Big-conductance Ca\(^{2+}\)-activated K\(^{+}\) channels in physiological and pathophysiological urinary bladder smooth muscle cells

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ABSTRACT
Contraction and relaxation of urinary bladder smooth muscle cells (UBSMCs) represent the important physiological functions of the bladder. Contractile responses in UBSMCs are regulated by a number of ion channels including big-conductance Ca\(^{2+}\)-activated K\(^{+}\) (BK) channels. Great progress has been made in studies of BK channels in UBSMCs. The intent of this review is to summarize recent exciting findings with respect to the functional interactions of BK channels with muscarinic receptors, ryanodine receptors (RyRs) and inositol triphosphate receptors (IP3Rs) as well as their functional importance under normal and pathophysiological conditions. BK channels are highly expressed in UBSMCs. Activation of muscarinic M3 receptors inhibits the BK channel activity, facilitates opening of voltage-dependent Ca\(^{2+}\) (CaV) channels, and thereby enhances excitability and contractility of UBSMCs. Signaling molecules and regulatory mechanisms involving RyRs and IP3Rs have a significant effect on functions of BK channels and thereby regulate cellular responses in UBSMCs under normal and pathophysiological conditions including overactive bladders. Moreover, BK channels may represent a novel target for the treatment of bladder dysfunctions.

KEYWORDS
big-conductance Ca\(^{2+}\)-activated K\(^{+}\) channel; inositol triphosphate receptor; muscarinic receptor; overactive bladder; ryanodine receptor; urinary bladder smooth muscle cell

Introduction

The primary functions of the urinary bladder are to relax and store urine during the filling phase and contract forcefully to empty the bladder during micturition.\(^1\) Overactive bladder (OAB), obstructive bladder, and urinary incontinence are common clinical disorders associated with bladder storage dysfunctions that cause a sudden urge to urinate and an increase in frequency of urination. The cost of treating these illnesses in the United States exceeds 10 billion dollars per year,\(^2\) and much research is being devoted to this problem. The current mainstay of OAB treatment is antimuscarinic pharmacotherapy. This therapy is limited in its efficacy and tolerability, since antimuscarinic drugs often cause adverse effects such as constipation, blurred vision and cardiac disturbances.\(^3-7\)

Bladder functions are facilitated by a coordinated contraction and relaxation of urinary bladder smooth muscle cells (UBSMCs) that make up the bladder wall.\(^1,8\) One of the major mechanisms in the regulation of UBSMC functions are implemented by various ion channels, particularly including the big-conductance Ca\(^{2+}\)-activated K\(^{+}\) (BK) channels.\(^9-15\) The UBSMC BK channels may represent a novel target for the treatment of bladder dysfunctions. Inhibition of BK channels triggers cell membrane depolarization leading to an increase in the activity of voltage-gated Ca\(^{2+}\) (CaV) channels and intracellular Ca\(^{2+}\) concentrations ([Ca\(^{2+}\)]\(_i\)) in UBSMCs, whereas the BK channel activation produces opposite effects.\(^9-13,15\) Thus, BK channels play a critical role in the control of cell contraction by providing a negative feedback to limit membrane depolarization and [Ca\(^{2+}\)]\(_i\) in UBSMCs.\(^1,8,16-18\) Signaling molecules and regulatory mechanisms involving ryanodine receptors (RyRs) and inositol triphosphate receptors (IP3Rs) that have a direct or an indirect effect on the activity of BK channels can control or regulate physiological and pathological functions in UBSMCs.

The present review aims to discuss the interactions of BK channels with muscarinic receptors, RyRs and IP3Rs in the regulation of functions in UBSMCs.

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Stimulation of muscarinic receptors provides a major neural control of contractile responses, thereby making a significant contribution to cellular functions in UBSMCs. In response to a variety of stimuli, the Ca$^{2+}$ release channels RyRs and IP$_3$Rs on the sarcoplasmic reticulum (SR) mediate the release of Ca$^{2+}$ from the SR into the cytoplasm of the cell. In UBSMCs, BK channels can be activated by Ca$^{2+}$ release from RyRs in the form of Ca$^{2+}$ sparks. These local Ca$^{2+}$ release events occur spontaneously in UBSMCs in the absence of stimulation and trigger transient BK currents (TBKCs), also known as spontaneous transient outward currents (STOCs), without affecting the global [Ca$^{2+}$]_i. Previous studies have also shown that Ca$^{2+}$ release channels on the SR contribute to muscarinic receptor-mediated contractions in human, pig, and mouse UBSMCs.

### Functional interactions of muscarinic receptors and BK channels in physiological and pathophysiological bladders

Acetylcholine (ACh) and adenosine triphosphate (ATP) are the 2 main neurotransmitters released from parasympathetic nerve endings that regulate UBSMC contractions in rodents. ACh is a major neurotransmitter triggering voiding contractions in UBSMCs by binding and activating muscarinic receptors. However, it is a controversial topic as to whether or not ATP acts as a neurotransmitter in human UBSMCs. Muscarinic receptor activation is a primary mechanism to enhance excitability and contractility of UBSMCs. Muscarinic M$_2$ and M$_3$ receptors (M$_2$Rs and M$_3$Rs) are predominantly expressed in rat and human UBSMCs. UBSMC contraction under physiological conditions is caused largely by activation of M$_2$Rs. The involvement of M$_3$Rs in UBSMC contractions has been examined by using the M$_3$R antagonist 4-diphenylacetoxy-N-methylpiperidine (4-DAMP) and the M$_2$R antagonist methoctramine. Four-DAMP has been shown to have a ~9 to 10-fold selectivity ratio for M$_3$Rs over M$_2$Rs. This antagonist inhibits M$_3$Rs with a higher affinity ($pA_2$) value of 8.5 to 8.9 (~1 nM) in rat UBSMCs. Methoctramine has been demonstrated to be the most selective M$_2$R antagonist and have a binding affinity 33-fold greater than that of M$_3$Rs. Four-DAMP has a large inhibitory effect on muscarinic contraction in UBSMCs.

In support of these pharmacological results, studies using M$_3$R and M$_2$R/ M$_3$R (double) knockout mice indicate that M$_3$Rs play a minor role in UBSMCs by enhancing the M$_2$R-mediated contraction. M$_3$Rs are the most valuable player for cholinergic contractile responses in both adult and newborn UBSMCs, while the M$_2$Rs are of lesser importance. The muscarinic agonist carbachol-induced UBSMC contraction has been reported to be attributed to M$_3$R activation, which leads to IP$_3$-induced Ca$^{2+}$ release and then to membrane potential depolarization. A study in guinea pig UBSMCs has shown that stimulation of M$_3$Rs with muscarinic agonists produces IP$_3$ and thereby induces UBSMC contractions. In contrast to this report, IP$_3$Rs do not control the nerve-evoked contractility in mouse UBSMCs.

TBKCs are controlled by Ca$^{2+}$ release from RyRs on the SR and directly suppressed by inhibition of RyRs with ryanodine in UBSMCs. Recent work by Parajuli and Petkov has shown that stimulation of muscarinic receptors with carbachol increases TBKCs followed by the current inhibition in rat UBSMCs. The inhibitory effect of carbachol on TBKCs is possibly caused by either an inhibition of RyRs or by a decrease in the SR Ca$^{2+}$ content due to the SR Ca$^{2+}$-pump blockade. Consistent with these findings, previous studies have shown a similar inhibitory muscarinic effect on BK channels in other SMCs.

In non-UBSMCs such as tracheal and gastrointestinal SMCs, activation of M$_2$Rs has been shown to cause a direct inhibition of BK channels. The functional contribution of M$_2$Rs in UBSMCs has been demonstrated under pathologic conditions such as neurogenic bladder dysfunctions and hypertrophy. It has been shown that the contractile responses to muscarinic agonists are significantly increased in diabetic UBSMCs. M$_3$R biosynthesis is upregulated in diabetic UBSMCs. In contrast, studies reveal that downregulation of M$_2$Rs and upregulation of M$_3$Rs occur in diabetic UBSMCs. Experiments further indicate that muscarinic contractile and Ca$^{2+}$ responses are enhanced in mild outlet obstruction bladders and decreased in severe outlet obstruction bladders. All these findings suggest that muscarinic receptors, in particular M$_2$Rs, show the increased expression and activity contributing to the enhanced UBSMC overactivity.

Using the perforated whole-cell patch clamp technique, Parajuli and Petkov have found that M$_2$Rs are
not involved in BK channel regulation in rat UBSMCs under physiological conditions. Noticeably, a study using the conventional whole-cell patch clamp technique has shown that activation of M3Rs causes BK channel inhibition. Previous experiments using animals and humans have revealed that muscarinic receptor agonist-induced UBSMC contractions are either due to Ca\(^{2+}\) entry through Ca\(_V\) channels or Ca\(^{2+}\) release from the SR. Since there is a considerable differences in relative contributions of muscarinic agonist-induced extracellular Ca\(^{2+}\) influx and intracellular Ca\(^{2+}\) release between human, porcine and murine UBSMCs, a better understanding into the mechanistic insight by which the intracellular Ca\(^{2+}\) handling impacts cellular functions in UBSMCs is of great importance.

It has been reported that after stimulation of M3Rs, protein kinase C (PKC) is activated and thus regulates multiple cellular responses in UBSMCs. Indeed, a number of studies have revealed that PKC-dependent signaling pathways may play a significant role in the regulation of functions in UBSMCs. An interaction of PKC with BK channels has been investigated at the molecular and functional level. A plausible explanation for this interaction is that stimulation of M3Rs leads to diacylglycerol production, which activates PKC (Fig. 1). PKC may cause direct inhibition of the SR Ca\(^{2+}\) pumps and/or RyRs, thereby resulting in suppression of BK channels in UBSMCs. However, the assumption of a direct PKC-BK channel interaction or an indirect mechanism involving the SR Ca\(^{2+}\) store needs to be tested directly. It has been shown that PKC can phosphorylate specific serine residues on the BK channel \(\alpha\) and \(\beta\) subunits, and a failure of phosphorylation of either subunit leads to a loss of the channel function when the serine residues were substituted for alanine.

Figure 1. A schematic diagram illustrating the functional interaction of IP3R, RyRs, and BK channels in urinary bladder smooth muscle cells. Activation of M3Rs leads to IP3 and DAG production via a pathway involving PLC and PIP2. IP3 activates IP3Rs, which releases Ca\(^{2+}\) from the SR. This IP3-induced Ca\(^{2+}\) release transiently activates the BK channels. Furthermore, depletion of the SR Ca\(^{2+}\) upon activation of M3Rs reduces Ca\(^{2+}\) spark activity, inhibits TBKCs and depolarizes cell membrane, which activates Ca\(_V\) channels, cause Ca\(^{2+}\) influx and thus increases contractility in UBSMCs. DAG activates PKC, leading to inhibition of Ca\(^{2+}\) sparks and TBKCs. Ca\(^{2+}\) release from the SR also activates melastatin transient receptor potential (TRPM) channels resulting in UBSMC contractions. (DAG, diacylglycerol; IP3, inositol triphosphate; M3R, muscarinic receptors type 3; PIP2, phosphatidylinositol 4,5-bisphosphate; PKC, protein kinase-C; PLC, phospholipase-C; SR, sarcoplasmic reticulum; Ca\(_V\), voltage-dependent Ca\(^{2+}\)).
specific PKC isoforms in BK channel-dependent excitability and contractility in UBSMCs remains to be elucidated. In spite of this fact, PKC may play an important role in maintaining the normal bladder storage and also bladder emptying force in a BK channel-dependent manner. To the best of our knowledge, the functional interaction between PKC and BK channels has not been studied in UBSMCs under a pathological condition such as OAB.

**Role of ryanodine receptors in BK channel-mediated physiological and pathophysiological functions in bladders**

Three subtypes of RyRs (RyR1, RyR2 and RyR3) are expressed in mammalian cells. RyR1 is chiefly expressed and required for physiological functions in skeletal muscle cells, RyR2 is primarily present and necessary for normal functions in cardiac myocytes, and RyR3 is predominantly located in brain and skeletal muscle cells with uncertain functions. It has been reported that RyR2, but not RyR1 or RyR3, mRNAs are expressed in freshly isolated and cultured human UBSMCs. However, RyR2 mRNA expression is undetected following culture, suggesting that properties of the RyR2 isoform in human UBSMCs may change when the cells are maintained in culture. The importance of RyRs to regulate the spontaneous phasic contractions has been demonstrated using a rat model of partial bladder outlet obstruction (PBOO). These animals develop overactive UBSMC contraction that is associated with a significant decrease in expression and activity of RyRs.

RyRs are an important modulator of excitation-contraction coupling in UBSMCs. In cardiac myocytes, \( \text{Ca}^{2+} \) channels on the SR are physiologically coupled to RyRs on the SR membrane whereby a small amount of extracellular \( \text{Ca}^{2+} \) influx through \( \text{Ca}^{2+} \) channels activates RyR2 to induce a massive \( \text{Ca}^{2+} \) release from the SR, i.e., \( \text{Ca}^{2+} \)-induced \( \text{Ca}^{2+} \) release (CICR), which is required for physiological cardiac functions. RyR2 may play a crucial role in the regulation of \( \text{Ca}^{2+} \) and contractile responses during the excitation-contraction coupling in UBSMCs. Reportedly, a loose coupling between \( \text{Ca}^{2+} \) channels and RyRs exists in UBSMCs, in which \( \text{Ca}^{2+} \) influx due to the opening of single \( \text{Ca}^{2+} \) channels is insufficient to activate RyRs to induce \( \text{Ca}^{2+} \) release; rather, aggregate \( \text{Ca}^{2+} \) influx via a number of \( \text{Ca}^{2+} \) channels must be large enough to open RyRs. An interpretation for this loose coupling is that RyR2 may show a lesser \( \text{Ca}^{2+} \) sensitivity in UBSMCs than in cardiac muscle. One of the most functional characteristics of RyRs is to form \( \text{Ca}^{2+} \) sparks. The sparks may develop into waves that spread across the cell to produce a global \( \text{Ca}^{2+} \) signal. Indeed, a loss of spontaneous contractile activity is associated with a significant decrease in RyR expression.

RyRs may act as a negative-feedback regulator of spontaneous contractile activity in normal UBSMCs, in which RyRs are localized with BK channels within a distance of ~20 nm. Because of this physical localization, \( \text{Ca}^{2+} \) release from RyRs may directly activate BK channels, which causes outward membrane currents and cell membrane hyperpolarization that leads to the inhibition of \( \text{Ca}^{2+} \) channels, extracellular \( \text{Ca}^{2+} \) influx and cell relaxation. On the other hand, simultaneous activation of massive RyRs following application of the classic channel agonist caffeine at a high concentration may simply elicit transient BK currents in UBSMCs. A similar response in UBSMCs has also been produced by a voltage depolarization.

Downregulation of BK channel activity is a common pathophysiological feature in hypertension. Similarly, the downregulated BK channels in vascular myocytes have also been shown in diabetes. The impaired functions of BK channels in hypertensive and diabetic vascular SMCs may primarily result from the downregulated \( \beta 1 \) subunits. It has been reported that diabetes causes a decrease in BK channel activity and \( \beta 1 \) subunit mRNA expression in diabetic UBSMCs, suggesting that the diabetic downregulation of BK channels may promote UBSMC excitability leading to OAB. In support of this finding, a previous study has shown that diabetic mice exhibit decreased voiding efficiency and other OAB characteristics. However, the molecular mechanisms by which diabetes causes a decrease in BK channel \( \beta 1 \) subunit expression, an impairment of BK channels, and a malfunction of \( \text{Ca}^{2+} \) signals in UBSMCs are not known.

**Interactive contribution of IP3 receptors in the regulation of functions of BK channels in physiological and pathophysiological bladders**

IP3 is a prime cytosolic messenger linking the plasma membrane events to the release of \( \text{Ca}^{2+} \) from the SR. Available literature indicates that 3 IP3 receptor isoforms (IP3R1–3) have been identified, each of which is encoded by a different gene. Expression of...
individual IP3R isoforms is based on the tissue of origin and developmental stage in mammalian SMCs. IP3R1 is the predominant isoform in vascular SMCs. Quantitative RT-PCR indicates that IP3R1 mRNA is the most abundant of the 3 isoforms in freshly isolated cerebral artery SMCs. IP3Rs are modulated by both cytosolic Ca\(^{2+}\) and SR luminal Ca\(^{2+}\) in SMCs. Depletion of the SR Ca\(^{2+}\) abolishes IP3R-mediated Ca\(^{2+}\) release. The SR Ca\(^{2+}\) may also determine the driving force for Ca\(^{2+}\) release and thus modulate the amplitude of IP3-mediated Ca\(^{2+}\) signals.

Stimulation of IP3R1 activates BK channels via their local interaction mechanism in cerebral arterial SMCs. It has been reported that activation of IP3Rs causes the opening of BK channels in coronary artery SMCs and consequently decreases the artery tone. Whether such a functional interaction between IP3Rs and BK channels exists in UBSMCs is not known. As in vascular SMCs, activation of IP3Rs may increase or decrease the excitability and contractility of UBSMCs by modulating BK channel activity. Emerging evidence indicates that stimulation of M3Rs causes phospholipase C activation, IP3 production, IP3R opening, and SR Ca\(^{2+}\) release in UBSMCs. However, it is not clear whether the Ca\(^{2+}\) release from IP3Rs modulates the BK channels to regulate the excitability and contractility of UBSMCs. In spite of this fact, intracellular application of IP3 can induce a substantial Ca\(^{2+}\) release in both freshly isolated and cultured human UBSMCs. Furthermore, it has been shown that Ca\(^{2+}\) release from IP3Rs activates melastatin transient potential 4 (TRPM4) channels and subsequently increases the membrane excitability. In support of the role of TRPM4 channels, a recent study has revealed that the TRPM4 channel inhibitor 9-phenanthrol attenuates spontaneous inward currents in the presence of the muscarinic receptor agonist carbachol in UBSMCs, thus reducing the cell excitability.

Treatment of cells with xestospongin C, an IP3R inhibitor, significantly decreases spontaneous transient inward currents (STICs) in human UBSMCs. These data indicate that activation of M3Rs results in IP3-induced Ca\(^{2+}\) release from IP3Rs, opening of TRPM4 channels, depolarization of cell membrane, Ca\(^{2+}\) influx via Ca\(_\text{v}\) channels, and contraction in UBSMCs. It has been demonstrated that UBSMC overactivity is associated with the downregulation of BK channels as evidenced by a decrease in BK channel expression and functions under neurogenic bladder conditions.

Presumably, the functional uncoupling between IP3Rs and BK channels may occur in UBSMCs under neurogenic bladder conditions or OAB syndromes, in which stimulation of IP3Rs may not be able to have a stimulatory effect on BK channels in UBSMCs. Another plausible functional interaction between IP3Rs and BK channels is that IP3R stimulation increases the basal Ca\(^{2+}\) level, leading to inhibition of Ca\(^{2+}\) sparks and BK channels, as shown in colonic SMCs.

In vascular SMCs, BK channels are gated by Ca\(^{2+}\) with a relatively low affinity. As a consequence of a large increase in [Ca\(^{2+}\)], following activation of IP3Rs, BK channels are activated, which causes hyperpolarizing K\(^+\) currents and inactivation of Ca\(_\text{v}\) currents, thereby preventing contraction in vascular SMCs. Despite very limited information on the role of IP3Rs in regulation of BK channels in various types of cells, whether stimulation of IP3Rs may modulate the BK channel activity in UBSMCs has never been studied. Conceivably, new strategies to modulate IP3Rs and BK channels in UBSMCs may have a significant clinical impact in the production of more effective therapeutics for bladder dysfunctions. OAB syndrome is one of the common complications of diabetes. Thus, it would be very interesting to investigate the interactive role of IP3Rs and BK channels in the development of OAB using an animal model of diabetes and bladder samples from diabetic patients.

**Conclusion**

Taken together, we propose a schematic diagram (Fig. 1) to summarize the important interactions of BK channels with the key signaling molecules and effectors including muscarinic receptors, DAG, PKC, IP3, RyRs, and IP3Rs in the control of cellular functions in UBSMCs. The antimuscarinic agents are the mainstay in treating OAB. Therefore, further studies are necessary to better understand the mechanistic details of muscarinic signaling pathways associated with RyRs and IP3Rs in physiological and pathophysiological bladders. Without doubt, additional investigations with respect to the role of BK channels, RyRs, and IP3Rs may help to generate novel and specific drugs to treat OAB and other bladder diseases.

**Disclosure of potential conflicts of interest**

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