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Uterine Excitability and Ion Channels and Their Changes with Gestation and Hormonal Environment

Susan Wray and Sarah Arrowsmith

Department of Women's and Children's Health, University of Liverpool, Liverpool L69 3BX, United Kingdom; email: s.wray@liverpool.ac.uk

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Abstract

We address advances in the understanding of myometrial physiology, focusing on excitation and the effects of gestation on ion channels and their relevance to labor. This review moves through pioneering studies to exciting new findings. We begin with the myometrium and its myocytes and describe how excitation might initiate and spread in this myogenic smooth muscle. We then review each of the ion channels in the myometrium: L- and T-type Ca^{2+} channels, K_{ATP} (Kir6) channels, voltage-dependent K channels (Kv4, Kv7, and Kv11), twin-pore domain K channels (TASK, TREK), inward rectifier Kir7.1, Ca^{2+}-activated K^+ channels with large (KCNMA1, Slo1), small (KCNN1–3), and intermediate (KCNN4) conductance, Na-activated K channels (Slo2), voltage-gated (SCN) Na^+ and Na^+ leak channels, nonselective (NALCN) channels, the Na K-ATPase, and hyperpolarization-activated cation channels. We finish by assessing how three key hormones—oxytocin, estrogen, and progesterone—modulate and integrate excitability throughout gestation.

EXCITATION

The Myometrium

The uterus is a muscular tissue; ~95% of its cells are smooth muscle myocytes, constituting the myometrium. This is bounded by endometrium that lines the uterine cavity, and perimetrium, the outer protective coat. Cells in the myometrium are arranged in bundles grouped in a species-specific manner. In rodents but not women there is a clear separation of outer longitudinal and inner circular muscle layers. In all species, the myocytes continue into the uterine cervix, but their numbers greatly decrease. The extracellular matrix of the uterus, but more notably the cervix, changes during gestation, with its collagen content decreasing and glycosaminoglycans and matrix proteins being degraded (1). A decrease in activity of the cervical myocytes may help with effacement and softening (increased hydration and distensibility) at term (2).

The Myocyte

Uterine myocytes are spindle-shaped cells with bleb-like protrusions when contracting. Their plasma membranes have an abundance of invaginations, called caveolae, leading to distinct, cholesterol-rich microdomains, which help in the sorting of signals impinging on the membrane (3). There is a large central nucleus, well-developed sarcoplasmic reticulum (SR), and gap junctions allowing for communication between cells and electrical coupling. The myocytes in the uterus hypertrophy in pregnancy to become the largest smooth muscle cells in the (female) body. Their diameter increases from 5 to 20 μm and length from 50 to 1,000 μm. Studies conducted mostly on rodents have provided understanding of how, during pregnancy, the myocytes proliferate (hyperplasia) before changing to a hypertrophic growth to accommodate the growing fetus. The extracellular matrix also becomes more elaborate to support the smooth muscle bundles. For further details of how mechanical and hormonal signals may be integrated to affect the uterine myocytes, see the review by Shynlova et al. (4). The uterine myocytes contribute to the synthesis of their matrix. When cells are cultured, this activity increases, leading to conversion from a contractile to synthetic phenotype and a change in the expression of ion channels. This reduces the usefulness of culture smooth muscle cells for the study of excitability. Details of muscle proteins and the cytoskeleton are reviewed elsewhere (5). Briefly, actin and myosin are the most abundant proteins in the cells and form the thin and thick filaments, respectively. Vimentin, desmin, and cytokeratin make a third filamentous structure in smooth muscle cells, the intermediate filaments. These are noncontractile and contribute to the cytoskeleton, giving structural integrity during contraction. When crossbridges are formed in these nonstriated cells, force is transmitted via α-actinin structures called dense bodies or plaques (6, 7). As described in Kajuluri et al.'s recent review (5), the force is then transmitted to the cytoskeleton and focal adhesions and thereby throughout the uterus.

Overview of Excitation-Contraction Coupling

The activity of the myometrium is regulated; myocytes are poorly active in the nonpregnant state, and throughout most of gestation, and then become increasingly excitable and contractile in the last days before parturition. The myometrium is a myogenic, phasically active smooth muscle. Excitation arises from the firing of action potentials initiated by Ca^{2+} ions entering through L-type, voltage-gated Ca channels and ends in attachment of myosin heads to the actin filaments, i.e., crossbridges, and cycling between attachment and detachment, linked to ATP hydrolysis. As shown in **Figure 1**, the increase in intracellular calcium concentration ($[Ca^{2+}]_i$) leads to the formation of 4Ca-calmodulin. This complex binds and activates myosin light chain

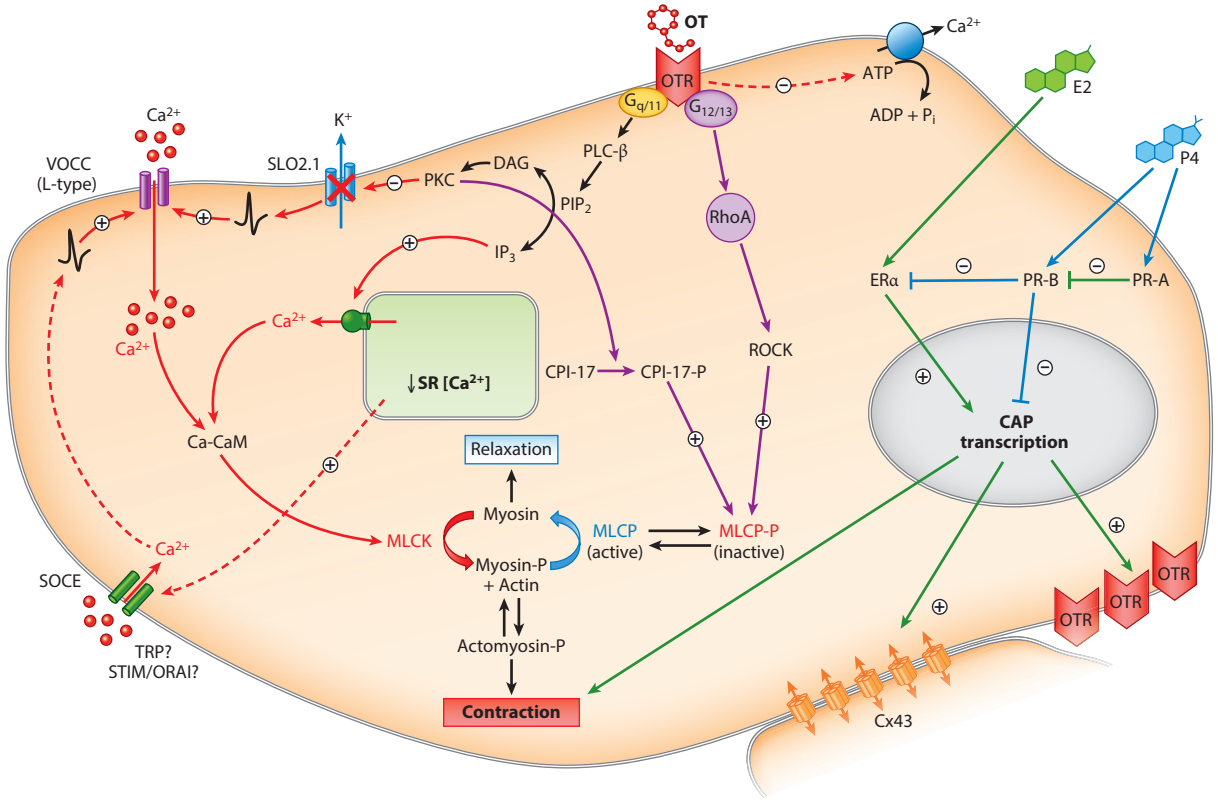

Figure 1

Hormonal signaling by estrogen, progesterone, and oxytocin in the myometrium leading to contraction. Red arrows indicate signaling pathways with direct influences on $[Ca^{2+}]_i$, whereas purple arrows indicate Ca-independent pathways to contraction, including Ca sensitization. Green arrows indicate genomic pathways promoting contraction and blue arrows denote those inhibiting them. Dashed lines indicate where mechanisms are not yet fully elucidated. Abbreviations: CaM, calmodulin; CAP, contraction-associated protein; CPI-17, protein kinase C–potentiated phosphatase inhibitor protein of 17 kDa; Cx43, connexin 43; DAG, diacylglycerol; E2, estrogen; ERα, estrogen receptor alpha; IP_3, inositol 1,4,5-trisphosphate; MLCK, myosin light chain kinase; MLCP, myosin light chain phosphatase; OT, oxytocin; OTR, oxytocin receptor; -P, phosphorylated; P4, progesterone; PIP_2, phosphatidylinositol 4, 5-bisphosphate; PKC, protein kinase C; PLC-β, phospholipase C beta; PR-A/B, progesterone receptor A/B; RhoA, Ras homolog family member A; ROCK, Rho-associated kinase; SLO2.1, sodium-activated, high-conductance, potassium leak channel; SOCE, store-operated calcium entry; SR, sarcoplasmic reticulum; TRP, transient receptor potential channel; VOCC, voltage-operated calcium channel. Figure adapted with permission from Reference 165.

kinase (MLCK). Unlike most kinases, MLCK is highly specific and only phosphorylates serine 19 on the light chains of myosin. Unlike striated muscles, this specific phosphorylation is necessary (and sufficient) for the actin activation of myosin MgATPase and subsequent crossbridge cycling and force output. Force declines as L-type channels inactivate, the membrane starts to repolarize, and myosin light chain phosphatase (MLCP) removes the phosphate from the light chains.

The magnitude of force will be affected by the membrane potential and pattern of action potentials, which in turn will alter the Ca signals (8). The role of the SR in myometrial cells is distinct and has been reviewed elsewhere (9, 10). In brief, the Ca that it accumulates via the activity of SERCA (sarco-endoplasmic reticulum ATPase) can only be released via inositol 1,4,5-trisphosphate (IP_3)

receptors. Ryanodine release channels are nonfunctional and, thus, there are no local releases of Ca (Ca sparks). To replenish the Ca released when agonists (e.g., oxytocin) bind to their receptor and generate IP_3, a channel is formed by combination of the STIM1-ORAI1 proteins, as occurs in most other cell types. This is known as store-operated calcium (SOC) entry. The SOC channel is a cationic channel and a mechanism for Ca^{2+} entry. Transient receptor potential canonical (TRPC) proteins are a family of seven (TRPC1–7) nonselective cation channels. TRPC1, TRPC3, TRPC4, TRPC5, and TRPC6 channels are expressed in smooth muscle cells, including the myometrium. These channels were thought to be the basis of SOC entry, but this is no longer considered to be the case (see, e.g., 11). A discussion of SOC and TRPC channels is beyond the scope of this review; further details on the myometrial TRPC channels can be found elsewhere (12–14). Unlike vascular smooth muscle, there is little evidence for Ca-sensitization, i.e., force increasing without a change in intracellular [Ca^{2+}] in myometrium.

Challenges to Our Understanding of Excitation

Excitation passes from cell to cell by current flow through low-resistance gap junctions (nexuses) (15, 16). As the number and permeability of gap junctions increase close to term, the speed of propagation also increases. The main gap junction protein in the myometrium is connexin 43 (Cx43). The number and size of myocytes, ion channels, and gap junctions they express are all under physiological control and subject to gestational (17) and developmental (18) state.

Accounts of excitation-contraction coupling in the myometrium become less convincing when more detailed questions are asked. How is electrical activity initiated, and what constitutes the uterine pacemaker? How does gestation change ion channel expression and control? These questions are of key importance as the myometrium transitions from quiescence to a highly contractile state, firing action potentials due to a depolarized membrane potential.

Pacemaking and Electrical Signaling

The myometrium is myogenic but does not have an anatomical pacemaker (19). The question is, how do millions of myocytes coordinate and produce a laboring uterine contraction?

No pacemaker cell type identified. If there is no fixed pacemaking site, then perhaps modified myocytes or cells like the classical gut pacemaker, interstitial cells of Cajal, exist throughout the uterus? Some uterine myocytes have atypical shapes, especially due to the long thin projections first described by Kao and colleagues (20). Some have named these teleocytes. To date, none of these cells from the uterus has been directly shown to be capable of firing action potentials, and our study showed they produce hyperpolarization (21). In cultured cells from human myometrium, T-type Ca channels (see below) have been found on teleocytes, potentially providing a network of cells contributing to depolarization (22). Further work is required to see whether these channels are also present in freshly isolated tissue and, importantly, whether they can contribute under physiological conditions.

One novel suggestion is that we are looking in the wrong place for the pacemaker. Vink (2) has hypothesized that the cervical myocytes react to stretch and pass electrical signals to the myocytes in the uterus, via gap junctions, but this has not been directly investigated.

Individual myocytes express different ion channels. Because cells are coupled by gap junctions, which increase in number and conductance at the end of gestation, then connecting a depolarizing pacemaking myocyte to neighboring cells could lead to pacemaking and coordinated contractions. This assumes some minimum number of such pacemakers and that propagation can spread a

reasonable distance before attenuation (23). A subset of myocytes expressing a modified set of ion channels, leading to unstable, pacemaking membrane potentials, would fulfill such a model. In support of this, some freshly isolated myometrial cells spontaneously fire action potentials, while only certain myocytes possess Ca-activated Cl^- (24) or fast Na^+ channels (25); both are capable of depolarizing the cell.

Membrane potential and action potentials in myometrium. Pioneering electrophysiological studies showed slow waves (26) or gradual membrane (pacemaking) depolarizations (27) that trigger action potentials, leading to a spontaneous contraction (28). In myometrium, the challenge is to understand the molecular identity of the underlying ion channels and how their currents lead to contractions. Insights into which currents may do this are discussed below. The resting potential of uterine myocytes depolarizes from about −60 mV in a nonpregnant state to −45 mV at term (27, 29). Action potential characteristics also change. In early pregnancy they are simple spikes, carried by Ca^{2+} on the upstroke and K^+ currents during repolarization. This gradually shifts to complex plateau-type action potentials, presumably reflecting increases and prolongation of Ca^{2+} entry and expression of different K^+ channels.

Mapping uterine electrical signals. Another way to find possible pacemakers is to map electrical activity. In a variety of species, electromyographic (EMG) activity shows a chaotic pattern, originating at multiple sites, consistent with there being no anatomical pacemaker (30–32). Similarly, Ca signals also initiate and spread in a random manner (33).

In rat myometrium, contractile activity is more often found to initiate at the ovarian end of the uterine tubes and electrical activity in zones close to implantation or placental sites (34). In contrast, also in the rat, earlier electrophysiological studies concluded that the activity in the placental region was markedly suppressed until term (35). Perhaps the approaches such as those recently published by Lutton et al. (36), which integrated histological and electrophysiological data to make a reconstruction of rat myometrium, and more refined technology, such as using the magnetic field associated with the myometrial electrical activity, will lead to insights into the initiating sites of myometrial electrical activity. These, combined with clinical biomarkers for preterm birth, such as fibronectin, should increase its utility as a predictor of risk of preterm delivery (37, 38).

ION CHANNELS IN THE MYOMETRIUM

Uterine excitability depends on the ion channels expressed on the myocytes. Pioneering microelectrode and sucrose gap work provided an understanding of the changes in membrane potential with contraction. Then patch-clamp studies and pharmacology allowed us to characterize the ion channels underlying the electrical changes. Now gene studies have revealed the molecular identity of the channels, and we can model their structure and relate this to function. Our review of these channels focuses on their physiological roles and changes with gestation and briefly gives their encoding genes and structure. **Figure 2** provides an overview of these channels. The reader is also referred to the modeling paper of Atia et al. (39) for a list of ion channels and their relation to uterine excitability.

Calcium Channels

An increase in intracellular $[Ca^{2+}]$ is the crucial link between excitation and contraction in uterine myocytes. This arises due to changes in the plasma membrane's permeability to Ca^{2+}, brought about by the opening of Ca channels.

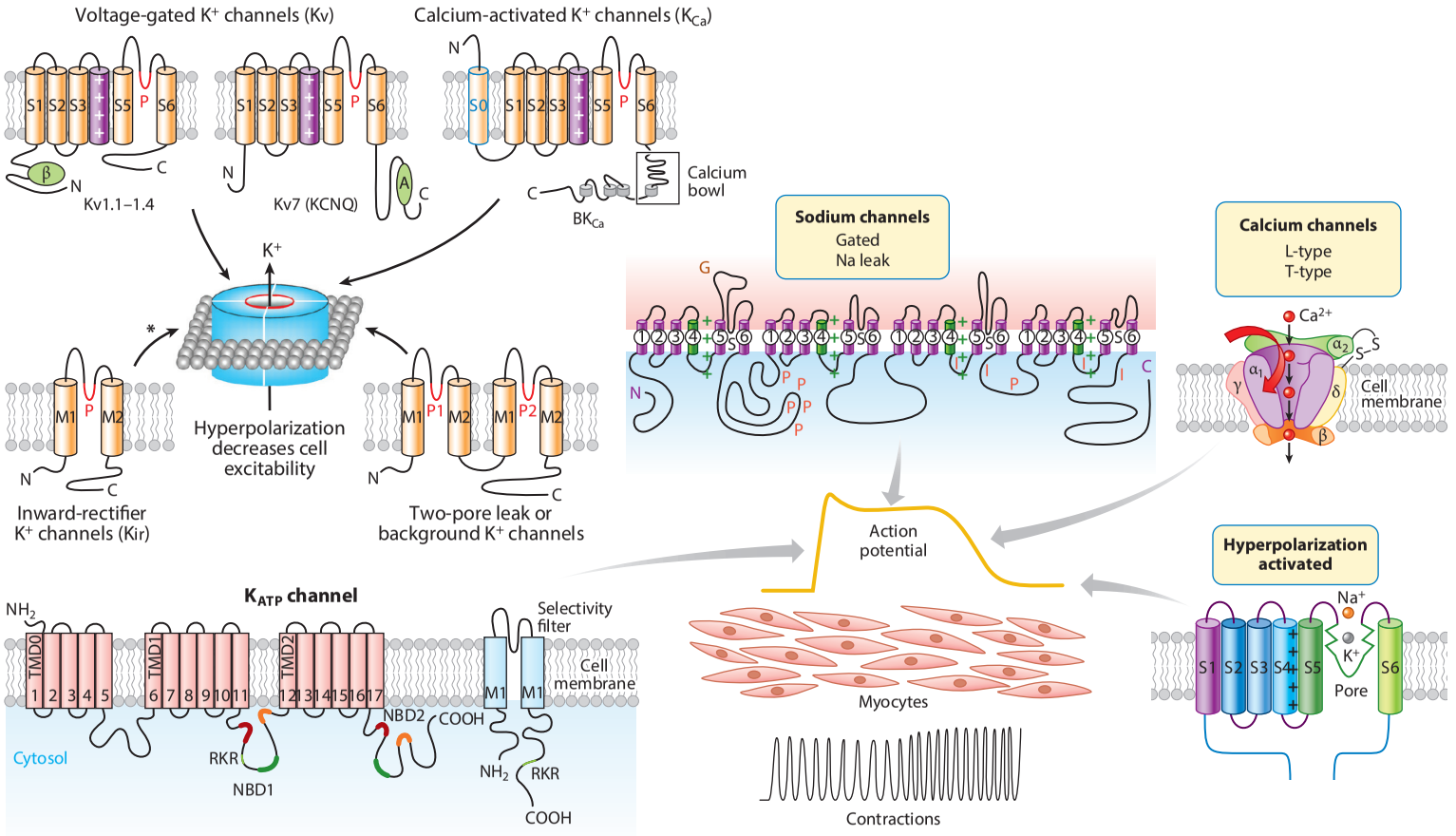

Figure 2

Ion channels in uterine myocytes. The channels underlie excitability, action potentials, and contractility. Abbreviations: NBD2, nucleotide-binding domain 2; TMD, transmembrane domain.

L-type Ca^{2+} channels. The dominant inward current in myometrium arises from the opening of high-voltage-activated, long-lasting, L-type Ca^{2+} channels. These channels are composed of a pore-forming α1 subunit, which is organized in 4 repeated domains, each of which contains 6 transmembrane segments, with the pore loop between S5 and S6 (40). The α1 subunit is sufficient to produce functional channels, as it has calcium selectivity and the voltage sensor, as well as regulatory binding sites. Without its auxiliary subunits, however, expression is low and channel kinetics and voltage dependence are abnormal. A review summarizing the function of the auxiliary β, ϒ, and α/ḋ subunits in anchorage, trafficking, and regulatory functions should be consulted for more details on this topic (40). The α1 pore of the channel can arise from isoforms $Ca_V1.1$ to $Ca_V1.4$, with myometrium expressing $Ca_V1.2$, encoded by *CACNA1C*. The channel is opened by depolarization, with an activation threshold of −55 to −50 mV. When opened, the channel conducts a robust inward current that can be of the order of nanoamperes (41). Inward current can be increased by increasing the number of L-type channels on the myometrial membrane and is modulated by cyclic nucleotides, gaseotransmitters (42), steroid hormones (discussed below), and metabolic inhibition (43).

Our group was the first to directly show the changes in $[Ca^{2+}]$ with channel opening and the resulting calcium transients (41). The L-type channel blocker nifedipine abolishes Ca^{2+} transients and phasic contractions (44); agonists, such as BayK8644, stimulate both (45). Cytoplasmic calcium binding to the channel inactivates it, especially at depolarized potentials (46), thereby contributing to uterine relaxation.

Frustratingly, there is no direct evidence concerning channel expression and activity around term in human myometrium, and animal data are sparse and contradictory, which should be addressed (47–50). Hormonal effects on mRNA levels of the myometrial α1 and β subunits have been examined and are discussed in the final section of this review. Both increased in rats treated with antiprogesterone drugs, and the β subunit, but not the α1 subunit, increased at term (48). Tezuka et al. (51) showed a gradual increase in the α1 subunit through gestation, which reversed at labor, whereas they found that the β subunits markedly increased at parturition. In the guinea pig, increased mRNA expression of the α1 and β subunits occurred as term was approaching, but this was not mirrored in protein levels.

T-type Ca^{2+} channels. T-type, low-voltage-activating Ca^{2+} channels are expressed in myometrium (52). There are differences in the molecular structure of the α1 subunits: $Ca_V3.1$, $Ca_V3.2$, and $Ca_V3.3$. In human myometrium, Blanks et al. (53) identified $Ca_V3.1$ T-type currents; nickel blocked these currents and slowed frequency. Unlike L-type channels, T-type channels are not essential for Ca^{2+} signals and contraction in myometrium, presumably because they are mostly inactive at resting membrane potentials (53, 54). Blanks et al. found no gestational changes in channel expression. In rat myometrium, changes in channel isoforms at term were noted (55).

Potassium Channels

The myometrium expresses many different K^+ channels. These can be gated by ions (Ca^{2+}, Na^+), metabolites (ATP, pH), stretch, and voltage. In addition, K^+ channels are modulated by many factors, but notably for the myometrium, by hormones. Change in the expression and regulation of K^+ channels, including their ancillary subunits (described below), is an effective way to modulate contractility, and their activity at term is often found to be decreased, as the uterus shifts from being quiescent to contractile. If K^+ channels could be targeted to remain active, then this could have potential applications for treating threatened preterm labors. If, however, these channels

are not present near term, then this strategy will be problematic. Nevertheless, given the toll of preterm birth, it makes sense to have a complete as possible understanding of their role in human myometrium (56).

K_{ATP} channels: the Kir6 family. ATP-sensitive potassium (K_{ATP}) channels are composed of sulfonylurea receptors (SURs), which convey selectivity for ATP and drugs, and K^+ inward rectifiers (Kirs; other Kirs are discussed below) that assemble to form large octameric channels. The K_{ATP} channels open when [ATP] falls and are modulated by [ADP] and pH; they are therefore well placed to sense metabolic changes and convert them into protective excitability changes. They are abundant in myometrium (57). Studies on animal and human myometrium (58, 59) suggest that Kir6.1 and 6.2 and SUR1 and 2B form functional K_{ATP} channels. Expression of K_{ATP} channels decreases at term in animal and human myometrium (60, 61). Du et al. (62) reported upregulation of SUR2B/Kir6.1 as women age and suggested that changes in expression levels of SURs and Kirs may contribute to more difficult labors in first-time, older mothers.

Given the ATP dependence of these channels, they are unlikely to be active during resting conditions or contribute to resting membrane potential. Glibenclamide, an antagonist of K_{ATP} channels, has little effect on spontaneous, oxytocin-stimulated, or in vivo contractions (63). In pregnant human myometrium, K_{ATP} channel openers inhibit spontaneous and oxytocin-stimulated contractility (50) but less potently in tissue from women in labor than those not in labor (61). Xu et al. (61) also found differences in subunit expression between the groups, with Kir6 units being less abundant in labor, SUR2B unchanged, and SUR1 increased. It is worth noting that these channels may also be important in the uterine arteries as they become compressed and ischemic as labor progresses.

When, however, there is a metabolic stress in the myocyte, such as ischemia in labor, [ATP] falls, [ADP] and [H^+] rise (64), and the channels open. We tested this in rat myometrium, using cyanide to reduce [ATP]. The decrease of contractility produced was accompanied by increased K^+ (^{86}Rb) efflux (65). The effects of force on other metabolic changes, such as acidification, were excluded (66). About 50% of the increase of K^+ efflux occurred through K_{ATP} channels, and the expected hyperpolarization would contribute to the fall in force. In studies on human myometrium, Modzelewska et al. (67) suggested that nitric oxide reduces myometrial contractility by directly activating K_{ATP} channels, and glibenclamide reduced the effects of H_2S in contractions, suggesting the channels are a target (42, 68).

Overview of voltage-dependent K channels. There are 12 families of voltage-dependent K channels, referred to as Kv1–Kv12 (69). Four α subunits, each with six transmembrane segments, form the pore with the voltage sensor in segment 4. There are a variety of auxillary β subunits that modify channel properties. In myometrium, Kv4, Kv7, and Kv11 have been detected and there is no doubt concerning their importance (70, 71).

Voltage-gated Kv4 channels. Kv4.3 is encoded by the *KCND3* gene. The four α subunits form the pore, which is modified by subunits, including Kv channel–interacting proteins (KChIPs), a family of cytosolic proteins that modify the ḍ pore-forming subunits; e.g., coexpression with Kv4 channels leads to higher current density, modulation of channel inactivation, and faster recovery from inactivation (72). Members of this rapidly inactivating K^+ channel play a prominent role in action potential repolarization in nerves and heart. Consequently, work in myometrium has focused on this Kv4 family member.

Fast transient outward currents were reported in nonpregnant rats (73). The molecular identities of the channels were reported later, with Suzuki & Takimoto (74) finding all three Kv4

channels and KChIPs expressed and regulated throughout pregnancy in the rat. Others have also found expression of Kv4.3 and that it is downregulated by estrogen (75) and at term (70, 76); increases in KChIP mRNA were also detected. Suzuki and Takimoto showed that Kv4.3–KChIP4 channel complexes are the major form of the rapidly inactivating channels in nonpregnant animals but are replaced with Kv4.2–KChIP2 complexes during pregnancy (74). There appear to be few functional studies of Kv4 modulation and contractility, in part perhaps owing to the lack of specific inhibitors. Low concentrations (<1 mM) of 4-amino pyridine (4-AP) can be used to block these channels, although other Kv channels will be affected (77). Another study (70) found expression of Kv4.3 and other Kv channels in mouse myometrium but only found gestational changes for Kv4.3. The study showed that both expression of Kv4.3 α subunit protein and contractile responses to 4-AP and phrixotoxin-2, a selective blocker of Kv4.2/4.3 channels (78), are seen in nonpregnant and early pregnant myometrium but disappear in late-pregnant mouse myometrium. The authors therefore concluded that there is a role for Kv4.3 channels in the regulation of myometrial function, and additionally, that a loss of Kv4.3 channel function occurs in preparation for labor. Aaronson et al. (71), in their study comparing functional effects of Ca- or voltage-activated K^+ channels, found no evidence for the former (discussed in more detail below) but an important role for the latter. Furthermore, these voltage-dependent channels had the electrophysiological profile of Kv4.3 channels. Collectively, the data suggest that Kv4.3 channels are active at rest, contribute to resting membrane potential, and are important for contractility in myometrium.

Voltage-dependent Kv7 channels. There are five Kv7 α subunits, encoded by genes KCNQ1–5. The channels are modified by β-accessory proteins, encoded by KCNE genes 1–5. These channels are voltage-gated, slowly activating near resting potential, and produce noninactivating currents. The voltage-sensitive K^+ current, discovered in 2006, regulates spontaneous contractions of rat myometrium (71) and has the characteristics of Kv7 channels.

Kv7 channels have been identified in a variety of smooth muscles (79), including mouse and human myometrium. Human myometrium expresses KCNQ1–4, and mice express all five KCNQs (80). All 5 accessory proteins KCNE1–5 have been found in mice. Expression of KCNEs appears to have been little studied in human myometrium, with only KCNE2 having been reported (81). In mouse myometrium, Kv7.1 was the most abundantly expressed, followed by Kv7.5 and Kv7.3. The expression of the channels and subunits changed during gestation: Kv7.3 dominated in early pregnancy, and Kv7.1, Kv7.4, and Kv7.5 in late pregnancy; KCNE2 increased toward term, while KCNE3 and KCNE5 were downregulated at this time, and KCNE4 was highly expressed throughout gestation (82). When preterm labor was induced in mice, there was a generalized suppression of most of the KCNQ and KCNE isoforms (82). In human myometrium Kv7.1 is also most abundantly expressed, followed by Kv7.4.

In contrast to expression studies, establishment of functional roles for the different Kv7 channels in myometrial and other smooth muscles has not been straightforward and has depended upon a pharmacological approach (56). Kv7 channel activators retigabine and flupirtine decreased contractility and frequency of contractions, especially close to term, in mice. However, although the Kv7 inhibitor XE991 increased contractility, the specific Kv7.1 inhibitor chromanol 293B had no effect on frequency or amplitude of mouse myometrial contractions (82), indicating that Kv7 isoforms other than Kv7.1 functionally predominate, despite their expression profile. Using chromanol 293B, researchers have drawn similar conclusions for human vascular myocytes (83), suggesting that Kv7.1 homotetramers have no function in vascular myocytes. Functional data on human myometrium are limited to three samples of nonlaboring tissue (82), and the conclusions are similar to those obtained in mice, despite the different expression profiles. XE991 increased the frequency of spontaneous myometrial contractions but decreased their amplitude,

and retigabine decreased contractility (82). The effects of the specific Kv7.1 inhibitor chromanol 293B do not appear to have been investigated.

The reason for the puzzling finding concerning Kv7.1 could be because, in myometrium and other smooth muscle, over 90% of the Kv7.1 channels are retained in the endoplasmic reticulum. For further discussion about whether Kv7.1 is functional or not in smooth muscle, see Reference 79. Work by Oliveras et al. (84) has proposed that functional Kv7.1 channels only appear when they make a heterotetrameric channel with Kv7.5. This is an interesting area for further research in the myometrium: to determine Kv7.1 channel localization, identify the roles of homo- and heterotetrameric channels, and explore other mechanisms of specifically inhibiting the channel isoforms, perhaps using the approaches to study the inward rectifier (85). That these channels are important for myometrial physiology is strengthened by two further findings. First, in a mouse model of preterm delivery, KCNQ and KCNE isoform expressions were slightly downregulated, although gene expression data suggested that mRNA levels remained high, and functional Kv7 channels were likely to still be expressed. Second, incorporation of these channels into models of myometrial excitability greatly improved the resemblance of the computed action potential to experimental data (86).

Voltage-dependent Kv11.1 channels. Recent work has suggested that these channels are functionally important in myometrium, perhaps more so than the Kv7 channels (87). Mammalian ether-à-go-go (EAG) channels are inwardly rectifying, voltage-gated K^+ channels, encoded by the *KCNH* gene family and their ancillary subunits by KCNEs. There are three members of the EAG family, and one of these, called EAG-related genes (ERGs; Kv11), is found in smooth muscle. There are three ERGs, 1–3, encoded by KCNHs 2, 6, and 7, which produce Kv11.1, Kv11.2, and Kv11.3 channels. There is a splice variant of ERG1, giving ERG1a and ERG1b (88), which has been found in mouse myometrium. All of the ERGs produce low-threshold K^+ currents that regulate excitability. The history of their discovery and details of their kinetics are described elsewhere (88). The most notable finding was by Shibasaki (89), who described an initial "hook" during deactivation current recordings. This hook arises because, upon depolarization, the open channel state features a rapid voltage-dependent inactivation. With hyperpolarization, the inactivation is quickly removed and current increases. This is the hook, and it occurs while the channel is still open before the channel deactivates. The characteristics of ERG currents in native smooth muscle cells under physiological conditions were first described by Yeung & Greenwood (90). As in other tissues, the channel state transitions produce distinctive kinetics but have a particularly accelerated rate of deactivation in smooth muscle. In smooth muscle Kv11.1, channels contribute to maintaining resting potential, with an activation threshold of −60 mV, and suppress increases in membrane excitability.

Kv11.1 (but not Kv11.2 or Kv11.3) has been found in mouse myometrium, with unchanged expression level throughout gestation but decreased function at term (76). The ERG1a form was much more abundant than ERG1b. Blockade of the channels increased spontaneous and oxytocin-stimulated contractions, but this, and the inhibitory effects of channel openers, was lost near term. Interestingly, in labor Kv11.1 channels were still expressed, and the abundance of mERG1a and mERG1b transcripts was unchanged during gestation, but the channels were rendered nonfunctional, presumably due to increased expression of KCNE2 and KCNE4, especially KCNE2. This led the authors to note "fine-tuning of uterine activity in preparation for labor may rely on alterations in the expression of ion channel auxiliary proteins" (76, p. 2322). It has been suggested that microRNA expression could also account for the reduced role of the Kv11.1 channels at term, as work in cardiac tissue has shown that microRNAs are potent effectors of the protein expression of these channels, independent of transcript levels (88). ERG activity suppressed contraction

when studied in pregnant nonlaboring human myometrium, but during labor, due to KCNE2's increased expression, ERG activity decreased. This was associated with action potentials shifting from bursts to plateau type, presumably contributing to the electrophysiological mechanism producing strong contractions during labor (81).

Parkington et al. (81) also found that obese women have elevated ERG and increased K^+ conductance attributed to a lower expression of KCNE2. An increased K^+ conductance in women with high body mass index (BMI) is consistent with poor labors and increased risk of Caesarean section in obese women (91). These may result from the fact that cholesterol, which is often elevated in obese women, increases outward K^+ current (92).

External acidification inhibits activation of the Kv11.1 channel through EAG-specific residues in the voltage sensor; these acidic residues are at positions 5 and 6 in S2 and S3 and are unique to the EAG superfamily among K^+ channels (93). This and the contribution of acid sensitive TASK-2 K^+ channels (see below) are of particular interest, as extracellular acidification occurs in labor (94) and can transiently increase contractility (95). Thus, Kv11.1 inhibition by acid may be part of the mechanism, causing depolarization and increased contractility in labor.

Two-pore domain K^+ channels: *KCNK*. Two-pore domain K^+ (K2P) channels constitute a widely distributed channel family, coded for by *KCNK* genes (69). They are active as dimers and consequently have two ionic pores; they rapidly activate and are noninactivating and active at all membrane potentials. These properties make them candidates for the voltage-insensitive background K^+ leak currents, which stabilize membrane potential. Particularly exciting is their sensitivity to a range of physiological factors, including pH, oxygen, phospholipids, neurotransmitters, and stretch. Human and mouse myometrium express TASK-1 and -2 and TREK-1 K2P channels.

TASK channels. The TASK family members are encoded by *KCNK3* and *KCNK9* and are of interest in myometrium, as their conductance is decreased by extracellular acidification (96) and hypoxia. Early array screens found little evidence for TASKs in human myometrium (97, 98). In a subsequent study, specifically on pregnant human myometrium, Bai et al. (99) identified TASKs 1, 4, and 5 at mRNA level but only TASK-1 at protein level. The same study also showed that the myometrial distribution of TASK-1 was predominantly intracellular.

There have been few functional studies of TASK channels despite their interesting regulation, but two studies have now highlighted the role of TASK-2 channels in myometrium. Working on mouse circular myometrium, Hong et al. (100) showed (*a*) increased TASK-2 staining in pregnant compared to nonpregnant myometrium, (*b*) stimulation of contraction by TASK-2 inhibitors (in the presence of blocker of stretch-activated channels), (*c*) depolarization and spontaneous action potentials with TASK-2 inhibitors, and (*d*) increased contractility with acidic pH. A later study (101) found largely similar findings in longitudinal myometrium but added data showing that estrogen inhibited the channels. These studies illustrate a potential, but unexplored, importance of TASK-2 channels in human myometrium; further investigation will benefit from simultaneous measurements of pH and contractility (102).

TREK channels. TREK K2P channels are mechanosensitive (as well as voltage dependent) and therefore of special interest for myometrial physiology, as stretch influences contractions. TREK-1 (*KCNK2*) is expressed in human myometrium (99), upregulated during pregnancy, and downregulated in labor. Furthermore, TREK-1 channel antagonism increases contraction frequency (103). Another study concluded that prolonged stretch increases expression and activity of TREK-1 channels in myometrium from pregnant women and that TREK-1 expression is increased at term. In twin pregnancies, expression of TREK appeared increased and the effect of

blocker augmented, compared to singletons. Furthermore, the TREK antagonist L-methionine reversed the decrease of contraction produced by stretch, arguing that TREK has an important role in maintaining quiescence (104). There do not appear to have been any functional studies of TREK-1.

Inward rectifiers. Along with some members of the two-pore K^+ channel family, Kirs contribute to setting and restabilizing the resting membrane potential. As the name implies, these are unusual K channels, as their conductance for K^+ entry is greater than for exit. Seven families of Kir have been described (69). They consist of four subunits forming the α ionic pore and are regulated by phosphatidylinositol 4, 5-bisphosphate (PIP_2). At positive potentials the channels are blocked by spermine. They are encoded for by multiple *KCNJ* genes.

In an impressive study, McCloskey et al. (85) showed that Kir7.1 is expressed in mouse and human myometrium. Transcript levels increased in pregnant mouse uterus until mid-gestation and then declined toward term. In women, transcript levels were significantly lower in pregnant women in labor than those not in labor. To assess Kir7.1's role in regulating uterine contractility, lentiviral vectors were used to alter the expression of the Kir7.1 channel in murine myometrial smooth muscle, both in vitro and in vivo. Knockdown of Kir7.1 in vitro significantly increased contractility. The selective Kir7.1 inhibitor VU590 also increased excitability and force. The authors also showed that overexpression of Kir7.1 significantly decreased contractions. Computer modeling showed how Kir7.1 contributes to key stages of repolarization and the shape of the action potential, suggesting the channel could be a therapeutic target. However, another, more limited study using VU590 on nonlaboring term human myometrium only found short-lived increases in contractility, with or without oxytocin (105). This led the authors to question the usefulness of the channels as therapeutic targets. It is hard to reconcile these differing data. In addition, a structurally unrelated (to VU590) Kir7.1 antagonist, MRT2000769, greatly increased contractility when tested in mouse myometrium in the study by McCloskey et al. (85). We could not determine whether the human contractility data showed by them came from laboring or nonlaboring samples, or whether differences in patient demographics between the groups could account for the differences found. Clearly, these important studies should be repeated by others to help improve our understanding of this channel in the myometrium.

Calcium-activated K^+ channels: the Slo1/ KCNMA family. The myometrium expresses large-, intermediate-, and small-conductance Ca-activated K^+ channels, formerly designated BK, IK, and SK, respectively (71, 106, 107). They have six (IK and SK) or 7 (BK) transmembrane spans and a pore loop between segments S5 and S6 (as do the voltage-gated channels).

K_{Ca}1.1: large-conductance (BK) channels. The best-studied Ca-activated K^+ channel is BK, also known as maxiK, Slo, and K_{Ca}1.1, the product of the *KCNMA1* (*hSlo*) gene. It senses both $[Ca^{2+}]_i$ and membrane potential, has a large single-channel conductance of 100–300 pS, and forms a channel from four α subunits. Splice variants and β subunits modify its gating properties, leading to different Ca^{2+} sensitivities (69). In neuronal and vascular smooth muscle, BK channels provide a negative feedback on excitability, countering the excitability produced by Ca^{2+} entry (108). While these channels are expressed in animal and human myometrium (107), most electrophysiological, pharmacological, and functional studies have failed to demonstrate a role for them in myometrial excitability (109). Thus, unlike vascular smooth muscles (110), Ca^{2+} sparks released from the SR do not activate Ca-activated K^+ channels in myometrium; indeed, the uterus has nonfunctional ryanodine receptors and cannot produce sparks (8). Blockers of these K_{Ca}1.1 channels, such as the specific compound iberiotoxin, have little or no effect on uterine contractions. Use of an opener

of the channel, NS1619, did not significantly affect the contractile activity of human term pregnant myometrium (111). Electrophysiological studies have also concluded that voltage-gated, but not Ca-gated, K^+ channels regulate excitability and contractility in myometrium (70, 71). The characteristics of the functionally important channels were consistent with Kv4.3 channels.

The BK channel is, however, coupled to agonists, e.g., oxytocin and β2-adrenergic receptors, adding to their tocolytic efficacy (112). It has a microdomain localization and complex intracellular trafficking (92, 113, 114). It receives inputs from, and can be modified by, a vast number of signaling entities and pathways, which, as along with steroid hormones (discussed below), include haem binding, gaseotransmitters, redox and reactive oxygen species (ROS), cyclic nucleotides and their protein kinases, Src tyrosine kinase, fatty acids and lipids such as PIP_2, cholesterol and omega-3 fatty acids, and arachidonic acid metabolites. All of these suggest that BK channels may function more as signaling hubs, sensing local Ca^{2+} and voltage, but also myriad other inputs (115). One recent study perhaps provides an example to support this suggestion. The BK channel has been found to modulate NF-κB translocation in the nucleus of an immortalized human myometrial cell line (hTERT). The NF-κB pathway stimulates inflammatory genes and cyclooxygenase-2, resulting in the production of cytokines and prostaglandins, respectively (116). The mechanism by which inhibition of the BK channel induces NF-κB translocation and synergizes with inflammatory mechanisms to retain NF-κB in the nucleus is unknown, but this is certainly an exciting area for further research.

Small-conductance channels. The distribution, expression, and functional roles of small-conductance, Ca^{2+}-activated K^+ (SK) channels have been determined in rat and mouse myometrium (106, 117, 118). Three isoforms (SK1–3; $K_{Ca}2.1$–2.3) are produced by three different genes (*KCNN1–3*). All are voltage independent and have a conductance of ~10 pS. In contrast to $K_{Ca}1.1$, which is directly activated by $[Ca^{2+}]_i$, activation of SK channels involves calmodulin. As with the $K_{Ca}1.1$ channel, splice variants are found and can lead to nonfunctional channels (69). Consistent expression of all $K_{Ca}2$ isoform transcripts and clear immunostaining of SK1–3 were found in the rat myometrium at three different gestational stages and at unchanged levels at labor. In mouse, one study reported expression of SK3 channels to be decreased from mid-gestation onward (118), whereas a later study found expression of $K_{Ca}2.2$ and $K_{Ca}2.3$ in approximately equal amounts in nonpregnant tissue, and then $K_{Ca}2.3$ decreasing and $K_{Ca}2.2$ increasing in pregnancy. Immunofluorescence labeling of intact strips revealed that $K_{Ca}2.2$ was diffusely distributed within the cytosol, whereas $K_{Ca}2.3$ was focused at the plasma membrane, and these distributions did not change with gestation (117). Inhibition of SK channels with apamin reduced the outward current and induced some quiescent cells to start firing action potentials (106). This suggests that at least a proportion of SK channels is activated by resting $[Ca^{2+}]$ and contributes to the resting membrane potential and Ca^{2+} signaling. Also in mice, an activator of $K_{Ca}2.2$ and $K_{Ca}2.3$, CyPPA, which increases their calcium sensitivity, was found to inhibit contractions, and in a model of preterm birth, it delayed preterm delivery by several hours (117). As discussed below, estradiol lowers mRNA for SK3 in rat myometrium (119, 120).

Functional studies showed that, in contrast to the lack of effect of inhibitors of BK channels, the SK channel inhibitors apamin and scyllatoxin increased myometrial contractions, and as demonstrated by Noble et al. (106), this was accompanied by an increase in Ca transients in pregnant rat myometrium. Similar conclusions, i.e., that SK channels limit Ca influx, were drawn from a study of $K_{Ca}2.3$ channels in mice. Of particular interest, in vivo work on mice showed that overexpressing $K_{Ca}2.3$ resulted in difficult labor and fetal demise (121, 122), but it protected against preterm labor (118).

Intermediate-conductance channels. The IK channels, $K_{Ca}3.1$, are encoded by KCNN4 and have a conductance of 20–80 pS (note that some nomenclatures have IK as being SK4). These channels have a high affinity for Ca^{2+} that, like SK, must bind to calmodulin for activation. They are mainly found in nonexcitable cells, and little work has been conducted in myometrium. Using measurements of mRNA by real-time PCR, $K_{Ca}3.1$ was weakly detected in myometrium from two patients with fibroids (123). Application of TRAM34, a blocker of IK channels, had no functional effects on contracting human pregnant or nonpregnant myometrium (124).

Sodium-activated K (K_{Na}) Slo2 channels. The reasons why Ca rather than other cations was selected as the preeminent ion for cell signaling are beyond the scope of this review (125). We make the point here only to stress how unusual it is to have Na^+ ions as messengers. Potassium channels gated by Na^+ were first described in cardiac myocytes in the 1980s. In myometrium, the first and only account of them appears to be that of Ferreira et al. in 2019 (126). They found that Slo2.1 (KCNT) is expressed in human myometrium and that the channel is open at resting membrane potentials, with a high K^+ conductance, suggesting that it can contribute to maintaining resting membrane potential. They also found that oxytocin can inhibit the Slo2 channels and cause depolarization (discussed below). It would be interesting to know whether these Slo2 channels are in microdomains adjacent to clusters of Na_V channels or Na-Ca exchangers, providing a mechanism for a local rise of $[Na^+]$ to regulate them. In neuronal preparations there is evidence that the Na_V channel is inhibited by ATP and has a binding site for NAD^+ (69), leading us to speculate that they may constitute a hitherto unknown mechanism in myometrium, linking metabolism to excitability.

Sodium Channels

Despite some early reports of Na^+ currents in uterine myocytes, once calcium was identified as carrying the inward depolarizing current and upstroke of the action potential, interest in sodium waned. In addition, the advent of fluorescent indicators sensitive to intracellular $[Ca^{2+}]$ led to an explosion of interest in Ca signaling. This contrasts with the problems of developing and using Na-sensitive indicators (127). Recently, there has been fresh interest in Na^+ channels, and we now reassess this field. There is no evidence for ligand-gated (e.g., ACh) Na^+ channels in myometrium.

Voltage-gated (fast) sodium channels (Na_V). These channels show much less diversity than other cation-gated ones. They are designated Na_V1 (or SCNs), as there is only one gene family (SCN) and nine isoforms, $Na_V1.1$–1.9. The channel consists of one α pore subunit (SCN) and one or more nonessential β subunits (SCN1–4B). A molecular characterization of Na_Vs in nonpregnant rat myometrium found four α subunits—Scn2a1, Scn3a, Scn5a, and Scn8a—in the cDNA from longitudinal muscle, the mRNAs of the β subunits Scbn1b, Scbn2b, and Scbn4b, and traces of Scn3b (128).

Early electrophysiological and molecular studies of uterine myocytes indicated that some cells possessed fast Na^+ channels, particularly near term (129–131). The channels have been recorded from isolated myometrial smooth muscle (128, 130), and the density of these currents increases in late pregnancy. We still lack a full description of how Na_V contributes to functional activity. One reason why these channels had been discounted was that blocking Na_V channels with tetrodotoxin did not prevent inward currents from developing or contractions in myocytes (132). (It should be noted that Na^+ leak channels, described below, are not sensitive to tetrodotoxin.) These negative findings are perhaps counterbalanced by arguments that there is a proportion of myocytes expressing Na_V channels (128, 133), which can aid excitability and possibly pacemaking, as championed by Kao et al. (134). In addition, removal of Na^+ prevents the regenerative bursting

activity in animal models (135). In nonpregnant myometrium Na_V agonists could initiate and maintain phasic contractions (128). In cardiac myocytes acidosis reduces the channel's conductance, but this has not been studied in myometrium. In summary, even in 2020, we struggle to properly understand the role of Na_V channels in myometrium (136).

Sodium leak channels: NALCN. A third member of the four-domain ion channel family, after voltage-gated Ca^{2+} channels and voltage-gated Na^+ channels, is the resting sodium conductance family. So far there is only one mammalian member of this family, the Na^+ leak channel, nonselective (NALCN). In 2007, Lu et al. (137) were able to show, for the first time, that the NALCN channel encodes a constitutively activated, nonselective cation channel. This is the long-hypothesized background Na^+ "leak" conductance. Unlike the other two members of this family, the NALCN current is voltage insensitive and resistant to both tetrodotoxin and Cs^+ but is blocked by Gd^{3+}. It can conduct Na^+, Ca^{2+}, K^+, and Cs^+. There are several alternatively spliced isoforms, but their functions remain unclear (138). NALCN is associated in a complex that can include G protein–coupled receptors, UNC-79, UNC-80, NLF-1, and the Src family of tyrosine kinases (139).

NALCN has been well documented in nerves, but not smooth muscle. However, in 2014, RNA sequencing data from human myometrial tissue revealed the expression of NALCN (133). This Na^+ leak current strongly resembles that described by Miyoshi et al. in 2004 in pregnant rat myometrium (140). More recently, its role in myometrium has been further explored with an investigation of how it might contribute to electrogenesis (141). This showed that NALCN contributes ~50% of the Na^+ leak in uterine myocytes from nonlaboring women (142). England's group (141) proceeded to demonstrate that mice lacking NALCN in smooth muscle had cells with shorter burst durations and fewer spikes than wild-type controls and suffered dysfunctional labors. It therefore seems reasonable to add NALCN as a contributor to both myometrial excitability and labor outcome, with studies on human myometrium required.

Na K-ATPase. The Na K-ATPase (Na pump) plays a pivotal role in setting myometrial ionic gradients, specifically that of K^+, which is the main contributor to resting membrane potential. The Na K-ATPase subunits and ancillary proteins (FYXD) change throughout gestation (143). All α and β subunit isoforms (1, 2, 3) and FXYD1 were detected, but FXYD2 was absent at all gestational stages. These developmental changes in isoforms allow Na K-ATPase function to be tightly regulated, as they control sensitivity to ions and inhibitors (144). These changes in expression correlated with increased functional sensitivity to ouabain and parallel changes in intracellular Ca^{2+}. An interesting study on human myometrium found that after prostaglandins E_2 and $F_{2\alpha}$ had stimulated contraction, their effects were curtailed by a hyperpolarization that was due to the Na K-ATPase (145). It is not clear exactly how the Na K-ATPase is stimulated, but the authors suggest that prostaglandin activating the adenylyl cyclase cyclic AMP system may play a role, as cAMP can activate the pump, or that intracellular $[Na^+]$ rises due to increased activity of Na-H and/or Na-Ca exchangers. The authors showed a gestational sensitivity of the hyperpolarization, with ouabain-sensitive hyperpolarization decreasing at term/labor, allowing contraction frequency to increase. In epithelial cells Kir7.1 colocalizes with the Na K-ATPase, and a role in K^+ recycling has been suggested (146). We conclude that the largely overlooked changes in Na K-ATPase should be better investigated, as they may be important for successful pregnancy and delivery.

Calcium-Activated Chloride Channels

A recent review of these channels in myometrium (147) should be consulted for a fuller account. Before the molecular identity of Ca-activated Cl^- channels (CaCCs) was established (148), a

variety of studies showed that smooth muscle contractility, including myometrial, is enhanced when these channels open (24). In smooth muscle the relatively high intracellular [Cl^-] (equilibrium potential of approximately −15 mV) means that opening of these channels will cause depolarization and activate L-type Ca channels. In rat myometrial cells, CaCCs have been recorded, and inhibitors of this channel, such as niflumic acid, attenuate myometrial contractility (24). The currents were present in around one-third of cells, leading us to propose that they may represent a population of pacemaking cells.

The molecular basis of the channels is now known to be the anoctamins and bestrophins. Human and animal myometrium express anoctamin 1 (ANO1 or TMEM16A)-encoded Ca-activated Cl^- channels (148, 149) and bestrophins (150). Hyuga et al. (151) showed that three ANO1-blocking drugs decreased the frequency of contraction in human myometrial strips. However, recent data gave the puzzling finding that, with deletion of ANO1 in mouse myometrial cells, there was no effect on $[Ca^{2+}]_i$, contractions, and pregnancy. In such technically challenging techniques, it may be that the channel was not knocked out completely, or the choice of control mice (which were not wild-type) led to animals with low ANO1 levels (see 147 for a fuller critique). In modeling excitability, ANO1 activation by local Ca transients was able to generate the initial depolarizing stimulus for pacemaking, within relevant, physiological parameters (39). It is still unclear how the channels are activated given their low Ca^{2+} sensitivity. In airway and vascular smooth muscle, a link to Ca^{2+} sparks from ryanodine receptors has been proposed (see 152 for a review). However, because these are not features of myometrial cells, other Ca^{2+} sources have to be considered, such as nonspecific cation channels. It has also been found that ANO1 localizes to caveolar (lipid raft) regions of the membrane (153). Thus, it may be that ANO1 is in a microdomain, where Ca^{2+} levels are elevated sufficiently to activate it, or regulators of the channels are present. Little is known, however, of what these regulators are in smooth muscle. The fact that anoctamins share no significant sequence similarity with any other ion channels or other membrane proteins, and the lack of structure function relation, means that it may be some time before the CaCC in the myometrium and other smooth muscles is better understood.

Hyperpolarization-Activated Cation Channels

Coded for by HCN genes, hyperpolarization-activated cation currents are voltage-gated channels, but, unusually, they are activated by hyperpolarization (154). They are composed of four subunits, HCN1–4, and form homo- or heterotetramers and are activated at approximately −60 mV, close to the resting membrane potential of smooth muscle myocytes. They are the major currents contributing to pacemaking potentials in cardiac and neuronal cells; when activated, their opening allows net cation entry, including a small Ca^{2+} component, depolarization, and action potential firing. These channels are modulated by cAMP, acidic lipids, intracellular H^+, and PIP_2.

Hyperpolarization-activated currents (I_h) have been identified in isolated pregnant rat uterine myocytes (155, 156), with activation thresholds of −70 to −60 mV. Both papers reported an inhibition of the current by Cs^+ and a decrease in frequency of contractions. Little work appears to have been conducted on uterine myocytes. A recent study showed that ZD7288, a blocker of I_h, significantly reduced contractions of pregnant rat myometrium, irrespective of how it was produced (157). The reductions in force were predominantly on amplitude and not, as might be expected, on frequency. The authors point to a desynchronization of activity as the channels are blocked as a possible explanation. We can find no studies of activity or expression of these channels in human myometrium. It is also worth noting that only a small fraction of I_h will be activated at the membrane potentials of term myometrium.

HORMONAL ENVIRONMENT AND EXCITABILITY

Having considered many ion channels expressed in myometrium, we finish by presenting a physiological perspective on their integrated activity. We have selected the hormones most closely associated with parturition: oxytocin and the two steroid sex hormones, estrogen and progesterone.

Oxytocin

The nonapeptide hormone oxytocin plays a central role in the regulation of parturition largely via its stimulatory actions on myometrial contraction. Levels of oxytocin remain fairly constant during pregnancy, with increases predominantly seen during labor (158). Instead, oxytocin receptors are upregulated, increasing sensitivity to the hormone (159).

Classically, oxytocin binding to its surface membrane receptor leads to the release of Ca from the SR via the canonical pathway involving PIP_2 hydrolysis and formation of the second messengers, IP_3 and diacylglycerol (DAG) (**Figure 1**). However, oxytocin can also increase $[Ca^{2+}]_i$ through other mechanisms, including Ca^{2+} entry via the L-type calcium channel (160), but this requires a change in membrane potential. The mechanism by which oxytocin achieves this was recently uncovered; activation of protein kinase C by DAG inhibits the sodium-activated, high-conductance, potassium leak channel Slo2.1 and thereby depolarizes the cell (126). This depolarization will open L-type Ca channels, allowing Ca^{2+} entry.

Oxytocin may also augment Ca^{2+} entry via SOC entry, where depletion of internal Ca^{2+} stores activates Ca^{2+} entry pathways (10). Downstream signal effectors including IP_3 and DAG are activated in response to oxytocin and contribute to SOC entry. The identity of the channels that facilitate SOC entry is unclear, but transient receptor potential (TRP) and STIM and ORAI proteins are expressed in myometrium and may contribute (161). The resulting influx of cations will facilitate membrane depolarization and L-type Ca channel opening. Oxytocin may also increase $[Ca^{2+}]_i$ by inhibiting Ca^{2+} extrusion at the plasma membrane Ca^{2+} ATPase (162–164). Mechanisms for increasing force of contraction independently of $[Ca^{2+}]_i$ (Ca sensitization) have also been proposed and are discussed elsewhere (165, 166).

Estrogen and Progesterone

In addition to their genomic effects, promoting and repressing uterine activation, respectively, estrogen (E2) and progesterone (P4) can differentially influence ion channel activity and hence membrane excitability and contraction.

Effects on L-type Ca^{2+} channels. Gestational and hormonal effects on the subunits of the L-type channel have been reported. The mRNA levels of the myometrial α1 and β subunits both increased in rats treated with antiprogesterone drugs, and the β subunit but not the α1 subunit increased at term (48). Tezuka et al. (51) showed a gradual increase in the α1 subunit through gestation, which reversed at labor, but found that the β subunits markedly increased at parturition, whereas Mershon et al. (48) found that the β subunit, but not the α1 subunit, increased at term. In the guinea pig, increased mRNA expression of the α1 and β subunits occurred as term was approaching, but this was not mirrored in protein levels (47). Application of P4 in rats reduces gestational increases in L-type expression, whereas application of antiprogesterone drugs promotes it (51). The relative expression of α1 isoforms is regulated by the ratio of P4 and E2. An α1C long isoform, which may be inhibitory on Ca flux compared to the shorter form, has been reported (167). In studies of ovariectomized rats, administration of P4 enhanced the expression of the α1C long isoform,

while E2 or a low P4/E2 ratio favored the expression of α1C short form (167). During pregnancy, the elevated plasma P4 levels observed would therefore facilitate α1C long form expression and channel inactivation and thus promote uterine quiescence.

Despite E2's genomic activities being procontractile, acute application of E2 can inhibit contraction and excitation in myometrium (168). This is partly due to effects on the voltage dependence of inactivation (169). Therefore, acute effects of E2 may contribute to the quiescence of the myometrium during most of pregnancy. Teleocytes may also respond to changes in steroid hormones by altering ion channel expression (170).

Effects on K^+ channels. The effect of acute and prolonged applications of E2 and P4 has been examined on several K^+ channels in myometrium. Consistent with the procontractile and proquiescence role for E2 and P4, respectively, during pregnancy, long-term (but not acute) exposure to E2 decreases K^+ channel activity, whereas P4 has an opposite effect (171).

BK channel expression decreases toward parturition, and E2 may participate in this process (172). In addition, expression of the BK channel β subunits is thought to be regulated throughout gestation by E2, as long-term exposure to 17β-estradiol upregulates the β1 transcript (173). Estrogen is also responsible for the dramatic reduction in Kv4.3 expression and function close to parturition. This is thought to be mediated by a direct reduction of channel transcription, as well as by reducing channel delivery to the plasma membrane (75).

Studies in mice and humans (119, 174, 175) but not rats (120) have found that SK3 is downregulated by E2, possibly via activation of SP1 and SP3 transcription factors that compete to regulate the channel expression in response to E2 (119). Therefore, the overall effect of long-term exposure to E2 is to depress K^+ channel function and increase cellular excitability. Estrogen was also shown to inhibit noninactivating outward K^+ currents, carried via TASK2 channels in myometrial cells (101).

Unlike E2, however, P4 does not affect K^+ channel expression (120, 173). Acute application of P4 to contracting strips of human myometrium in vitro rapidly decreases contraction (176, 177). However, acute application of P4 or E2 to myocytes had no effect on K^+ current (171). Moreover, the application of several potassium channel blockers including apamin (SK inhibitor) and iberiotoxin (BK inhibitor) to spontaneously contracting strips of human myometrium failed to counteract the inhibitory effect of progesterone on contractions (176), further suggesting that when applied acutely, P4 does not have any direct effect on K^+ channel activity. Further information on the involvement of sex hormones and regulation of K^+ channels in muscle function can be found elsewhere (178).

Other channels. Recent data suggest that E2 and P4 also regulate the NALCN in human myometrium. NALCN mRNA and protein levels are downregulated by E2 and upregulated by P4, consistent with the finding that E2 decreases and P4 increases the Na^+ current (179).

In addition to effects on ion channels, E2 and P4 also regulate myometrial contraction via changes in Cx43 expression and subsequent gap junction formation (**Figure 1**). The expression of Cx43 and gap junctions is positively correlated with plasma E2 levels (180) and decreased by P4 treatment (181). Consequently, changes in Cx43 expression mirror the E2:P4 ratio. Progesterone inhibits Cx43 synthesis directly, via PRs, by repressing Cx43 transcription and indirectly by blocking E2's action. Specifically, P4 acting via progesterone receptor B (PR-B) inhibits Cx43 trafficking and gap junction formation (and maintaining quiescence), whereas P4 stimulated PR-A promotes it (182).

CONCLUSIONS

By reviewing excitation in the myometrium, it is possible to see where our knowledge is firmly evidenced, and where lacunae or a lack of consensus exists. The field is moving forward thanks to scientists across the globe studying the myometrium, applying the latest technologies and collaborating. We sincerely regret not being able to cite more contributions from colleagues. We think the field has progressed well. In fact, it is developing so well that there are now excellent models of electrical activity, and together the data and the models will be powerful tools for taking us from isolated myocytes to the behavior of the whole organ. From this comes the renewed hope of bringing our science to bear on the problems experienced by women in labor, day after day, throughout the world.

DISCLOSURE STATEMENT

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