Agonist-stimulated contraction of smooth muscle, including that of the uterus, involves the integration of many signal transducing events linking receptor occupation at the plasma membrane with varied intracellular effectors and eventual alteration of the activation state of the myofilaments in the cell cytoplasm. Precise co-ordination of these myometrial signalling events at term is paramount to ensuring efficient and powerful contractile activity necessary for expeditious expulsion of the fetus. A comprehension of the complex intracellular mechanisms of action of uterotonic agents is thus essential not only to our understanding of parturition but also for the management of situations involving dysfunctional uterine performance including, for example, those associated with pre-term labour, non-labouring uteri and post-partum haemorrhage. As such, in this paper we review recent studies that shed light on many of the mechanisms contributing to the efficient coupling of extracellular contractile stimuli and intracellular effectors in uterine smooth muscle. Special consideration is given to the processes of receptor-coupled recruitment to the plasma membrane of intracellular proteins important for agonist-induced alterations in Ca^{2+} sensitivity of contraction such as rhoA and ROK (rho-associated kinase); evidence for the involvement of caveolins, proteins integral to plasma membranous caveolae, in this signal transduction cascade is discussed.

Receptor activation of uterine smooth muscle

Agonist-stimulation of uterine smooth muscle primarily involves an elevation of intracellular Ca^{2+} ([Ca^{2+}]) via increased trans-sarcolemmal influx and/or release of Ca^{2+} from intracellular stores especially those responsive to generation of IP_3 (inositol 1,4,5-trisphosphate; Taggart & Wray, 1998). The elevation in [Ca^{2+}], results in activation of the Ca^{2+}- and calmodulin-dependent enzyme myosin light chain kinase and subsequent phosphorylation of the regulatory myosin light chains (MLC_20; Word et al. 1991; Taggart et al. 1997). This increased phosphorylation of MLC_20 allows for enhanced actomyosin Mg-ATPase activity and subsequent contractility. Smooth muscle relaxation is preceded by dephosphorylation of MLC_20 allows for enhanced actomyosin Mg-ATPase activity and subsequent contractility. Smooth muscle relaxation is preceded by dephosphorylation of MLC_20 by a myosin phosphatase (MLCP; Khromov et al. 1995) which has been cloned from uterine tissue (Johnson et al. 1997). However, a prominent additional mechanism whereby agonists can stimulate smooth muscle contractility is by sensitising the myofilaments to the activating [Ca^{2+}]. This occurs in both intact (Taggart & Wray, 1998) and permeabilised (Izumi et al. 1996; Somlyo & Somlyo, 1998; Taggart et al. 1999) uterine smooth muscle and, in other smooth muscles, has been associated with corresponding elevations of MLC_20 phosphorylation.
This agonist-induced Ca\(^{2+}\) sensitisation of MLC\(_{20}\) phosphorylation and force is thought to result from inhibition of MLCP (Kureishi et al. 1996; Swärd et al. 2000) although an involvement of thin filament-linked regulation has also been suggested for other smooth muscles (Horowitz et al. 1996).

**Agonist Ca\(^{2+}\) sensitisation of single uterine smooth muscle cell force production**

As first reported by Taggart et al. (1999), and illustrated in Fig. 1, the contractile force of permeabilised single isolated uterine smooth muscle cells from pregnant rat can be monitored in response to agonist application. Brief permeabilisation of these cells with saponin allows the concentration of activating Ca\(^{2+}\) surrounding the myofilaments to be strictly controlled by that in the bathing milieu. At suprabasal, but sub-maximal activating Ca\(^{2+}\) (pCa 6.7; Crichton et al. 1993) application of the muscarinic agonist carbachol results in pronounced contraction of these single uterine cells. Carbachol application resulted in contractions of magnitude 4.30 ± 0.30 µN (n = 5) similar to that reported previously for uterine smooth muscle (Taggart et al. 1999) but significantly greater than that observed for agonist stimulation of isolated vascular smooth muscle cells (Collins et al. 1992; Lee et al. 1999). Wortmannin, an inhibitor of myosin light chain kinase (MLCK), completely reversed carbachol-induced Ca\(^{2+}\) sensitisation (n = 5) – and even slightly decreased the basal force in pCa 6.7 (Fig. 1) – indicating an important role of an active MLCK to the sensitisation process. It is to be noted, however, that wortmannin is also capable of inhibiting several other kinases (Linseman et al. 1999; Duan et al. 2000).

One molecule suggested to be important for agonist-induced Ca\(^{2+}\) sensitisation is the small G-protein rhoA which acts downstream of receptor occupancy. Inactivation of rhoA in a variety of smooth muscles by ADP-ribosylation (Otto et al. 1996; Fujihara et al. 1997) or monoglucosylation (Otto et al. 1996; Lucius et al. 1998) results in inhibition of Ca\(^{2+}\) sensitisation. The Ca\(^{2+}\)-sensitising effects of rhoA activation appear mediated, in part at least, by its effector molecule ROK. As shown in Fig. 1, pharmacological inhibition of ROK with Y-27632 (Ueheta et al. 1997) results in 91 ± 5.6% (n = 4) reduction of carbachol-induced Ca\(^{2+}\) sensitisation of single uterine smooth muscle cell force. This is in agreement with the abrogation of oxytocin-induced Ca\(^{2+}\) sensitisation in multicellular preparations (Somlyo & Somlyo, 1998) indicating that multiple uterotonic agents may sensitise the myofilaments to Ca\(^{2+}\) via a rhoA/ROK-mediated reduction of MLCP activity. Notably, uterine ROK expression is increased with pregnancy (Niiro et al. 1997; Moore et al. 2000) as is agonist-induced Ca\(^{2+}\) sensitisation of force (Taggart & Wray, 1998). However, the assumed inhibition of MLCP activity in uterine single smooth muscle cells by activation of ROK, at least in response to muscarinic activation, is unlikely to be complete as application of the phosphatase inhibitor microcystin increases Ca\(^{2+}\) sensitisation to carbachol by a further 60.5 ± 12.4% (n = 3; see Fig. 1).

Additionally, in light of these results and also the rather steep

![Figure 1](image-url)

**Figure 1**

Agonist-induced Ca\(^{2+}\) sensitisation of single uterine smooth muscle cell force production. Single uterine smooth muscle cells were enzymatically isolated from 19-day pregnant rat, briefly permeabilised with saponin and prepared for measurement of force as described by Taggart et al. (1999). Permeabilised cells incubated in pCa 6.7 solution exhibited prominent agonist-induced Ca\(^{2+}\) sensitisation by contracting strongly to 100 µM carbachol (CCh). A, enhancement of CCh-induced Ca\(^{2+}\)-sensitised contractions by the phosphatase inhibitor microcystin-LR. B, inhibition of agonist-induced contractions by MLCK inhibitor wortmannin. C, reduction of CCh-induced contractions by ROK inhibitor Y-27632.
relationship reported between agonist $\text{Ca}^{2+}$ sensitisation of MLC$\text{20}$ phosphorylation and force in smooth muscle (Buus et al. 1998; Swärd et al. 2000), alternative possible pathways of rhoA/ROK-mediated $\text{Ca}^{2+}$ sensitisation, such as alteration of actin filament dynamic equilibrium (Mehta & Gunst, 1999), require fuller investigation. For example, treatment with cytochalasin D, an inhibitor of F-/G-actin dynamic equilibrium, results in attenuation of intact uterine smooth muscle contractions in response to carbachol without altering $[\text{Ca}^{2+}]_i$ responsiveness (Taggart et al. 2001).

### Agonist-stimulated cellular redistribution of rhoA and ROK

In resting uterine cells rhoA is diffusely distributed throughout the cytoplasm (Yu & López Bernal, 1998; Taggart et al. 1999) most probably existing in a rhoA-GDI (guanine nucleotide dissociation inhibitor) complex. Muscarinic stimulation of isolated uterine smooth muscle cells results in the translocation of rhoA from the cytoplasm to the cell periphery (Taggart et al. 1999). Membranous relocation of rhoA is essential for its $\text{Ca}^{2+}$-sensitising actions in a variety of smooth muscles (Gong et al. 1996; Fujihara et al. 1997). Notably, ROK$\alpha$ is similarly redistributed from the cytoplasm to the plasma membrane of intact uterine smooth muscle cells following identical conditions of receptor activation that result in maintained $\text{Ca}^{2+}$-sensitised contractions of permeabilised cells (Taggart et al. 1999). $\text{Ca}^{2+}$ sensitisation thus occurs without significant relocalisation of ROK away from submembranous regions of the cell. The precise subcellular distribution of the trimeric MLCP, or the

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**Figure 2**

Caveolin and rhoA localisation in uterine smooth muscle. Laser scanning confocal microscopy of single uterine smooth muscle cells isolated from pregnant rat was used to establish the localisations of caveolins and rhoA. Central cell sections are illustrated. A, caveolin-1, -2 and -3 all exhibit a predominantly plasma membranous distribution in single myometrial cells. B, RhoA localisation before and after cellular introduction of the fluorescein isothiocyanate (FITC)-labelled (green) caveolin-1 scaffolding domain peptide. RhoA shows a stimulus-dependent relocalisation from the cytosol to the cell periphery in control cells. RhoA translocation is inhibited by the caveolin-1 scaffolding domain peptide. Scale bars represent 10 $\mu$m. Adapted from Taggart et al. (2000b).
targeting subunit of MLCP (MYPT) that is a major substrate for ROK, is unknown in smooth muscle. Although most MLCP activity is found in myofibrillar cell fractions (Alessi et al. 1992) substantial MYPT is present in membrane fractions (Kimura et al. 1996; Ito et al. 1997), has been shown to be regulated by interactions with acidic phospholipids (Ito et al. 1997) and, in confluent cultured cells, has been localised close to the plasma membrane (Hirano et al. 1999). On balance, it would appear, therefore, that the major subcellular site(s) of rhoA/ROK-mediated Ca\(^{2+}\) sensitisation in uterine smooth muscle lies in close proximity to the plasma membrane. Indeed, the ultrastructural arrangement of the smooth muscle cell – where contractile and cytoskeletal filaments exit membrane attachment sites at an acute angle to the cell axis – is consistent with a significant proportion of contractile load being sensed by the plasma membrane (Small & Gimona, 1998). In this manner it may be feasible for the effects of discrete changes in the activation state of myofilaments close to the plasma membrane to be transduced across the whole cell.

What might be the nature of the membrane structure(s) that co-ordinates the recruitment of downstream signalling molecules such as rhoA and ROK? Electron micrography of uterine smooth muscle illustrates that the membrane consists of periodic electron dense material – the dense plaques where actin filaments insert into the membrane – interspersed with rows of \(\Omega\)-shaped invaginations known as caveolae (Taggart et al. 2000b). Caveolins are a family of proteins – three main mammalian isoforms exist (termed caveolin-1, -2 and -3) – critical to caveolar formation that, \textit{in vitro}, interact with a variety of signal transducing molecules (reviewed by Okamoto et al. 1998 and Shaul & Anderson, 1998). A short cytoplasmic region of caveolin appears essential for the interaction with signal transduction molecules and has thus been termed the scaffolding domain. The possibility exists, therefore, that caveolae, as a result of the properties of caveolins, may be specialised plasmalemmal regions involved in the co-ordination of receptor-coupled signalling events.

**Caveolins and uterine smooth muscle signal transduction**

Use of isoform-specific antibodies indicates that each caveolin isoform is expressed in rat and human uterine smooth muscle irrespective of gestational status (Taggart et al. 2000a,b). Laser scanning confocal microscopy of single isolated rat uterine smooth muscle cells indicates that caveolins-1, -2 and -3 are all localised predominantly at the plasma membrane (Fig. 2). Increased intensity of staining is often observed at the cell extremities and membranous areas closely apposing the nucleus (Taggart et al. 2000b). Thus caveolin localisation is suitable for any potential interaction with signalling molecules redistributed to the plasma membrane following cell stimulation.

Introduction to single isolated uterine smooth muscle cells of the caveolin-1 scaffolding domain peptide completely inhibited rhoA translocation following muscarinic stimulation (Fig. 3). PKC\(_{\alpha}\), suggested to be important in agonist-induced Ca\(^{2+}\) sensitisation of other smooth muscles (Lee et al. 1999), also shows a receptor-coupled relocalisation to the plasma membrane of uterine smooth muscle cells (Taggart et al. 1999) that is inhibited by the caveolin-1 scaffolding domain peptide (Taggart et al. 2000b). Translocation was unaffected in sham-treated cells, or in cells treated with a scrambled version of the caveolin scaffolding domain peptide (Taggart et al. 2000b). These data suggest that both rhoA and PKC\(_{\alpha}\) interact with caveolins via the N-terminal proximal scaffolding domain in intact uterine smooth muscle, results consistent with \textit{in vitro} biochemical studies (Oka et al. 1997; Gingras et al. 1998). Furthermore, Couet et al. (1997) described caveolin binding motifs typical of many signalling molecules found to interact with caveolin and both PKC\(_{\alpha}\) and rhoA contain corresponding sequences. In particular, the sites of monoglicosylation (T\(^{37}\)) and ADP-
ribosylation (N45) on rhoA are both contained within the putative caveolin binding motif (136YVPTVFEN41); this motif also overlaps with a region important for determining rhoA binding with ROK (Fujisawa et al. 1998). It is presently unknown if caveolin directly interacts with ROK but it too contains putative caveolin binding motifs in the catalytic domain of the molecule (131WVVQLFCAF159 and 164YLYMVMES172). Collectively, these data suggest that caveolin interacts with, and regulates activity of, rhoA (and possibly ROK). Consistent with such a mechanism of action is the observation that introduction of the caveolin-1 scaffolding domain peptide to intact uterine smooth muscle resulted in inhibition of contractile function: contractions in response to carbachol (but not those in response to KCl) were significantly reduced whilst responses in sham-treated tissues remained unaltered (Fig. 3).

Conclusions
Receptor-stimulated membranous recruitment of key signalling molecules rhoA and ROK appears to be crucial for agonist-induced Ca2+ sensitisation in single uterine smooth muscle cells. Caveolae, as a result of the regulatory actions of caveolins, are suggested to be specialised plasmalemmal regions involved in this integration of extracellular signals and intracellular effectors. Such discrete membranous localisation of signal transducing events is likely to contribute to the efficacy of receptor-coupled contractile activation of uterine smooth muscle.


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