Ion channels of the mammalian urethra

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The mammalian urethra is a muscular tube responsible for ensuring that urine remains in the urinary bladder until urination. In order to prevent involuntary urine leakage, the urethral musculature must be capable of constricting the urethral lumen to an extent that exceeds bladder intravesicular pressure during the urine-filling phase. The main challenge in anti-incontinence treatments involves selectively-controlling the excitability of the smooth muscles in the lower urinary tract. Almost all strategies to battle urinary incontinence involve targeting the bladder and as a result, this tissue has been the focus for the majority of research and development efforts. There is now increasing recognition of the value of targeting the urethral musculature in the treatment and management of urinary incontinence. Newly-identified and characterized ion channels and pathways in the smooth muscle of the urethra provide a range of potential therapeutic targets for the treatment of urinary incontinence. This review provides a summary of the current state of knowledge of the ion channels discovered in urethral smooth muscle cells that regulate their excitability.

Role of the Urethra in the Lower Urinary Tract

The filtration of blood by glomeruli in the kidney leads to the formation of urine, which is then transported to the urinary bladder by specialized muscular tubes called ureters that undergo finely-tuned peristaltic waves to prevent urine backflow toward the kidneys. The bladder is a hollow muscular organ capable of receiving and storing urine as it is propelled by the ureters into the kidneys. The bladder is a hollow muscular organ capable of receiving and storing urine as it is propelled by the ureters into its interior. The compliant bladder expands as urine fills its interior and raises intravesicular pressure on the bladder walls. The impulse/desire to urinate is thought to result from the high firing rate of afferent sensory nerves stimulated by mechanoreceptors in the bladder wall, which are activated by the rise in intravesicular pressure in the bladder. These afferent sensory nerves project to the bladder neck and connects the bladder interior to the exterior environment (Fig. 1). It is a structurally-complex multi-layered tissue comprising the lamina propria, including both mucosa and submucosa, as well as longitudinal and circular layers of smooth muscle. The urethra also contains striated muscle proximal to the pelvic floor, often referred to as the external urethral sphincter. Contraction of this muscle is commonly associated with the “guarding reflex” experienced during times of high bladder intravesicular pressure (e.g., sneezing, coughing). The adult female urethra is embedded in the anterior vaginal wall and typically ranges 3-4 cm in length and ~0.6 cm in luminal diameter. Although the male urethra is ~20 cm in length, it is mainly the prostate and pre-prostatic regions that contribute to the true internal urethral sphincter.

In healthy individuals, the process of urination is a coordinated voiding mechanism involving the contraction of the detrusor muscle lining the bladder, combined with the relaxation of the urethral smooth muscle, also known as the internal urethral sphincter (Fig. 1). During the urine filling/storage phase, urine outflow from the bladder does not occur by virtue of the fact that the bladder musculature is largely relaxed and electrically quiescent, while the urethra, a conduit muscular tube extending from the base of the bladder, maintains a constant tone and is effectively closed (excellent reviews are available describing bladder and urethral physiology/pharmacology). Early studies utilizing catheters in humans reported a time-delay ranging from 5-15 seconds between the relaxation of urethral musculature and contraction of the bladder detrusor muscle.

It is well-established that the inability of the urethral musculature to maintain sufficient tone can result in involuntary urine leakage. Damage to urethral smooth musculature may be in the form of acute trauma (e.g., surgical intervention, childbirth complications) or from aging-related diseases. Recently there has been an increasing recognition of the value of targeting the urethral musculature in the clinical management of urinary incontinence. The array of ion channels located in urethral smooth muscle membranes play a crucial role in determining internal urethral sphincter excitability and therefore the overall function of the urethra.

Studying electrical activity in the urethra

The mammalian urethra is known to exhibit spontaneous mechanical activity, but during the urine-storage phase, muscle activity is mostly tonic in nature. The complex nature of urethral tissue has presented many challenges in studying its function in detail. Most current knowledge regarding the role of ion channels in the urethra has come from isometric tension

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 recordings, sharp microelectrode recordings of intact preparations, and the patch clamp technique. Earlier studies measuring electrical and mechanical (i.e., force) activity in rabbit urethral smooth muscle reported that the spontaneous activity was in fact myogenic in nature, resembling the activity seen in the GI tract. Thus, the excitability appeared to be driven by a form of pacemaker originating within the muscle itself. It is important to apply caution when interpreting data from intact preparations, such as from tension and microelectrode recordings, since (i) the urethral smooth musculature contains a mixed population of cells that includes a range of non-contractile interstitial cells of Cajal (ICC)-like cells (Fig. 1B, see focused reviews), and (ii) the urethra receives innervation from sympathetic and parasympathetic nervous systems. It is extremely difficult, therefore, to reliably isolate the role of ion channels in a given cell type using such intact preparations, particularly with limited pharmacology. The patch clamp technique, remains the only reliable method currently available to study the behavioral properties of ion channels expressed on the surface membranes of the ICCs and smooth muscle cells of the urethra. Recent advances in isolating toxins and selective pharmacological agents have greatly aided in identifying and profiling the roles of ion channels, as discussed below.

### K⁺ Channels in Urethral Smooth Muscle Cells

The Brading research group successfully applied the patch clamp technique to urethral smooth muscle cells isolated from pigs in the late 1990s, reporting a number of different K⁺ channel currents that exhibited small and large conductance properties. One conductance in particular showed characteristics consistent with ATP-sensitive K⁺ (KₐTP) channels, and was the focus of the early studies principally using the pig model.

#### KₐTP Channels

KₐTP channels are formed by the combination of 4 inwardly-rectifying K⁺ (Kᵢᵣ) channel subunits, comprising the pore-forming core, surrounded by a ring of 4 regulatory sulfonylurea subunits (Fig. 2 and review). The KₐTP channels identified in the urethral smooth muscle cells have a single channel conductance of 43 pS and their activation by pharmacologic agents was reported to hyperpolarize the membrane potential. Thus, K⁺ efflux from these channels drives the membrane potential in the negative direction, decreasing excitability. Since then, Kᵢᵣ6.1 and Kᵢᵣ6.2 subunit transcripts have been detected and it has been suggested that the KₐTP channel comprises a “mixed” (i.e. heterotetrameric) channel of Kᵢᵣ6.1 and Kᵢᵣ6.2 subunits, since the observed conductance value of 43 pS was intermediate between those measured for homomeric channels consisting of either Kᵢᵣ6.1 or 6.2 subunits. It has recently been definitively reported that the pore-forming region of the urethral smooth muscle KₐTP channel is in fact a heterotetrameric complex of Kᵢᵣ6.1 and Kᵢᵣ6.2 subunits, arranged in a 3:1 ratio (Fig. 2). In addition, transcripts for the regulatory sulfonylurea receptors SUR1 and SUR2B have also been detected, and are known to functionally co-assemble.

![Figure 1. (A) A representation of the anatomy of the lower urinary tract including bladder and urethral structures, with inputs from various nervous systems illustrated. (B) A smooth muscle bundle depicting smooth muscle cells in close contact with an ICC-like cell and nerve. Abbreviations: ACh, acetylcholine; NO, nitric oxide; ATP, adenosine 5’-triphosphate; ICC, interstitial cell of Cajal.](image-url)
Thorough studies by Teramoto and colleagues outlining the biophysical properties of K\textsubscript{ATP} channels in the pig urethra has provided valuable experimental evidence that K\textsubscript{ATP} channels contribute to setting the resting membrane potential ($\sim$ $-37$ mV).\textsuperscript{38,46} Important physiological implications result from the particular heterotetrameric arrangement of the channel complex identified in the urethra that distinguish it from other smooth muscle types such as those found in vascular tissues.\textsuperscript{52,53} The gating properties (i.e., activation) of the heterotetrameric K\textsubscript{ATP} channel complex found in the urethra can be dynamically modulated by protein kinase C (PKC),\textsuperscript{38} which is distinct from the mechanism observed in vascular smooth muscle for K\textsubscript{ATP} channels.\textsuperscript{53}

**Voltage and Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels**

Following elucidation of the role of K\textsubscript{ATP} channels in urethral smooth muscle cells, the next series of breakthroughs in characterizing urethral smooth muscle cell ion channel properties originated from a collaboration among the Hollywood, McHale and Thornbury laboratories investigating K\textsuperscript{+} channels and voltage-gated Ca\textsuperscript{2+} channels (VGCCs, Fig. 2). Urethral smooth muscle cells were reported to have large conductance, Ca\textsuperscript{2+}-activated K\textsuperscript{+} (BK\textsubscript{Ca}) channels and voltage-gated K\textsuperscript{+} (K\textsubscript{v}) channels carrying outwardly-rectifying K\textsuperscript{+} current (Fig. 3).\textsuperscript{40} However the lack of K\textsubscript{v}-specific inhibitors/enhancers at that time hampered further identification and profiling of the K\textsubscript{v} conductances. The subsequent isolation and development of toxins, such as the tarantula spider venom peptide, stromatoxin 1 (ScTx),\textsuperscript{54,55} has provided a valuable selective pharmacological tool to study certain K\textsubscript{v} channels. ScTx targets the voltage sensor domain in homotetrameric K\textsubscript{v} channels K\textsubscript{v}2.1, K\textsubscript{v}2.2 and K\textsubscript{v}4.2.\textsuperscript{30,54} In addition, ScTx has been shown to inhibit heterotetrameric K\textsubscript{v}2.1/6.3\textsuperscript{56} and K\textsubscript{v}2.1/9.3-containing K\textsubscript{v} channels.\textsuperscript{30,54,57,58} Using the ScTx

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**Figure 2.** A schematic illustration of the various ion channels identified in urethral smooth muscle. The architecture of the transmembrane $\alpha$ and $\beta$ subunits is shown in the left panel. Dimeric and trimeric arrangements can be seen for K\textsubscript{ATP} and P2X channels, respectively. Most channels have tetrameric structures and several have $\beta$ subunits associated. Abbreviations: N, amino-terminus; C, carboxyl-terminus; K\textsubscript{ATP}, ATP-sensitive K\textsuperscript{+} channel; C\textsubscript{A}, positively-charged residues; BK\textsubscript{Ca}, large conductance, Ca\textsuperscript{2+}-activated K\textsuperscript{+} channel; K\textsubscript{v}, voltage-gated K\textsuperscript{+} channel; CaM, calmodulin; K2P, 2-pore domain K\textsuperscript{+} channel; IK\textsubscript{Ca}, intermediate conductance K\textsuperscript{+} channel; SK\textsubscript{Ca}, small conductance K\textsuperscript{+} channel; VGCC, voltage-gated Ca\textsuperscript{2+} channel; CaCC, Ca\textsuperscript{2+}-activated Cl\textsuperscript{−} channel.
peptide in combination with biophysical analysis. Hollywood and colleagues reported that the majority of outwardly-rectifying Kv current in urethral smooth muscle cells was carried by Kv2.1-containing channels.\(^{30}\) By comparing the deactivation properties of currents “native” to the urethra with those of Kv2.1 and Kv2.2 channels “cloned” from the urethra in the same report, it was suggested that the native outward current was mediated by Kv2.1 subunits co-assembled with a “silent” Kv channel subunit (i.e., either Kv 6, 8 or 9). RT-PCR and immunocytochemical analyses strongly suggested that the Kv2.1 subunit is co-expressed with a Kv9.3 subunit, in a manner similar to previous reports characterizing native Kv channels in vascular smooth muscle cells\(^{57}\) and more recent reports in bladder.\(^{58}\) Kv9.3 subunits do not form functional channels by themselves. They must be expressed in conjunction with a member of the Kv2 family.\(^{59}\)

Intriguingly, while it was firmly established that Kv2 channels carried the majority of the delayed rectifier Kv current in the urethra, these channels apparently contributed very little to action potential repolarisation (Fig. 3A).\(^{30,31}\) Rather, the repolarisation phase of the action potential was heavily dependent on K\(_{\text{Ca}}\) efflux via BKCa channels.\(^{30,31,40}\) However, if BKCa channels were pre-inhibited, the role of the Kv2 channels became apparent, suggesting that Kv2.1-containing channels may represent a possible “fail-safe” brake mechanism for repolarising the cellular membrane potential if the BKCa channels were not properly activated, or otherwise suppressed. Isometric tension studies indeed demonstrated an increase in excitability when the Kv current was inhibited in urethral tissue, particularly when BKCa channels were pre-inhibited.\(^{30,31}\) However, it is worth noting that it has not yet been determined if Kv2.1-containing channels and/or BKCa channels are also present in ICCs within urethral smooth muscle, which could contribute to the observed effect(s).

BKCa channels are unique members of the K\(_{\text{Ca}}\) channel family due to their dual Ca\(^{2+}\)- and voltage-sensitive features (see reviews).\(^{60,61}\) In addition, their large single channel conductance makes them formidable players in the regulation of urethral smooth muscle excitability. While most of the transmembrane region of the BKCa
channel α subunit superficially resembles that of the Kᵥ channel subunits (Fig. 2), the BKCa channel has a considerably larger C-terminal regulatory domain that is key to its overall function in smooth muscle. This region contains Ca²⁺-binding sites and can be dynamically regulated by intracellular signaling pathways (e.g., phosphorylation). 

While not yet directly reported, it is likely that the BKCa channels expressed in the urethral smooth muscle plasma membrane co-associate with the smooth muscle BKCa channel β subunit, BKCa B1 (Fig. 2), which has been identified in bladder smooth muscle cells. 

Electrophysiological studies in urethral smooth muscle cells using the perforated patch (amphotericin-mediated), voltage clamp configuration have provided evidence for an interesting transient-like or A-type outward K⁺ current during membrane depolarization (Fig. 3B) that was not carried by Kᵥ channels. It was determined that this transiently-activating current was in fact carried by BKCa channels (i.e., iberiotoxin-sensitive), and required Ca²⁺ influx via L-type voltage-gated calcium channels (VGCCs), leading to Ca²⁺-induced Ca²⁺ release (CICR) via ryanodine receptors (RyRs) on the sarcoplasmic reticulum (SR) store during depolarization of the cell membrane. 

RyK-mediated Ca²⁺ release was found to be crucial for the transient activation of BKCa current and this proved important for repolarising the membrane potential, thus shortening the duration of action potential spike events. It was also reported that this transient current could be inhibited by muscarinic receptor activation (i.e., M₃), by altering the SR store Ca²⁺ release mechanisms and mitigating the contribution of the BKCa current to action potential repolarization. These data also suggested that the BKCa current could be suppressed during parasympathetic stimulation, as evidenced by broadened action potential events and isometric tension studies. Such findings also supported the idea that the delayed rectifier Kᵥ current was functionally important during BKCa current suppression, as may occur during parasympathetic stimulation.

Possible roles for other K⁺ channels in urethral smooth muscle

Comprehensive investigation into urethral smooth muscle cell ion channels has lagged behind its bladder smooth muscle cell counterpart, particularly with regard to K⁺ channels. The reasons for this are multifactorial, and exacerbated by the difficulties associated with obtaining adequate urethral tissue from small rodents including rats and mice. The more readily available bladder tissue, however, has provided a valuable reference point for candidate ion channels in urethral smooth muscle since both bladder and urethra cell types superficially and electrically resemble each other.

The residual urethral smooth muscle delayed rectifier Kᵥ current (i.e., non-Kᵥ2) has not yet been fully characterized. However, parallel studies in bladder smooth muscle cells indicate that this current may be carried by Kᵥ7-containing subunits in either homo- or heteromeric combinations. While this possibility would present an alternative therapeutic Kᵥ target in urethral smooth muscle, the role of this third conductance would appear to be less significant than those mediated by BKCa or Kᵥ2 channels during action potential events, based on results obtained using currently-available pharmacology.

Studies involving bladder smooth muscle cells have definitively reported expression of BKCaβ1 subunits in functional BKCa channel complexes. Interestingly, BKCaβ4 subunit transcript and protein also appear to be present in bladder smooth muscle cells, although its role, if any, has not yet been elucidated. It has not yet been reported in urethral smooth muscle cells.

It is certainly worth noting that the Kᵥ2P channels (Fig. 2) TASK-1 and TASK-2 have been reported by Sang Don Koh and co-workers to be present in bladder smooth muscle cells. These “leak” K⁺ channels were found to be functionally important in terms of regulating the resting membrane potential. Similar investigations focusing on urethral smooth muscle cells would constitute a logical next step in understanding the mechanisms responsible for setting the resting membrane potential. It has also been reported that there is a role for small-conductance, Ca²⁺-activated K⁺ (SKCa) channels in bladder smooth muscle cells using patch clamp recordings in combination with intact preparations. These channels have also been implicated in bladder ICC function. Intermediate-conductance, calcium-activated K⁺ (IKCa) channels have not yet been found to be functionally important in bladder smooth muscle cells, although they have been detected at the transcriptional and protein levels.

VGCCs in Urethral Smooth Muscle

Most forms of mammalian smooth muscle employ Ca²⁺ entry through VGCCs as a key process to elevate intracellular Ca²⁺ levels. This has certainly proved to be consistent with action potential spike events observed in urethral smooth muscle cells.

It is well-established that the L-type (long-lasting) VGCCs activate at more depolarized potentials than their T-type (transient-lasting) counterpart. It was also previously demonstrated that L-type VGCC current was crucial for generating and maintaining urethral tone in intact preparations. However, characterization of VGCCs in the individual smooth muscle cells has required extensive biophysical and pharmacological studies utilizing the patch clamp technique. In the early 2000s, urethral smooth muscle cell VGCC currents were examined and it was reported that human and rabbit urethral smooth muscle cells functionally contained both L- and T-type VGCC conductances. This was followed up by studies demonstrating that the L-type VGCC current was essential not only for the upstroke in the action potential, but for the transient nature of the BKCa channel current. While detailed biophysical data are available for the L- and T-type VGCCs, the channel subunit isoforms and regulatory subunits have not yet been identified or described.
P2X Channels

Studies examining purinergic stimulation of urethral smooth muscle have been controversial for a number of reasons. Briefly, ATP activates purinergic receptors that include P2X (ionotropic) ion channels, and the G protein-coupled purinergic P2Y (metabotropic) receptors (see excellent reviews on purinergic receptors). Both sub-families of these purinergic receptors are known to be expressed in urethral smooth muscle tissue, which further complicates the interpretation of data arising from intact preparations, as discussed previously. This helped contribute to apparently conflicting reports by several groups characterizing purinergic stimulation as both inhibitory and excitatory under their respective recording conditions.

Recent patch clamp studies undertaken by Sergeant and coworkers has provided clarifying evidence that purinergic compounds released by nerves in urethral smooth muscle tissue activate the cation-selective P2X receptors expressed on smooth muscle cell membranes and depolarize the membrane potential. This was found to be in contrast to the ATP-induced activation of ICCs from urethral tissue, which was mediated by P2Y receptors. This area of research is ongoing and will likely prove to be more difficult than traditional ion channel studies due to the complex array of purinergic receptors, some of which can “desensitize” to a stimulus during the course of an experiment.

CaCC Channels

Early studies using intact preparations including microelectrode recordings strongly indicated a functional role for Ca-electrotonic activated Cl⁻ channels (CaCCs) in regulating the excitability and function of urethral smooth muscle. Patch clamp studies on cells isolated from the urethra provided evidence that the CaCC currents were tightly-linked with the pace-making mechanism exhibited by ICCs. While CaCC currents could be activated upon depolarization of urethral smooth muscle cells, the vast majority of cells isolated from human and rabbit urethra show no spontaneous activity, unlike ICCs. The most likely candidate CaCC expressed in urethral smooth muscle cells is anoctamin-1 (ANO-1), also known as TMEM16A. The possible functional role(s) of ANO-1 in urethral smooth muscle is now undergoing greater scrutiny.

Concluding Remarks and Future Directions

Selectively-regulating the excitability and tone of the smooth muscles in the lower urinary tract remains the most difficult challenge in battling urinary incontinence. Over the last 2 decades, research examining the functionality of urethral smooth muscle, which provides a crucial “internal sphincter” mechanism, has greatly advanced. It is well-established that the behaviors of ion channels expressed at the surface membrane are closely-tied to smooth muscle excitability and tone. Most therapies for urinary incontinence currently available attempt to selectively-target bladder function and achieve only limited success. These treatments have notorious system-wide side-effects that promote poor patient-compliance and reduce quality of life. Unfortunately, this is a consequence of the limited range and bioavailability of therapeutic targets in the bladder, combined with a lack of tissue-specific available pharmacologic tools. Novel developments in understanding the respective roles of several K⁺ channels (BKCa, K,2 and K,7 families) in regulating bladder excitability has generated interest in developing selective K⁺ channel agonists for overactive bladder treatments. While it is likely that this would reduce detrusor smooth muscle excitability, parallel advances in urethral smooth muscle studies indicate that similar channels are also present in this tissue, and that enhancing their activity may promote undesired relaxation in the urethra.

Recently a range of novel therapeutic targets have been identified in urethral smooth muscle cells that include several members of the K⁺ channel families, VGCCs, purinergic receptors and CaCCs. In addition, several members of their signaling pathways have been reported (e.g., RyRs, muscarinic receptors), adding to the richness of potential therapeutic targets. In addition to directly targeting these channels and their respective signaling pathways with activators and/or inhibitors, other approaches to manipulate ion channel behavior may be available. For instance, determining that a specific subunit is contained in a heterotetrameric ion channel (e.g., K2.1/9.3 channels or K6.1/6.2), or an associated accessory subunit (BKCaB1), may serve as a signature and provide new opportunities to selectively-target ion channel complexes expressed primarily in the bladder and/or urethra. Additionally, selectively-targeting established signaling pathways may promote desired ion channel behavior. Such a strategy, for instance, is exploited by sildenafil, the active ingredient in Viagra, in erectile tissues (i.e. PDE5 inhibition).

Strategies involving molecular biology (e.g., genetic) can now be explored with greater confidence in urethral smooth muscle in the form of directly and selectively upregulating/downregulating ion channel expression (e.g., plasmid-infused). Similarly, by targeting tissue-specific ion channel subunit chaperones and regulatory proteins (e.g., BKCa channel chaperones) it is possible to selectively increase or decrease a particular ion channel expression at the surface membrane. Controlling BKCa channel protein turnover is a powerful determinant of the cell excitability, but more study will be required to understand the trafficking and chaperoning mechanisms of these channel proteins in urethra and bladder smooth muscle before a useful strategy can be utilized.

Given the prominent role of BKCa channels in regulating urethral smooth muscle excitability, it is likely that this channel will become a primary therapeutic target in the near future, similar to current bladder research and development projects. In the short term, it is likely that successful strategies will make use of the cell’s existing machinery that dynamically regulate ion channel excitability (e.g., PDEs, PKC, PKG). PDE4 has recently emerged as an upstream BKCa channel modulator in bladder smooth muscle cells, and thus may be a
candidate for similar modulation in urethral smooth muscle. While there has been much recent progress in understanding urethral smooth muscle regulation, it is disappointing that no clear candidate protein/channel has emerged that is distinguishable from bladder smooth muscle. Although K<sub>2P</sub>, K<sub>7</sub> and BK<sub>Ca</sub>β4 protein expression has been reported in bladder, it remains to be seen whether they will be found to be functionally present in urethral smooth muscle.

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Disclosure of Conflicts of Interest

No potential conflicts of interest were disclosed.

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