Relationship of Ion Channel Activity to Control of Myometrial Calcium

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This article reviews the contribution of ion channels to membrane potential, the ion channels expressed in myometrium, and the effect of ion channel activity on the control of myometrial intracellular free calcium. Plasma membranes constitute barriers to permeability that establish concentration gradients of ions inside versus outside the cell. Na^+, Ca^{2+}, and Cl^- are normally in higher concentration outside than inside cells, whereas K^+ is higher inside. In myometrium, Ca^{2+} entry into cells mediates myometrial membrane potential changes and serves as the internal signal for contraction. K^+ efflux is thought to promote repolarization after an action potential and to participate in setting the resting membrane potential. Ions cross the cell membrane through channels that have different regulated properties and selectivities. Ion movement has been measured by a number of techniques, including radiolabeled ion flux, use of intracellular indicators, and patch-clamp methodology. A number of myometrial Ca^{2+} channels have been described, including voltage-regulated L-type channels and Ca^{2+} entry in response to intracellular Ca^{2+} store depletion. Fast Na^+ channels may contribute to cation entry late in pregnancy. K^+ channels in myometrium include Ca^{2+}-activated channels, a delayed rectifier, and an inward rectifier. A Ca^{2+}-activated Cl^- channel is also present in myometrium. In addition to being regulated by Ca^{2+}, the activity of a number of these channels can be regulated by uterine contractants and relaxants. Regulation of ion channel activity can affect intracellular free Ca^{2+} concentrations in the myometrium. Therefore, control of ion channel activity represents one of several approaches for controlling myometrial contractile activity. (J Soc Gynecol Investig 2000;7: 4–11) *Copyright © 2000 by the Society for Gynecologic Investigation.*

KEY WORDS: Myometrium, calcium, ion channel, action potential.

A rise in intracellular Ca^{2+} is central to control of contractile activity in myometrium.[1] In nervous tissue, the entry of Na^+ is in large part responsible for altering membrane potential.[2] In myometrium, Ca^{2+} is considered the major charge carrier, except at the end of pregnancy when Na^+ may also play a role.[2–6] Thus, Ca^{2+} mediates myometrial membrane potential changes and serves as the internal signal for contraction (electromechanical coupling). Hence, understanding the basis for regulation of myometrial electrical activity is key to understanding the regulation of myometrial Ca^{2+} and contractile activity. Importantly, control of ion movement may provide a target for the management of uterine contractions.

MEMBRANE PROPERTIES AND ION PERMEABILITY

Plasma membranes constitute barriers to permeability that establish concentration gradients of ions inside versus outside the cell. The unequal distribution of ions and the presence of large intracellular biomolecules with net negative charge inside the cell create a potential difference across the membrane and a negative intracellular environment relative to the cell exterior.[2–7] Ions cross the cell membrane through channels that have different regulated properties and selectivities. The direction of flux for a given ion is determined by the concentration gradient for that particular ion and the membrane potential difference. The equilibrium potential for ion X is given by the Nernst equation $E_x = (RT \ln [X]_o/[X]_i)/zF$, where R represents the gas constant, T the temperature, $[X]$ the concentration (more strictly, activity) of ion X outside (o) and inside (i) the cell, z the charge and valency on ion X, and F the Faraday constant. The equilibrium potential for a given ion is that potential at which the force of the concentration gradient is balanced by the electric force generated as a result of the electrical potential difference across the membrane. In simplest terms, if the membrane potential is more positive than the equilibrium potential for a given ion, efflux of the ion from the cell is favored. Conversely, membrane potentials more negative than the equilibrium potential favor net influx of the ion.

The resting potential of myometrium has been estimated to range between −60 and −35 mV.[3–5] Resting potential is determined primarily by the permeabilities and relative concentrations of Na^+, K^+, and Cl^-. Direct measurements with ion-sensitive electrodes have not been made in myometrium, but estimated intracellular and extracellular concentrations of

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Supported in part by National Institutes of Health grant no. HD09618.

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1071-5576/00/$20.00
PII S1071-5576(99)00051-9

Table 1. Estimated Ion Concentrations Inside and Outside Myometrial Cells*

	Outside	Inside	Ratio outside/ Inside
Na^+	137 mmol/L	40 mmol/L	3.4
Ca^{2+}	1.5 mmol/L	0.13 μmol/L	10^4
K^+	6 mmol/L	169 mmol/L	0.03
Cl^-	134 mmol/L	65 mmol/L	2.1

* Mean of values from rat, cat, and guinea pig, from Parkington and Coleman.[5]

these ions are summarized in Table 1. Whereas Na^+, Ca^{2+}, and Cl^- are higher in concentration outside than inside cells, the opposite is true for K^+. By far, the greatest differential pertains to Ca^{2+}, with extracellular concentrations in the millimolar range and resting intracellular free Ca^{2+} concentrations in the nanomolar range. The equilibrium potentials for Na^+ and Ca^{2+} are more positive than the resting potential or the action potential, so that these ions flow in the direction of their concentration gradients into the cell when the respective ion channels open. The equilibrium potentials for K^+ and Cl^- are less than zero, and the direction of ion flux depends on the membrane potential.

The movement of ions in and out of cells depends on changes in ion channel permeability. Ion channel permeability is regulated by a number of factors including ions and metabolites, ligand-stimulated receptors, covalent modification, and membrane potential itself. Therefore, changes in ion channel permeability can alter membrane potential, which in turn can alter ion permeability. In addition to these mechanisms, ions can move by passive diffusion and can be pumped across membranes against their concentration gradient by adenosine triphosphate (ATP)-requiring pumps that will not be discussed in detail here.

MEASUREMENT OF ION MOVEMENT

This brief discussion will be limited to techniques generally in use; there are a number of more detailed discussions of these topics to which the reader is directed for more information.[2–7]

Isotope Flux Measurements

Ion movement can be measured by isotope flux techniques. In these approaches, the movement of radiolabeled ions into or out of whole cells or organelles is measured. An example of such a measurement is shown in Figure 1A, where the ability of the hormone relaxin to increase $^{45}Ca^{2+}$ efflux from myometrial cells preloaded with $^{45}Ca^{2+}$ is demonstrated. This method is useful for studying changes in net ion movement as a whole and requires no specialized equipment other than a means to measure radioactivity. However, it measures effects on net flux and provides a minimum of mechanistic insight.

Intracellular Indicators

The development of intracellular indicators and dual-wavelength fluorescence spectrometers capable of collecting and analyzing data gathered at millisecond to second intervals has permitted the measurement of net changes in ion concentrations in cellular compartments in both cell suspensions and single cells. There are now indicators for Ca^{2+}, K^+, Mg^{2+}, and Cl^- ions, as well as many other molecules and cell functions.[8] Specifically with respect to Ca^{2+}, the most widely used indicators are Fura-2, Fluo-3, Indo-1, and aequorin (bioluminescent).[8–12] Most of these dyes cross the plasma membrane as esters and are hydrolyzed by intracellular esterases.

Most of the dyes used in ion channel measurements change their spectral properties upon binding the ion. Changes in the concentration of the ion under study are calculated from knowledge of the affinity of the dye for the ion.[8,13,14] Factors that influence measurements include the degree of dye loading and dye leakage (usually compensated for by using ratio measurements) and dye accumulation in inappropriate intracellular organelles. Figure 1B shows an example of changes in intracellular free Ca^{2+} in response to oxytocin in myometrial cells, as measured by Fura-2, and demonstrates that the transient is partially reduced in the absence extracellular Ca^{2+}. The experimental techniques can easily be mastered by competent laboratory personnel, but specialized equipment is required. Even more specialized equipment is needed to measure rapid subcellular changes in ion concentration with these indicators.[13–15]

Patch-Clamp Techniques

In the patch-clamp technique, a glass suction electrode is used to form a tight seal on an individual cell and the amount and properties of the current resulting from the flow of ions are measured while the membrane potential is held or "clamped" at some value.[2,5–7] Patch clamping can be done in several modes (Figure 1C). In the whole cell (also perforated patch) mode, the cell interior is perfused with buffer from the patch pipette and the current measured represents the sum contributions of active channels in the entire cell membrane. In the cell-attached mode, the cell interior remains intact and the contributions of individual channels active in the patch are measured. In the excised mode, the contributions of individual channels in inside-out or outside-out configurations are measured. In all of these cases, the investigator manipulates the membrane potential, ion composition of the extracellular and patch pipette buffers, and presence of specific channel inhibitors or enhancers to study specific channels. The patch-clamp technique requires highly specialized electrophysiologic equipment and a high level of experimental expertise. Figure 1(C) shows examples of both whole-cell and cell-attached patch-clamp experiments measuring K^+ currents and channel activity, respectively, in myometrial cells.

CONTRIBUTION OF ION PERMEABILITY TO MYOMETRIAL ACTION POTENTIAL

The basal membrane potential in myometrium undergoes rhythmic changes called slow waves.[3–6] In the myometrium, it is thought that rhythmic changes in the permeability to Ca^+ contribute to depolarization, and changes in the permeability to K^+ contribute to repolarization or hyperpolarization.[3–6,16,17] In support of these assertions, decreasing external Na^+ makes human pregnant myometrial membrane potential more nega-

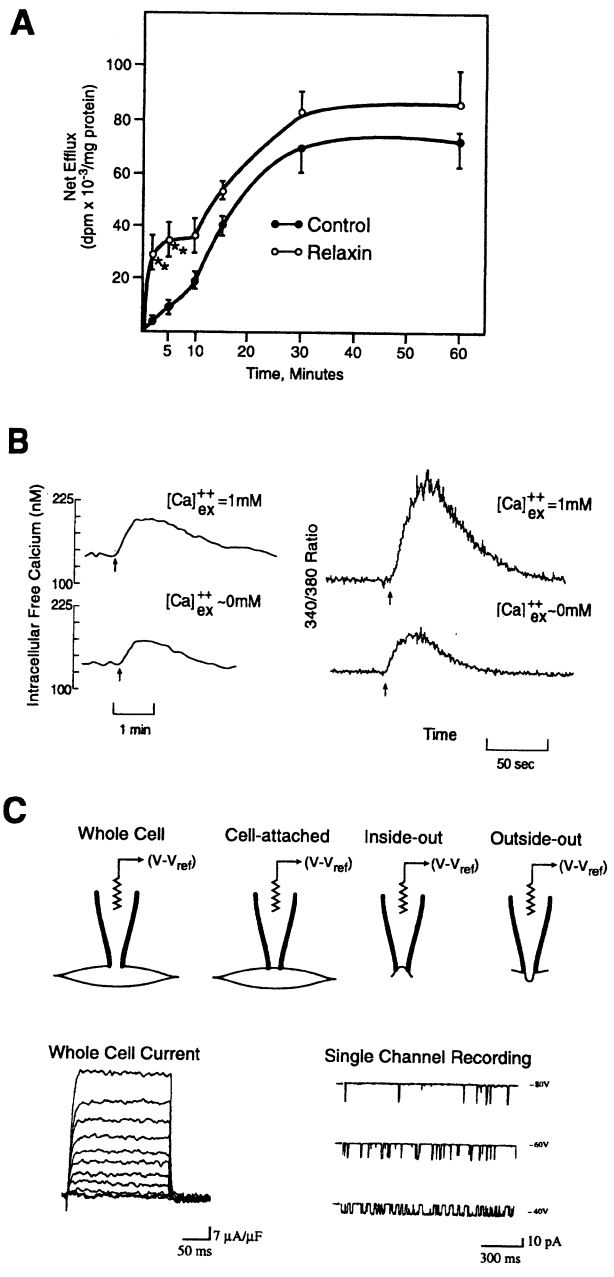

Figure 1. A) $^{45}Ca^{2+}$ efflux from rat myometrial cells preloaded with $^{45}Ca^{2+}$ is increased by treatment with porcine relaxin (2 μg/mL). Adapted from Rao and Sanborn[69] with permission. B) Intracellular free Ca^{2+} transients in rat (*left*) and human (*right*) myometrial cells, as measured with Fura-2. Cells were exposed in the presence ($[Ca]^{++}_{ex}$ = 1 mmol/L) or absence ($[Ca]^{++}_{ex}$ = 0 mmol/L) to oxytocin (*arrow*). From Anwer et al[9] with permission, and Monga and Sanborn (unpublished data). C) Schematic representation of patch-clamp modes. An automated voltage source keeps the pipette potential at the reference potential, and this adjustment is recorded. In the whole cell and outside-out modes, the composition of the pipette solution ultimately determines the composition of the intracellular compartment. In the cell-attached mode, cellular contents remain intact, whereas in the inside-out mode, the intracellular face of the membrane is exposed to the bathing solution. Representative recordings of whole-cell and single-channel recordings of K^+ channel activity in rat (*left*) and human (*right*) myometrial cells are shown. In the single-cell recordings, the channel is opening downward. Adapted from Sanborn[6] with permission.

tive[18] and iberiotoxin, a K^+ channel blocker, makes the membrane potential more positive.[19] At the threshold potential, there is a faster depolarization that is attributed primarily to entry of Ca^{2+} (and Na^+ in late pregnancy), generating the action potential.[3–6,18] The downstroke of the action potential is also thought to involve the opening of K^+ channels.[5,6,20]

The evidence for the presence of specific ion channels in myometrium has been reviewed elsewhere.[6] During pregnancy, the membrane potential ranges between −40 and −50 mV but becomes more negative at midpregnancy (~60 mV) and increases near term to about −45 mV.[3–5] As pregnancy progresses, depolarizations leading to an action potential change from small, irregular spikes to regular sustained activity. In some cases, this change is accompanied by a prolonged plateau phase in which the activity of Cl^- channels have been implicated.[5] The formation of gap junctions near term increases the electrical coupling between muscle cells and promotes coordinated contractile activity.[21] At this time, action potentials are seen on top of the slow waves, and these correlate with contractions.[6,17,22]

Ca and Na Channels

A number of Ca^{2+} channels are expressed in the myometrium, including L-type voltage sensitive channels and T-type voltage-inactivated Ca^{2+} channels.[6,18,23–25] The L-type Ca^{2+} channel exhibits voltage-dependent inactivation, with half-inhibition at approximately 45 mV, but can be returned to the resting state at more negative potentials.[5,23,24] Therefore, both membrane potential changes and increased intracellular Ca^{2+}, which also inactivates the channel,[26] can provide negative feedback controls to regulate channel activity. L-type Ca^{2+} channels are inhibited by Mg^{2+} and by dihydropyridines such as nifedipine, both of which are used to inhibit uterine contractions in premature labor.[23,24,27] Data on the regulation of the expression of these channels are variable. Some investigators find a progressive increase in channel subunit mRNA and/or channel density in rat myometrium[23,28]; others find little evidence for an increase in channel density after the first half of pregnancy.[24,25]

A new class of channels involved in Ca^{2+} entry has recently been described, and several of these proteins are present in myometrial cells. Members of the Trp family are plasma membrane proteins implicated in Ca^{2+} entry into cells in response to signals resulting from inositol 1,4,5-triphosphate (IP_3) generation and/or intracellular Ca^{2+} store depletion.[29] Mammalian Trp proteins have been cloned from a number of species; full or partial sequences are available for human Trp 1, 3, 4, and 6.[29–31] Using reverse transcriptase–polymerase chain reaction, we recently obtained evidence for hTrp1 mRNA, several splice variants of hTrp1 mRNA, and several other Trp forms in a human myometrial cell line.[32] Evaluation of the role of these

recently discovered putative channel proteins should lead to new insights into mechanisms for Ca^{2+} entry in myometrium.

Although the major charge carrier in myometrium is thought to be Ca^{2+}, fast Na^+ currents have also been measured in pregnant human and rat tissue and cells.[6,23,24,33,34] The number of cells possessing these channels increased before parturition in the rat.[24,25] This type of channel may contribute to inward current in late pregnancy.[3] Voltage-gated Na^+ channel mRNAs are expressed in human and rat uterus.[35,36]

K^+ and Cl^- Channels

K^+ channels contribute to repolarization after an action potential and may play a role in setting the resting membrane potential. A number of studies have recorded fast and slow outward currents in myometrium,[6,37,38] and a number of specific K^+ channel types have been described in myometrium.[6] One of the best characterized is the Ca^{2+}-activated K^+ or maxi-K channels (K_{ca}) detected in myometrial cells from a number of species, including humans.[6,19,37–44] Figure 1 shows examples of whole-cell and single-cell K^+ currents in myometrial cells attributed to K_{ca} channel activity on the basis of physical properties and inhibition by iberiotoxin.

Iberiotoxin increases contractile activity in rat and human myometrium and depolarizes human myometrial cells in association with an increase in intracellular Ca^{2+} as a result of activation of voltage-sensitive L-type Ca^{2+} channels.[19] These data suggest that K_{Ca} channels can influence both resting membrane potential and the shape and duration of the action potential in myometrium. During labor, a large K^+ channel insensitive to voltage or Ca^{2+}, which may permit an increase in intracellular Ca^{2+} without hyperpolarization, has been described in human myometrium.[45]

Adenosine triphosphate–sensitive K^+ channels (K_{ATP}) have not been measured directly in myometrium, but their action has been implicated in myometrial function. K_{ATP} channel activators depressed contractant-stimulated uterine contractions, although they were more potent in nonpregnant than in pregnant human myometrium.[6,46–48] K_{ATP} channels are inhibited by steady-state ATP concentrations. K^+ channel activity in myometrium increased following exposure to cyanide and the effect could be partially inhibited by a K_{ATP} channel inhibitor.[49] Because dystocia is associated with hypoxia, activation of K_{ATP} channels may be associated with this condition.[49]

A number of other K^+ channels and K^+ currents have been described in myometrium. These include a slowly activated delayed rectifier ($Kv_{1.5}$), the mRNA for which exhibits estrogen-dependent regulation, and an inward rectifier (ROMK-1), the mRNA for which is highest in midpregnancy in rat uterus.[50–53] A number of other K^+ currents and channels have been described in myometrium.[6,41] All of these channels may be active in the repolarization phase of the action potential and their relative contributions may be hormone and pregnancy stage dependent.

The plateau of the action potential in uterine cells can be close to the equilibrium potential for Cl^- channels. Myometrial Cl^- currents and large channels with the properties of Cl^- channels have been reported[54,55] and can be activated by oxytocin in pregnant rat myometrium, leading to depolarization.[55] Other nonselective cation channels have also been reported, but their functional role is not yet known.[6]

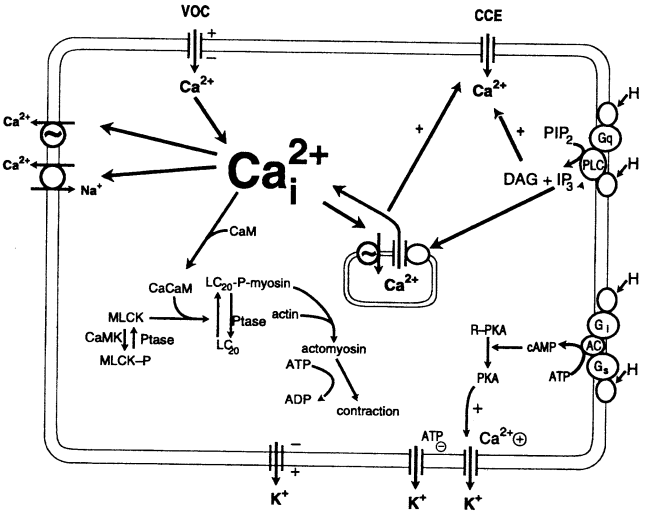

Figure 2. Schematic representation of pathways leading to control of $Ca^{2+}{}_i$ in smooth muscle such as myometrium. Components include calmodulin (CaM), myosin light chain kinase (MLCK), myosin light chain (LC_{20}), protein phosphatase (Ptase), L-type voltage-operated Ca^{2+} channel (VOC), capacitative channel entry (CCE), heterotrimeric G proteins (G), phospholipase C (PLC), inositol 1,4,5-triphosphate (IP_3), diacylglycerol (DAG), adenylyl cylase (AC), protein kinase A (PKA), Ca^{2+} transport adenosine triphosphate (ATP)ases, Na/Ca exchanger, Ca^{2+}-activated K^+ channel, ATP-sensitive K^+ channel, and voltage-sensitive K^+ channel. Modified from Sanborn et al with permission.[57]

ION CHANNEL ACTIVITY AND CONTROL OF MYOMETRIUM CALCIUM

The balance between uterine contraction and relaxation is extremely important throughout pregnancy and during labor. A rise in intracellular free Ca^{2+} initiates contraction in myometrium as a result of activation of myosin light chain kinase (shown schematically in Figure 2).[1,6,56] Relaxation is facilitated by decreasing intracellular free Ca^{2+} and by covalent modification of key components of the contractile apparatus. Therefore, to understand and regulate uterine contraction and relaxation, it is important to understand the mechanisms that control intracellular free Ca^{2+}.

The receptors for a number of contractant hormones couple to effectors such as phospholipase C through heterotrimeric guanosine triphosphate–binding proteins (G proteins) composed of α and $\beta\gamma$ subunits.[57] Phospholipase C isoforms stimulate the production of IP_3. IP_3 triggers Ca^{2+} release from intracellular stores, where concentrations (as in the extracellular environment) far exceed those in the cytosol. Adenosine triphosphate–sensitive Ca^{2+} pumps in the plasma membrane and endoplasmic reticulum actively lower intracellular free Ca^{2+} by pumping it out of the cell or back into the endoplasmic reticulum, respectively. Exchangers and other means of

passive transport also affect ion movement but will not be discussed here.

Although contractant-stimulated IP_3-mediated Ca^{2+} release is a major mechanism for increasing intracellular free Ca^{2+} in myometrial cells, Ca^{2+} entry from the extracellular environment also contributes significantly to the total intracellular free Ca^{2+} pool and to the refilling of intracellular stores.[58–60] This refilling is essential for continued contractile activity in response to a stimulus. The uterus does not maintain maximal contractile activity in the absence of extracellular Ca^{2+}. Several mechanisms have been proposed for regulation of Ca^{2+} entry, including activation of receptor-operated cation channels, L-type voltage-operated Ca^{2+} channels, second messenger–operated channels, and Ca^{2+} entry triggered as a result of depletion of intracellular stores.

Blockage of L-type Ca^{2+} channels in myometrium inhibits prolonged spontaneous and agonist-stimulated contractions. However, there are conflicting data regarding whether oxytocin increases L-type channel activity in myometrial cells.[60–62] In the pregnant human myometrial cell line PHM1-41, oxytocin-stimulated intracellular free Ca^{2+} rise was not affected by nifedipine,[59] indicating little contribution from L-type channels in these cells. Holda et al[63] reported a similar finding in primary cultures of human cells. Nonetheless, it has been suggested that stimulation of L-type channel activity may involve activation of Ca^{2+}-dependent protein kinase C,[64] which could occur as a consequence of oxytocin-stimulated phospholipase C activation and the concomitant formation of diacylglycerol and increase in intracellular free Ca^{2+}.

Capacitative calcium entry (also store-operated Ca^{2+} entry) refers to Ca^{2+} entry triggered by the release of Ca^{2+} from intracellular stores or the depletion of intracellular stores as a result of specific inhibition of the smooth endoplasmic reticulum Ca-ATPases by agents such as thapsigargin (Figure 2).[29,65] The mechanisms signaling such entry are still highly debated. Diacylglycerol or arachidonic acid produced from diacylglycerol as a result of receptor-stimulated phospholipase C activation may target some of the same entry mechanisms.[30,66]

Several findings provide evidence for capacitative Ca^{2+} entry in human myometrial cells. Whereas removal of extracellular Ca^{2+} reduced the oxytocin-stimulated increase in intracellular free Ca^{2+} by ~50% in PHM1-41 cells, phospholipase C inhibitors reduced the increase by 80–90% (Figure 3A).[59] These data suggest that the pathways of oxytocin-stimulated intracellular Ca^{2+} release and entry of extracellular Ca^{2+} are interdependent. Furthermore, the oxytocin-stimulated intracellular free Ca^{2+} increase due to Ca^{2+} entry was blocked by prior depletion of intracellular Ca^{2+} stores by thapsigarin. Also, after depletion of the intracellular stores with thapsigargin in the absence of extracellular Ca^{2+}, addition of extracellular Ca^{2+} elicited a larger rise in intracellular free Ca^{2+} in cells treated with thapsigargin than in untreated cells (Figure 3[B]). These data are consistent with store depletion-mediated capacitative Ca^{2+} entry and are further supported by the finding of Trp protein expression in myometrial cells.[31] It should be noted, however, that the presence of Trp proteins

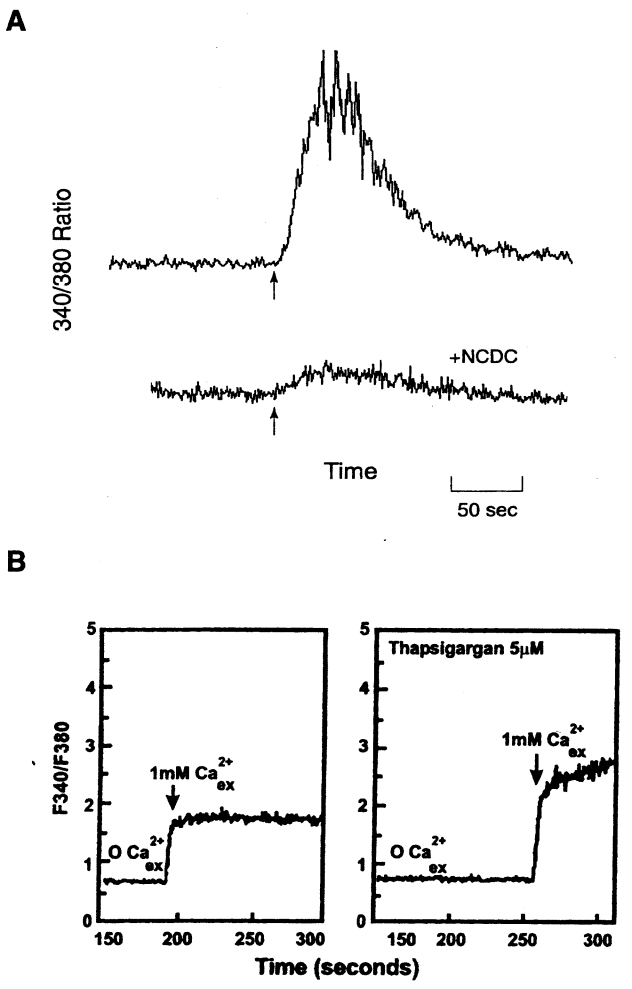

Figure 3. A) The Ca^{2+} transient stimulated by 20 nmol/L oxytocin in human myometrial cells is almost completely attenuated by 100 μmol/L NCDC, a phospholipase C inhibitor. B) In human myometrial cells cultured in the absence of extracellular Ca^{2+}, the increase in intracellular Ca^{2+} after addition of 1 mmol/L Ca^{2+} to the medium is greater in cells pretreated with 5 μmol/L thapsigargin. Adapted from Monga et al[59] with permission.

does not prove capacitative channels exist, as individual Trp proteins exhibit only some of the properties of capacitative Ca^{2+} entry.

As Ca^{2+} increases in myometrial cells and promotes contraction, it will activate the K_{Ca} channels, resulting in K^+ efflux and promoting repolarization. Inhibition of K_{Ca} channels in myometrium promotes Ca^{2+} entry and contraction,[19] suggesting that K_{Ca} channel activity can affect the shape and duration of the action potential and the Ca^{2+} transient. Notably, these channels are activated by agents that increase cyclic adenosine monophosphate (cAMP) and protein kinase A activity, including β-adrenergic agents and relaxin.[39,44,58,67] Figure 4 shows the activation of K_{Ca} channel activity by relaxin and cAMP-dependent protein kinase in human myometrial cells. This mechanism is one of several ways that tocolytics such as ritodrine, which stimulate β-adrenergic receptors that activate adenylate cyclase, are thought to function.[27] Similar to

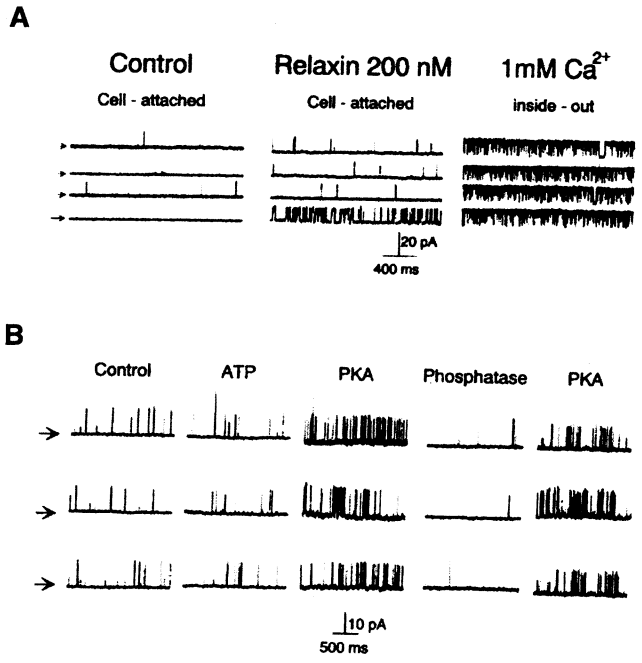

Figure 4. A) Relaxin activates the Ca^{2+}-activated K_{Ca} channel in human myometrial cells, measured in a cell-attached patch. The holding potential was +40 mV; the *arrows* indicate the closed state of the channel. B) K_{Ca} channel activity is activated by 20 U/mL protein kinase A (PKA) and inhibited by 100 U/mL alkaline phosphatase in an inside-out patch from a myometrial cell. Adapted from Meera et al[44] with permission.

activation of K_{ca}, Ca^{2+}-activated Cl^- currents could also contribute to depolarization.[55] In addition, a recent report suggests that in tissue from late term pregnant women and women in labor, hyperpolarization after a single prostaglandin-stimulated response is due primarily to activation of the Na^+/K^+ ATPase pump and not activation of K^+ channels.[68] This may reflect a modification of response at term.

SUMMARY AND CONCLUSIONS

In summary, ion channels play an important role in the regulation of ion flow in and out of cells. In the myometrium, Ca^{2+}, Na^+, K^+, and Cl^- channels contribute to the regulation of the resting potential and the action potential. Ion channel activity contributes both directly and indirectly to the regulation of intracellular free Ca^{2+} concentrations in myometrium. Intracellular free Ca^{2+} concentrations affect the contractile apparatus and influence contraction and relaxation mechanisms. Ca^{2+} entry from the extracellular environment involves the opening of voltage-activated Ca^{2+} channels and the activation of store-operated or agonist-stimulated entry channels, perhaps composed of Trp proteins. After the action potential, K^+ channels, activated by Ca^{2+}, depletion of ATP, or membrane potential changes, contribute to the regulation of the resting membrane potential and to repolarization. Therefore, K^+ channels can influence the duration of contractions as well as membrane excitability. Cl^- channels can also contribute to repolarization, as may the Na^+/K^+ ATPase. A number of tocolytic approaches target ion channel activity. It is likely that a combination of approaches targeting more than one aspect of intracellular Ca^{2+} control will be more effective than a single approach in reducing inappropriate uterine contractile behavior.

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