing effective urethral closure mechanisms. In animal models there is also evidence to suggest that exogenous estrogens increase the collagen to DSM ratio which may lead to an increase in OAB type symptoms,¹⁵ by reducing bladder compliance.

The most recent meta-analysis of the effect of estrogen replacement therapy on LUTD has been performed by the Cochrane group and includes 19,676 women.¹⁶ Systemic administration of unopposed oral estrogens and combination therapy resulted in worse UI than placebo; (RR 1.32; 95%CI: 1.17–1.48) and (RR 1.11; 95%CI: 1.04–1.18), respectively. However, local estrogen therapy may improve UI (RR 0.74; 95%CI: 0.64–0.86) and has been shown to be safe and effective.¹⁷

Vaginal estrogens cause changes in autonomic and sensory vaginal innervation density,^{18,19} and evidence from the ovariectomized rat model suggests that estrogen may reverse urothelial damage, inflammatory cell infiltration, and muscular atrophy.²⁰ Further animal studies have also demonstrated the effects of estradiol on morphology, vascular endothelial growth factor expression, and reversal of bladder atrophy.²⁰ Consequently, vaginal estrogen replacement therapy may lead to an improvement in physiological voiding function, while at the same time reducing the risk of developing symptoms of OAB. There is also evidence supporting the synergistic use of vaginal estrogens with antimuscarinic therapy in the management of postmenopausal women with OAB,²⁰ although not in patients with an urodynamic diagnosis of DO.²¹

Whilst there are no current data to support the use of estrogens alone in postmenopausal women with stress UI, the evidence regarding vaginal estrogens in postmenopausal women with OAB is more robust and there may be a role for synergistic therapy with antimuscarinics. Therefore, further basic science studies are required to better understand the underlying molecular and cellular mechanisms.

Research proposals.

- Is there a role of adjuvant vaginal estrogen therapy in postmenopausal women with OAB in addition to antimuscarinic or β 3-adrenoceptor agonist therapy? Is this synergistic or additive?
- Is there a role for adjuvant vaginal estrogen therapy in women with stress UI in addition to pelvic floor muscle training and duloxetine?
- What is the effect of vaginal estrogen therapy in the conservative and surgical management of urogenital prolapse?

Are Novel Non-Genomic Targets for Estrogens, Such as BK channels, Potential Therapeutic Targets for the Treatment of OAB?

The major entry pathway for Ca^{2+} that initiates DSM contractions is via L-type voltage-dependent Ca^{2+} channels (VDCC).^{22,23} As a negative feedback regulatory mechanism, Ca^{2+}

entry via L-type VDCC also activates large conductance voltage- and Ca^{2+} -activated K^{+} (BK) channels, and the BK channel hyperpolarizing K^{+} current induces L-type VDCC closure, thereby reducing DSM contractility.^{22–25} This negative feedback makes the BK channels the most important physiologically-relevant K^{+} channels that regulate human DSM function.^{22,23} Recent data from the Petkov laboratory reveal that estradiol, at nanomolar concentrations, that are close to the physiological estrogen plasma levels in women, can directly activate BK channels in human DSM cells, thus reducing DSM excitability and contractility (Fig. 1).²⁶ However, the precise molecular and cellular mechanisms by which estrogens and androgens regulate BK channel activity in human DSM have not yet been investigated. Defects in DSM BK channel proteins, or in the molecules involved in their regulatory pathways, may underlie certain forms of LUTD, offering the opportunity for novel therapeutic intervention²³ which highlights the significance of new studies in this area. Indeed, recent breakthrough findings indicate that neurogenic DO is associated with decreased BK channel expression and function in DSM.²⁷ The Petkov research group proposes the novel hypothesis that BK channels are key determinants of age- and sex-related differences in bladder physiology, and therefore changes in BK channel expression, function, or regulation may underlie age/sex-specific DSM dysfunction and resultant LUTD.

Preliminary experiments by the Petkov laboratory show that estradiol reduces spontaneous phasic contractions of human DSM isolated strips in a concentration-dependent manner which may involve a direct interaction of estrogens with BK channels, independent of estrogen receptors, as demonstrated in excised membrane patches.²⁶ This direct action could potentially be mediated by the BK channel regulatory $\beta 1$ -subunit,²⁸ which regulates the Ca^{2+} sensitivity of BK channels.²⁹ This mechanism can be addressed by using the BK channel $\beta 1$ -subunit knockout mouse (KO) model (Fig. 1).²⁹ In addition, sex hormones, including estradiol, can potentially influence BK channel activity through modulating the expression of BK channel subunits, splice variants, or their regulatory proteins.

Research proposals.

- An integrated, multi-level approach profiling BK channel molecules in native, freshly-isolated, human DSM cells using molecular and cellular physiology, electrophysiology, Ca^{2+} imaging, as well as functional studies of the impact of pharmacological modulators on DSM contractility, should be applied to elucidate human DSM regulation by BK channels in health and disease.
- Comparative experiments should be performed with estrogen and testosterone to examine for a potential sex-specific influence on BK channel activity.
- Consideration of BK channel sex variables is critical to the accurate interpretation of basic science findings and to the development of personalized approaches for treating LUTD.

A significant outcome of the proposed studies would be to prove that BK channels play a critical role in human bladder sex-related LUTD, as recently demonstrated for neurogenic DO.²⁷ If this is the case, the studies can be extended to search for alternative therapeutic interventions such as gene therapy that might be helpful to treat OAB with associated DO. Indeed, BK channel gene transfer is in a phase 1 clinical trial for treatment of erectile

dysfunction, and a similar trial is planned for OAB.³⁰ Modulation of BK channel activity or their regulatory mechanisms by sex hormones may provide the basis for developing novel therapeutic interventions to help a large population of patients suffering from OAB/DO.

Do We Need to Know More About the Effects of Sex Hormones on Diuresis?

Diuresis is influenced by three different renal mechanisms as follows: (1) the glomerular filtration rate (GFR); (2) osmotic diuresis in the loop of Henle; and (3) water diuresis mainly in the collecting duct.³¹ GFR is the net result of the renal plasma flow and arterial pressure and the glomerular pressure. Osmotic diuresis in healthy individuals is mainly determined by sodium and to a lesser extent by urea concentrations (in diabetes it can be glucose). The excretion of sodium is regulated by atrial natriuretic peptide (ANP) and the renin-angiotensin-aldosterone (RAAS) system, where ANP is involved in sodium and water excretion and the RAAS system in sodium and water reabsorption.³² Water diuresis is regulated by antidiuretic hormone (ADH) or vasopressin, in response to the input from osmotic and volume receptors. GFR, osmotic diuresis, and water diuresis can be easily measured by determining a renal function profile which can be a useful tool to study the pathophysiological cause of changes in urine production.³¹

GFR is higher in men compared to women but there is no direct link with sex hormones.³³ The hormone that regulates the circadian rhythm in our body is melatonin and a nocturnal peak in melatonin secretion causes a decrease in nocturnal GFR. Whether this is a direct effect or rather an indirect result of central vasodilatation, through modulation of vasopressin and RAAS, remains to be established.^{34,35} ANP on the other hand is known to increase the GFR and is stimulated by sex hormones (estrogens and progesterone), resulting in an increased urine production due to an increased sodium and water excretion.³⁶ Additionally, ANP inhibits vasopressin and induces water diuresis.³⁷ Activation of the RAAS system results in sodium and water retention in the body and lowers the urine production.^{32,38} When the RAAS system is inhibited, one can expect an increase in urine production; however, it has been suggested that after a while a steady-state is reached resulting in a decrease in urine production. Premenopausal women have a lower RAAS activation compared to men, which might be related to the fact that androgens increase RAAS activation. The net effect of estrogens and androgens is sodium and water reabsorption while the net effect of progesterone is sodium and water excretion. What impact these hormones have on urine production over time is another point of interest.^{38,39}

Many studies allude to sex differences in men and women: women have a decreased circadian rhythm of “arginine vasopressin,” respond to lower doses of desmopressin and are more prone to hyponatremia.³⁹ A genetic cause for this sex difference has been suggested in recent literature, as the vasopressin receptor gene is located on the X-chromosome.⁴⁰ Furthermore, men have a higher evening fluid consumption whereas women have a significantly higher mean 24 hr urine output and are more often polyuric compared to men.⁴¹ Sex hormones also have an influence on vasopressin secretion: estrogens and androgens increase vasopressin (lowering diuresis) while progesterone antagonizes vasopressin (increasing diuresis).^{42–44}

The net effect of all sex hormones is anti-diuretic, which might indicate a reason for post-menopausal hormone substitution in patients with bothersome nocturia.

Research proposals.

- Further evidence on the effect of castration and hormonal substitution on (nocturnal) polyuria is needed.
- In addition, given the multiple described relations between sex hormones and diuresis, we are convinced that a more thorough investigation is needed using a renal function profile.

Do We Need to Know More About the Effects of Circadian Hormones on the LUT?

The hormonal system plays a critical role in the regulation of endogenous circadian rhythms which have a periodicity of ~24 hr and are vital for survival and health. The circadian system is complex with a central circadian clock located in the hypothalamus. The major mammalian “clock” genes are the period genes, *Per1* and *Per2*, the cryptochrome genes, *Cry1* and *Cry2*, and the clock genes, *Bmal1* and *clock*. Similar genes have been found in peripheral tissues such as the gastrointestinal tract (GIT), liver, muscle, adipose tissue⁴⁵ and the urinary bladder.⁴⁶ Human lifestyle involves voluntary shifts in daily activity, such as shift-working, resulting in circadian dysfunction, thought to contribute to a wide range of clinical conditions including sleep disorders, gastrointestinal diseases, metabolic syndrome, inflammation, and cancer.⁴⁷ Little is known about the impact of circadian dysfunction on the LUT and whether it might play a causative role in LUT disorders.

Melatonin, which is secreted by the pineal gland, plays a major role in the regulation of circadian rhythms. Chronodisruption due to exposure to artificial light at night causes decreased melatonin secretion with a consequential increase in reproductive hormone levels and several studies have reported an association between night work and breast,⁴⁸ prostate and bladder⁴⁹ cancers. In addition to being a potent anti-oxidant, melatonin acts through the regulation of intracellular proteins (can pass through the cell membrane) and by binding to its membrane receptors MT1 and MT2 which are expressed both in the central nervous system and in peripheral organs including the GIT, kidney, bladder, and prostate.

In animal models melatonin has been shown to protect the bladder from oxidative damage as a result of ischemia.⁵⁰ In vitro studies have shown that melatonin can impact DSM contractility with reports of melatonin-increased⁵¹ mediated and decreased⁵² contractile responses to selective stimuli. Administration of melatonin to nocturia patients reduced the number of episodes per night but it is unknown if the beneficial effects are due to a direct action of the hormone on the bladder.⁵³ In animal studies, melatonin inhibited cholinergic- and KCl-induced contractions of DSM strips in vitro. However, the exact mechanism responsible for the effects of melatonin on DSM is unknown.⁵⁴ Melatonin has been associated with the maintenance and replacement of the mucosal lining of the GIT.⁵⁵ Little is known about a role for “circadian hormones” such as melatonin in the barrier and signaling function of the bladder mucosa (urothelium) and whether deficiencies in melanotropic signaling might underlie pathophysiologies of the bladder such as interstitial cystitis/painful bladder syndrome (IC/PBS).

The hormonally active form of vitamin D, 1 α , 25-dihydroxyvitamin D₃ (VitD₃), is reported to participate in the maintenance of circadian rhythms through the regulation of circadian genes.⁴⁵ The principal mechanism for acquisition of vitamin D is cutaneous ultraviolet-B photosynthesis of VitD₃ (cholecalciferol) from 7-dehydrocholesterol. Seasonal changes in length of sunlight, protective clothing, air pollution and sunscreen impair optimal cutaneous vitamin D production. In a cross-sectional, population-based survey of US men, vitamin D insufficiency was highly prevalent and associated with the presence of LUTS and moderate-severe UI.⁵⁶ Vitamin D receptors are present in both the DSM and the urothelium of the human bladder. Treatment with the VitD₃ analogue elocalcitol reduced the number of non-voiding contractions in a rat model of partial bladder outflow obstruction, however, it failed to significantly suppress non-voiding contractions in OAB patients in clinical trials.⁵⁷ Antimicrobial peptides such as defensins and cathelicidins play an important role in the first line of mucosal immunity. Similar to its effect on cells of the immune system, VitD₃ has been reported to enhance production of the cathelicidin, LL-37 by the bladder urothelium during uropathogenic *E. coli* infection. As serum levels of VitD₃ are reported to be lower in post-menopausal women, this may account for the higher susceptibility to urinary tract infections in this age group.⁵⁸ Urinary tract infections can in turn cause disruption to the urothelial barrier leading to bladder dysfunction. Further, it might potentially impact the resident bladder microbiome leading to LUTD.

Research proposals.

- What is the role of melatonin signaling in the regulation of DSM contractile activity especially in relation to the aging bladder?
- Is melatonin signaling (other than its antioxidant activity) important in the maintenance of urothelial barrier function in the bladder?
- Does circadian disruption (shift work) alter the microbiome “signature” of the LUT?
- Is the LUT microbiome impacted by changes in serum VitD₃ levels and/or VitD₃ receptor polymorphisms?

CONCLUSIONS

In order to advance our understanding of the role of hormones in LUTD and improve clinical practice, further basic science studies at the molecular, cellular, and tissue level are urgently needed as outlined by this ICS-RS Think Tank report.

ACKNOWLEDGMENTS

The authors would like to thank the participants in this Think Tank during the 2014 ICI-RS meeting. The study was supported by a grant from the National Institutes of Health R01 DK084284 to Georgi V. Petkov.

Grant sponsor: National Institutes of Health; Grant number: R01 DK084284

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Fig. 1.

Proposed mechanism of estrogen effects on BK channels in DSM cells. Estrogens activate the BK channels in DSM cells by directly binding to the channel, presumably to the regulatory β1-subunit. This causes membrane potential hyperpolarization, closure of the L-type VDCC, and subsequent DSM relaxation. Theoretically, estrogens should activate BK channels only in the presence of the regulatory β1-subunit and not in DSM from β1-subunit knockout mouse (KO).