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Potassium channels and uterine function

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Abstract

Ion channels are effector proteins that mediate uterine excitability throughout gestation. This review will focus primarily on the role of potassium channels in regulating myometrial tone in pregnancy and labor contractions. During gestation, potassium channels maintain the uterus in a state of quiescence by contributing to the resting membrane potential and counteracting contractile stimuli. This review summarizes the current knowledge about the significance of the potassium channels in maintaining a normal gestational period and initiating labor contractions at term.

Keywords

Myometrium; Potassium channel; Pregnancy; Labor

1. Introduction

Potassium efflux from myometrial cells results in membrane repolarization and this efflux is the primary ionic current responsible for maintaining the resting membrane potential. Potassium channels comprise one group of proteins that contribute significantly to uterine quiescence during pregnancy. In myometrial smooth muscle cells (MSCs), changes in the expression or activity of K⁺ channels can translate into an inadequate repolarization leading to aberrant uterine activity. Thus, K⁺ channel alterations may contribute to certain pathophysiological conditions such as dystocia, preterm labor and post-term labor. These proteins are the focus of extensive research given their role in maintaining uterine quiescence throughout pregnancy and their potential role in the induction of myometrial contractions during labor.

In myometrial cells, K⁺ channels are an underlying component of the mechanism that allows for adaptation of the gravid uterus to increases in stretch and intrauterine pressure. To date, several types of K⁺ channels have been identified in the myometrium. The most abundant and well studied include the large-conductance calcium- and voltage-sensitive K⁺ channel (BKCa channel), the ATP-sensitive K⁺ channel (K_{ATP}), the *Shaker*-like voltage-gated potassium channels (Kv), and small-conductance calcium-sensitive potassium channels (SK). The variety of K⁺ channels in this tissue reflects the multiplicity and complexity of the mechanisms involved in the regulation of uterine tonus. Because K⁺ channels are widely represented in the myometrium, molecular mechanisms that regulate potassium channels in pregnancy will be the main focus of this review; however other channels will be described briefly.

2. Potassium channel regulation and function

2.1 BKCa channels

The BKCa channels (also known as maxi-K and slo) are large-conductance, voltage- and calcium-sensitive K^+ channels. They are one of the most extensively studied ion channels in uterine smooth muscle due to their abundance and significant repolarizing current. Relatively few BKCa channels need to be activated to produce uterine relaxation; thus these channels can have profound effects on myometrial activity. Multiple cellular regulators with uterotonic actions modulate this channel, suggesting that the channel's major physiological role is to induce smooth muscle relaxation in response to depolarization and to mediate the action of uterotonic substances.

The contribution of the BKCa channel to the total cell K^+ current varies depending upon gestational stage. In rat non-pregnant myocytes, BKCa channels contribute approximately 35% to whole cell repolarizing K^+ current; however by late gestation, electrophysiological measurements show that loss of BKCa-generated currents is concomitant with an increased contribution of other K^+ channel types to maintain uterine quiescence [1]. Contractile studies in mid- and late pregnant rats show that the BKCa opener NS1619 and inhibitor iberiotoxin (IbTX) do not alter spontaneous contractile activity induced by prostaglandins [2]. Complementary studies have demonstrated that BKCa has a more pronounced relaxant effect in mid-gestation versus late gestation after activation with adenylyl cyclase [3]. These data all indicate an attenuated role for these channels in maintaining uterine quiescence in term gravid uterus from rats. Similar to rat myometrium, BKCa current is suppressed in late-pregnant mice compared to non-pregnant mice, however BKCa transcript and protein expression was enhanced suggesting that multiple mechanisms regulate BKCa channel function [4].

The modulation of BKCa current during pregnancy in many of these studies may result from altered sensitivity to cell signaling molecules known to activate channel activity. BKCa channels mediate uterine relaxation in response to adenylyl cyclase (AC) activation, which was present only in mid-term but not late pregnancy or labor [3]. Thus, differential expression of AC in gestation (*see Lopez Bernal 2007, this issue*), or altered sensitivity of the BKCa channel to downstream signaling of AC, may explain differential activity of BKCa during uterine quiescence in the rat model of pregnancy. Nitric oxide (NO) also mediates uterine relaxation in pregnancy via activation of BKCa channels, and this effect depends on gestational stage. Nitric oxide was less effective in inducing uterine relaxation in late compared to mid-gestation rats [5]. In human pregnant myocytes, the open state probability of the BKCa increases in response to an NO donor [6]. Currently, it is not known whether NO activation of myometrial BKCa channels is by direct interaction and/or indirect activation via cyclic cGMP dependent pathways [6,7]; however it is apparent that BKCa channels are altered during pregnancy to hone their response to signaling molecules.

Hormonal regulation of myometrial tone in gestation depends, in part, on modulation of activity and/or expression of ion channels. Specifically, various hormones modulate BKCa channels to aid in the maintenance of pregnancy. Sex hormones and hormones of the hypothalamus-pituitary-adrenal axis affect uterine tone in part by regulating expression of BKCa gene products [8]. Carvajal et al [9] observed that chorion exerted a relaxant effect in pregnant myometrium pre-contracted with oxytocin. This relaxation depended on activation of BKCa channels, suggesting that relaxin generated by the feto-placental unit and ovaries during gestation activates BKCa channels. This result was verified in cultured human myometrial smooth muscle cells where relaxin increased open state probability of BKCa channels via a PKA-mediated pathway [10].

Molecular and cellular studies have elucidated that differential regulation of BKCa channels during pregnancy results from various mechanisms including alternative splicing, association with accessory β -subunits, post-translational modification, and association with proteins that regulate channel expression and current phenotype. The BKCa channel is encoded by a single gene, *KCNMA1* (also referred to as *slowpoke*) and derives its diversity by alternative splicing of its pre-mRNA. Splicing of the BKCa transcript results in functionally distinct variants (Figure 1A), which differ in their calcium and voltage sensitivity [11], hormonal sensitivity [12,13], ability to be phosphorylated [14] and trafficking [15].

Alternative splicing of the BKCa channel may be a molecular mechanism by which this channel is able to respond to a wide variety of regulatory substances in a tissue specific manner. Multiple splice variants have been identified; however few studies have focused on their function in myometrium during pregnancy. Starting at mid-gestation, BKCa isoforms with decreased sensitivity to Ca^{2+} increase in mouse myometrium [4]. While human BKCa isoforms with decreased sensitivity do not show a higher level of expression until the onset of labor, they allow the uterus to be in a more excitable state during labor [11,16]. Recent studies indicate that specific BKCa isoforms may function as myometrial-specific proteins. In humans, the mK44 isoform contains 44 additional amino acids in the first intracellular loop and is less sensitive to Ca^{2+} and voltage compared to BKCa channels lacking these 44 amino acids [11]. Although the levels of BKCa channel transcript decrease towards term in humans [17], the proportion that mK44 transcript comprises is up-regulated at late-term, suggesting that this isoform may modulate uterine activity near labor [16]. Recent studies in human myometrial cells have shown that mK44 is proteolytically cleaved, resulting in membrane localization of the N-terminus with retention of the C-terminus in the endoplasmic reticulum [18]. In response to an excitatory Ca^{2+} signal, the C-terminus translocates to the plasma membrane and reconstitutes with the N-terminus to produce a repolarizing current that induces uterine relaxation, underscoring the potential for myometrial specific regulation of BKCa channels [18].

Other splice variants have been identified that are not myometrial specific but could play an important role in myometrial excitability during gestation. The STREX isoform introduces 59 amino acids into the linker between S8 and S9 [13] and is regulated during pregnancy [19] by ACTH, 17β -estradiol, and progesterone [12],[8,20]. This variant introduces a second consensus PKA phosphorylation motif, which allows PKA phosphorylation to inhibit the activity of the channel [21]. Although alternative splicing is a way to gain variability from single gene products, it may also regulate protein expression. A splice variant termed SV1, in which 33-amino acids are inserted in the S1 transmembrane domain, caused retention of the channel in the ER, acting as a dominant-negative isoform [15]. Expression of BKCa isoforms with lower sensitivity to Ca^{2+} would be another explanation for Ca^{2+} -insensitive BKCa currents in laboring myometrium [19].

Dynamic association of the BKCa channel with an accessory beta subunit (β -subunit) is another molecular mechanism that provides diversity of BKCa function in MSCs. Four distinct genes, *KCNMB1-4*, encode the BKCa channel beta subunits with the $\beta 1$ subunit being the predominant isoform present in myometrium [22]. The $\beta 1$ subunit of the BKCa channel increases the voltage and calcium sensitivity of the channel [23] resulting in enhanced activation of BKCa channels to dampen uterine excitability and attenuate contractions. In addition, the $\beta 1$ subunit is regulated by 17β -estradiol [4] and facilitates binding of 17β -estradiol to the BKCa channel, but the exact mechanism remains controversial [24]. In mouse myometrium, the $\beta 1$ subunit transcript and protein are up-regulated at mid-gestation with post-partum expression returning to non-pregnant levels [4]. In human gravid uterus, expression of the $\beta 1$ subunit was lower during term and preterm labor compared to a non-laboring state [17]. This protein may play a role in maintaining uterine quiescence in mid- and late pregnancy

when intrauterine pressure and myometrial stretch gradually reach maximum. Uncoupling of BKCa channels from their $\beta 1$ subunits would generate channels with low sensitivity to Ca^{2+} . During human term and pre-term labor, however, a decrease in expression of both proteins, but not uncoupling, appears to be one mechanism to induce contractions [17].

Variability in channel behavior among distinct BKCa isoforms can also be in part due to disparate post-translational modification, which may underlie changes in uterine relaxant activity discussed earlier in this chapter. Enhanced activity of BKCa channels in pregnancy is often due to differential phosphorylation rather than an increase in BKCa expression levels. The membrane-bound PKG activates BKCa channels in human non-laboring myometrium thus contributing to myometrial quiescence during gestation [25]. Similarly, in rats, BKCa isoforms inhibited by PKA phosphorylation decreased throughout pregnancy whereas expression of isoforms activated by PKA peaked at mid-gestation [20].

Association of BKCa channels with membrane and intracellular proteins is currently emerging as a novel mechanism to regulate uterine relaxation. Beta-receptor agonists are commonly used in clinical practice to postpone pre-term labor. In myometrium, $\beta 2$ - and $\beta 3$ -receptors have been reported to form a functional complex with BKCa channels to induce channel activation and uterine relaxation in response to activation of beta-receptors [26,27]. Inhibition of BKCa channels with the specific blocker iberiotoxin (IbTX) abolished the effects of the $\beta 3$ -receptor activation in both myometrial cells and uterine tissue [26]. Immunoprecipitations suggest a physical association of receptors between BKCa channels and $\beta 2$ -receptors, however how this alters BKCa current directly is not known [27]. Both studies implicate an important role for this channel in tocolytic therapy with β -adrenergic agents. Also, myometrial BKCa channels are present in caveolae and clustering of receptors and channels within these signaling structures may be one mechanism for receptor-channel interactions in the absence of direct association [28].

Another explanation for diminished BKCa current at late gestation is attenuated membrane channel expression [29]; however this appears to be species-specific. In pregnant rats, unlike in mice, inhibition of BKCa current was due to down-regulation of channel transcript and protein expression [29]. In humans, increased levels of BKCa channels in late pregnant non-laboring were observed yet decreased BKCa channel expression was seen in pre-term and term laboring uterus indicating that these channels may be a part of the mechanism to induce spontaneous labor contractions [17,30]. However, channel expression and intracellular regulation are not mutually exclusive. Myocytes isolated from human laboring myometrium express BKCa channels with low sensitivity to Ca^{2+} and inhibitors [31]. Recent studies have discovered that the current phenotype measured in laboring tissue can be mimicked by measuring BKCa current in the presence of actin depolymerizing agents [28]. The recent linkage of myometrial BKCa channels activity with the cytoskeletal components may elucidate other mechanisms to regulate uterine contraction [28,31].

Alternative splicing, β -subunit expression, post-translational modifications and differential expression on the cell surface are only some of the mechanisms identified that regulate BKCa channel function in MSCs. Association with certain membrane and intracellular structures, such as caveolae and interaction with receptors are also mechanisms that will need to be further explored to understand the complex nature of BKCa channel regulation in uterine function.

K_{ATP} channels—Functional studies indicate that the ATP-sensitive inward rectifying potassium channels (K_{ATP}) have an important role in regulation of myometrial quiescence during pregnancy. The inward rectifier K⁺ channel family Kir6 comprises the potassium channel component of the K_{ATP}, while the sulfonylurea receptor (SUR) is responsible for the ATP sensitivity, pharmacological properties, and trafficking of this channel [32-34] (Figure

2). The predominant myometrial isoform is Kir6.1/SUR2B, although Kir6.2/SUR1 has been detected at the transcript level [35,36]. K_{ATP} in smooth muscle maintains basal membrane potential despite a relatively low open state probability [37]. Studies have also shown that Kir6.1/SUR2B is down regulated late in pregnancy, which could facilitate enhanced myometrial activity necessary for labor contractions [36]. This channel is inhibited by intracellular ATP and stimulated by MgADP, thus coupling metabolic state to changes in cellular excitability [38]. In other smooth muscle types, these channels have also been linked to smooth muscle relaxation in hypoxia due to a precipitous drop in ATP [39], however whether this occurs in myometrium is not known.

Over-expression of K_{ATP} subunits Kir.6.1 and SUR2B contribute to an inhibition of oxytocin-induced contractions in late pregnant but not term-pregnant rats [40]. Similarly, in the human uterus, K_{ATP} channels comprised primarily of Kir6.1 and SUR2B subunits, are expressed at higher levels in non-pregnant myometrium compared to late gestation [36]. These findings may indicate that in humans, down-regulation of K_{ATP} channels may contribute to increased uterine excitability and induction of labor contractions in term pregnancy. In human myometrium, the expression of K_{ATP} depends on both the gestational stage and the presence of labor contractions [41]. Pinacidil, a K_{ATP} opener, inhibits oxytocin-induced contractions in late pregnant non-laboring myometrium [41]. This effect was attenuated in laboring myometrium from term and preterm uteri. Between the two latter groups, inhibition was more pronounced in term labor uterine strips. The differential effects of pinacidil may reflect altered activity and/or expression levels of K_{ATP} channels in gestation and labor [41].

The regulation of K_{ATP} channels in the myometrium may also differ in spontaneous versus agonist-induced contractions. Kafali et al [42] found that in late pregnant rats, K_{ATP} channels may mediate the relaxation phase of spontaneously induced contractions. In mid- and late pregnant rat myometrium neither the K_{ATP} opener levcromakalim or K_{ATP} inhibitor glibenclamide had an effect on PGF E_2 and PGF I_2 -induced contractions [2]. These authors concluded that in mid- and late pregnant rats, K_{ATP} are not a part of the mechanism to regulate prostaglandin-induced uterine contractions. K_{ATP} channel activity during pregnancy also may depend on second-messengers. In late pregnant rat myometrium K_{ATP} channels mediate uterine relaxation in response to activation of adenylyl cyclase (AC) and guanylyl cyclase (GC) [3]. GC was expressed at all stages of gestation with AC present only in midterm but not during late pregnancy or labor. Thus, differential regulation of AC and GC in gestation may explain the regulation in uterine quiescence by K_{ATP} channels in a rat model of pregnancy. K_{ATP} activation resulted in equally potent relaxation in midterm, late term or laboring uterus in rats [3]. This finding suggests that, unlike in humans, K_{ATP} is not down-regulated during labor in rats. Inhibition of contractions by K_{ATP} depended both on AC and GC. Further molecular and cellular characterization of this channel in combination with functional experiments will aid in understanding the regulation of this channel during pregnancy.

Small conductance Ca^{2+} sensitive potassium channels—Small conductance Ca^{2+} sensitive and voltage insensitive potassium channels (SK) generate a hyperpolarizing current in excitable cells following action potential generation, and thus may induce relaxation of smooth muscle. SK channels belong to the KCNN family of potassium channels and derive their molecular diversity from both being encoded by multiple genes and utilizing alternative start codons within each gene [43] (Figure 1B). SK channels constitutively associate with calmodulin, which mediates gating of the channels through binding of calcium [44]. Even though SK channels do not possess a consensus calmodulin-binding motif, a calmodulin binding site has been mapped to a conserved stretch of amino acids in the cytoplasmic C-termini [45]. In addition to gating, calmodulin is also involved in the trafficking of SK channels to the plasma membrane [46].

SK channels recently emerged as critical regulators of myometrial contractility during gestation and labor. Transgenic mice over-expressing isoform 3 of SK channels (SK3) have a distinct phenotype of compromised labor possibly due to inefficient labor contractions [47]. In human non-pregnant and pregnant myometrium, apamin, an inhibitor of SK channels, attenuated relaxation induced by NO [48,49]. Recent studies have demonstrated that SK3 expression depresses phasic contractions in mouse uterus by limiting the influx of Ca^{2+} [50]. In PC12 cell line, the SK3-1B splice variant of the SK3 channel silences endogenous SK current by acting in a dominant-negative fashion [51]. Another splice variant of the SK3 channel, SK3-1C, suppressed the membrane expression of SK1, SK2, SK3 and intermediate conductance Ca^{2+} -activated K^{+} channels by sequestering the protein intracellularly [52]. Modulation of SK channels expression and function during pregnancy has not been studied to date. Thus, further investigation into the function of SK channels in modulating uterine function and parturition will be intriguing.

Voltage-gated potassium channels (Kv channels)—Voltage-dependent K^{+} channels (Kv) are widely expressed in uterine smooth muscle. In response to depolarization, efflux of K^{+} through these channels induces repolarization of the MSCs to resting membrane potential. Voltage-gated potassium channels contribute to resting membrane potential in myocytes and may play a role in maintaining uterine quiescence before labor [53]. In MSCs, small depolarizations open L-type Ca^{2+} channels in MSCs resulting in enhanced Kv channel activity, which is important for dampening cell excitation and maintaining basal uterine tone. Phenotypically, there are several types of voltage-gated K^{+} currents, which differ in their kinetics and pharmacological sensitivity. In human myometrium, two main types of Kv currents have been identified using electrophysiological methods, delayed rectifiers and rapidly inactivating currents [1,54,55].

The Kv channel subfamily that appears to play the most predominant role in the myometrium during pregnancy has been the Kv4 subfamily. Electrophysiological studies have described rapidly inactivating K^{+} current in rat pregnant myometrium generated by the Kv4 family of K^{+} channels [56]. Of this channel subfamily, Kv4.2 and their accessory subunits increased before parturition, whereas Kv4.1 and Kv4.3 expression declined during gestation suggesting that soluble factors, possibly hormones, regulate Kv4 channels in pregnancy [56]. This was further supported by research showing that in ovariectomized rats, estrogen down-regulated membrane localization, protein and current expression of Kv4.3 channels [57]. These findings suggested that prior to parturition elevated estrogen levels increase myometrial contractility by inhibiting function of Kv4.3 channels. Similarly, in cultured human myometrial cells, 17β -estradiol, but not progesterone, inhibited currents generated by Kv channels thus increasing excitability of the MSC membranes [55].

Kv4 channels associate with many different subunits, most of which have been studied in tissues other than the myometrium [58-60]. One of these subunits is the Kv channel interacting proteins (KChIPs), which are calcium-binding proteins [61]. Two isoforms, KChIP2 and 4 have been found in the myometrium [56]. In rats, KChIP2 is upregulated in late gestation, and enhances Kv4 channels cell membrane density by trafficking channels from the endoplasmic reticulum to the cell membrane. KChIP also alters channel kinetics to allow greater Kv4 channel current [61,62]. The KChIP proteins may play a significant role in reducing myometrial excitability and potentially dampening uterine contractions.

Two pore channels—The role of other potassium channels in regulation of myometrial contractility in gestation and parturition remains unresolved. Two pore domain channels (2P) are inward rectifying K^{+} channels sensitive to pH, hypoxia and stretch. Similar to K_{ATP} and Kv channels, they may set a resting membrane potential in myometrial smooth muscle cells. The isoforms 1, 4 and 5 of TWIK-related acid-sensitive K^{+} channels (TASK) and isoform 1

of TWIK-related K⁺ channels (TREK) have been identified in human pregnant myometrium [63], however their role during pregnancy needs further elucidation.

3. Regulation of Na⁺ and Cl⁻ channels present in the myometrial smooth muscle

Other channels discovered in pregnant myometrium are chloride (Cl⁻), sodium (Na⁺), non-specific cation, and calcium (Ca²⁺) channels, the latter being covered by other reviews in this journal. Functionally, Na⁺ channels generate fast inward current resulting in membrane depolarization. The expression of the two voltage-gated Na⁺ channels (Nav2.1, Nav2.3) has been reported in human and mouse myometrium [64,65], however the role for Na⁺ channels in regulating uterine tone in pregnancy is not well understood since the majority of these channels are inactivated at the resting membrane potential. Several studies in rats have shown an increase in Na⁺ current during gestation. Martin C. et al [66] reported that both functional and non-functional Na⁺ channels are present in rat pregnant uterus based on identification of high and low affinity binding sites for the Na⁺ channel inhibitors, tetrodotoxin (TTX) and saxitoxin. However other studies using the same preparations of rat uterine cells, did not find Na⁺ channels with a low sensitivity to TTX [67,68]. Electrophysiological studies detected both fast inactivating and TTX-sensitive Na⁺ currents in myocytes of pregnant rats that increased throughout gestation, and these studies also reported a subpopulation of myocytes lacking Na⁺ channels. These authors concluded that in gestation, Na⁺ current density increases towards term in part due to a higher number of cells expressing Na⁺ channels. They also suggest that, besides faster action potential propagation in labor, Na⁺ channels may augment uterine excitability through an elevation of intracellular Ca²⁺ due to activation of Na⁺/Ca²⁺ exchanger in response to an increase in transmembrane transport of Na⁺. These hypotheses remain untested.

Yoshino M. et al [69] have demonstrated the presence of Na⁺ channels in non-pregnant uterine myocytes following estrogen stimulation with augmentation and faster kinetics as pregnancy progressed. These currents peaked at term and were nearly undetectable postpartum. This study concluded that a combination of mechanisms, including increased Na⁺ current density and inhibition of expression of K⁺ channels result in a more excitable myometrium starting mid-gestation and promotes coordinated uterine contractions during labor. While there is disagreement about the existence of the subpopulation of uterine myocytes lacking Na⁺ channels, there is general consensus that high Na⁺ current density close to labor may contribute to generation of coordinated uterine contractions necessary for expulsion of the fetus. The distinct roles of Na_v2.1 and Na_v2.3 channels in gestation and labor is unclear since these channels have not been functionally characterized and the study of these channels in pregnancy remains an area for further investigation.

The contribution of chloride ions to the regulation of myometrial tone has been acknowledged in the past. The Ca²⁺-dependent Cl⁻ channels have been identified in human myometrial smooth muscle cells [70-72]. In response to an increase in intracellular Ca²⁺, Cl⁻ channels generate an outward current, which results in depolarization of the cell membrane and increased excitability of the myometrium. These channels may regulate the pacemaker activity, thus consequently affecting spontaneous uterine contractions by increasing their amplitude and frequency [71,72]. However, a role for Cl⁻ channels in regulation of uterine contractions remains unclear due to the absence of selective inhibitors/openers of Cl⁻ channels, the lack of selectivity of the channels for Cl⁻ ions, and the uncertainty concerning their molecular structure [73].

SUMMARY

Ion channels are a part of the molecular mechanism(s) regulating uterine smooth muscle excitability in gestation and labor (Figure 2). Potassium channels, in particular, are crucial in maintaining myometrial quiescence in pregnancy. Dysfunction or decreased expression of K^+ channels would diminish MSC repolarizing current resulting in induction of premature uterine contractions and pre-term labor. Over-expression of K^+ channels current late in pregnancy may suppress coordinated myometrial activity needed for term labor contractions. The voltage-sensitive Na^+ and Cl^- channels may contribute to spontaneous uterine contractions by supporting a pacemaker activity of uterine smooth muscle cells, and thus, also be important in sustaining labor contractions. Myometrial ion channels represent a promising subject for therapeutic manipulation to treat and/or prevent pre- and post-term labor. However, many other tissues, besides myometrium, express these channels. Therefore it is important to discover uterine-specific mechanisms that regulate K^+ , Na^+ and Cl^- channels in order to aid in the alleviation of conditions leading to uterine dysfunction.

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A **B** **Figure 1.**

(A) Isoforms of the BKCa channel, mK44, SV1, and STREX are the protein products resulting from alternative splicing of the KCNMA gene. S0-S6 represents hydrophobic transmembrane spanning domains, while S7-S10 represents intracellular hydrophobic domains. (B) Isoforms of the SK3 channel derived from alternative start codons (adapted from 51 and 52).

Figure 2.

A schematic figure of a myometrial smooth muscle cell showing a variety of ion channels that are involved in regulation of membrane potential and cell excitability. Protein phosphatase (Ptase), myosin light chain kinase (MLCK), phosphorylated myosin light chain kinase (MLCK-P), calcium calmodulin-dependent protein kinase (CaMK), Kv channel interacting protein (KChIP).