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Hormonal signaling and signal pathway crosstalk in the control of myometrial calcium dynamics

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Abstract

Understanding the basis for the control of myometrial contractant and relaxant signaling pathways is important to understanding how to manage myometrial contractions. Signaling pathways are influenced by the level of expression of the signals and signal pathway components, the location of these components in the appropriate subcellular environment, and covalent modification. Crosstalk between these pathways regulates the effectiveness of signal transduction and represents an important way by which hormones can regulate phenotype. This review deals primarily with signaling pathways that control Ca^{2+} entry and intracellular release, as well as the interplay between these pathways.

Keywords

myometrium; calcium; signaling; cAMP; ion channels

1. Introduction

Interest in understanding hormonal signaling in myometrium stems from a need to control uterine contractile activity in pregnancy. Signaling pathways are influenced by the level of expression of the signals and the respective signaling components, the location of these signaling components in the appropriate subcellular environment, and covalent modification (previously reviewed in [1–5]). Hormonal influences can alter one or more of these parameters. Furthermore, more than one signaling pathway may be used to achieve the desired result, thus providing redundancy that may be essential for propagation of the species but complicating the assessment of the importance of any one pathway. This review summarizes what is currently known about the signaling pathways that control myometrial Ca^{2+} entry and intracellular release, as well as the interplay between these pathways.

2. Contractant and relaxant signaling pathways

2.1 Multiple pathways lead to effects on calcium homeostasis

Figure 1 depicts components involved in regulating Ca^{2+} homeostasis in the myometrium. Intracellular Ca^{2+} concentrations $[Ca^{2+}]_i$ increase as a result of Ca^{2+} entry through plasma membrane cation channels and release from intracellular stores, primarily endoplasmic reticulum (ER). Many uterine contractants stimulate plasma membrane G-protein-coupled receptors (GPCRs) (see Table 1, López Bernal, 2007, this issue). These GPCRs, in turn, stimulate trimeric GTP-binding proteins (G-proteins), such as $G\alpha_qG\beta\gamma$, that activate multiple

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pathways to generate intracellular signals. Some of these signaling molecules impact Ca^{2+} homeostasis directly, whereas others stimulate feedback loops or initiate crosstalk between pathways that can be either positive or negative in nature and impact Ca^{2+} homeostasis indirectly.

In a pathway used by a number of contractants, $G\alpha_q$ activates phospholipase Cβ (PLCβ) isoforms, resulting in the hydrolysis of plasma membrane phosphatidylinositide (PI) bisphosphate (PIP_2) to produce inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 binds to endoplasmic reticulum (ER) IP_3 receptors, stimulating an increase in release of ER Ca^{2+} and a rise in $[Ca^{2+}]_i$. Other hormones signal through receptor tyrosine kinases, directly activating PLCγ and generating the same downstream intracellular intermediates. Ca^{2+} is the primary charge carrier in the myometrium and is responsible for stimulating the contractile apparatus (see Taggart, 2007, this issue). Contractant hormones may indirectly stimulate a change in membrane potential, thus triggering Ca^{2+} entry through voltage-operated ion channels (VOC). In addition, the signals generated by PLC activation, i.e., IP_3 and DAG, stimulate other types of channels and contribute to capacitative/store-operated or receptor-operated Ca^{2+} entry [6,7]. This type of Ca^{2+} entry will be broadly referred to here as signal-regulated Ca^{2+} entry (SRCE); it is dependent on hormone-generated signals and is potentially related to hormonal effects on contractile activity. The presence of this pathway in myometrium has been documented [8–10], but its physiological importance is only now being investigated.

$[Ca^{2+}]_i$ concentrations decrease as a result of the actions of the plasma membrane (PMCA) and endoplasmic reticulum (SERCA) Ca^{2+}-ATPases and Na/Ca^{2+} exchangers (NCX). $[Ca^{2+}]_i$ is also affected indirectly by mechanisms such as the activation of K^+ channels that hyperpolarize the cell and decrease VOC-mediated Ca^{2+} entry. These pathways are regulated by $[Ca^{2+}]_i$ itself, but also by components of relaxant and contractant signaling pathways.

2.2 Contractant and relaxant signaling pathways stimulate multiple downstream actions

Myometrial contractant signaling pathways that result from stimulation of specific receptors are complex and bifurcate to influence many downstream pathways, as illustrated by the following examples.

Oxytocin—The oxytocin GPCR (OTXR) couples to several G proteins (reviewed in [2,5]). In rat myometrial cells and membranes, almost all oxytocin-stimulated increases in $[Ca^{2+}{}_i]$, membrane GTPase and PLC activity were inhibited by anti-$G\alpha_q$ antibodies, suggesting that this was the predominant signaling pathway. Nonetheless, OTXR can also activate $G\alpha_i$ and can couple to $G\alpha_h$ to stimulate PLCδ. The relative importance of different G-protein pathways in oxytocin signaling may vary between species and cell type. The DAG and IP_3 produced in response to OTXR activation each stimulate components of the SRCE mechanism. Oxytocin also stimulates the TNFα-enhanced cyclic ADP-ribose (cADPR) pathway, leading to intracellular Ca^{2+} release via RyR receptors [11,12].

Oxytocin stimulates additional downstream signaling pathways in myometrium, including ERK1/2 phosphorylation [2,5]. This pathway is independent of effects on $[Ca^{2+}]_i$. The sensitivity to pertussis toxin of oxytocin-mediated ERK stimulation in rat myometrium suggests $G\alpha_iG\beta\gamma$-mediated activation. However, this inhibition by pertussis toxin is reversed in human myometrial cells by a PKA inhibitor. This is inconsistent with covalent modification of $G\alpha_i$, and supports involvement of $G\beta\gamma$ derived from $G\alpha_q$ activation itself, which is known to be inhibited by PKA [13]. This pathway requires the participation of the EGF receptor and Ca^{2+}. Truncation of the C-terminus of rat OTXR resulted in loss of the ability to stimulate ERK phosphorylation but not pertussis toxin-sensitive increases in $[Ca^{2+}]_i$, suggesting that $G\alpha_i$ coupling may activate a Ca^{2+} release/entry pathway independent of $G\alpha_q$ [14]. Recently, OTXR signaling through $G\alpha_iG\beta\gamma$, resulting in enhanced production of cAMP, has been reported in

rat pregnant myometrium [15]. Other OTR-$G\alpha_i$-linked pathways resulting in ERK activation involve Src/EGR receptor and/or PLC/Src/PI3-kinase [16]. ERK activation plays a role in the regulation on contractile activity (see Taggart, 2007, this issue).

ERK activation by oxytocin has also been linked to the stimulation of cyclooxygenase-2, PGF synthase, cfos and RGS2 gene expression in myometrial cells [2,17–19]. Oxytocin also increases total protein synthesis, as well as eukaryotic elongation factor 2 dephosphorylation, in myometrial cells [20].

Prostaglandin F2α (PGF2α)—The uterus produces several prostaglandins and expresses a number of prostaglandin receptors (reviewed in [4]). PGF2α binds to FP and TP receptors; some differences in reported responses may be due to use of different signaling pathways by these two receptor types. Prostaglandin receptors can be located on both plasma and nuclear membranes and their location can change during gestation. PGF2α increases $[Ca^{2+}]_i$ by stimulating entry and release mechanisms in human myometrium [21,22], with a significant dependence on extracellular Ca^{2+} [23]. PGF2α activates ERK through the SHC/SOS/Ras pathway in rat myometrial cells via a Gβγ mediated mechanism [24].

EGF—The EGF receptor possesses tyrosine kinase activity and directly stimulates PLCγ by tyrosine phosphorylation. In myometrium, this results in IP_3 generation and an increase in $[Ca^{2+}]_i$, consistent with intracellular Ca^{2+} release [25]. Activation of the EGF receptor has been implicated in myometrial $[Ca^{2+}]_i$ oscillations and also in OTXR signaling [13,26]. EGF also activates the PI-3 kinase/Akt pathway in other tissues, synergizing with PLCγ activity in mediating EGF effects [27]. EGF activation of ERK in myometrium is PKC-independent and involves the conventional Ras pathway [28]. EGF has also been implicated in myometrial cell proliferation in a pathway involving PKC and eicosanoids [29].

Relaxant signaling pathways—Myometrial relaxants such as β-adrenergic agents, CGRP and relaxin stimulate GPCR-coupled pathways that activate $G\alpha_s$ which activates adenylyl cyclase, generating cAMP and activating PKA. PKA is localized in various parts of the cell as a result of interaction with A-Kinase-Anchoring Proteins (AKAPs). PKA-mediated phosphorylations affect a number of the components regulating $[Ca^{2+}{}_i]$, as discussed in more detail by López Bernal (2007, this issue). Another relaxant signaling pathway in the myometrium is the nitric oxide (NO) guanylyl cyclase/cGMP/protein kinase G (PKG) pathway also discussed in depth by López Bernal (2007, this issue).

Other signaling pathways—Another potentially important signaling pathway in myometrium involves sphingosine kinase-1, which produces sphingosine-1-P. Endothelin-1 affects Rho-/Rho kinase-dependent pathways via activation of this pathway [30].

Ca^{2+} itself is an intracellular signal that influences the activity of a number of ion channels and, through its effect on the Ca^{2+} binding protein calmodulin, can activate not only the contractile apparatus through myosin light chain kinase activation (Taggart, 2007, this issue), but also CaM kinases, calcineurin (protein phosphatase PP2B) and transcription factors such as NFAT. Many of these potential Ca^{2+}-dependent mechanisms have yet to be studied in detail in the myometrium.

3. Proteins responsible for membrane calcium entry and exit pathways

3.1 Nifedipine-sensitive voltage-dependent channels

Entry of extracellular Ca^{2+} through L-type voltage-dependent Ca^{2+} channels is clearly important for sustaining myometrial contractions (reviewed in [2,5,31]). The $alpha_1$ subunit ($Ca_v1.1$–1.4) contains the channel pore, voltage sensor and drug binding sites; beta, $alpha_2$–

delta and gamma subunits modulate channel activity [32]. The concentration of myometrial $Ca_v1.2$ alpha and beta subunit mRNAs are expressed differentially in gestation and labor and in longitudinal versus circular muscle [33–36]. Two $Ca_v1.2$ isoforms are generated by post-translational processing; the longer form, reported to generate lower inward currents but to contain the regulatory PKA phosphorylation site, predominates in late pregnant rat myometrium [37]. The link between differential expression of L-type voltage-dependent Ca^{2+} channel isoforms and myometrial function remains to be determined.

The role of L-type Ca^{2+} entry in myometrium has been assessed using agonists and channel blockers. BAY K 8644, an L-type channel agonist, markedly increased the frequency but not the amplitude of human pregnant myometrial contractions [38]. L-type channel blockers reduced both spontaneous and stimulus-induced myometrial contractions [39,40]. Nifedipine (5 nM) inhibited the amplitude of oxytocin-stimulated contractions with less effect on frequency [41] and at μM concentrations completely eliminated contractile activity [42]. The effect of nifedipine on human myometrial strips was more marked in term not-in-labor than in term-labor specimens [43]. A significant proportion of oxytocin and PGF2α-induced contractions and increases in $[Ca^{2+}]_i$ in late pregnant guinea pig myometrium was attributed to Ca^{2+}entry inhibited by L-type Ca blockers [44], as was the response in pregnant rat myometrial cell [45]. In contrast, no effect of nifedipine on oxytocin- stimulated increases in $[Ca^{2+}]_i$ was observed in nonpregnant human myometrial cells [46], and L-type blockers had no effect on oxytocin-stimulated PI turnover [2,47].

Effects of contractants on myometrial membrane potential in relation to Ca^{2+}entry are complex. Oxytocin caused a slight depolarization in myometrial cells [48], suggestive of Ca^{2+}entry, but the mechanism was not established. In contrast, L- type current was inhibited by oxytocin in pregnant rat myometrial cells [49]. In other smooth muscles, L-type channels can be stimulated by GPCR pathways involving Gβγ, PI-3K and PKC [50]. Oxytocin has been implicated in the opening of other cation channels (consistent with SRCE) and of Ca^{2+}-activated Cl^- channels in myometrium, leading indirectly to depolarization and opening of voltage-dependent Ca^{2+} channels [51].

In pregnant guinea pig myometrium, PGF2α stimulated sustained depolarization and biphasic increases in $[Ca^{2+}]_i$ at mid to late-pregnancy; nifedipine inhibited most of the former but only some of the latter [52]. Verapamil, an L-type channel inhibitor, significantly reduced PGF2α-stimulated PI turnover in late pregnant human myometrium [2,47]. In apparent contrast to a stimulation of Ca^{2+} entry, PGF2α has been reported to induce hyperpolarization in pregnant human myometrium, which may involve effects on the Na/K ATPase [53].

3.2 T-type cation channels

Low voltage-activated T-type Ca^{2+} channels have been implicated in Ca^{2+} oscillations, pacemaker activity and contractility in smooth muscle. Rat myometrium expresses T-type channels $Ca_v3.1$ and 3.2 mRNA (multiple splice variants) in patterns that differ between longitudinal and circular muscle and with stage of pregnancy [36,54].

T-type currents have been observed in human myometrial cells (reviewed in [55]). The relatively specific T-type channel inhibitor mibefadril exhibited effects different from those of nifedipine on human myometrial contractile activity [41] and on spontaneous and OT-stimulated mid-pregnant goat myometrial contractions [56]. In contrast, mibefradil affected rat myometrial cell contractile responses in a complex manner, suggesting effects on both Ca^{2+} entry and intracellular release [57].

3.3 Signal-regulated (capacitative) channels and other cation channels

Cells have additional mechanisms to allow Ca^{2+} entry. While, in the past, the focus was on the properties of the currents or stimuli resulting in changes in $[Ca^{2+}]_i$ (i.e., capacitative, receptor- or store-operated, ICRAC, or non-selective cation currents, collectively termed SRCE here), as more of the proteins have been cloned, the focus has shifted to the properties of particular protein channel complexes. The TRP (transient receptor potential) channel superfamily is comprised of the TRPM, TRPC, TRPV, TRPA, TRPP, TRPML, and TRPN subfamilies. A number of the proteins in this family have been shown, in many different cell types, to contribute to nonselective cation and Ca^{2+} entry in response to one or more signals [6,7,58]. To date, TRPC, TRPM and TRPV proteins have been implicated in smooth muscle Ca^{2+} entry and responses to conditions such as hypoxia and stretch. TRPC channels are reported to influence cell migration, growth and differentiation, depolarization and modulation of membrane potential, slow wave activity and tone. The seven TRPC proteins (TRPC1-7) form separate subgroups that assemble into homo- and heterotetramers; the properties of individual channels are influenced by the nature of the subunits forming these tetramers. TRPC channels respond to a variety of stimuli, including GPCR activation, IP_3 receptor activation, DAG and intracellular Ca^{2+} store depletion resulting from exposure to SERCA inhibitors.

Interest in the role of TRPs in myometrium stems largely from the fact that hormonal signals play a role in stimulating this type of Ca^{2+} entry, thus raising the possibility that TRP channels may have roles in mediating hormonal effects on uterine contractile activity. From Fig. 1, one can see that several signals resulting from activation of GPCRs linked to PLC (e.g., IP_3 and DAG) could trigger SRCE in myometrium. IP_3- and thapsigargin (SERCA inhibitor)-mediated ER Ca^{2+} release in the absence of extracellular Ca^{2+} has been demonstrated in primary and immortalized human myometrial cells [9,10] (Fig. 2A). Upon addition of extracellular Ca^{2+}, these treatments evoke Ca^{2+} entry of a magnitude not seen in the absence of either stimulus. This entry is inhibited by SKF96365 and gadolinium, in concentrations relatively specific for SRCE, but not by nifedipine. The diacylglycerol oleyl-acetyl-glycerol (OAG) also evokes calcium entry in myometrial cells independent of the actions of PKC [59]. Therefore, all of the major stimulants of SRCE attributed to TRPC channels have been observed in myometrium. In addition, overexpression of TRPC3 in myometrial cells elicits enhanced Ca^{2+} entry in response to oxytocin, thapsigargin and OAG [10]. In less direct studies, a verapamil-resistant $[Ca^{2+}]_i$ elevation consistent with oxytocin-induced SRCE was noted in human myometrial cells [22]. Oxytocin also elicited a long-lasting nonselective cation current consistent with SRCE in late pregnant rat myometrium [60]. Furthermore, the sustained phase of $[Ca^{2+}]_i$ elevation after PGF2α-induced contractions in pregnant guinea pig myometrium was not affected by nifedipine [52], consistent with SRCE.

TRPC mRNA and protein have been detected in rat and human myometrium [9,61–63]. In human myometrium and myometrial cells, the relative abundance of TRPC mRNAs is TRPC1=TRPC4>TRPC6≫TRPC3>TRPC5>TRPC7 [63] (Fig. 2B). TRPC2 is a pseudogene in humans. Interestingly, rat myometrium expresses predominantly hTRPC4 mRNA, although all the other TrpCs except TrpC3 were also detected [62].

Expression of TRPC proteins may be regulated in ways that affect myometrial responsiveness. One study reports no significant differences in TRPC1, TRPC3, TRPC4, TRPC6 or TRPC7 mRNAs between human not-in-labor and in-labor samples but significant increases in TRPC3, TRPC4 and TRPC6 protein concentrations after the onset of labor [64]. In another study, fundal myometrium TRPC4 mRNA decreased between not-in-labor and in-labor samples, with no changes in the other TRPC mRNAs and no change in TRPC1, TRPC3, TRPC4, or TRPC6 membrane protein [63]. Only TRPC4 and TRPC5 mRNA and protein changed during gestation in rat myometrium, decreasing after d13 [62]; no changes in TRPC mRNAs were seen with

labor. Clearly, complex relationships exist between TRPC mRNA and protein expression in myometrium that are yet to be fully understood.

To date, understanding of the functional role of TRPs in Ca^{2+} homeostasis and contraction has come largely from other smooth muscle systems. Endothelial-dependent regulation of vascular tone is altered in TrpC4−/− mice, whereas TrpC6 −/− mice exhibit increased vascular muscle contractility attributable to upregulation of TrpC3 channels in these animals [65,66]. TRPCs have been implicated as mechanosensors linked to PP2B/NFAT signaling pathways responsible for gene activation [67]. In human myometrium, interleukin 1-β increased exclusively TRPC3 protein, suggesting a possible role for this protein in labor [64]. Recently, mechanical stretch of late pregnant human myometrial cells in culture has been found to enhance basal and store-operated Ca^{2+} entry and inward currents, accompanied by increases in TRPC3 and TRPC4 mRNA and TRPC3 protein [68]. This study provides additional evidence that labor-associated events could alter the expression of TRPCs and enhance SRCE in labor.

Other mechanisms have been proposed for store-operated Ca^{2+} entry. One involves the Stim1 protein, which acts as an ER Ca^{2+} sensor and interacts with Orai1, a plasma membrane protein implicated in Ca^{2+} entry [69,70]. Stim1 is reported to bind to TRPC1, TRPC2 and TRPC4 and to stimulate TRPC1 channel activation [70]. Orai1 interacts with TRPC3 and TRPC6; low concentrations of Orai1 enhance store-operated Ca^{2+} entry in cells overexpressing these TRPCs, whereas high concentrations inhibit inward current Ca^{2+} entry, suggesting that Orai1 may act as a regulatory subunit of TRP channels [71]. Stim1 and Orai1 are expressed in human myometrium (Fig. 2C), but their function there remains to be elucidated. An alternative store-operated mechanism involves production of a Ca^{2+} influx factor, Ca-dependent phospholipase A2 activation, and generation of lysophospholipids that activate store-operated channels [72]; this has not been studied in myometrium.

It is also very likely that, in addition to the channels discussed here, other types of cation entry channels exist in myometrium. Clearly, this is an important area for future investigation.

3.4 Calcium pumps, exchangers and intracellular release channels

PMCAs, SERCAs and NCXs—The plasma membrane Ca^{2+} ATPase (PMCA) extrudes Ca^{2+} from the cell, thus lowering $[Ca^{2+}]_i$ (Fig. 1). PMCA has been detected in human myometrium and expression is reported to increase in labor [73]. PMCA1b, 4a and 4b mRNA/or protein are detectable in rat uterus [74]; there is significant expression of PMCA1 and PMCA4b but not PMCA4a mRNA in human myometrial cells (Fig. 2C). Both PMCA4 and NCX have been implicated in attenuation of depolarization-induced $[Ca^{2+}]_i$ transients and associated membrane currents in mouse and rat myometrial cells [75,76].

The ER Ca^{2+} ATPase (SERCA) is of critical importance in refilling the ER Ca^{2+} store (Fig. 1). SERCA isoforms 2a and 2b have been detected in myometrium; the refilling of the ER Ca^{2+} store is essential for sustained myometrial contractions and plays an important role in modulating myometrial intracellular $[Ca^{2+}]_i$ (see Wray, 2007, this issue).

Ryanodine receptors and IP_3Rs—Human myometrium expresses all 3 RyR mRNAs, with RyR3 being the most abundant; expression is regulated during pregnancy [77,78] (see also Wray 2007, this issue). Nonetheless, there are only variable reports of effects of ryanodine or caffeine on myometrial $[Ca^{2+}]_i$ and no evidence of Ca^{2+} sparks that would be indicative of Ca^{2+} -induced Ca^{2+} release, suggesting an absence of productive coupling. However, RyR may be important for establishing localized Ca^{2+} elevations in myometrial cells (Wray, 2007, this issue). Human myometrium expresses IP_3R1 and rat myometrium expresses IP_3R1-3, which presumably mediate IP_3-stimulated Ca^{2+} release from the ER [77,78].

4. Crosstalk between contractant and relaxant pathways

There is significant potential for crosstalk between contractant and relaxant signaling pathways in the systems described here. This crosstalk may regulate the effectiveness of a given pathway as well as the subcellular localization of the signaling components themselves. This may be an important way in which hormones regulate and fine tune the control of contractile activity at various stages in gestation.

4.1 Pathway crosstalk at the level of GPCRs, G proteins and effectors

Both PKC, stimulated by contractant pathways, and PKA and PKG, stimulated by relaxant pathways, exert effects on components of their own and other signaling pathways. PKA and PKC phosphorylation can affect GPCR desensitization and recycling directly or indirectly. G-proteins, RGS proteins and adenylyl cyclase isoforms are also phosphorylation targets (López Bernal, 2007, this issue).

Further downstream in the $G\alpha_q$ pathway, PLCβ1 is phosphorylated and inhibited by PKC but not PKA, PLCβ2 is phosphorylated and inhibited by PKA, and PLCβ3 is phosphorylated and inhibited by PKA, PKC and PKG (reviewed in [2,5]). Notably, in addition to regulation by negative crosstalk from the PKA and PKG pathways, the effect of PKC on PLCβ constitutes potential negative feedback by the OTR pathway itself. Both PKCα and PKCδ have been identified as downstream components of oxytocin receptor activation in pregnant human myometrium [79]. In rat myometrium, PLCβ1, PLCβ2 and PLCβ3 increase at term [80] whereas PLCβ2 was not detected in human myometrial cells [81]. Prelimnary data indicate that phosphorylation of PLCβ3 on Ser^{1105} occurs in intact myometrial cells in response to both activation of PKA and PKC pathways (Zhong and Sanborn, unpublished observations), raising the possibility that rapid regulation of hormonal signaling responses impacts temporal fluctuations in $[Ca^{2+}{}_i]$ and influences the pattern of contractile activity. A recent report confirms the importance of the PKA inhibitory pathway in the regulation of myometrial PLC and provides evidence for a Gβγ-mediated enhancement of this effect by oxytocin in pregnancy [15].

Importantly, cAMP and cGMP are less potent inhibitors of contractile pathways at the end of gestation. There are multiple reasons for this, ranging from changes in the ability to generate and sustain the signals to changes in pathway component expression and in the sublocalization of the signaling components. These are discussed in more detail elsewhere (England, 2007, this issue; López Bernal, 2007, this issue).

4.2 Pathway crosstalk at the level of ion channels and calcium pumps

Calcium channels—Cav1.2a and 1.2b subunits are phosphorylated on comparable Ser residues by CaMKII, PKA and PKC [82]. PKA-mediated phosphorylation shifts the Cav1 channel into a gating mode where its activation occurs under physiological conditions. Smooth muscle $Ca_v1.2b$ currents are inhibited by 8-Br-cGMP, while PKC has variable effects on channel activity, ranging from activation to inhibition [32]. The divergent effects may depend on the type of PKC, the specific channel splice variant expressed or phosphorylation of other subunits. PKC elicited a stimulatory effect on L-type Ca^{2+} currents in pregnant rat myometrium [83].

In other systems, the activity of T-type channels, particularly $Ca_v3.2$, which predominates in myometrium, is modulated by Gβγ, PKA, PKG, PKC, CaMKII and tyrosine kinases [84]. Whether this type of regulation has functional significance in myometrium remains to be determined. The role of covalent modification in TRP channel function is not fully understood at present.

Other channels—ATP-dependent, BK_{Ca}, small conductance (SK), voltage-gated (K_v) and 2-pore K^+ channels that promote membrane repolarization are also regulated in part by the action of kinases stimulated in $G\alpha_s$, $G\alpha_q$ and $G\beta\gamma$ signaling pathways. All of these types of K^+ channels are expressed in myometrium and, in many cases, regulation is dependent on stage of pregnancy (see England, 2007, this issue). Ca^{2+}-activated Cl^- channels are stimulated by GPCR activation but inactivated by CaMKII; Ca^{2+}-activated chloride inward currents have been observed in myometrium (England, 2007, this issue).

Calcium pumps and exchangers—Potential phosphorylation sites for PKC and PKA have been identified in all PMCAs. Phosphorylation of PMCA1b by PKA increased both affinity for Ca^{2+} and catalytic activity, whereas phosphorylation by PKC increased maximal activity. SERCA activity in smooth muscle can be enhanced by PKA-mediated inhibition of the endogenous inhibitor phospholamban [85]. Whether similar effects pertain and have functional significance in the myometrium remains to be determined. NCX has been reported to be phosphorylated in smooth muscle, but there is no information on this regulation in myometrium.

IP_3 and ryanodine receptors—IP_3R1 is phosphorylated by PKA, PKG, PKC, CaMKII, ERK and tyrosine kinases with variable effects on Ca^{2+} release, but the effect of these signaling pathways has not been studied extensively in myometrium. In myometrium and other smooth muscle, IP_3Rs are associated with a PKG substrate (IRAG); overexpression of IRAG suppresses ER Ca^{2+} release, consistent with a relaxation mechanism [86]. While RyR2 is phosphorylated by PKA, there is little information about phosphorylation of RyR3, which predominates in myometrium.

4.3 Importance of subcellular localization

Many events involved in signal transduction are dependent on subcellular co-localization of the components into microdomains and “signalplexes”. Some GPCRs, G-proteins, effectors, PKA and PKC, PMCA and PIP_2 have been localized to membrane lipid microdomains [87, 88]. Some TRPC proteins associate with caveolin-1, lipid rafts, $G\alpha_{q/11}$, PLCs, IP_3R and scaffolding proteins [58,89,90]. L-type Ca channels exist in and are stimulated by a complex containing the β_2-adrenergic receptor, a G-protein, adenylyl cyclase, PKA and protein phosphatase 2A [91]. $PLC\beta_3$ interacts with E3ARP, a protein that enhances GPCR stimulation of PLC and with other scaffolding proteins [92]. The plasma membrane PKA anchoring protein AKAP79 binds cell membranes via interaction with PIP_2 and associates with GPCRs and other scaffolding proteins [93]. AKAP79 also binds PKC and PP2B, thus facilitating localized phosphorylation/dephosphorylation cycles. Besides influencing subcellular localization, PIP_2 can also influence activity of a number of signaling components affecting $[Ca^{2+}]_i$ [94]. PMCA, NCX, K_{ATP} and other inward rectifier channels have been reported to be activated by PIP_2, whereas TRP and IP_3R are inhibited [58,94].

Although estrogen has been reported to down-regulate caveolin and caveolae in rat myometrium, caveolin concentrations increase in late pregnancy [95]. Caveolin may help coordinate myometrial signaling pathways, as evidenced by the observation that a caveolin-1 peptide inhibits carbachol-stimulated myometrial contractions [96]. OTXR can exist in and outside of membrane microdomains, and signaling to different pathways is determined by location [97,98]. The inhibitory effect of PKA on PI turnover in myometrium is AKAP-dependent [99]. The PKA/plasma membrane AKAP79 association decreases markedly near term in rat myometrium, coincident with a decrease in inhibitory effects of cAMP on contractant-stimulated PI turnover that occurs in the membrane [100]. Effects are significant but less dramatic in human myometrium near or at term [101] (see also López Bernal, 2007, this issue).

The presence of closely associated signaling complexes supports the concept of the generation, in response to stimuli, of localized changes in signals such as Ca^{2+}, cyclic nucleotides, PIP_2 and IP_3 in the myometrium that can rapidly influence effectors in the immediate environment. The challenge in understanding the function of such signaling is to demonstrate that it makes an impact on myometrial physiology.

5. Conclusions

While a number of the pathway components of hormonal signal transduction relating to myometrial Ca^{2+} homeostasis have been identified and some of the respective roles defined, there are still many undefined components. In addition, the challenge remains understanding both the regulation of the assembly and activity of these signaling pathways, the interrelationship of the complex crosstalk and feedback loops that integrate signals over time, and the relation of these influences to the pattern and control of uterine contractile activity. Only in doing so can we understand fully how uterine contractions are regulated and better determine how to control them.

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References

1. Challis J. Mechanism of parturition and preterm labor. Obstet Gynecol Surv 2000;55:650–660. [PubMed: 11023206]
2. Sanborn BM. Hormones and calcium: mechanisms controlling uterine smooth muscle contractile activity. The Litchfield Lecture Exp Physiol 2001;86:223–237.
3. Lopez-Bernal A. Mechanisms of labour - biochemical aspects. BJOG 2003;110:39–45. [PubMed: 12763110]
4. Myatt L, Lye S. Expression, localization and function of prostaglandin receptors in myometrium. Prostaglandins Leukot Essent Fatty Acids 2004;70:137–148. [PubMed: 14683689]
5. Sanborn BM, Ku CY, Shlykov SG, Babich LG. Molecular signaling through G protein coupled receptors and the control of intracellular calcium in myometrium. J Soc Gynecol Investig 2005;12:479–487.
6. Putney JW Jr. Physiological mechanisms of TRPC activation. Pflugers Arch 2005;451:29–34. [PubMed: 16133266]
7. Minke B. TRP channels and Ca2+ signalling. Cell Calcium 2006;40:261–275. [PubMed: 16806461]
8. Monga M, Campbell DF, Sanborn BM. Oxytocin-stimulated capacitative calcium entry in human myometrial cells. Am J Obstet Gynecol 1999;181:424–429. [PubMed: 10454695]
9. Yang M, Gupta A, Shlykov SG, Corrigan R, Tsujimoto S, Sanborn BM. Multiple Trp isoforms implicated in capacitative calcium entry are expressed in human pregnant myometrium and myometrial cells. Biol Reprod 2002;67:988–994. [PubMed: 12193412]
10. Shlykov SG, Yang M, Alcorn JL, Sanborn BM. Capacitative cation entry in human myometrial cells and augmentation by hTrpC3 overexpression. Biol Reprod 2003;69:647–655. [PubMed: 12700192]
11. Thompson M, Barata da Silva H, Zielinska W, White T, Bailey J, Lund F, et al. Role of CD38 in myometrial Ca2+ transients: modulation by progesterone. Am J Physiol Endocrinol Metab 2004;287:E1142–E1148. [PubMed: 15339743]
12. Dogan S, Deshpande D, White T, Walseth T, Kannan M. Regulation of CD 38 expression and function by steroid hormones in myometrium. Mol Cell Endocrinol 2006;246:101–106. [PubMed: 16388888]
13. Zhong M, Yang M, Sanborn BM. Extracellular signal-regulated kinase 1/2 activation by myometrial oxytocin receptor involves Gα(q)Gβγ and epidermal growth factor receptor tyrosine kinase activation. Endocrinology 2003;144:2947–2956. [PubMed: 12810550]

14. Hoare S, Copland JA, Strakova Z, Ives K, Jeng Y-J, Hellmich MR, et al. The proximal portion of the COOH terminus of the oxytocin receptor is required for coupling to G_q, but not G_i. Independent mechanisms for elevating intracellular calcium concentrations from intracellular stores. J Biol Chem 1999;274:28682–28689. [PubMed: 10497238]
15. Zhou X, Lutz S, Steffens F, Korth M, Wieland T. Oxytocin receptors differentially signal via Gq and Gi proteins in pregnant and nonpregnant rat uterine myocytes: implications for myometrial contractility. Mol Endocrinol 2007;21:740–752. [PubMed: 17170070]
16. Rimoldi V, Reversi A, Taverna E, Rosa P, Francolini M, Cassoni P, et al. Oxytocin receptor elicits different EGFR/MAPK activation patterns depending on its localization in caveolin-1 enriched domains. Oncogene 2003;22:6054–6060. [PubMed: 12955084]
17. Park E, Echetebu C, Soloff S, Soloff M. Oxytocin stimulation of RGS2 mRNA expression in cultured human myometrial cells. Am J Physiol Endocrinol Metab 2002;282:E580–E584. [PubMed: 11832360]
18. Franczak A, Woclawek-Potocka I, Oponowicz A, Kurowicka B, Kotwica G. Oxytocin stimulates prostaglandin F2alpha secretion and prostaglandin F synthase protein expression in porcine myometrial tissue. Reprod Biol 2004;4:177–184. [PubMed: 15297891]
19. Oldenhof AD, Shynlova OP, Liu M, Langille BL, Lye SJ. Mitogen-activated protein kinases mediate stretch-induced c-fos mRNA expression in myometrial smooth muscle cells. Am J Physiol Cell Physiol 2002;283:C1530–C1539. [PubMed: 12372814]
20. Devost D, Girotti M, Carrier M, Russo C, Zingg H. Oxytocin induces dephosphorylation of eukaryotic elongation factor 2 in human myometrial cells. Endocrinology 2005;146:2265–2270. [PubMed: 15661857]
21. Luckas JJ, Taggart MJ, Wray S. Intracellular calcium stores and agonist-induced contractions in isolated human myometrium. Am J Obstet Gynecol 1999;181:468–476. [PubMed: 10454702]
22. Fu X, Liu Y, Ciray N, Olovsson M, Ulmsten U, Gylfe E. Oxytocin-induced oscillations of cytoplasmic Ca2+ in human myometrial cells. Acta Obstet Gynecol Scand 2000;79:174–179. [PubMed: 10716297]
23. Molnar M, Hertelendy F. Signal transduction in rat myometrial cells: comparison of the actions of endothelin-1, oxytocin and prostaglandin F2 alpha. Eur J Endocrinol 1995;133:467–474. [PubMed: 7581972]
24. Ohmichi M, Koike KKA, Masuhara K, Ikegami H, Ikebuchi Y, Kanzaki T, et al. Role of mitogen-activated protein kinase pathway in prostaglandin F2a-induced rat puerperal uterine contraction. Endocrinology 1997;138:3103–3111. [PubMed: 9231756]
25. Anwer K, Monga M, Sanborn BM. Epidermal growth factor increases phosphoinositide turnover and intracellular free calcium in an immortalized human myometrial cell line independent of the arachidonic acid metabolic pathway. Am J Obstet Gynecol 1996;174:676–681. [PubMed: 8623806]
26. Barhoumi R, Awooda I, Mouneimne Y, Safe S, Burghardt R. Effects of benzo-a-pyrene on oxytocin-induced Ca2+ oscillations in myometrial cells. Toxicol Lett 2006;165:133–141. [PubMed: 16567066]
27. Wang Y, Wu J, Wang Z. Akt binds to and phosphorylates phospholipase C-gamma1 in response to epidermal growth factor. Mol Biol Cell 2006;17:2267–2277. [PubMed: 16525023]
28. Robin P, Boulven I, Bole-Feysot C, Tanfin Z, Leiber D. Contribution of PKC-dependent and -independent processes in temporal ERK regulation by ET-1, PDGF, and EGF in rat myometrial cells. Am J Physiol Cell Physiol 2004;286:C798–C806. [PubMed: 14644778]
29. Kornyei J, Li X, Lei Z, Rao C. Analysis of epidermal growth factor action in human myometrial smooth muscle cells. J Endocrinol 1995;146:261–270. [PubMed: 7561638]
30. Leiber D, Banno Y, Tanfin Z. Exogenous sphingosine 1-phosphate and sphingosine kinase activated by endothelin-1 induced myometrial contraction through differential mechanisms. Am J Physiol Cell Physiol 2007;292:C240–C250. [PubMed: 16956968]
31. Wray S, Burdyga T, Noble K. Calcium signalling in smooth muscle. Cell Calcium 2005;38:397–407. [PubMed: 16137762]
32. Keef K, Hume J, Zhong J. Regulation of cardiac and smooth muscle Ca(2+) channels (Ca(V)1.2a,b) by protein kinases. Am J Physiol Cell Physiol 2001;281:C1743–C1746. [PubMed: 11698232]

33. Mershon J, Mikala G, Schwartz A. Changes in the expression of the L-type voltage-dependent calcium channel during pregnancy and parturition in the rat. Biol Reprod 1994;51:993–999. [PubMed: 7849200]
34. Tezuka N, Ali M, Chwalisz IK, Garfield RE. Changes in transcripts encoding calcium channel subunits of rat myometrium during pregnancy. Am J Physiol 1995;269:C1008–C1017. [PubMed: 7485440]
35. Collins P, Moore J, Lundgren D, Choobineh E, Chang S, Chang A. Gestational changes in uterine L-type calcium channel function and expression in guinea pig. Biol Reprod 2000;63:1262–1270. [PubMed: 11058528]
36. Ohkubo T, Kawarabayashi T, Inoue Y, Kitamura K. Differential expression of L- and T-type calcium channels between longitudinal and circular muscles of the rat myometrium during pregnancy. Gynecol Obstet Invest 2005;59:80–85. [PubMed: 15564792]
37. Helguera G, Olcese R, Song M, Toro L, Stefani E. Tissue-specific regulation of Ca2+ channel protein expression by sex hormones. Biochim Biophys Acta 2002:59–66. [PubMed: 11853958]
38. Poli E, Rusagara J, Coruzzi G, Bertaccini G. Dihydropyridine receptors in the pregnant human uterus in vitro. Pharmacology 1989;39:309–316. [PubMed: 2482513]
39. Ulmsten U, Andersson K, Forman A. Relaxing effects of Nifedipine on the nonpregnant human uterus in vitro and in vivo. Obstet Gynecol 1978;52:436–441. [PubMed: 714325]
40. Forman A, Andersson K, Maigaard S. Effects of calcium channel blockers on the female genital tract. Acta Pharmacol Toxicol (Copenh) 1986;58:183–192. [PubMed: 3521196]
41. Young R, Zhang P. Inhibition of in vitro contractions of human myometrium by mibefradil, a T-type calcium channel blocker: support for a model using excitation-contraction coupling, and autocrine and paracrine signaling mechanisms. J Soc Gynecol Investig 2005;12:e7–e12.
42. Phillippe M, Basa A. Effects of sodium and calcium channel blockade on cytosolic calcium oscillations and phasic contractions of myometrial tissue. J Soc Gynecol Investig 1997;4:72–77.
43. Longo M, Jain V, Vedernikov Y, Hankins G, Garfield R, Saade G. Effects of L-type Ca(2+)-channel blockade, K(+)(ATP)-channel opening and nitric oxide on human uterine contractility in relation to gestational age and labour. Hum Reprod 2003;9:159–164.
44. Maiggard S, Forman A, Brogaard-Hansen K, Andersson K. Inhibitory effects of nitrendipine on myometrial and vascular smooth muscle in human pregnant uterus and placenta. Acta Pharmacol Toxicol (Copenh) 1986;59:1–10.
45. Arnaudeau S, Lepretre N, Mironneau J. Oxytocin mobilizes calcium from a unique heparin-sensitive and thapsigargin-sensitive store in single myometrial cells from pregnant rats. Pflugers Arch 1994;4284287:51–59. [PubMed: 7971161]
46. Holda JR, Oberti C, Perez-Reyes E, Blatter LA. Characterization of an oxytocin-induced rise in $[Ca2+]_i$ in single human myometrium smooth muscle cells. Cell Calcium 1996;20:43–51. [PubMed: 8864570]
47. Maka F, Breuiller-Fouche M, Geny B, Ferre F. Prostaglandin F2 alpha stimulates inositol phosphate production in human pregnant myometrium. Prostaglandins 1993;45:269–283. [PubMed: 8484012]
48. Mironneau J. Effects of oxytocin on ionic currents underlying rhythmic activity and contraction in uterine smooth muscle. Pflugers Arch 1976;363:113–118. [PubMed: 945544]
49. Inoue Y, Shimamura K, Sperelakis N. Oxytocin actions on voltage--dependent ionic channels in pregnant rat uterine smooth muscle cells. Can J Physiol Pharmacol 1992;70:1597–1603. [PubMed: 1284486]
50. Callaghan B, Zhong J, Keef K. Signalling pathway underlying stimulation of L-type Ca2+ channels in rabbit portal vein myocytes by recombinant Gβ{γ} subunits. Am J Physiol Heart Circ Physiol 2006;291:H2541–H2546. [PubMed: 16877561]
51. Arnaudeau S, Lepretre N, Mironneau J. Oxytocin mobilizes calcium from a unique heparin-sensitive and thapsigargin-sensitive store in single myometrial cells from pregnant rats. Pflugers Arch 1994;428:51–59. [PubMed: 7971161]
52. Coleman HA, Hart JD, Tonta MA, Parkington HC. Changes in the mechanisms involved in uterine contractions during pregnancy in guinea-pigs. J Physiol (Lond) 2000;523:785–798. [PubMed: 10718755]

53. Parkington HC, Tonta MA, Brennecke SP, Coleman HA. Contractile activity, membrane potential, and cytoplasmic calcium in human uterine smooth muscle in the third trimester of pregnancy and during labor. Am J Obstet Gynecol 1999;181:1445–1451. [PubMed: 10601927]
54. Ohkubo T, Inoue Y, Kawarabayashi T, Kitamura K. Identification and electrophysiological characteristics of isoforms of T-type calcium channel Ca(v)3.2 expressed in pregnant human uterus. Cell Physiol Biochem 2005;16:245–254. [PubMed: 16301824]
55. Sanborn BM. Relationship of ion channel activity to control of myometrial calcium. J Soc Gynecol Investig 2000;7:4–11.
56. Mandi G, Sarkar S, Mishra S, Raviprakash V. Effects of calcium channel blocker, mibefradil, and potassium channel opener, pinacidil, on the contractile response of mid-pregnant goat myometrium. Indian J Exp Biol 2005;43:795–801. [PubMed: 16187530]
57. Asokan K, Sarkar S, Mishra S, Raviprakash V. Effects of mibefradil on uterine contractility. Eur J Pharmacol 2002;455:65–71. [PubMed: 12433596]
58. Ambudkar I. Ca2+ signaling microdomains: platforms for the assembly and regulation of TRPC channels. Trends Pharmacol Sci 2006;27:25–32. [PubMed: 16337693]
59. Shlykov SG, Sanborn BM. Stimulation of interacellular Ca 2+ oscillations by diacylglycerol in human myometrial cells. Cell Calcium 2004;36:157–164. [PubMed: 15193863]
60. Shimamura K, Kusaka M, Sperelakis N. Oxytocin induces an inward current in pregnant rat myometrial cells. Can J Physiol Pharmacol 1994;72:759–763. [PubMed: 7530160]
61. Dalrymple A, Slater DM, Beech D, Poston L, Tribe RM. Molecular identification and localization of Trp homologues, putative calcium channels, in pregnant human uterus. Mol Hum Reprod 2002;8:946–951. [PubMed: 12356946]
62. Babich LG, Ku CY, Young HW, Huang H, Blackburn MR, Sanborn BM. Expression of capacitative calcium TrpC proteins in rat myometrium during pregnancy. Biol Reprod 2004;70:919–924. [PubMed: 14627551]
63. Ku C, Babich L, Word R, Zhong M, Ulloa A, Monga M, et al. Expression of transient receptor channel proteins in human fundal myometrium in pregnancy. J Soc Gynecol Investig 2006;13:217–225.
64. Dalrymple A, Slater DM, Poston L, Tribe RM. Physiological induction of transient receptor potential canonical proteins, calcium entry channels, in human myometrium: influence of pregnancy, labor, and interleukin-1 β. J Clin Endocrinol Metab 2004;89:1291–1300. [PubMed: 15001625]
65. Freichel M, Philipp S, Cavalie A, Flockerzi V. TRPC4 and TRPC4-deficient mice. 2004:189–203.
66. Dietrich A, Mederos Y, Gollasch M, Gross V, Storch U, Dubrovska G, et al. Increased vascular smooth muscle contractility in TRPC6−/− mice. Mol Cell Biol 2005;25:6980–6989. [PubMed: 16055711]
67. Bush E, Hood D, Papst P, Chapo J, Minobe W, Bristor M, et al. Canonical transient receptor potential channels promote cardiomyocyte hypertrophy through activation of calcineurin signaling. J Biol Chem 2006;281:33487–33496. [PubMed: 16950785]
68. Dalrymple A, Mahn K, Poston L, Songu-Mize E, Tribe R. Mechanical stretch regulates TRPC expression and calcium entry in human myometrial smooth muscle cells. Mol Hum Reprod 2007;13:31–39.
69. Smyth J, DeHaven W, Jones B, Mercer J, Trebak M, Vazquez G, et al. Emerging perspectives in store-operated Ca2+ entry: roles of Orai, Stim and TRP. Biochim Biophys Acta 2006;1763:1147–1160. [PubMed: 17034882]
70. Huang G, Zeng W, Kim J, Yuan J, Han L, Muallem S, et al. STIM1 carboxyl-terminus activates native SOC, I(crac) and TRPC1 channels. Nat Cell Biol 2006;8:1003–1010. [PubMed: 16906149]
71. Liao Y, Erxleben C, Yildirim E, Abramowitz J, Armstrong D, Birnbaumer L. Orai proteins interact with TRPC channels and confer responsiveness to store depletion. Proc Natl Acad Sci U S A 2007;104:4682–4687. [PubMed: 17360584]
72. Csutora P, Zarayskiv V, Peter K, Monje F, Smani T, Zakharow S, et al. Activation mechanism for CRAC current and store-operated Ca2+ entry: calcium influx factor and Ca2+-independent phospholipase A2beta-mediated pathway. J Biol Chem 2006;281:34926–34935. [PubMed: 17003039]
73. Tribe RM, Moriarty P, Poston L. Calcium homeostatic pathways change with gestation in human myometrium. Biol Reprod 2000;63:748–755. [PubMed: 10952916]

74. Penniston J, Enyedi A. Modulation of the plasma membrane Ca2+ pump. J Membr Biol 1998;165:101–109. [PubMed: 9744998]
75. Matthew A, Kupittayanant S, Burdyga T, Wray S. Characterization of contractile activity and intracellular Ca2+ signalling in mouse myometrium. J Soc Gynecol Investig 2004;11:207–212.
76. Shmigol A, Eisner D, Wray S. Properties of voltage-activated [Ca2+]i transients in single smooth muscle cells isolated from pregnant rat uterus. J Physiol 1998;511:803–811. [PubMed: 9714861]
77. Martin C, Chapman KE, Thornton S, Ashley RH. Changes in the expression of myometrial ryanodine receptor mRNAs during human pregnancy. Biochim Biophys Acta 1999;1451:343–352. [PubMed: 10556588]
78. Mesonero JE, Tanfin Z, Hilly M, Colosetti P, Mauger JP, Harbon S. Differential expression of inositol 1,4,5-trisphosphate receptor types 1, 2, and 3 in rat myometrium and endometrium during gestation. Biol Reprod 2000;63:532–537. [PubMed: 10906061]
79. Bermeo M, Fomin V, Ventolini G, Gibbs S, McKenna D, Hurd W. Magnesium sulfate induces translocation of protein kinase C isoenzymes alpha and delta in myometrial cells from pregnant women. Am J Obstet Gynecol 2004;191:1051–1052. [PubMed: 15467591]
80. Mhaouty-Kodja S, Houdeau E, LeGrand C. Regulation of myometrial phospholipase C system and uterine contraction by beta-adrenergic receptors in midpregnant rat. Biol Reprod 2004;70:570–576. [PubMed: 14585815]
81. Dodge KL, Sanborn BM. Evidence for inhibition by protein kinase A of receptor/G alpha(q)/phospholipase C (PLC) coupling by a mechanism not involving PLCbeta2. Endocrinology 1998;139:2265–2271. [PubMed: 9564832]
82. Catterall W. Structure and regulation of voltage-gated Ca2+ channels. Annu Rev Cell Dev Biol 2000;16:521–55. [PubMed: 11031246]
83. Kusaka M, Sperelakis N. Stimulation of Ca2+ current by phorbol esters in rat myometrial cells is dependent on intracellular Ca2+ concentration. Reprod Fertil Dev 1996;8:1147–1152. [PubMed: 8981638]
84. Chemin J, Traboulsie A, Lory P. Molecular pathways underlying the modulation of T-type channels by neurotransmitters and hormones. Cell Calcium 2006;40:121–134. [PubMed: 16797700]
85. Paul RJ, Shull GE, Kranias EG. The sarcoplasmic reticulum and smooth muscle function: evidence from transgenic mice. Novartis Found Symp 2002;246:228–238. [PubMed: 12164311]
86. Schlossmann J, Ammendola A, Ashman K, Zong X, Huber A, Neubauer G, et al. Regulation of intracellular calcium by a signalling complex of IRAG, IP3 receptor and cGMP kinase lbeta. Nature 200;404:197–201. [PubMed: 10724174]
87. Ostrom RS, Post SR, Insel PA. Stoichiometry and compartmentation in G protein-coupled receptor signaling: implications for therapeutic interventions involving G_x1. J Pharm Exp Therapeutics 2000;294:407–412.
88. Daniel E, El-Yazbi A, Cho W. Caveolae and calcium handling, a review and a hypothesis. J Cell Mol Med 2006;10:529–544. [PubMed: 16796818]
89. Brazer SC, Singh BB, Liu X, Swaim W, Ambudkar IS. Caveolin-1 contributes to assembly of store-operated Ca2+ influx channels by regulating plasma membrane localization of TRPC1. J Biol Chem 2003;278:27208–27215. [PubMed: 12732636]
90. Kwiatek A, Minshall R, Cool D, Skidgel R, Malik A, Tiruppathi C. Caveolin-1 regulates store-operated Ca2+ influx by binding of its scaffolding domain to transient receptor potential channel-1 in endothelial cells. Mol Pharmacol 2006;70:1174–1183. [PubMed: 16822931]
91. Davare MA, Avdonin V, Hall DD, Peden EM, Burette A, Weinberg RJ, et al. A b2 adrenergic receptor signaling complex assembled with the Ca2+ channel Cav 12. Science 2001;293:98–101. [PubMed: 11441182]
92. Suh P, Hwang J, Ryu S, Donowitz M, Kim J. The roles of PDZ-containing proteins in PLC-b-mediated signaling. Biochem Biophy Res Comm 2001;288:1–7.
93. Gardner L, Naren A, Bahouth S. Assembly of an SAP97-AKAP79-cAMP-dependent protein kinase scaffold at the type 1 PSD-95/DLG/ZO1 motif of the human beta(1)-adrenergic receptor generates a receptosome involved in receptor recycling and networking. J Biol Chem 2006;282:5085–5099. [PubMed: 17170109]

94. Hilgemann D, Feng S, Nasuhoglu C. The complex and intriguing lives of PIP2 with ion channels and transporters. Sci STKE 2001 2001:RE19.

95. Turi A, Kiss AL, Mullner N. Estrogen downregulates the number of caveolae and the level of caveolin in uterine smooth muscle. Cell Biol Int 2001;25:785–794. [PubMed: 11482902]

96. Taggart MJ, Leavis P, Feron O, Morgan KG. Inhibition of PKCalpha and rhoA translocation in differentiated smooth muscle by a caveolin scaffolding domain peptide. Exp Cell Res 2000;258:72–81. [PubMed: 10912789]

97. Gimpl G, Fahrenholz F. Human oxytocin receptors in cholesterol-rich vs. cholesterol-poor microdomains of the plasma membrane. Eur J Biochem 2000;267:2483–2497. [PubMed: 10785367]

98. Reversi A, Rimoldi V, Brambillasca S, Chini B. Effects of cholesterol manipulation on the signaling of the human oxytocin receptor. Am J Physiol Requl Integr Comp Physiol 2006;291:R861–R869.

99. Dodge KL, Carr DW, Sanborn BM. Protein kinase A anchoring to the myometrial plasma membrane is required for cyclic adenosine 3′,5′-monophosphate regulation of phosphatidylinositide turnover. Endocrinology 1999;140:5165–5170. [PubMed: 10537145]

100. Dodge KL, Carr DW, Yue C, Sanborn BM. A role for AKAP (A kinase anchoring protein) scaffolding in the loss of a cyclic adenosine 3′,5′-monophosphate inhibitory response in late pregnant rat myometrium. Mol Endocrinol 1999;13:1977–1987. [PubMed: 10598575]

101. Ku CY, Word A, Sanborn BM. Differential expression of protein kinase A, AKAP79 and PP2B in pregnant human myometrial membranes prior to and during labor. J Soc Gynecol Investig 2005;12:1509–1515.

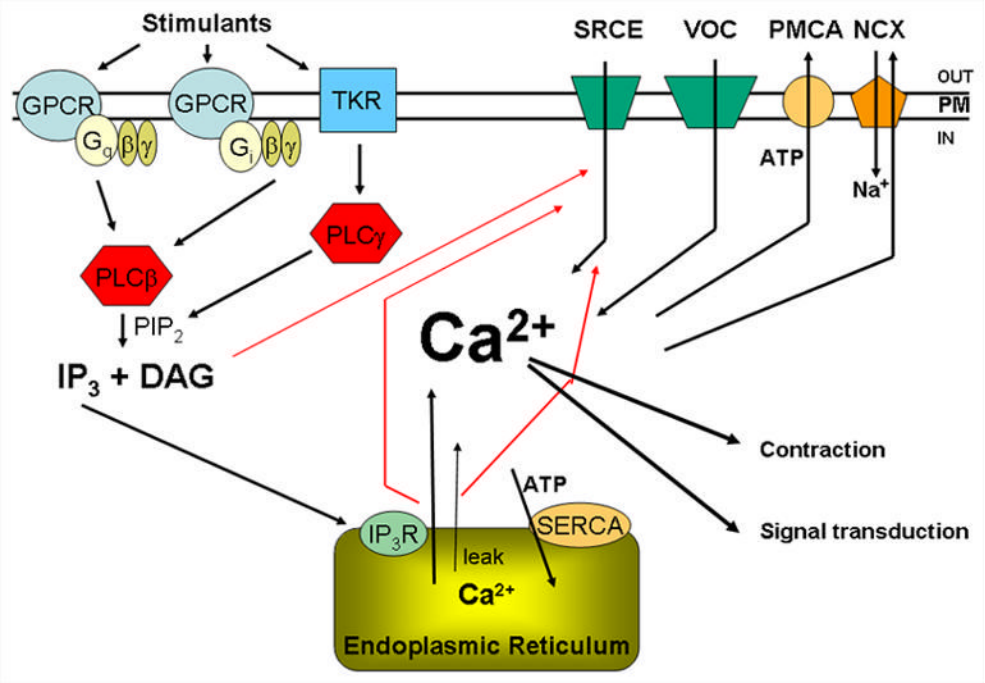

Figure 1.

Schematic diagram showing the major components that control $[Ca^{2+}]_i$ in myometrium. These include the contractant hormone pathways that stimulate G-protein coupled (GPCR) and tyrosine kinase (TyKR) receptors, G-proteins composed of Gα subunits ($G\alpha_q$, $G\alpha_i$ and (not shown) $G\alpha_h$), the associated Gβ and Gγ subunits, phospholipase C β and γ, which convert PIP_2 to IP_3 and diacylglycerol (DAG). IP_3 binds to IP_3 receptors (IP_3R) in the endoplasmic reticulum (ER), releasing Ca^{2+} from this intracellular store. Another ER release mechanism involves ryanodine receptors (RyR). Other Ca^{2+} entry mechanisms include cation channels responsive to stimuli such as IP_3R activation, DAG and release of Ca^{2+} from the ER (SRCE) and voltage-operated Ca^{2+} channels (VOC). The plasma membrane Ca^{2+} ATPase (PMCA) and the Na/Ca exchanger (NCX) are responsible for moving Ca^{2+} out of the cell; the ER Ca^{2+} ATPase (SERCA) pumps Ca^{2+} back into the ER. There is also a passive leak of Ca^{2+} from the ER. Ca^{2+} has stimulatory effects on the contractile apparatus, resulting in contraction, and also acts as an intracellular signal influencing a number of pathways in the myometrium.

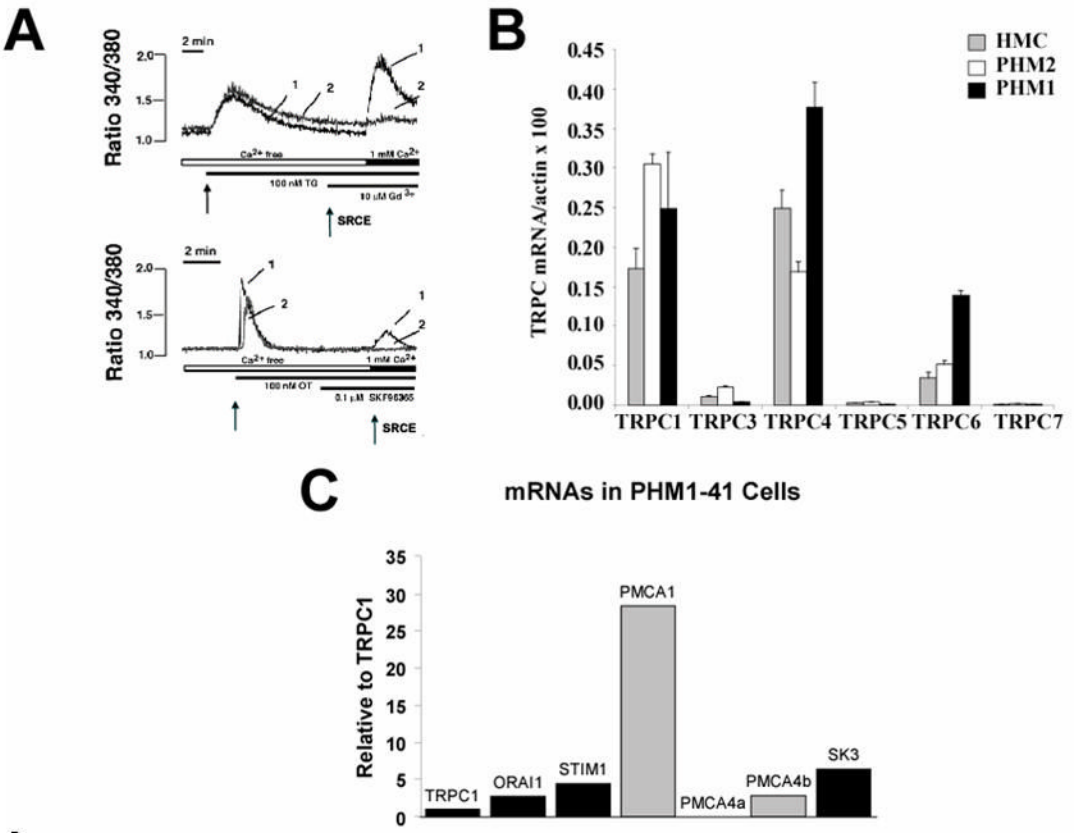

Figure 2.

A. Both oxytocin and thapsigargin increase PHM1 human myometrial cell $[Ca^{2+}]_i$, measured with Fura-2, in the absence of extracellular Ca^{2+} (first arrow), presumably by release of Ca^{2+} from intracellular stores. When extracellular Ca^{2+} is increased to 1 mM, a second increase in $[Ca^{2+}]_i$ is observed in cells treated with oxytocin or thapsigargin (SRCE), but not if cells are not exposed to these agents (not shown). SRCE was inhibited by SKF96365 or gadolinium. Data from [10] with permission. **B.** Relative expression of TRPCs in human myometrial cells, expressed relative to TRPC4 (HMC: primary non-pregnant human myometrial cells; PHM1 and PHM2: immortalized myometrial cells prepared from myometrial samples from two pregnant women. Modified from data in [63]. **C.** Expression of myometrial Orai1, Stim1, PMCA isoforms and SK3 K^+ channel mRNAs, determined by quantitative RT-PCR in PHM1 cell RNA by the ΔΔCt method, normalized to HMBS and expressed relative to TRPC1 mRNA [63]. Data represent mean of determinations using two different RNA preparations (J. Phillips and B. Sanborn, unpublished observations).