Heterotrimeric G proteins are actively involved in intracellular signalling in the myometrium and play important roles in regulating myometrial contraction and relaxation. Increases in intracellular calcium can be induced by agents that stimulate uterine contractions. In a number of instances, these increases in intracellular calcium are attributed to stimulation of phospholipase C by either Gs or Gβγ subunits as a result of activation of G protein-coupled plasma membrane receptors. This mechanism also stimulates calcium entry through calcium release-activated channels, either directly or indirectly. Thus, while phospholipase C can be activated by other pathways and calcium can enter myometrial cells through other channels, G proteins play a major role in these processes. Similarly, activation of protein kinase A and protein kinase C are consequences of G protein activation. Protein kinase A and protein kinase C exert a number of regulatory influences on phospholipase C, ion channel activity and other processes in the myometrium. The mitogen-activated protein kinase pathway can also be activated directly or indirectly by the action of G proteins in myometrium. Responsiveness to G proteins can be altered during pregnancy and depends on the relative expression of all of the components of the signalling pathways involved. The balance between G protein-mediated stimulatory and inhibitory signalling pathways has important consequences for the control of myometrial contractile activity.

Heterotrimeric G proteins are of the G protein family that mediate intracellular signalling by coupling to a class of ligand-stimulated plasma membrane receptors possessing seven-transmembrane domains (G protein-coupled receptors, GPCRs) (Conklin and Bourne, 1993; Exton, 1996; Hedin et al., 1993). Three subunits (α,β,γ) comprise the G proteins (Fig. 1). In the inactive state, the Gα subunit binds GDP. Receptor activation by ligand stimulates GDP–GTP exchange on the Gα subunit, and the activated GTP-bound Gα subunit dissociates from the Gβγ complex. Both the Gα and Gβγ subunits are capable of influencing the activity of effector molecules in cells. GTPase activity in the Gα subunit promotes reassociation of GDP-bound Gα subunit with Gβγ.

The G protein subunits are classified into subfamilies that couple to different effectors (Wickman and Clapham, 1995; Exton, 1996) (Table 1). A useful tool in G protein classification is the effect of pertussis and cholera toxins that either inhibit or activate specific Gα subunits as a result of ADP-ribosylation. The specificity of the intracellular response to a given extra- cellular ligand depends on several interactions. In general, elements in the intracellular domains of GPCRs determine both the G proteins with which the receptors interact and the efficacy of effector activity modification (Hedin et al., 1993; Exton, 1996). A major determinant for Gα protein interaction with receptors is found in the C-terminal Gα sequence, while effector interaction sites map to several regions on Gα proteins (Conklin and Bourne, 1993; Exton, 1996; Hepler et al., 1996). Hence, the specificity of response to a given ligand in a given cell type depends on the expression and coupling of specific receptors, G proteins, and effectors.

In addition to direct stimulation by G proteins of effectors that initiate signalling pathways, there can be cross-talk among downstream effectors of these pathways. For example, the tyrosine kinase-activated mitogen-activated protein kinase (MAPK) pathway can be activated by Gβγ acting at the Shc protein, by Gαs-simulated protein kinase C (PKC) acting at Raf, and by a pathway independent of PKC (van Biesen et al., 1996; Lopez-Illasaca et al., 1997). Conversely, PKA, activated as a result of Gαs stimulation of adenyl cyclase, can inhibit both PLC activation and the MAPK pathway (Chen and Iyengar, 1994; Sanborn et al., 1995). In addition, ion channels can be regulated by phosphorylation catalysed by PKA, PKC and other components involved in G protein signalling pathways (Wickman and Clapham, 1995).

Although G protein signalling pathways could potentially affect both contractile activity and the growth and differentiation of the myometrium, the majority of studies to date have focused on the regulation of intracellular calcium and contraction-relaxation. It is helpful to briefly review the components of the regulatory systems involved to place these studies in context (Fig. 2) (reviewed in more detail in Sanborn et al., 1994, 1995).
diacylglycerol activates PKC. The increase in intracellular free calcium \([\text{Ca}^2+]\) increases the concentration of the calcium-calmodulin complex, which activates myosin light chain kinase. This enzyme stimulates phosphorylation of myosin regulatory light chain, which facilitates activation by actin of myosin ATPase, resulting in contraction. Relaxation is facilitated by decreasing \([\text{Ca}^2+]\) and by covalent modification of key components of the contractile apparatus. Calcium pumps in the plasma membrane and endoplasmic reticulum actively extrude or sequester calcium, respectively.

Calcium entry from the extracellular environment can also contribute in a significant way to the total intracellular free calcium pool and to the refilling of intracellular stores. Several mechanisms can regulate calcium entry, including activation of receptor-operated and second-messenger-operated cation channels, L-type voltage-operated calcium channels, and calcium release-activated calcium entry triggered as a result of depletion of intracellular stores (Berridge, 1995; Sanborn, 1995). Modulation of the activity of potassium and other ion channels can potentially affect calcium homeostasis through effects on membrane potential.

**G proteins and phospholipase C regulation in myometrium**

**Oxytocin receptor activation of G proteins stimulates PLC activity**

PLC\(\beta_1-4\) comprise the currently known mammalian phosphatidylinositide-specific PLC\(\beta\) subfamily. Members of the \(\text{G}_{\alpha_\text{q}}\) family and \(\text{G}_{\beta_\gamma}\) can stimulate PLC\(\beta\) activity (Exton, 1996; Singer et al., 1997). \(\text{G}_{\alpha_\text{q}}, \text{G}_{\alpha_\text{11}}, \text{G}_{\alpha_{1-3}}, \) and \(\text{G}_{\beta_\gamma}\) are found in myometrium (Table 1). While all PLC\(\beta\)s are activated by \(\text{G}_{\alpha_\text{q/11}}, \text{PLC}_{\beta_2}\) and PLC\(\beta_3\) are also stimulated by \(\text{G}_{\beta_\gamma}\). On the basis of relative abundance and pertussis toxin sensitivity, \(\text{G}_{\alpha_\text{q}}\) stimulation is thought to be the major source of activated \(\beta_\gamma\) subunits that stimulate specific PLC\(\beta\) isomers. PLC\(\beta_2\), but not PLC\(\beta_1\), has been detected in rat myometrium, while PLC\(\beta_1\) was found in some but not all preparations (Ku et al., 1995; Lajat et al., 1996). An immortalized pregnant human myometrial cell line expressed only PLC\(\beta_1\) and PLC\(\beta_3\) (Dodge and Sanborn, 1998), while PLC\(\beta_1\), PLC\(\beta_2\), and PLC\(\beta_3\) were detected in human myometrium (Phaneuf et al., 1996).

Several lines of evidence indicate that the uterine contractile hormone oxytocin stimulates myometrial PLC activity by coupling through its receptor with \(\text{G}_{\alpha_\text{q/11}}\) subfamily members. In rat and human myometrial membranes, oxytocin stimulated both GTPase and PLC activities, and this stimulation was attenuated almost completely by an antibody directed against the carboxyl terminal sequence of the GTP-binding proteins, \(\text{G}_{\alpha_\text{q}}\) and \(\text{G}_{\alpha_\text{11}}\) (Ku et al., 1995) (Fig. 3a). A similar antibody delivered into a myometrial cell in the whole cell patch mode inhibited the oxytocin-stimulated increase in \([\text{Ca}^2+]\) completely (Arnaudeau et al., 1994). Furthermore, when COS cells were transfected with a plasmid expressing the oxytocin receptor, oxytocin-stimulated phosphatidylinositol turnover was increased in a dose-dependent manner when \(\text{G}_{\alpha_\text{q}}\) was also overexpressed (Qian et al., 1998). These data show both that the major pathway for coupling of the oxytocin receptor to PLC involves coupling to \(\text{G}_{\alpha_\text{q/11}}\) and that this is the primary mechanism for increasing \([\text{Ca}^2+]\).

In a number of GPCRs, amino acids in the third intracellular domain of the GPCR determine the specificity of coupling (Hedin et al., 1993). However, data obtained using vasopressin \(\text{V}_{1A}-\text{V}_2\) hybrid receptors indicated that the third intracellular domain of the \(\text{V}_2\) receptor contained the determinants for specifying coupling to \(\text{G}_{\alpha_\text{q}}\), while the second intracellular domain of the \(\text{V}_{1A}\) receptor contained the determinants necessary and sufficient for coupling to \(\text{G}_{\alpha_\text{q}}\) (Liu and Wess, 1996). The situation appears to be more complicated for the oxytocin receptor. Co-expression of each of the oxytocin receptor intracellular domains into COS cells overexpressing the oxytocin receptor and \(\text{G}_{\alpha_\text{q}}\) inhibited oxytocin-stimulated phosphatidylinositol turnover, with the third intracellular domain having the greatest effect (Qian et al., 1998) (Fig. 3b). Expression of the third intracellular domain of the \(\alpha_\text{q}\)-adrenergic receptor, which also couples to \(\text{G}_{\alpha_{11}}\), also inhibited phosphatidylinositol turnover, while expression of the comparable domain from the dopamine \(\text{D}_{1A}\) receptor, which couples to \(\text{G}_{\alpha_\text{q}}\), had no effect. While these data indicate a functional role for the oxytocin receptor third intracellular domain in G protein stimulation of PLC, they also suggest that interactions with more than one intracellular domain mediate the coupling of this receptor to the \(\text{G}_{\alpha_{11}}\) class of GTP-binding proteins. Work underway using oxytocin-\(\text{V}_2\) receptor chimaeras should clarify which elements determine coupling specificity.

Partial pertussis toxin sensitivity has been cited as evidence that oxytocin can also stimulate PLC through \(\text{G}_{\beta_\gamma}\) released from activated \(\text{G}_{\alpha_\text{q}}\). \(\text{G}_{\alpha_\text{q}}\) is inactivated by pertussis toxin-stimulated ADP ribosylation. The oxytocin-stimulated increase in phosphatidylinositol turnover has been reported to be pertussis toxin-sensitive in nonpregnant rats, partially sensitive in women, and insensitive in guinea-pigs, pigs and late pregnant rats (reviewed in Sanborn et al., 1995; see also Carrasco et al., 1996; Do Khac et al., 1996; Molnar et al., 1996; Phaneuf et al., 1996). However, in an immortalized pregnant human myometrial cell line, the inhibition of the oxytocin-stimulated
increase in phosphatidylinositide turnover was reversed by the addition of a protein kinase A inhibitor (Dodge and Sanborn, 1998), suggesting that pertussis toxin can also have an indirect effect (see below). The varied responses to pertussis toxin in different species and hormonal states indicate that the degree of this pathway contributes to myometrial phosphatidylinositide turnover, and the role of cellular context in this coupling, remain to be clarified.

The human myometrial oxytocin receptor also co-associated with and stimulated the photolabelling of an 80 kDa G protein characterized as Gaq (Baek et al., 1996). Gaq couples a variety of receptors to a 69 kDa PLC distinct from PLCβ that is present in human myometrial membranes. At present it is not known how much this pathway contributes to myometrial phosphatidylinositide turnover.

Stimulation of the MAPK pathway through Gaq by oxytocin

Oxytocin rapidly stimulated MAPK activity and MAPK substrate phosphorylation in cultured human uterine myometrial cells in a pertussis toxin-sensitive manner (Ohmichi et al., 1995, 1997). These data suggest that activation of MAPK by oxytocin was mediated through coupling of the oxytocin receptor to a Gaq type protein in human myometrial cells. Additional support for this hypothesis stems from the demonstration that an oxytocin receptor complex could be isolated from pregnant rat myometrial membranes associated with both Gaq11 and Gaq13 (Strakova and Soloff, 1997). Pertussis toxin inhibited oxytocin-stimulated GTPase activity in these membranes and, conversely, oxytocin inhibited the pertussis toxin-stimulated ADP ribosylation of Gaq. These data are consistent with stimulation by oxytocin of MAPK via a Gβγ mechanism. However, since this pathway can also be stimulated indirectly by PKC as a result of Gaq activation, it will be important to determine more directly that Gβγ is involved. The MAPK pathway is mainly involved in cell proliferation and differentiation. However, a recent report suggests that it may also be involved in the regulation of uterine contraction (Ohmichi et al., 1997).

Activation of G proteins by other uterine contractants

Prostaglandin F2α activates PLC, presumably through prostaglandin FP receptor stimulation of Gaq/11 (Harbon et al., 1994; Carrasco et al., 1996; Ohmichi et al., 1997). However, some studies indicate a primary effect on calcium influx (Molnar and Hertelendy, 1995; Molnar et al., 1996). Prostaglandin F2α can also activate MAPK in the uterus, both by a Gaq-stimulated pathway presumably involving protein kinase C activation, and by a Gβγ-stimulated pathway presumably involving Gαq (Ohmichi et al., 1997). The effects of other prostaglandins are even more complicated, since compounds like PGE2 interact with prostaglandin EP1–4 receptors that couple to several Gα subtypes, have been linked to PLC in myometrium, but the nature of the coupling has not been characterized in detail (Fuchs, 1995).

Table 1. General families of G proteins and their actions1,2

<table>
<thead>
<tr>
<th>G-protein subfamily</th>
<th>Family members</th>
<th>Properties</th>
<th>Activities</th>
<th>Found in myometrium</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gaq</td>
<td>Gaq11, Gaq15, Gaq16</td>
<td>Cholera toxin activates</td>
<td>Stimulate adenyl cyclase</td>
<td>Gaq</td>
<td>3,4,5</td>
</tr>
<tr>
<td>Gaq/10</td>
<td>Gaq11, Gaq15</td>
<td>Pertussis toxin inhibits</td>
<td>Inhibit adenyl cyclase</td>
<td>Gaq11, Gaq12, Gaq13</td>
<td>3,5</td>
</tr>
<tr>
<td>Gaq(2.3)</td>
<td>Gaq12, Gaq13</td>
<td>Pertussis toxin inhibits</td>
<td>Close calcium channels</td>
<td>Gaq2</td>
<td>3</td>
</tr>
<tr>
<td>Gaqx</td>
<td>Gaq2, Gaq4, Gaq11</td>
<td>Pertussis, chola toxin sensitive</td>
<td>Activate cGMP phosphodiesterase</td>
<td>Gaq11</td>
<td>3,5,6</td>
</tr>
<tr>
<td>Gaqq</td>
<td>Gaq5, Gaq13</td>
<td>?</td>
<td></td>
<td>Gaq12, Gaq13</td>
<td>7</td>
</tr>
<tr>
<td>Gαq</td>
<td>Gaq5</td>
<td></td>
<td></td>
<td>Gaq5 (80 kDa)</td>
<td>8</td>
</tr>
<tr>
<td>Gβγ</td>
<td>Gβ1–5, Gγ1–7</td>
<td></td>
<td></td>
<td>Gβγ, Gγ1</td>
<td>3,5</td>
</tr>
</tbody>
</table>

1(Coeklin and Bourne, 1993); 2(Singer et al., 1997); 3(Phaneuf et al., 1996); 4(Elwardy-Merezak et al., 1994); 5(Cohen-Rennoudji et al., 1995); 6(Ku et al., 1995); 7(Strathmann et al., 1991; total uterus); 8(Baek et al., 1996).
Regulation of contractile agent-stimulated phospholipase C coupling in pregnancy

The increased efficacy of contraction at term could result, at least in part, from the upregulation of the components in GPCR-G protein–PLCβ pathways or from enhanced efficiency of coupling. Oxytocin receptor mRNA and protein concentrations increase during the latter part of pregnancy and during labour in a number of species (Fuchs, 1995; Kimura et al., 1996; Wu et al., 1996). The ability to extract the oxytocin receptor associated with G proteins was also greater at the end of gestation (Strakova and Soloff, 1997). Although there was no change in uterine M₃ muscarinic receptors during pregnancy in rats, carbachol-stimulated phosphatidylinositol turnover was increased at days 12 and 21 of gestation in a pertussis-insensitive manner (Lajat et al., 1996). These data suggest an increased efficiency of coupling of M₃ receptors to Ga₃ during late pregnancy. In contrast, both M₂ and M₃ receptors declined in the same proportion during pregnancy in rabbits (Brandes and Ruggieri, 1995).

There was a gradual increase in the total number of uterine α₁ receptors during pregnancy in guinea-pig myometrium and in late pregnancy in women and rats (Legrand et al., 1987; Arkinstall and Jones, 1989; Dahle et al., 1993). The nature of
prostaglandin receptor coupling also changes during pregnancy in the rat myometrium. FP prostaglandin receptors mediated putative coupling to G\textsubscript{aq} in nonpregnant and pregnant tissue, while putative EP\textsubscript{1} coupling to G\textsubscript{aq} increased in late pregnant myometrium (Harbon et al., 1994). mRNA encoding G\textsubscript{aq} increased near term in rat myometrium (Cohen-Tannoudji et al., 1995). Consistent with this finding, G\textsubscript{aq} protein concentration was increased at both days 12 and 21, coincident with enhanced agonist- and aluminium fluoride-stimulated PLC activation (Lajat et al., 1996). The concentration of PLC\textsubscript{b3} was similar in day 0 and day 21 rat myometrium, but was decreased by 75% at mid-gestation. In contrast, GTP\textsubscript{gS}-stimulated PLC activity in pregnant guinea-pig myometrial membrane was decreased significantly throughout late gestation, suggesting a suppression of G protein coupling that extends to term in this species (Arkinstall and Jones, 1990).

The relative importance of different PLC\textsubscript{b} isoforms with respect to efficiency of coupling to specific G proteins needs to be clarified to understand the physiological relevance of changes in PLC expression during pregnancy.

Little is known about the regulation of \(\beta\gamma\) subunits and their role in PLC activation during pregnancy. Since expression of G\textsubscript{q}\gamma increases during the late stage of pregnancy (see below), it is possible that activation of G\textsubscript{q}\gamma results in the concerted down-regulation of adenylyl cyclase activity and increased stimulation of PLC by G\textsubscript{q}\beta\gamma subunits, thereby facilitating an increase in contraction near term.

Other effects of G protein-stimulated pathways that favour contraction

PKC activation is a consequence of PLC activation and diacylglycerol production. PKC has an inhibitory effect on PLC activity and oxytocin-stimulated contractions in myometrium (Phillipe, 1994). PKC phosphorylated PLC\textsubscript{b3} and inhibited its calcium dependence but did not phosphorylate PLC\textsubscript{b2} in vitro (Litosch, 1997). However, phosphorylation of PLC\textsubscript{b3} by PKC may be involved in G\textsubscript{q}-coupled receptor desensitization \textit{in vivo} (Ali et al., 1997) and, hence, there is a potential for feedback loops in this system.

Stimulation of phospholipase D and phospholipase A\textsubscript{2} can result directly or indirectly from G protein activation (Exton, 1996; Naze et al., 1997). These enzymes, in turn, generate other signalling intermediates that can have important influences on signalling pathways in myometrium. These are important areas for future research.

G proteins and adenylyl cyclase regulation in myometrium

Effect of G protein modulation of adenylyl cyclase activity on signalling pathways in myometrium

\(\beta\)-Adrenergic agents, relaxin, some prostaglandins and neuropeptides relax myometrium and increase cAMP but
direct receptor-mediated stimulation of $G_\alpha$ has only been demonstrated in a few cases (see Fig. 2) (Sanborn and Anwer, 1990; Harbon et al., 1994; Sanborn et al., 1994; Fuchs, 1995; Grammatopoulos et al., 1996; Casey et al., 1997). Increases in uterine cAMP and activation of PKA have been linked to relaxation in some but not all studies (Sanborn and Anwer, 1990; Harbon et al., 1994; Sanborn et al., 1994; Perez-Vallina et al., 1997). None the less, cAMP pathways have been targeted by some drugs used in suppressing preterm labour (Monga and Creasy, 1995). cAMP acts through PKA at a number of points to promote relaxation in the uterus (reviewed in Sanborn et al., 1990, 1994). In general, myosin phosphorylation, contractile agent-induced PLC activation and calcium entry mechanisms are inhibited, while calcium-activated potassium currents and calcium pumps are activated.

PLC activation by agents that contract the uterus is inhibited in the oestrogen-primed nonpregnant rat uterus and in a pregnant human myometrial cell line by isoproterenol, relaxin, forskolin and chlorophenylthio-cAMP (CPT-cAMP). The mechanisms presumably involve PKA since they were inhibited by PKA inhibitors (Haynes et al., 1993; Sanborn et al., 1994; Dodge and Sanborn, 1998) (Fig. 4a). In both the myometrial cell line and COS cells, the cAMP inhibitory mechanism was neither cell- nor receptor-specific (Dodge and Sanborn, 1998). PKA also inhibited the ability of GTPS to stimulate PLC activity in myometrial membranes (Wen et al., 1992). These data suggest that cAMP inhibits G protein–PLC coupling independent of the GPCR. PKA can phosphorylate PLC$_{\beta_2}$ in transfected COS cells (Liu and Simon, 1996). However, data from this laboratory are more consistent with inhibition by cAMP of the coupling of $G_\alpha$ to PLC$_{\beta_2}$ in the myometrium (Dodge and Sanborn, 1998; Yue et al., 1998). PLC$_{\beta_2}$ is phosphorylated by PKA, whereas $G_\alpha$ and PLC$_{\beta_1}$ are not phosphorylated (Kim et al., 1989; Yue et al., 1998). PKA-stimulated phosphorylation of PLC$_{\beta_2}$ inhibits $G_\alpha$ coupling in COS cells (Yue et al., 1998), while in basophilic leukaemia cells inhibition was limited to G$_{\alpha_q}$-stimulated PLC activity (Ali et al., 1997). PLC$_{\beta_1}$ phosphorylation in response to PKA activation does occur in myometrial cells (Yue et al., 1998), but whether this accounts entirely for the inhibitory effect of cAMP on phosphatidylinositide turnover is not yet known. There is increasing evidence for the influence of additional proteins on G protein–effector coupling that could also be targets of PKA phosphorylation.

Pertussis toxin could also promote PKA activation indirectly. Indeed, inhibition of $G_\alpha$ by pertussis toxin resulted in an increase in cAMP in rat myometrium (Singh et al., 1992). Consistent with an effect of PKA, a protein kinase inhibitor reversed the inhibitory effect of pertussis toxin on oxytocin-stimulated phosphatidylinositide turnover. Similar results were obtained in a cultured human myometrial cell line (Dodge and Sanborn, 1998) (Fig. 4b).

Other data are less supportive of an inhibitory effect of cAMP on the G protein–PLC pathway. Forskolin did not inhibit the oxytocin-stimulated increase in phosphatidylinositide turnover in human myocytes (Phaneuf et al., 1993). Neither did forskolin inhibit PLC in day 21 pregnant rat myometrium, although it did increase cAMP, while an inhibitory effect of isoproterenol was observed in the absence of an increase in cAMP (Do Khac et al., 1996).

At present the basis for the differences observed are not clear but it may involve differences in cell-specific expression of one or more proteins involved directly or indirectly in G protein–effector coupling and regulation.

Fig. 4. (a) Relaxin (R, pig, 1 µg ml$^{-1}$) inhibited oxytocin (OT, 100 nmol l$^{-1}$)-stimulated phosphatidylinositide turnover in the PHM1-41 human pregnant myometrial cell line, and this was reversed by pretreatment for 1 h with 30 µmol H-89 l$^{-1}$, a protein kinase A inhibitor. (b) Preincubation for 3 h with 0.3 µg pertussis toxin (PTX) ml$^{-1}$ partially inhibited oxytocin-stimulated phosphatidylinositide turnover in PHM1-41 cells, and H-89 reversed this inhibition. C, control. Groups with different letters are significantly different from each other at $P < 0.05$. (Data from Dodge and Sanborn, 1998.)
Changes in G protein–adenyl cyclase signalling during pregnancy

It has been hypothesized that the balance of influences on contraction and relaxation in the myometrium shift to favour contraction at the initiation or progression of labour (Europe-Finner et al., 1994; Cohen-Tannoudji et al., 1995). The inhibition of contraction by β-adrenergic agonists decreased, as did their ability to increase cAMP at the end of pregnancy in myometrium (Litine et al., 1989; Cohen-Tannoudji et al., 1995; Yeagley et al., 1996; Engstrom et al., 1997). This decrease in response may involve a decrease in any of the signalling molecules involved in cAMP generation or a loss of the cAMP inhibitory mechanism itself. Understanding the nature of this decrease in response relates directly to the design of better tocolytics for the prevention of premature labour, as well as to an understanding of factors affecting the initiation of parturition.

The balance between adrenergic receptors with stimulatory and inhibitory effects on adenylyl cyclase changes during pregnancy (Fuchs, 1995). This balance favours cAMP generation in mid-pregnancy but a diminished effectiveness at term. mRNA encoding stimulatory β-adrenergic receptor decreased throughout gestation and protein decreased at term in the rat myometrium, with mRNA encoding β1 receptor changing more than mRNA encoding β2 receptor (Engstrom et al., 1997; Principe et al., 1997). Inhibitory α2 adrenergic receptor concentrations increased at mid-pregnancy and decreased sharply near term in rats and guinea-pigs (Legrand et al., 1987; Arkinstall and Jones, 1988).

In addition to changes in the concentration of stimulatory and inhibitory receptors, the ability to stimulate cAMP is influenced by the relative concentrations of stimulatory (Gαs) and inhibitory (Gαi) G proteins regulating adenylyl cyclase activity. In humans, the concentrations of Gαs and Gαi3 did not significantly change between nonpregnant and pregnant myometrium, but Gαi5 concentrations were increased in pregnancy (Europe-Finner et al., 1994). Moreover, in myometrium from women in spontaneous labour, Gαs concentrations and Gαs-coupled adenylyl cyclase activity decreased compared with those observed in nonpregnant tissue. Another study described a decrease in agonist-stimulated but not total adenylyl cyclase activity near term, consistent with a decrease in receptor-cyclase coupling at that time (Litine et al., 1989).

In the rat myometrium, the ability of a β-adrenergic agonist to stimulate adenylyl cyclase declined between day 15 of pregnancy and term, while the ability of a Gαs-coupled β-adrenergic agonist to inhibit adenylyl cyclase increased (Cohen-Tannoudji et al., 1995). Moreover, inhibition of Gαi by pertussis toxin increased adenylyl cyclase activity by 50%, suggesting that some of the decrease in β-adrenergic response may be due to inhibition of adenylyl cyclase (Engstrom et al., 1997). Rat myometrial Gαs concentration decreased at term, while Gαi3 decreased from mid-pregnancy to term and Gαs increased progressively during pregnancy (Cohen-Tannoudji et al., 1995). In contrast, another group reported maximal concentration of Gαs at day 12 of pregnancy, a return of Gαs to nonpregnant concentrations at term, and a decrease in Gαi3 throughout pregnancy (Harbon et al., 1994). In this study, the maximal concentration of Gαs at day 12 correlated with minimal agonist-stimulated adenylyl cyclase activation at that time and a progressive increase in stimulated adenylyl cyclase activity later in pregnancy. The differences among these findings have not been resolved to date.

The expression of adenylyl cyclase isoforms during pregnancy has been studied in most detail in rats. Maximal basal and cholera toxin-stimulated cAMP production, indicative of total adenylyl cyclase activity, was found at day 15 of pregnancy (Cohen-Tannoudji et al., 1995). Types 2, 4, 6, 7 and 9 adenylyl cyclase were found in the pregnant rat myometrium, with type 6 being the most abundant (Suzuki et al., 1997). All subtypes of adenylyl cyclase are stimulated by Gαs and inhibited by Gαi, but types 2 and 4 can also be activated by Gβγ subunits (Tassig and Gilman, 1995). Type 2 and type 4 adenylyl cyclases may mediate the α2-β-adrenergic receptor stimulation of cAMP production in midpregnancy in rats through Gβγ, whereas α2-β-adrenergic inhibition of cyclase via Gαi predominates at term (Mhawnty et al., 1995). Expression of all adenylyl cyclase forms increased during the course of pregnancy, with the highest expression on day 17 and decreased expression near term. This correlated with changes in both basal and forskolin-stimulated activity over pregnancy (Suzuki et al., 1997). These data suggest that the relative expression of adenylyl cyclase forms is an important factor in G protein-mediated effects on uterine quiescence in rats. However, in human myometrium, total adenylyl cyclase activity was unchanged in nonpregnant, pregnant and labouring tissue (Litine et al., 1989; Europe-Finner et al., 1994). Further study is required to determine whether subtypes change in these tissues.

G proteins and ion movement in myometrium

Regulation of calcium-activated potassium channels by cAMP-dependent mechanisms presumably involving Gαs

Ion channel regulation by G proteins can occur by several mechanisms (Sanborn, 1995; Wickman and Clapham, 1995). The G protein can alter ion channel activity directly (so called membrane-delimited regulation). Alternatively, the action of the G protein on an effector can generate or degrade a product (for example, cAMP, cGMP, a lipid) that directly activates a channel. Finally, the product of effector activation could itself activate another molecule in the pathway (for example, PKA, PKC) which could alter the activity of the channel. In some cases, more than one type of regulation pertains for a given channel, making analysis difficult.

Temporal aspects of calcium mobilization and sequestration may relate to phasic contractions of the myometrium (Sanborn et al., 1994; Sanborn, 1995). Large calcium-activated potassium (KCa) channels play an important role in the regulation of myometrial membrane potential and calcium homeostasis. In a human myometrial cell line, blockage of these channels with iberiotoxin resulted in depolarization and an increase in [Ca2+] (Anwer et al., 1995). The increase in [Ca2+] was blocked by nifedipine, suggesting that calcium was entering the cells through L-type voltage-operated calcium channels (VOC) in response to depolarization. Iberiotoxin also increased phasic contractions in human and rat myometrium. These results provide strong evidence that large conductance KCa channels participate in the control of myometrial cell membrane potential and [Ca2+]. By repolarizing the cell, KCa channels may decrease the entry of calcium through voltage-sensitive channels and, thereby,
influence the shape of the [Ca\textsubscript{i}] transient and contractile properties. Since contractants increase [Ca\textsubscript{i}], the activation of KCa channels may constitute a feedback repolarizing system. In a similar way, PKC stimulates potassium currents in pregnant myometrium (Satoh, 1996).

Myometrial KCa channels are activated by agents known to oppose uterine contractions, such as β-agonists, relaxin and PKA (Meera et al., 1995; Sanborn, 1995). In pregnant rat myometrial cells, isoproterenol increased whole cell currents representing these channels (Anwer et al., 1992). In cell-attached patches from a human myometrial cell line, relaxin increased the mean open probability of the KCa channel (Meera et al., 1995). Consistent with the use of a second messenger pathway, the stimulating effect of relaxin was prevented by the PKA antagonist Rp-cAMP[S]. Moreover, the channel could be activated by forskolin via a mechanism that does not involve cAMP (Inoue et al., 1993). The inhibition by isoproterenol of oxytocin-stimulated potassium currents, forskolin and ritodrine both activated KCa channels, as did GTP and PKA (Hamada et al., 1994). This may indicate the direct activation of KCa channels by G proteins in addition to PKA mechanisms.

Other potassium channels, such as delayed rectifiers and ATP-sensitive channels, are also potential targets for direct regulation by GPCRs or the second messengers generated by GPCR-linked pathways (Sanborn, 1995; Wickman and Clapham, 1995). Stimulation of ATP-sensitive potassium channels relaxed the uterus, but it is not clear if G protein mechanisms mediate hormonal influences (Sanborn, 1995; Hughes and Hollingsworth, 1996). In pregnant human myometrial cells, ritodrine, forskolin, and PKA activated an ATP-sensitive potassium current, but GTP did not, suggesting that phosphorylation may be a primary regulator (Hamada et al., 1994). A delayed rectifier in late pregnant myometrium is inhibited by forskolin via a mechanism that does not involve cAMP (Inoue et al., 1993). The inhibition by isoproterenol of oxytocin-stimulated phosphatidylinositol turnover is thought to involve pertussis toxin-sensitive activation of a voltage-operated potassium channel (Do Khac et al., 1996). Although details are not available at present, G\textsubscript{q} proteins can stimulate potassium channels directly (Wickman and Clapham, 1995), and this action may be involved.

\textbf{Regulation of voltage-operated and calcium release-activated calcium channels, calcium pumps, and calcium release mechanisms}

Removal of extracellular calcium decreases oxytocin-stimulated [Ca\textsubscript{i}] increases in myometrial cells, suggesting an effect of oxytocin on calcium entry (Sanborn et al., 1994, 1995). Dihydropyridine blockers of L-type voltage-operated calcium channels have little (<30%) or no effect on oxytocin-stimulated [Ca\textsubscript{i}] in myometrial cells by some, but more significant effects by others (reviewed in Sanborn et al., 1995). At present, it is not clear whether these effects are mediated directly by the oxytocin receptor through G protein coupling, or whether they are indirect effects resulting from intracellular signalling. Prostaglandin E\textsubscript{2}, stimulated calcium influx but details of the mechanism are not available (Molnar and Hertelendy, 1995; Molnar et al., 1996).

Another mechanism for calcium entry is through calcium release-activated channels (CRACs) as a consequence of depletion of intracellular calcium (Berridge, 1995). A CRAC expressed in S9 cells is stimulated by GPCRs known to couple to PLCβ (Harteneck et al., 1995). In both pig and human myometrium, oxytocin-stimulated influx is blocked by inhibiting PLC, providing evidence for capacitative calcium influx secondary to intracellular calcium release (Zhuge et al., 1995; M. Monga and B. M. Sanborn, unpublished). These data are consistent with a contribution from CRACs as a major portion of oxytocin-stimulated calcium entry in myometrium through a G protein-stimulated mechanism. Whether this pertains for other contractants remains to be determined.

Plasma membrane calcium transport ATP\textsubscript{ases} are expressed in myometrium (Sanborn et al., 1994). While relaxants stimulate calcium efflux and contractants, such as oxytocin, inhibit calcium transport ATP\textsubscript{ases}, at present there is no available information on whether these actions involve the action of G proteins, either directly or indirectly. Similarly, uterine endoplasmic reticulum calcium pumps and their regulation by agents increasing cAMP have been described (Sanborn et al., 1994), but the biochemical pathways involved have not been studied in detail.

Calcium release mechanisms are profoundly affected indirectly by G protein-stimulated pathways. IP\textsubscript{3} receptors have been described in myometrium and are regulated directly by IP\textsubscript{3} produced as a result of PLC stimulation by GPCRs (Sanborn et al., 1994). In addition, these receptors may be regulated by cAMP–PKA pathways, but the details have not been determined in myometrium.

\textbf{Future directions}

G proteins play important roles in signalling pathways regulating myometrial contraction and relaxation. It follows from this discussion that the balance between stimulatory and inhibitory signalling pathways, and hence the control of myometrial contractile activity, depends not only on the expression of stimulatory and inhibitory signalling ligands, but also on the nature of the receptors, G proteins and effectors expressed in the tissue. Changes in these proteins during differentiation, the nonpregnant hormonal cycle, pregnancy, labour, and the postpartum state may play significant roles in determining the contractile state of the myometrium at these stages. Only some of these parameters have been investigated in this context, and as yet none have definitively been proven to play a functional role. Furthermore, the mechanisms of action of other uterine relaxants and contractants need to be determined.

The role of G protein-coupled pathways in myometrial cell growth and differentiation, perhaps via the interaction with the MAPK pathway, represents an interesting and virtually unexplored area of investigation. In addition, the regulation of sodium and chloride channels in myometrium by G protein-mediated mechanisms has received little attention to date.

Space constraints precluded discussion of the direct or indirect role of G protein pathways in the regulation of the contractile apparatus itself or of the role of small (non-heterotrimeric) G proteins in increasing the calcium sensitivity of myofilaments and promoting contraction. Furthermore, the role of G proteins in other tissues of the uterus, such as...
the uterine epithelium and vasculature, was not discussed. These areas are important for the understanding of total uterine biology.

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*Exton JH (1996) Regulation of phosphoinositide phospholipases by hormones, neurotransmitters, and other agonists linked to G proteins. Annual Reviews of Pharmacology and Toxicology 36 481–509


References

Key references are identified by asterisks.


*Conklin BR and Bourne HR (1993) Structural elements of Gα subunits that interact with Gβγ receptors, and effectors. Cell 73 631–641


Singh SP, Anwer K, Wilen Y and Sanborn BM (1992) Inhibition of oxytocin-stimulated phosphoinositide turnover in rat myometrium by pertussis and cholera toxin may involve protein kinase C activation. Cellular Signalling 4 619–625


Strathmann MP and Simon MI (1991) Gα12 and Gα13 subunits define a fourth class of G protein α subunits. Proceedings of the National Academy of Science USA 88 5582–5586


