The cytoskeleton of human myometrial cells

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Eukaryotic cells have an internal cytoskeletal scaffolding, giving them their distinctive shapes. The cytoskeleton enables cells to transport vesicles, undergo changes in shape, migrate and contract. This dynamic structure is formed by three classes of filamentous assembly: actin microfilaments, intermediate filaments and microtubules. In this investigation the cytoskeleton of cultured human myometrial cells was studied by immunohistochemistry using specific antibodies against vinculin, cytokeratin, vimentin, tubulin and RhoA, covalently labelled with a fluorescent tag. Polymerized actin was visualized with fluorescein-conjugated phalloidin. Myometrial cells were very rich in actin fibres, which generally appeared as parallel bundles along the longest axis of the cells. There was a strong expression of vinculin which concentrated at actin–vinculin focal adhesion sites. By contrast, intermediate filaments (vimentin and cytokeratin) were organized in a dense cytoplasmic meshwork which excluded the nuclear space. A similar pattern was observed for tubulin. RhoA had a diffuse distribution and was associated with actin fibres. Exposure of the cells to oxytocin provoked a 10% shortening of actin stress fibres. These results demonstrate that myometrial smooth muscle cells have a rich cytoskeletal structure and that agonists that stimulate myometrial activation provoke measurable changes in actin fibres which may be important for efficient contractility.

Introduction

The cytoskeleton is a dynamic structure that allows eukaryotic cells to adapt to a variety of shapes and to carry out co-ordinated and directed movements. Moreover, transport of vesicles inside the cell requires the interaction of cytoskeletal proteins and intracellular signalling systems such as Rho (Murphy *et al.*, 1996). Rho belongs to the family of small GTP-binding proteins (Hall, 1994) that have been implicated in the signalling processes that activate actin fibres during the chemotactic response of neutrophils and control morphological changes in fibroblasts. The cytoskeleton is a sensor of cell volume and is essential during cell division; it may also be involved in the development of tumours (Dartsch *et al.*, 1994; Symons, 1996). Contraction of cells involves the cytoskeleton not only for generating force within each cell, but also for transmitting contractile forces through attachment and linkage of groups of cells and interaction with tissue matrix (Hall, 1994). The diverse activities of the cytoskeleton depend on actin filaments, intermediate filaments and microtubules. Polymerized actin filaments (F-actin) elongate by stepwise addition of actin monomers to exposed ends through ATP hydrolysis (Tardif *et al.*, 1995). However, a significant proportion of the actin is not polymerized because of the presence of actin-binding proteins (Cunningham, 1992).

Vinculin is one of several interacting focal adhesion proteins involved in anchoring F-actin to the membrane through

adhesion plaques (Geiger *et al.*, 1987). An adhesion plaque is a complex structure formed as a result of integrin heterodimers binding to extracellular matrix ligands (Pavalko and La Roche, 1993). Integrin β -subunits bind to talin which binds to vinculin, which, in turn, interacts with α -actinin (Stromer, 1995). Since α -actinin is known to bind to and crosslink actin filaments, vinculin represents a key element in the transmembrane linkage of the extracellular matrix to the cytoplasmic microfilament system (Stromer, 1995).

Intermediate filaments, such as cytokeratins and vimentins, are cytoplasmic structural proteins present in most vertebrate cells. Cytokeratins, a group made up of at least 29 different proteins are characteristic of epithelial cells (Moll, 1994). Vimentin is a widely distributed cytoplasmic intermediate filament protein and is present in many cells of mesodermal origin, including fibroblast, endothelial and white blood cells (van-den-Hurk *et al.*, 1995). There is little knowledge about distribution of these proteins in myometrial smooth muscle cells.

Microtubules are structural and mobile elements involved in mitosis, intracellular transport, flagellar movement and other functions mediated by the cytoskeleton. Tubulin, the major building block of microtubules is a heterodimer (α - and β -subunits). Structurally different tubulin subunits are the product of different genes and the microtubular system contains at least three α -tubulin isoforms (Burns and Surridge, 1994).

In previous studies we have investigated second messenger pathways involved in receptor activation of myometrial cells

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(Asbóth *et al.*, 1996; Phaneuf *et al.*, 1996; Carrasco *et al.*, 1996). In this investigation we studied the presence of cytoskeletal elements that provide the structural support for efficient myometrial contractions. The attachment of the cells to different types of extracellular matrix protein was compared, and the close association of RhoA to F-actin was demonstrated.

Materials and Methods

Materials

Fluorescein (FITC)-conjugated phalloidin was from Molecular Probes (Eugene, OR, USA). Monoclonal mouse anti-human vinculin antibody, monoclonal mouse anti- α -tubulin antibody, monoclonal mouse anti-cytokeratin peptide 18 antibody, monoclonal mouse anti-vimentin antibody, purified mouse immunoglobulins, sheep anti-mouse IgG FITC-conjugated antibody, EGF, fibronectin, collagens I, III and IV were obtained from Sigma Chemical Company (Poole, Dorset). Texas Red-conjugated goat anti-rabbit IgG antibody and Texas Red-conjugated goat anti-mouse IgG antibody were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA). Rabbit anti-RhoA polyclonal antibody, RhoA blocking peptides and FITC-conjugated anti-rabbit IgG were obtained from Santa Cruz Biotechnology (Autogen-Biolar, Calne, Wiltshire). Oxytocin was purchased from Calbiochem-Novabiochem (Nottingham). Human chorionic gonadotrophin (hCG, Profasi) was obtained from Serono Labs UK Ltd (Herts). All other reagents were commercial preparations of the highest available purity. Laminin and heparin-binding epidermal growth factor (HB-EGF) were gifts from H. Mardon (Nuffield Department of Obstetrics and Gynaecology, Oxford).

Isolation and culture of myocytes

Myometrium was obtained from six premenopausal women at hysterectomy. The operations were indicated for benign gynaecological disorders (menorrhagia, dysmenorrhoea) in patients of mixed (1–3) parity. All patients gave informed consent. Samples were taken from the middle of the uterine wall, about 5 mm from endometrial or serosal surfaces. Uterine leiomyomas (fibroids) were not included in this study. The tissues were collected in ice-cold Krebs solution, washed and processed for cell dispersion within 60 min of excision. Pieces of myometrium were incubated with gentle shaking for 2 h at 37°C in Hanks' balanced salt solution (HBSS) containing 2.5 mmol $\text{CaCl}_2 \text{ l}^{-1}$, 0.9 mmol $\text{MgCl}_2 \text{ l}^{-1}$ and 10 mg dispase ml^{-1} . The softened pieces of tissue were then washed in Ca^{2+} -free and Mg^{2+} -free HBSS and cell dispersion was achieved in the same solution supplemented with collagenase (300 U ml^{-1}), elastase (2 U ml^{-1}), DNase I (30 U ml^{-1}) and 1 mg FAF-BSA ml^{-1} at 37°C with gentle shaking. After 2 h, the cell suspension was aspirated with a Pasteur pipette, filtered through 250 μm sterile gauze and washed three times with HBSS by centrifugation at 600 g. The final pellet was resuspended in culture medium: Waymouth MB752/1 containing 10% (v/v) fetal calf serum, 10 000 iu penicillin ml^{-1} and 10 mg streptomycin ml^{-1} . Viability was checked with Trypan blue

and was consistently higher than 80%. Myocytes were plated at densities between 0.5 and 2×10^4 cells cm^{-2} in plastic culture flasks. The myocytes were kept at 37°C in a humidified atmosphere of 95% air and 5% CO_2 until confluent (6–10 days). The medium was changed after 24 h and then every other day.

Cell attachment

Glass coverslips (13 mm) were sterilized by baking overnight and were precoated with either collagen I, collagen III, collagen IV or fibronectin, all at a concentration of 10 $\mu\text{g ml}^{-1}$, or with laminin at 1 $\mu\text{g ml}^{-1}$. The coverslips were then seeded with myometrial cells at 2×10^4 cells per well in Waymouth's medium with and without 10% (v/v) fetal calf serum and the cells were allowed to attach for 2, 6, 16, 24 and 48 h at 37°C in a humidified atmosphere containing 5% CO_2 .

Growth factors and hormones

Myometrial cells were incubated with EGF, HB-EGF (10–200 ng ml^{-1}) or hCG (0.1–1.0 iu ml^{-1}), for 1 h, 6 h and 16 h before fixing and staining for cytoskeletal proteins. In separate experiments, the cells were challenged with oxytocin (1 $\mu\text{mol l}^{-1}$) for 45 s, 5 min and 3 h before fixing and staining.

Immunohistochemistry

At the times indicated the coverslips were washed twice with warm (37°C) phosphate-buffered saline (PBS). The cells were then fixed in 3% (w/v) paraformaldehyde diluted in PBS at room temperature for 5 min, followed by incubation in permeabilization buffer (10 mmol Hepes l^{-1} pH 7.4, 200 mmol sucrose l^{-1} , 3 mmol $\text{MgCl}_2 \text{ l}^{-1}$, 50 mmol NaCl l^{-1} , 0.5% (v/v) Triton X-100, 0.2% (w/v) sodium azide) for 5 min. Finally, the coverslips were washed twice in PBS containing 3% (w/v) BSA and the cells were exposed to the appropriate conjugates or antibodies for 45–60 min in the dark at room temperature. After staining, the coverslips were mounted on glass slides with polyvinyl alcohol ready for analysis under the fluorescence microscope.

Actin. Polymerized F-actin was visualized with FITC-conjugated phalloidin (dilution 1:40), based on the ability of phalloidin to bind actin (Allen *et al.*, 1996).

Vinculin. Vinculin was stained with a monoclonal antibody to human vinculin (dilution 1:400), derived from mouse ascites fluid. The coverslips were washed in PBS/0.05% (w/v) BSA for 5 min and visualized with Texas-Red conjugated goat-anti-mouse IgG antibody.

Cytokeratin. A monoclonal FITC-conjugated mouse anti-cytokeratin peptide 18 antibody was used in the presence of 2% normal human serum at 1 in 100 dilution.

Vimentin. The cells were incubated with the primary antibody to vimentin (dilution 1:40), washed in PBS and then incubated with sheep anti-mouse IgG FITC-conjugate.

Tubulin. The cells were incubated with a monoclonal antibody against α -tubulin (dilution 1:2000), washed in PBS and then incubated with sheep anti-mouse IgG FITC-conjugate.

RhoA. The cells were incubated with primary antibody against RhoA (dilution 1:100), washed in PBS and then incubated in Texas-red conjugated goat anti-rabbit IgG antibody.

Negative controls. These were carried out by omitting the primary antibodies and by blocking with purified mouse immunoglobulins. Furthermore, RhoA antibodies were preabsorbed with cognate peptides.

Image analysis. Cells were visualized with a Leica DMRBE epifluorescence microscope and images were captured using the Biovision software package (Improvision, Warwick Science Park, Coventry) on an Apple Macintosh computer. Images were captured in standard formats and the cells were counted and measured using calibrated image estimations. Red and green fluorescent images were taken from the same fields using appropriate light frequencies and filters.

Statistical analyses

Experiments were carried out using four coverslips for each condition and were repeated at least twice using different cell cultures. The effect of the various extracellular matrices or hormones on cytoskeletal structures was assessed in a minimum of 100 cells per different time or condition. Quantitative differences were assessed by Student's *t* test.

Results

Effect of extracellular matrix on cell attachment

The attachment of cells was studied comparing several matrices (collagen I, collagen III, collagen IV, fibronectin and laminin), with and without serum in the medium. Under all conditions, cell attachment was very poor for at least 6 h. However, after incubation for 16 h there were clear differences. Cells incubated in the presence of serum attached much better than in serum-free medium and tended to align themselves in parallel, reminiscent of myometrial smooth muscle cell bundles in fresh tissue (Fig. 1). Collagen IV and, to a lesser extent, fibronectin, improved cell attachment in serum-free conditions whereas collagens I and III and laminin had no significant effect. In the presence of serum, collagen IV and fibronectin accelerated the rate of cell attachment, although after 24–48 h there was excellent attachment irrespective of the matrix used, probably because in serum-rich conditions myometrial cells were able to synthesize or deposit enough matrix proteins. For further experiments, myometrial cells were routinely allowed to attach for 24 h on a matrix of collagen IV.

Distribution of actin, vinculin and RhoA

Myometrial cells were very rich in actin stress fibres which were generally oriented along the longest axis of the cells (Fig. 2). Actin fibres often spanned the whole length of the cell. There was a strong expression of vinculin (Fig. 3) which concentrated at adhesion plaques. It was possible, using a double staining technique, to demonstrate the colocalization of vinculin and the termini of actin fibres in focal adhesion plaques (Fig. 4).

By contrast, RhoA had a diffuse, granular distribution and tended to accumulate in the centre of the cell. Nevertheless there was also an intimate association between RhoA protein and actin fibres (Fig. 5). Thus, vinculin–actin focal adhesion complexes provide the basis for myometrial cell attachment to the extracellular matrix and for the architectural integrity of the cells, whereas RhoA may be a link between hormonal and other signals reaching myometrial cells and the regulation of actin dynamics.

Intermediate filaments

The intermediate filaments cyokeratin and vimentin revealed a complex and dense mesh of cytoskeletal structures which occupied the cytoplasmic compartment but excluded the nucleus, providing a clear 'negative' impression of the nuclear space. There was stronger staining for vimentin than for cyokeratin, but both components of the intermediate cytoskeleton followed a similar pattern (Figs 6 and 7). The presence of cyokeratin was somewhat surprising as this protein is usually found in cells of epithelial origin (O'Keane *et al.*, 1992).

Microtubules

Myometrial cells had a dense basketwork of microtubules as revealed with the tubulin antibody. Microtubules were seen in greatest density around the nucleus where they radiated out into the cell periphery in fine lacelike threads. This tubular arrangement is essential for the transport of vesicles and organization of cytoplasmic organelles in the cells. Again, the nuclear space was clearly delineated (Fig. 8).

Effect of growth factors and hormones

When myometrial cells were incubated in the presence of EGF, we observed an increase of cytoplasmic extensions resembling filopodia, more evident at the leading edge of the cells, but also in areas of cell to cell contact; otherwise the morphology of the cells was very similar to serum control. The effect of EGF was evident only when the cells were treated with 200 ng l^{-1} for 16 h (Fig. 9). HB-EGF at the same dose had little effect. Exposure of the cells to 1 iu hCG l^{-1} for 16 h provoked a small increase in filopodia; otherwise there was no apparent effect on cell morphology.

Oxytocin provoked a rapid but transient effect on actin organization, which resulted in apparent shortening of the cytoskeleton. For quantitating the effect of oxytocin, the length of the actin cytoskeleton of individual cells was measured along their longest axis. The results showed that oxytocin provoked

Fig. 1. Attachment of human myometrial cells to glass coverslips. Cells were allowed to attach in the presence of 10% fetal calf serum: (a) and in serum free medium: (b) for 24 h. F-actin stress fibres visualized by the phalloidin method. Scale bars represent 20 μ m.

Fig. 2. Actin stress fibres in human myometrial cells. Note the abundance of filaments in a predominately longitudinal orientation. The cells were allowed to attach in 10% serum for 24 h. Scale bar represents 20 μm .

a significant shortening of the actin fibres after 45 s and 5 min of exposure, but the effect was not seen after 3 h (Table 1).

Discussion

This is the first comprehensive demonstration of cytoskeletal proteins in human myometrial cells. In the uterus, myometrial smooth muscle cells are arranged in bundles that are embedded in a matrix of connective tissue consisting mainly of collagen. The human uterus has collagens types I, III, IV, V and VI (Karkavelas *et al.*, 1988) which form a dense matrix that facilitates the transmission of the contractile forces generated by the individual myometrial cells. We compared several matrix components and found that, under our *in vitro* conditions, collagen IV resulted in accelerated cell attachment. Type IV collagen molecules have a more flexible structure than the fibrillar collagens. Their triple-stranded helix is interrupted in 26 regions, allowing multiple bends (Eyre *et al.*, 1984). Electron microscope studies of preparations of assembling type IV collagen molecules suggest that these molecules associate by

their carboxyl termini to form head-to-head dimers, which then form an extended lattice via amino-terminal associations with three other collagen molecules (Eyre *et al.*, 1984). Disulphide and other covalent cross-links between the collagen molecules stabilize these associations. The resulting meshwork forms an insoluble scaffolding to which other components of the basal lamina bind via their specific associations with type IV collagen molecules (Shimizu *et al.*, 1997). The ability of type IV collagen to assemble into a multilayer meshwork might provide better attachment of myometrial cells onto the coverslips. The cells had a tendency to align themselves in bundle-like structures resembling their distribution in fresh myometrium (Scoutt *et al.*, 1991).

The cytoskeleton is a structure primarily involved in linking or connecting protein filaments to each other and to anchoring sites in the adhesion plaques (Gilmore and Burridge, 1996). Our data demonstrate a complex array of cytoskeletal structures in myometrial cells. There was a high density of parallel actin fibres usually in the direction of the longest axis, and their presence is in good agreement with the contractile function of these cells. The actin filaments provide strong cytoskeletal

Fig. 3. Vinculin at focal adhesion plaques. Human myoblasts were allowed to attach for 16 h to a matrix of collagen IV in serum free medium. Note the high density of plaques over the entire area of cell matrix contact: (a). In the absence of collagen IV: (b). There was poor attachment and vinculin tended to have a peripheral distribution. Vinculin was visualized using a specific monoclonal antibody and a Texas Red conjugated secondary antibody. Scale bars represent 20 μ m.

Fig. 4. a and b. Colocalization of actin fibre terminus and vinculin at focal adhesion plaques in human myometrial cells, demonstrated by double staining. Cells were allowed to attach for 24 h on a matrix of collagen IV. Vinculin was visualized using a specific monoclonal antibody and a Texas Red conjugated secondary antibody. F-actin in green and vinculin in red. Scale bars represent 20 µm.

Fig. 5. Texas Red fluorescent immunochemistry of human myometrial cells using anti RhoA polyclonal antibodies. Cells were allowed to attach for 24 h on a matrix of collagen IV. Note the association between Rho A and actin stress fibres which can be visualized even though there was no staining for F-actin (a). (b) Negative control in which the Rho antibody was preabsorbed with blocking peptide. Scale bars represent 20 μ m.

Fig. 6. Fluorescent immunocytochemistry of human myometrial cells using fluorescein (FITC)-conjugated anti-cytokeratin monoclonal antibodies. Cells were allowed to attach for 24 h on a matrix of collagen IV. Note the meshwork distribution of cytokeratin fibres in the cytoplasm and the nuclear exclusion (a); (b): Negative control using blocking immunoglobulins. Scale bars represent 20 μ m.

Fig. 7. Fluorescent immunocytochemistry of human myometrial cells using fluorescein-ITC-conjugated anti-vimentin monoclonal antibodies. Cells were allowed to attach for 24 h on a matrix of collagen IV. Note the dense hair-like structure within the cytoplasm (a). (b) Negative control without the primary anti-vimentin antibody. Scale bars represent 20 μm.

Fig. 8. Microtubules in human myometrial cells. Tubulin was visualized by anti α -tubulin monoclonal antibody and fluorescein (FITC) conjugated secondary antibody (a). Cells were allowed to attach for 24 h on a matrix of collagen IV. (b) Negative control without the primary antibody. Scale bar represents 20 μ m.

bridges between focal adhesion points and we were able to show by double immunostaining an intimate relationship between vinculin, a major component protein of focal adhe-

sions (Geiger *et al.*, 1987), and the distal ends of actin fibres at the cell membrane. Obliquely organized contractile filaments belonging to a longitudinal cytoskeletal framework and a

Fig. 9. Effect of (a) epidermal growth factor (EGF), (b) heparin-binding EGF and (c) hCG on cultured human myometrial cells; (d) control. The cells were allowed to attach to a matrix of collagen IV in the presence of serum for 24 h. After attachment they were washed and exposed to growth factors and hormones. The arrows point to actin-rich filopodia-like extensions. Scale bar represents 20 μ m.

multitude of vinculin attachments allow the cells to exert tension on the surrounding extracellular matrix over their entire length. Actin-associated membrane complexes are essential for co-ordinated cell motility in fibroblasts and other cell types (Cunningham, 1992), and it is likely that actin/vinculin attachments to the collagen matrix via integrins are required for effective uterine smooth muscle activity. Moreover, actin-associated proteins are known to interact with the contractile machinery and may be actively involved in the maintenance of tension. For example, calponin has been implicated in the regulation of smooth muscle contraction through its interaction with F-actin and inhibition of the

actin-activated Mg^{2+} -ATPase activity of phosphorylated myosin (Borovikov *et al.*, 1996).

Contractions in myometrial cells are known to be activated primarily by the activation of Ca^{2+} /calmodulin-dependent myosin light chain kinase (Word *et al.*, 1994). Oxytocin stimulates myometrial contractility by increasing intracellular calcium. This involves coupling of the oxytocin receptor to phospholipase C- β by GTP-binding proteins (G proteins) of the Gq and Gi families (Phaneuf *et al.*, 1993; Ku *et al.*, 1995; Phaneuf *et al.*, 1996). Moreover, oxytocin can activate small G proteins of the Rho family and preliminary data suggest that oxytocin provokes translocation of RhoA to the cytoskeletal

Table 1. Effect of oxytocin on the maximum length (μm) of polymerized actin fibres in human myometrial cells

	Basal	45 s	5 min	3 h
Experiment 1	92.8 $\pm$ 27.3	84.8 $\pm$ 27.1*	84.4 $\pm$ 21.6*	N.D.
Experiment 2	94.5 $\pm$ 24.8	83.3 $\pm$ 26.7*	82.8 $\pm$ 22.9*	93.8 $\pm$ 25.7

The cells were challenged with $1 \mu\text{mol oxytocin l}^{-1}$ for the times indicated.

* $P < 0.01$ compared with control cells; means $\pm$ SD; $n = 100$ – 105 for each.

ND: not determined.

compartment in rat myometrium (Ruzycky, 1997a). RhoA is thought to link cell membrane receptors to the organization of actin into stress fibres and the phosphorylation of thick filament components (Ridley, 1996). Double immunostaining of actin and RhoA showed a strong association between the two. In human myometrial cells RhoA is thought to enhance the calcium sensitivity of smooth muscle contractions (Ruzycky, 1997b). There are several mechanisms by which Rho can enhance myosin activation; for instance, Rho inhibits myosin phosphatase favouring myosin phosphorylation indirectly (Kimura *et al.*, 1996) and it can also promote direct myosin light chain phosphorylation via stimulation of Rho kinase (Amano *et al.*, 1996). Recent data have demonstrated a direct contractile effect of Rho kinase in vascular smooth muscle through myosin light chain phosphorylation (Kureishi *et al.*, 1997); whether a similar system operates in myometrium requires investigation. It is possible that oxytocin initiates myometrial activation through the G protein calcium pathway, with further potentiation of contractions involving a RhoA-mediated increase in actin polymerization (Ruzycky, 1997a).

Myometrial cells show a marked increased in protein phosphorylation in response to EGF (Carrasco *et al.*, 1996) which operates through the intrinsic tyrosine kinase activity of its receptor (Wahl *et al.*, 1989). EGF has mitogenic effects and may be involved in uterine growth in pregnancy and in fibroid formation (Harrison-Woolrych *et al.*, 1994). Furthermore myometrial EGF receptors increase during spontaneous labour (Gargiulo *et al.*, 1997) and it is thought that EGF may favour parturition by increasing prostaglandin production by intra-uterine cells (Pollard and Mitchell, 1996). Our data suggest that EGF increases the presence of actin-associated cytoplasmic projections, reminiscent of filopodia and other structures which appear at points of neuronal cell growth (O'Connor and Bentley, 1993) and during movement in fibroblasts (Small, 1994). The function of filopodia in myometrial cells is not clear, but they may serve to increase cell to cell contact to provide a synchronized response to hormonal and electrical stimuli during uterine activation.

Intermediate filaments (cytokeratin and vimentin) were very dense and prominent in myometrial cells which was somewhat unexpected as such complexity is usually ascribed to cells of neuronal and epithelial origin (Stagaard and Mollgard, 1989). Moreover, there was strong cytoplasmic staining for tubulin. It is now accepted that apart from their specialized contractile function, myometrial cells have secretory properties, for example they release interstitial collagenase (Jeffrey *et al.*, 1991). Microtubules are long stiff polymers found throughout

the cytosol which govern the location of membrane-bound organelles and other cell components. Microtubule formation is essential during mitosis, and although in this study we have not investigated cell division, the strong presence of tubulin may be related to the enormous capacity of myometrial cells for hypertrophy and hyperplasia during pregnancy.

In summary, we have demonstrated a complex cytoskeletal structure in human myometrial cells. Although there are many studies on myometrial function at the level of receptor/second messenger activation and electrophysiological mechanisms, the role of the cytoskeleton has received little attention. Our findings emphasize the need to address the interaction of cytoskeletal proteins with extracellular matrix components and the response of these proteins to signalling events during myometrial activation, in order to achieve a full understanding of the mechanism of contractility.

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