SYMPOSIUM REPORT

Lipid rafts, the sarcoplasmic reticulum and uterine calcium signalling: an integrated approach

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The pathways involved in Ca^{2+} signalling in the uterus remain incompletely understood, impairing our ability to prevent preterm and difficult labours. In this review we focus on two elements in the pathway of Ca^{2+} signalling that have recently emerged as playing important roles: membrane lipid rafts and the sarcoplasmic reticulum. We examine the evidence for lipid rafts in the uterus and discuss their functional role. We suggest that the increases in cytosolic $[Ca^{2+}]$ and contractility that occur with raft disruption are due, at least in part, to effects on large conductance Ca^{2+}-activated K^+ (BK) channels that are localized to rafts. The role of the SR in contributing to subsarcolemmal cytosolic microdomains in uterus is evaluated, along with its interactions with ion channels on the plasma membrane. Thus, signalling microdomains play an important, but incompletely understood, role in the uterus, and integrating them into other Ca^{2+} signalling pathways is a challenge for further research. We suggest that the role of the SR changes in pregnancy, from promoting quiescence via BK channels or SR Ca^{2+} uptake, to promoting Ca^{2+} entry and contractility at term, and relate data on lipid rafts to clinical outcome in obese pregnant women.

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Introduction

Understanding the control of uterine contractility remains a key goal of many researchers. The motivation arises from both the richly complex nature of the problem and the need to prevent the enormous toll of preterm labours on neonatal mortality and morbidity. It is recognized that intracellular Ca^{2+} changes and the signals arising from these changes, are of paramount importance to contractility and its control (Wray *et al.* 2003). Much has been learnt in the last decade concerning Ca^{2+} signals in the myometrium and how these relate to uterine contractility. Two treatments for threatened preterm labour are based on affecting the change of intracellular Ca^{2+}, the Ca^{2+} channel antagonists (e.g. nifedipine) and magnesium chloride. The global Ca^{2+} signal recorded from uterine cells arises from the opening of voltage-gated L-type Ca^{2+} channels. In this review we examine two regulators of this Ca^{2+} rise, identified by recent work as possible important physiological mechanisms by which uterine activity is influenced: lipid raft microdomains, and the internal Ca^{2+} store, the sarcoplasmic reticulum (SR). As we will show, interactions between these two structures also occur, further adding to their role in modulating signalling in uterine cells. Answers to some of the questions raised by these latest findings may provide new loci for therapeutic interventions for both preterm labours and dysfunctional labours, i.e. those characterized by small infrequent contractions, necessitating delivery by caesarean section.

Membrane microdomains and lipid rafts

The plasma membrane of mammalian cells has long been known to be composed of a variety of lipids and proteins and contain ion channels, pumps and exchangers. Studies of many different cell types have shown that these components of the membrane are not evenly distributed, and the notion of membrane microdomains has been developed. The best characterized such microdomains are those known as lipid rafts, domains enriched in cholesterol and sphingolipid. The decreased fluidity as a consequence of the high cholesterol, gives rise to these regions becoming 'rafts', floating in the membrane (Simons & Ikonen, 2000;

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Laude & Prior, 2004). While there may be speculation about the exact physical nature of rafts (Laude & Prior, 2004), it is accepted that they have associated with them (or excluded from them) a range of signalling components, including ion channels, receptors and enzymes, and hence the interest in them as modulators of contractility (Simons & Toomre, 2000; O'Connell *et al.* 2004). Caveolae, invaginations of the surface membrane, may be regarded as a type of raft, where the structure has been stabilized by the protein caveolin (Quest *et al.* 2004). If we are to translate these exciting emerging concepts into medical benefit, the challenge is to elucidate the physiological importance of these microdomains and increase understanding of the underlying mechanisms. As described below, it may be anticipated that both membrane and cytosolic microdomains are playing an important role in uterine signalling.

Uterine caveolae

Caveolae are a particular feature of the membranes of uterine smooth muscle. The uterus expresses all three isoforms of caveolin (Taggart *et al.* 2000) and caveolae may increase in number towards the end of pregnancy (Turi *et al.* 2001; but also see Ciray *et al.* 1995). There is evidence that caveolae numbers are under hormonal control. Thus, oestrogen down-regulates the number of caveolae and the level of caveolin in rat uterine smooth muscle (Turi *et al.* 2001). In addition the oestrogen receptor may well be localized in caveolae, as their disruption with methyl β-cyclodextrin (MCD; a chelator of cholesterol) or caveolin-1 down-regulation has been found to activate oestrogen receptor α expression, suggesting that when it is localized to caveolae it is inhibited. Impairment of this inhibitory mechanism has been linked to 17β-oestradiol-stimulated mammary tumorigenesis (Zhang *et al.* 2005). Another powerful uterotonic hormone, oxytocin also appears to act via lipid rafts; the activity of its receptor is reduced if rafts are disrupted (Klein *et al.* 1995). A marked dependence of its binding function on cholesterol content suggests that the receptor exists in it high-affinity state only when it is in caveolae. It seems pertinent then to ask, what is the functional relevance of lipid rafts in the uterus?

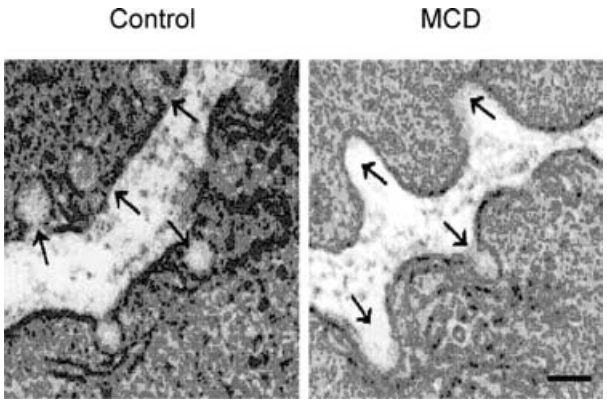

Figure 1. Uterine caveolae and cholesterol
Electron micrograph of rat myometrium, showing caveolae in control (untreated) uterus, and the disruptive effect of cholesterol extraction with methyl β-cyclodextrin (MCD). Scale bar 0.2 μm. From Smith *et al.* (2005).

Lipid rafts and the uterus

Figure 1 shows an electron micrograph of a uterine cell, and the arrows indicate caveolae before and after treatment with MCD (Smith *et al.* 2005). As can be seen, cholesterol extraction, which can be verified biochemically, disrupts caveolae. We have used MCD to explore the functional consequences of this raft disruption to uterine Ca^{2+} signalling and contractility (Kendrick *et al.* 2004; Smith *et al.* 2005). As Fig. 2 shows, modulation of cholesterol content of the myometrial plasma membrane has a profound effect on both Ca^{2+} and force. The

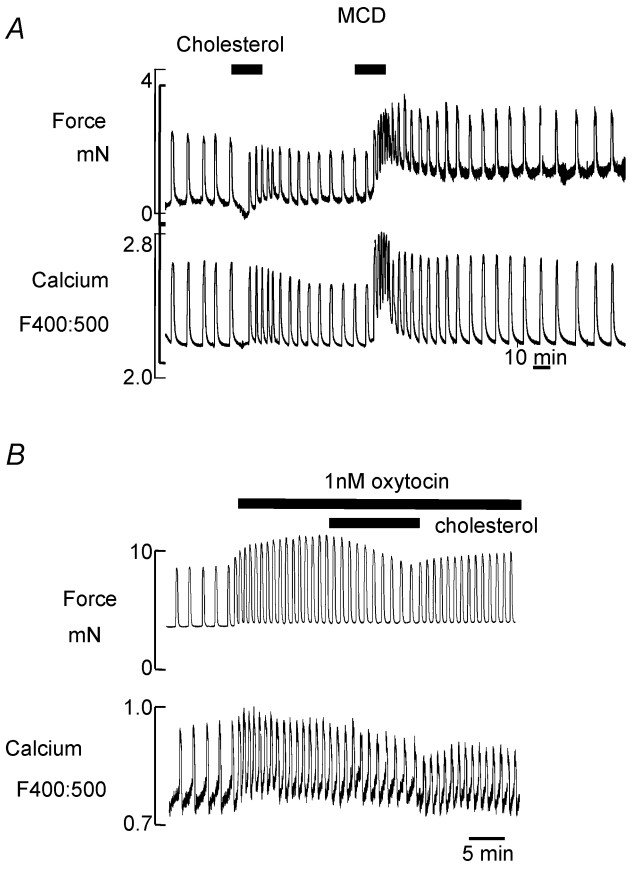

Figure 2. Cholesterol manipulation and uterine function
A, simultaneous recording of force and Ca^{2+} (indo-1 ratio) in a strip of myometrium taken from human myometrium. Methyl β-cyclodextrin (MCD, 15 mM) was used to extract cholesterol, and cholesterol was replenished using 0.5 mM cholesterol (Zhang & Wray, unpublished data). *B*, the effect of increasing cholesterol in the presence of oxytocin (1 nM) in myometrium form pregnant rat. Taken from Smith *et al.* (2005)

spontaneous contractions and associated Ca^{2+} transients of longitudinal muscle, from rat and human myometrium, were greatly enhanced by cholesterol extraction and raft disruption (Fig. 2*A*). The same result was found if cholesterol was reduced using the bacterial enzyme, cholesterol oxidase (Smith *et al.* 2005). Interestingly if cholesterol is increased in the membrane, force and Ca^{2+} are markedly reduced, or even abolished. This was the case even in preparations stimulated with oxytocin, to increase the contractile drive on the tissue (Fig. 2*B*). Application of low density lipoproteins (LDLs), which will also increase cholesterol, was also detrimental to myometrial force and Ca^{2+} signalling (Smith *et al.* 2005). Recent studies on both pregnant and non-pregnant human myometrium have shown the same findings; elevation of cholesterol is deleterious for uterine Ca^{2+} signalling and reduces the force of contraction; the clinical implications of these findings are discussed later.

Lipid rafts and function in other smooth muscles

The effects of lipid raft disruption appear to be smooth muscle and agonist specific. Thus in ureteric smooth muscle, MCD treatment selectively reduced Ca^{2+} signalling and phasic contractions (Babiychuck *et al.* 2002). Tonic contraction to 40 mM K^+ solution or carbachol were not affected by MCD, but the phasic components of these stimulants were abolished. This selective effect of cholesterol depletion is in agreement with an earlier study in blood vessels that showed MCD reducing efficacy of some but not all agonists (Dreja *et al.* 2002). Elevated cholesterol is considered to underly much vascular dysfunction, but a direct effect via rafts remains to be confirmed. High cholesterol is also thought to impair gall bladder contractility (Chen *et al.* 1999) and be a key factor in the pathogenesis of cholesterol gallstones, but the effects of raft disruption do not appear to have been examined. Further functional studies in other smooth muscles are required before any general conclusions can be drawn.

As mentioned above, rafts have been suggested to serve as platforms for signal transduction (Bastiaanse *et al.* 1997; O'Connell *et al.* 2004) and so the question arises, which specific targets can explain the functional effects on the uterus?

The effects on uterine Ca^{2+} signalling were found in the presence and absence of oxytocin, and in pregnant and non-pregnant animals and women. This suggests then that the mechanism is one fundamental to Ca^{2+} signalling (Smith *et al.* 2005).

BK channels

The enhancement of cytosolic uterine Ca^{2+} signalling by MCD suggests that lipid rafts contain elements of signalling pathways that reduce force; thus when rafts are disrupted these inhibitory signals are reduced. Such a mechanism would be analogous to the inhibitory control of eNOS and oestrogen receptors (Goligorsky *et al.* 2002), and would be explicable if K^+ channels were the target. Although there is evidence in other tissues for both K_{ATP} (Sampson *et al.* 2004) and K_V (Martens *et al.* 2004) channels being localized to lipid rafts, in myometrium the large conductance, Ca^{2+}-sensitive K^+ (BK) channel may be more important.

There is growing evidence from biochemical and molecular biology studies that the BK channel is located in caveolae (Babiychuck *et al.* 2002; Brainard *et al.* 2005). The opening of BK channels produces membrane hyperpolarization and relaxation of the uterus, as opening of voltage-gated Ca^{2+} channels is reduced (Anwer *et al.* 1993; Wray *et al.* 2003). Furthermore in freshly dispersed myocytes from rat myometrium, we have recently found that MCD reduces outward current due to BK channels, consistent with BK channels being located in rafts (Shmygol & Wray, 2005).

Recent data have suggested that coupling between myometrial β_2-adrenoreceptors and BK channels facilitates uterine relaxation (Chanrachakul *et al.* 2004). If localization of BK channels to rafts is necessary for their proper functioning, then this may be an additional mechanism to regulate uterine contractility. It has already been shown that BK expression changes with gestation in many species (Khan *et al.* 1993; Eghbali *et al.* 2003) and may also be regulated by clustering at the surface membrane (Eghbali *et al.* 2003); transient movement into and out of rafts would add another element to their control.

From microdomains to microsignals

One of the main regulators of BK channels is intracellular Ca^{2+}, and in particular Ca^{2+} release from the SR has been implicated in activating these channels, as well as Ca^{2+}-activated Cl^- channels. We have recently shown that the SR plays a pivotal in controlling the refractory period in ureteric smooth muscle (Burdyga & Wray, 2005). Thus Ca^{2+} release from the SR may be anticipated to have a marked influence on excitability and hence contractility of the uterus (Wray *et al.* 2003) and this will now be discussed. As we will also describe, microdomains in the cytoplasm, under the microdomains in the plasma membrane, are also thought to play a substantial role in regulating contraction and Ca^{2+} signalling.

Sarcoplasmic reticulum structure

Modern imagining techniques have allowed an appreciation of the beautiful reticular pattern of the SR of the uterine myocytes (Fig. 3): an interconnecting membrane system of tubules and cisternae found

throughout the cytoplasm. In uterus it makes up around 6% of the total smooth muscle cell volume (Shmygol & Wray, 2004) and increases in volume during pregnancy.

The SR is able to take up Ca^{2+} against the electrochemical gradient due to ATP-dependant Ca^{2+} pumps (SERCA) in the SR membrane. In the uterus there is evidence of physiological regulation of pump activity; expression of the dominant isoform (SERCA2b) is up-regulated during pregnancy (Khan *et al.* 1993; Tribe *et al.* 2000*a*).

In smooth muscle cells, Ca^{2+} is released from the SR by IP_3- and Ca^{2+} (ryanodine)-dependant channels. In myometrium, all three isoforms of the IP_3 channel (Morgan *et al.* 1996) and the ryanodine channel (RyR) (Martin *et al.* 1999) have been found. Golovina & Blaustein (1997) first proposed that although the smooth muscle SR appeared continuous, its Ca^{2+} stores were organized into spatially distinct units that could have specific physiology. There is indeed evidence for this occurrence in myometrium and it is discussed in more detail in Shmygol & Wray (2004).

SR compartments

In pregnant rat uterine myocytes, fluorescent labelling of ryanodine and thapsigargin (which binds to SERCA) is non-homogeneous and is clearly concentrated around the nucleus (deep SR) and close to the cell membrane (superficial SR; Shmygol & Wray, 2004). It is not yet known if these regions differ in their expression of either IP_3 or RyR release channels or SERCA, or if they are the functionally distinct store identified by some workers in other smooth muscles (e.g. Flynn *et al.* 2001; Wray *et al.* 2005). Van Breemen *et al.* (1986) first suggested from work on vascular tissue that the superficial SR had a specific function, that it buffered Ca^{2+} by taking up a fraction of the Ca^{2+} that enters the cell through the plasmalemma, and that the deep SR supplies Ca^{2+} for the contractile machinery. Studies on rat (Shmigol *et al.* 1999) and human (Young *et al.* 2001; Young & Zhang, 2004) myocytes lend support to this functional separation between deep cytosolic and subplasmalemmal calcium concentrations in the myometrium. The close apposition between superficial SR and parts of the plasma membrane has led to the suggestion that it is a special signalling domain (Blaustein *et al.* 2002) and will result not only in different $[Ca^{2+}]$ from bulk cytosol, but also different $[Na^+]$, due to preferential expression of the $\alpha 2$ subunit of the Na^+ pump. Although little explored in the uterus to date, we have preliminary evidence of gestational changes in Na^+ pump in myometrium (Floyd *et al.* 2003).

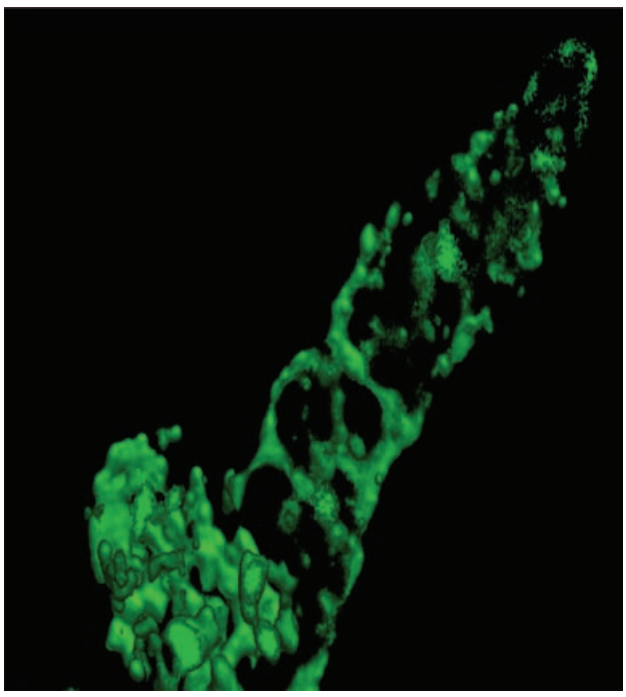

Figure 3. Uterine SR
An *xyz* image of the sarcoplasmic reticulum (SR) from a rat uterine myocyte, obtained on a Perkin-Elmer Ultraview confocal system, and outlined with bodipy–ryanodine (Shmygol & Wray, unpublished work).

SR and local cytosolic Ca^{2+} releases

Van Breemen's model required that for SR Ca^{2+} to be maintained at steady state, accumulated Ca^{2+} is released into the subplasmalemmal space (referred to as 'vectorial Ca^{2+} release') and extruded through the cell membrane. There is currently a great deal of interest in how smooth muscle activity might be regulated by interaction of vectorial Ca^{2+} release and specific membrane microdomains. These studies have been greatly helped by confocal microscopy allowing the direct visualization of Ca^{2+} release from the SR, Ca^{2+} sparks from RyRs and Ca^{2+} puffs from IP_3Rs.

Spontaneous transient outward currents (STOCs) are associated with Ca^{2+} sparks and BK channels activation in many smooth muscles (Burdyga & Wray, 2005). STOCs have been recorded in some uterine myocytes but no reports of Ca^{2+} spark activity appear to have been made, perhaps because Ca^{2+} release channels are not sufficiently clustered. It is however, clear that the SR has an important role in controlling myometrial excitability and contraction.

SR and uterine contraction

In the mouse, rat and human myometrium pharmacological inhibition of the SR Ca^{2+} pump, e.g. by cyclopiazonic acid (CPA), causes depletion of Ca^{2+} from the SR and increases cytosolic $[Ca^{2+}]$ and contraction (Taggart & Wray, 1998; Tribe *et al.* 2000*b*; Noble & Wray, 2002; Kupittayanant *et al.* 2002; Matthew

et al. 2004). In rats, these studies have also provided evidence of physiological regulation of the myometrial SR; 60th developmental regulation (inhibition of the SR caused greater stimulation of neonatal uterus compared with adult uterus; Noble & Wray, 2002) and during pregnancy when SR Ca^{2+} depletion caused increased stimulation in late compared with early pregnancy (Taggart & Wray, 1998). These data are supported by Tribe *et al.* (2000*b*) who found in human myometrium that SR inhibition caused greater stimulation in labouring compared with non-labouring uterus. In order to understand how the SR might limit contraction *in vivo* we must question how SR Ca^{2+} depletion stimulates contraction.

SR and ion channels

Depletion of SR Ca^{2+} was shown to depolarize and contract vascular smooth muscle in a manner similar to agents which inhibited BK channels (Nelson *et al.* 1995). However, in uterus Taggart & Wray (1998) found that prior inhibition of BK channels with iberiotoxin did not inhibit further potentiation with CPA, and in cultured human uterine myocytes, Young & Zhang (2004) have recently shown that depletion of SR Ca^{2+} with CPA increased BK channel activity.

An alternative explanation is that SR Ca^{2+} depletion activates store-operated calcium entry (SOCE). SOCE is well described in non-excitable cells and increasingly in smooth muscle. SOCE has been demonstrated in primary cultured (Tribe *et al.* 2000*a*) and immortalized pregnant human myometrial cells stimulated with oxytocin (Monga *et al.* 1999), although caution must be used when applying these data on phenotypically altered cells to intact preparations of uterus. In rat myometrium, we have recently shown that a large component of the CPA-induced rise in basal Ca^{2+} is due to SOCE (Noble *et al.* 2005). The putative SOCE channels are thought to be members of the Trp C family and interestingly Dalrymple *et al.* (2004) have recently reported an up-regulation of specific Trp C mRNA and protein during human labour.

We know that oxytocin and prostaglandins are important myometrial agonists during labour and that they induce IP_3 receptor activation and SR Ca^{2+} release. We might therefore speculate that as pregnancy progresses SERCA is up-regulated, and SR Ca^{2+} uptake is the dominant process until labour, when agonist release of SR Ca^{2+} allows SOCE and increased uterine excitability.

Obesity, channels and labour – translational research

It is interesting to speculate that the elevation of cholesterol, which occurs in obese women, could contribute to poor uterine contractility *in vivo*. Obesity is associated with an increase of both cholesterol and LDLs, and there is a further elevation of both in pregnancy (Gostynski *et al.* 2004). There is an increased risk of caesarean section associated with pregnancy in obese women (Crane *et al.* 1997). The underlying reason for the increased risk of surgery is not known, and it is tempting to speculate that the dyslipidaemia in these women is causing poor contractility, leading to dysfunctional labour, and hence the need for surgical intervention. This is supported by our preliminary data (Kendrick *et al.* 2004), showing poor contractility with increasing body mass index, this, in turn, being the reason that a caesarean section is needed, rather than obstruction or fetal distress. During the onset of labour we conclude that changes in lipid rafts and caveolae may have a role, perhaps by their clustering or the sorting of regulatory components of cell signalling, into or out of them. We can also speculate that changes in channel expression and SR function needed for successful parturition may not occur adequately in those women suffering dysfunctional labour, but there are at present no data on this.

Summary

As our understanding of signalling becomes ever more complex, and as we learn more about local signals, domains and environments, it is important that the functional relevance, under physiological conditions, of putative pathways be tested. In this brief review we have attempted to relate some of the exciting findings from membrane biology and signalling biochemistry to the control of myometrial, and indicate future therapeutic potential.

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