The Myometrium: From Excitation to Contractions and Labour

10

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Abstract

We start by describing the functions of the uterus, its structure, both gross and fine, innervation and blood supply. It is interesting to note the diversity of the female's reproductive tract between species and to remember it when working with different animal models. Myocytes are the overwhelming cell type of the uterus (>95%) and our focus. Their function is to contract, and they have an intrinsic pacemaker and rhythmicity, which is modified by hormones, stretch, paracrine factors and the extracellular environment. We discuss evidence or not for pacemaker cells in the uterus. We also describe the sarcoplasmic reticulum (SR) in some detail, as it is relevant to calcium signalling and excitability. Ion channels, including store-operated ones, their contributions to excitability and action potentials, are covered. The main pathway to excitation is from depolarisation opening voltage-gated Ca^{2+} channels. Much of what happens downstream of excitability is common to other smooth muscles, with force depending upon the balance of myosin light kinase and phosphatase. Mechanisms of maintaining Ca^{2+} balance within the myocytes are discussed. Metabolism, and how it is intertwined with activity, blood flow and pH, is covered. Growth of the myometrium and changes in contractile proteins with pregnancy and parturition are also detailed. We finish with a description of uterine activity and why it is important, covering progression to labour as well as preterm and dysfunctional labours. We conclude by highlighting progress made and where further efforts are required.

Keywords

Uterus · Electrophysiology · Pace-making · Calcium signalling · Sarcoplasmic reticulum · Parturition

10.1 Functions and Structure of the Uterus

10.1.1 Roles of Uterine Contractions

The uterus is a myogenic organ, mostly known for nurturing and protecting a foetus and then delivering it after a lengthy period of contractile activity. The smooth muscle within the uterus, the myometrium, is responsible for generating the electrical activity and thence contractions. The intrinsic, phasic pattern of activity is modulated by hormonal,

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metabolic and mechanical factors. Although most apparent during parturition, a low level of this intrinsic contractile activity is present in females throughout their life. This intrinsic activity can be recorded using surface electrodes on the abdomen to pick up electrical signals from the uterus [1], while contractions can be recorded in vitro in myometrial biopsies from women well beyond the age of menopause [2]. By analogy to skeletal muscle, such activity may help stop the tissue atrophying. In addition to childbirth, there are also two other occasions when uterine contractions are important to a woman's reproductive life, menses and fertilisation.

10.1.1.1 Menses

Menses, periods or menstruation, is the sloughing of part of the uterine lining (endometrium) each month between puberty and menopause in non-pregnant women. The shedding of this menstrual debris is aided by uterine contractions. The muscular strength and activity of the non-pregnant myometrium change throughout the menstrual cycle [3]. Waves of contractions produce a rippling effect on the endometrial surface. The frequency and strength of these contractions depend upon the hormonal milieu. Measurements of muscle strength have confirmed that contractile strength values approaching those found at labour (50–200 mmHg) can occur in non-pregnant myometrium during menstruation. Not surprisingly, this muscular effort produces menstrual pain in most women. Uterine activity helps to both slough off dead tissue from the endometrium into the uterine cavity and propel it towards the cervix and beyond.

10.1.1.2 Endometriosis

Endometriosis is a puzzling and painful condition. Often associated with infertility, it occurs in 5–10% of otherwise healthy women and there is no cure. Endometriosis arises because cells from the sloughing uterine lining are deposited at sites in the body, starting with the pelvis. The cells can adhere and cause inflammatory reactions and other problems. The endometrial debris in the pelvis is thought to have been propelled there from the uterus by aberrant, misdirected contractions—so-called retrograde menstruation [4]. It is unknown why some women are susceptible to this and the condition can be hard to diagnose and treat.

10.1.1.3 Fertilisation

Uterine activity is also helpful in moving sperm up from the cervix and towards the fallopian tubes. Sperm are motile using flagella to propel themselves from the vagina to the uterine cavity. The additional directed flow to the fallopian tubes from uterine contractions is considered necessary for them to reach their destination. The tubes are the usual site for fertilisation if a healthy egg has been released by the ovaries and transported down the fallopian tubes. Post-fertilisation, the egg moves down into the uterus, where it will implant, if hormonal conditions have prepared the endometrium appropriately.

It is also believed that a lack of uterine contractile activity is best for implantation of the fertilised egg into the endometrium, although the evidence is not extensive [5]. Using modern imaging techniques, rippling can be seen on the endometrial surface, arising from myometrial activity. This has led to the use of anti-contraction medication in women undergoing IVF egg transfers, e.g. blockers of oxytocin receptors [6] to help increase success rates.

10.1.2 Uterine Anatomy

The anatomical appearance of the uterus is species dependent. While in women there is one pear-shaped cavity, with the cervix at the bottom and fallopian tubes (oviducts) at the top, this is not the structure seen in other mammals. Rodents, which are a frequently used animal model, have a bicornuate uterus—they have two uterine "horns" in which the ten or so embryos implant. Rabbits and marsupials have two horns as well as two cervical canals, and in the case of the latter, two vaginas. These differences occur during embryonic development, as for example the paramesonephric ducts fuse to a greater (e.g. human) or lesser (e.g. rodent) extent.

Although the structure of the uterus varies considerably between species, its basic cellular con-

tent and tissue components are rather similar. There will be an outer serosal coat made of connective tissue, which is continuous with the broad ligament. The next tissue, which itself can be in several layers, and is by far the greatest component of the uterine structure, is the myometrium. It can usually be separated into circularly and longitudinally orientated muscle layers. In women, in contrast to rodents, these two layers are highly interwoven and separating the circular and longitudinal smooth muscle layers is not so easily accomplished due to the presence of interconnections between the two layers. For a detailed analysis of the structure of human myometrium, see [7]. Running along and between these muscle bundles and layers are the uterine blood vessels and nerves.

10.1.2.1 Endometrium

The inner lining of the uterus is the endometrium which contains glands, as well as blood vessels. The endometrium is bounded on the luminal side of the uterus by a single layer of epithelial cells, which may or may not have cilia (depending on the stage of the menstruation cycle) and its basal lamina. Looping in from the epithelium are the uterine glands, and their extent and development also change with the menstrual cycle. The bulk of the tissue is a specialised, cell-rich connective tissue (stroma) containing a rich supply of blood vessels. The cells and glands of the endometrial lining will either grow or die depending on the cyclic changes in the female hormones, oestrogen and progesterone. Thus, it is the structures and cells of the endometrium, including the epithelium, that are lost each month during the menstrual cycle, or built up if pregnancy occurs. The endometrium also accommodates and provides the environment, i.e. constituents and support cells to develop the embryo. The basalis is the name given to the portion of the endometrium that is not shed each month, and from which the endometrium will be rebuilt during the next menstrual cycle. Menstruation only occurs in women, and some primates; in other species there are cyclical changes, referred to as oestrus. As with menstruation the appearance of the uterus can change considerably with the stage of oestrus, and there are accompanying moderate changes in excitability and contractility [8].

10.1.2.2 Cervix

The cervix is contiguous with the uterus and displays a decreasing muscle content and increasing connective tissue, especially collagen. During pregnancy, the cervix serves as a barrier, protecting the foetus from infection and retaining the foetus in utero. The non-pregnant cervix (~3 cm in length) is principally composed of dense fibrous extracellular matrix (predominantly collagen, but also elastin), although 10–15% consists of smooth muscle cells and vascular, immune and glandular cells [9]. In a normal pregnancy, the cervix remains closed until gestation reaches term. The process of labour and birth requires significant cervical remodelling. The prepartum phases of remodelling include (1) softening of the cervix, which starts in the first trimester of pregnancy and is characterised by decreased collagen crosslinking; (2) ripening of the cervix, where the collagen structure further degrades (increased spacing between fibres and a switch from straight to wavy fibres), associated with an increase in hyaluronic acid production and immune cell infiltration; and (3) cervical dilation, which occurs during active labour. The cervix must efface and dilate to a diameter of 10 cm to allow the delivery of the foetus. In the final post-partum repair phase of the remodelling, the cervix recovers its integrity (see [10–12] for detailed reviews of the remodelling process).

10.1.3 Innervation and Nerves in the Uterus

It is important to state at the start that, although the uterus receives both parasympathetic and sympathetic innervation, neither branch of the autonomic nervous system is required for uterine contraction. There is no motor innervation to the uterus. The uterus is a myogenic organ, meaning it can contract without nervous (or hormonal) stimulation. Thus, there are no synapses in the myometrium and no direct associations between axonal endings and myocytes. However,

neurotransmitters, i.e. noradrenaline from the sympathetic fibres and acetylcholine (ACh) from parasympathetic fibres, are released into the spaces between muscle bundles. In their recent review of autonomic innervation of the uterus, Tica et al. [13] conclude that "it is unclear the precise role of the nervous supply on myometrial activity". Furthermore, it has long been known, although not always appreciated, that there is a functional degeneration and hence denervation in myometrium from term-pregnant animals and women [14–17].

10.1.3.1 Innervation of the Cervix

The cervix, unlike the uterus, is well innervated and nerve density is sustained or increased at term [18, 19]. In the rat, if the sensory neuropeptidergic nerves that project to the cervix (and are involved in inflammation and local vasodilation) are transected, the ripening of the cervix and therefore birth are delayed and there is a reduction in immune cell infiltration [20]. Infection and subsequent premature cervical remodelling are recognised to be significant risk factors for preterm birth and are associated with 25–40% of all preterm births. The birefringence from collagen fibrils and impedance changes in the cervix can be used to detect cervical ripening, and therefore provide the basis for testing the risk of preterm labour [21].

10.1.3.2 Innervation of Uterine Blood Vessels

The blood vessels of the uterus are also innervated with sympathetic, parasympathetic and sensory neurons [22]. Parasympathetic stimulation produces vasodilators mainly via activation of muscarinic cholinergic receptors, and the sympathetic innervation produces vasoconstrictors via activation of α-adrenergic receptors [23]. Like the sympathetic denervation seen in the myometrium during pregnancy, fluorescence histochemistry of uterine adrenergic nerves shows abundant perivascular innervation in non-pregnant and early pregnant rats, and degeneration commences by day 15, so that perivascular nerves are practically absent by term [24, 25]. In the guinea pig uterine artery, while the number of noradrenaline-positive nerve fibres is significantly reduced in term pregnancy, the number of neuropeptide Y (NPY)-containing fibres is increased [26].

10.1.3.3 Neuromodulators

The myometrium and its blood vessels are innervated by sensory nerves identified as containing a range of neuromodulators, such as calcitonin gene-related peptide (CGRP), substance P (SP), vasoactive intestinal polypeptide (VIP), neuropeptide Y (NPY) and neurokinins.

Gnanamanickam and Llewellyn-Smith [22] describe in detail the distribution of nerves immunoreactive for CGRP, SP and NPY in non-pregnant rat uterus. Pregnancy affects this innervation. For example, nerve fibres containing CGRP are abundant in non-pregnant rat uterus but rare in term pregnant rat myometrium [27]. CGRP has a relaxant effect on non-pregnant uterus but no effect on the term pregnant rat myometrium. Circulating CGRP levels increase during pregnancy (human and rodent) but then decline sharply at term [28]. CGRP is also a potent vasodilator and sensitivity to CGRP increases during pregnancy [29]. CGRP may therefore have a significant role to play in uterine quiescence during pregnancy as well as the vascular remodelling that occurs in normal pregnancy (described below). CGRP is also implicated in the ripening of the cervix that must occur before cervical effacement and dilation to allow a successful birth [19, 30].

Substance P (SP)-positive nerve fibres are present in the non-pregnant rat myometrium, but there are contradictory reports of either an increase in SP-immunoreactivity in pregnancy [31] or a progressive decrease during pregnancy and absence at term [32]. The vasodilator action of SP on myometrial arteries does not seem to change with pregnancy [33]. While SP can induce contraction of uterine smooth muscle, it appears to have a more significant role in cervical ripening, promoting the inflammatory responses and tissue rearrangements required for cervical remodelling [30].

VIP-immunoreactive fibres are found throughout the uterus, cervix and uterine vasculature [34,

35]. VIP has vasodilatory properties, in addition to its ability to decrease myometrial contractility. However, its main role may involve its anti-inflammatory actions. Inflammation and activation of immune cells are an integral part of normal pregnancy and VIP appears to be involved in the modulation of the inflammatory and immune state throughout pregnancy [36].

10.1.4 Vasculature of the Uterus

Using tyrosine hydroxylase labelling of sympathetic nerves in whole-mount preparations of rat uterus, Gnanamanickam and Llewellyn-Smith were able to map the arterioles supplying blood to the uterus because the vessels are so densely innervated [22]. They showed that the vessels are not organised as a vascular tree, in which arteriole diameters get smaller and smaller until they become capillaries. Rather, the uterine arterioles are organised in an anastomosing network; that is, they are extensively interconnected across the whole circumference of the uterus. Such interconnected systems are associated with organs in which an assured supply of oxygenated blood is essential. In rats, blood is supplied to the uterus by the uterine arteries, which can receive blood from either their ovarian or cervical ends [37]. This potentially bidirectional supply and the anastomosing network ensure considerable redundancy and help maintain uterine perfusion. The human uterus has a similar bidirectional delivery of blood via the uterine arteries and uterine branches of the ovarian arteries. The uterine arteries have branches called arcuate arteries which are within the myometrium but near the surface of the uterus. Radial arteries branch out and traverse the myometrium radially towards the endometrium. At the myometrium/endometrium boundary, the radial arteries branch to form basal arteries and spiral arteries. Basal arteries remain unaltered by the menstrual cycle or pregnancy. Spiral arteries, on the other hand, are highly sensitive to oestrogen and progesterone. While sparsely distributed during the proliferative phase of the menstrual cycle, they undergo a rapid growth phase that outstrips the growth of the surrounding tissue, resulting in a tortuous, highly coiled path through the endometrium. During the secretory phase of the menstrual cycle, there is a constriction of the radial and/or spiral arteries, resulting in ischaemia and necrosis of the functional endometrium leading to its shedding during menstruation. The basal arteries then give rise to new spiral arteries and the cycle begins again.

During pregnancy, major adaptations occur to the uterine vasculature. Firstly, the spiral arteries undergo remodelling from tortuous, contractile vessels to wide conduits that do not respond to vasocontractile stimuli in the maternal system. This is vital to ensure adequate blood flow to the placenta and growing foetus. The remodelling extends up to a third of the way into the myometrium (into the radial arteries). The remodelling process has been reviewed in detail [38]. Briefly, immune cells (macrophages and uterine natural killer cells) initiate the breakdown of the arterial basal lamina and the dedifferentiation and disorganisation of the arterial smooth muscle cells. Interstitial trophoblasts invade and colonise the vessel walls, inducing apoptosis in the endothelial cells, so that ultimately the entire endothelium is removed and replaced with trophoblasts. This remodelling appears to be vital for a successful pregnancy, since a lack of spiral artery remodelling is associated with intrauterine growth restriction and pre-eclampsia [39].

The second major adaptation that occurs is that the larger arteries (uterine, arcuate, radial arteries) dilate to allow a greater blood flow to the uterus, accommodating an increase from ~45 mL/min in the non-pregnant uterus to ~750 mL/min at term. Maternal blood pressure remains the same or decreases in normal pregnancy; therefore this increase in flow is achieved by an increase in vessel size (outward remodelling) and a reduced reactivity to vasoconstrictive agents.

10.1.5 Myometrial Cells

The smooth muscle cells of the uterus have much in common with those in other smooth muscle tissues—spindle shape and central nucleus, mitochondria, sarcoplasmic reticulum (SR) and of course myoproteins (see Fig. 10.1a). What distin-

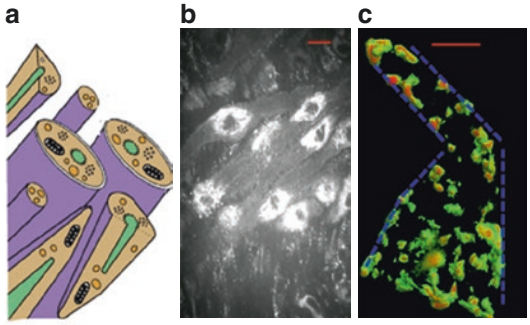

Fig. 10.1 Uterine myocytes. (**a**) Schematic representation of smooth muscle myocytes. (**b**) Uterine myocytes in bundle of longitudinal myometrium from pregnant rat. Bright central nuclei are clearly visible in the spindle-shaped cells. Red bar indicates 5 μm. (**c**) Part of a single-uterine cell, with sarcoplasmic reticulum calcium store revealed by intraluminal fluorescent, Ca-sensitive indicator Mag-Fluo3. Red bar indicates 10 μm. The dotted blue lines indicate the plasma membrane to show how close the SR approaches it

guishes the uterine myocytes is their relatively large size, up to 0.5 mm long (see Fig. 10.1b), large Ca^{2+} currents and powerful contractions.

The plasma membrane bounds the myometrial myocyte and houses the ion channels, pumps and exchangers necessary for uterine function and health. As with other smooth muscles, there are invaginations of the surface membrane, known as caveolae. The invaginations arise because of the insertion of the protein caveolin. We now appreciate that the caveolae are rich in cholesterol and sphingomyelin and constitute a form of lipid rafts, and are involved in cell signalling pathways [40].

10.1.5.1 Gap Junctions

The myocytes in the myometrium are connected by gap junctions. These connections contain mostly connexin 43 and allow for electrical coupling between the cells. The increase in myometrial gap junctions, and with it electrical synchronisation, is thought to play a significant role in the gradual development of uterine contractility during labour. Much of what we know about their role and regulation comes from the work of Garfield's group, which showed that endocrine conditions govern gap junction numbers, as well as conductance [41–43].

10.1.5.2 Sarcoplasmic Reticulum (SR)

The internal calcium store, the sarcoplasmic reticulum (SR), is a feature of all muscle types. Between smooth muscles, its size and role vary, and within the same smooth muscle its function may change with development, disease and physiological state [44]. The myometrium probably has one of the largest SRs, as judged by electron microscopy and X-ray microanalysis [45]. Confocal imaging has also helped show the extent and three-dimensional distribution of the SR in uterine smooth muscle (Fig. 10.1c), as well as identify Ca^{2+} release and uptake sites. Following from the development of Ca^{2+}-sensitive fluorescent indicators to monitor changes in the cytoplasmic concentration of Ca^{2+} ($[Ca^{2+}]$), low-affinity Ca^{2+} indicators were developed to measure SR Ca^{2+}. Mag-Fluo4 has worked particularly well in myometrium. These methodologies revealed that myometrial SR is not just a central store close to the myofilaments, but it also runs very close to the plasma membrane, as can be appreciated from Fig. 10.1c. This enables microdomains of higher than bulk cytosolic $[Ca^{2+}]$ to occur, as SR Ca^{2+} is released into a diffusion-limited space [46]. This is important as it helps to explain how Ca^{2+}-activated ion channels on the surface membrane are exposed to a high enough $[Ca^{2+}]$ to activate them. This link between the SR and excitability, via ion channels, is key to the now accepted view that the role of the SR is not simply a Ca^{2+} store, but also affects excitability in smooth muscle.

The SR can release Ca^{2+} via inositol trisphosphate (IP_3)- or Ca^{2+}-gated channels on its membrane; the latter release channels are also known as ryanodine receptors (RyR). Western blotting has shown that the myometrial SR contains both IP_3 receptors and RyR. The presence of RyR led to the assumption that Ca^{2+}-induced Ca^{2+} release (CICR) would be a feature of myometrial physiology. Thus Ca^{2+} entry upon excitation and depolarisation should elicit a large release of Ca^{2+} from the SR store, via RyR, as occurs in cardiac muscle. Despite thorough and intensive investigations, such a CICR process has not been demonstrated in the myometrium [47]. Furthermore,

caffeine, an agonist at RyR, does not elicit a release of Ca^{2+}; instead it relaxes myometrial tissue, due to its inhibition of phosphodiesterase and subsequent increase in cAMP. In addition, Ca^{2+} sparks, representing small, localised, transient releases of Ca^{2+} via RyR, could not be demonstrated in myometrium, using protocols and techniques that had revealed their presence and role in ureteric smooth muscle [48]. The answer to this puzzle came with more sophisticated molecular biology revealing that RyR expressed in the myometrium were non-functional splice variants [47]. This lack of Ca^{2+} sparks also means that they cannot stimulate Ca^{2+}-activated K^+ or Cl^- channels in myometrium. Calcium release through IP_3 receptors is stimulated by agonists binding to the plasma membrane G-coupled receptors and eliciting IP_3 production from phosphatidylinositol 4,5-bisphosphate (PIP_2) hydrolysis. This released Ca^{2+} augments cytosolic [Ca^{2+}] and helps strengthen uterine contractions. Such augmentation is considered to contribute to the actions of oxytocin, vasopressin and prostaglandins on the myometrium.

Sarco-Endoplasmic Reticulum Calcium ATPase *(SERCA)*

Active transport is required to drive the movement of Ca^{2+} into the SR in smooth muscle cells. The ATPase that catalyses this movement is sarcoendoplasmic reticulum calcium ATPase (SERCA) which is a P-type ATPase and the major protein associated with the SR. As described by Wray and Burdyga [44] in their review of the SR in smooth muscle, calcium-binding proteins enable large amounts of Ca^{2+} to be stored in the SR. In cardiac muscle, the auxiliary protein phospholamban is an important regulator of SERCA, with its inhibition being lifted when it is phosphorylated. In myometrium however, no role for phospholamban has been reported. Perhaps of more importance in the myometrium is regulation of SERCA activity by intracellular pH and metabolites. Using Western blotting, Tribe et al. [49] reported that isoforms 2a and 2b of SERCA are expressed in the myometrium. The expression of both isoforms was also increased in women in labour compared with those not in labour.

Contribution of the SR to Myometrial Contractions

Our group has extensively investigated the role of the SR in the myometrium [48, 50–52]. We have found that the SR Ca^{2+} load has a profound effect on intracellular Ca^{2+} signals. Using single uterine myocytes, we found that an increased SR Ca^{2+} load, produced by maintained depolarisation, inhibited spontaneous Ca^{2+} signals. In contrast, a decreased SR luminal Ca^{2+} load, obtained by inhibiting SERCA, activated intracellular Ca^{2+} spikes. Similar results were found in intact tissues where there was a potentiation of Ca^{2+} transients and contractions when SERCA was inhibited by cyclopiazonic acid (CPA). In pregnant rat uterus, CPA stopped the phasic contractions and transformed activity to be tonic-like, associated with a large increase in the baseline Ca^{2+}. Thus, during spontaneous uterine contractions there is no involvement of the SR, as Ca^{2+} entry and efflux across the plasma membrane account for these phasic contractions [53]. As mentioned above there is also no role for CICR in myometrium, and caffeine relaxes contractions. The lack of Ca^{2+} sparks means that there is no Ca^{2+} spark-STOC mechanism, where the Ca^{2+} released during a spark activates Ca^{2+}-sensitive K^+ channels and produces a spontaneous transient outward current, known as a STOC. The resulting hyperpolarisation leads to relaxation in vascular smooth muscle [54], and curtailment of the action potential in ureteric smooth muscle [55]. We have recently been able to show, using electrophysiology, force and intracellular Ca^{2+} measurements, that depletion of the SR in the myometrium produces depolarisation and Ca^{2+} entry through store-operated channels [56] which can increase contractility upon agonist stimulation.

10.1.5.3 Mitochondria

Uterine myocytes contain round or ovoid mitochondria. In human and rat, light and electron microscopies show these mitochondria to be distributed like "pearls on a string" close to the nuclei and rough endoplasmic reticulum or subsarcolemmally adjacent to caveolae [57]. In all cells, there is an electrical potential difference across the mitochondrial membrane, with their

inside being significantly more negative, (values range in 180–220 mV) than the cytoplasm [58]. As well as their obvious importance to oxidative phosphorylation, evidence has grown that mitochondria are important to Ca^{2+} signalling in smooth muscle [59]. Mitochondria interact with and may link with the SR [60, 61]. They also store significant amounts of Ca^{2+}. Early work conducted by Batra [62] showed rapid and substantial uptake of Ca^{2+} into uterine mitochondria in microsomal preparations. More recently, Gravina et al. [63] have shown that they may be important in modulating spontaneous activity in rat uterus. Work from the same group indicated that oxytocin causes depolarisation of the mitochondrial membrane potential, ψ_m [64]. This was suggested to be likely due to oxytocin inducing an increase in cytosolic [Ca^{2+}], "causing enhanced mitochondrial uptake of Ca^{2+} and resultant dissipation of the mitochondrial electrochemical gradient". The mitochondrial ATP synthase is also stimulated, which further contributes to a decrease in ψ_m. In a study comparing mitochondria from guinea pig uterus and heart, the uterine mitochondria were shown to have higher amounts of hexokinase and adenylate kinase, and to use them with mitochondrial creatine kinase, to enhance local [ADP], which will help the mitochondrial responses to energetic demands [65]. Investigators have looked for changes in mitochondrial appearance or activity in various pregnancy-related disorders, including pre-eclampsia, where small changes were noted [66], and obesity and diabetes, where no changes were found [57, 67]. Myometrial mitochondrial copy number was reduced in older mice, although there were no age-induced changes to the enzymatic activities of the mitochondrial electron transport chain complexes [68].

10.1.5.4 Other Cell Types: Are There Pacemaker Cells?

Neither removing the tissue from the body nor dissection down to the smallest of myometrial strips prevents it from rhythmically contracting. Even freshly dissociated single myocytes can be seen to contract. Despite many hunts for an anatomical pacemaker in the uterus, none has ever been found. This has led to the suggestion that some myocytes, perhaps a third of them based on data from Ca^{2+}-activated Cl^- channels (see later), or groups of cells have inherent pacemaking activity. This can be attributed to them possessing a different cassette of ion channels, leading to unstable membrane potentials. This in turn causes action potentials to fire and membrane depolarisation which spreads quickly through to adjacent myocytes via their gap junctions.

When electrical activity has been mapped in the myometrial strips, using techniques pioneered by Lammers, that involve arrays of multiple electrodes, an erratic, whirling spread of excitation can be seen [69]. Areas initiating excitation appear to be fluid and the pattern of spread of the ensuing depolarisation is also malleable. Similar findings have been described when calcium is used as a surrogate for depolarisation, i.e. mapping the rises of Ca^{2+} throughout myometrial cells and bundles, to illustrate where excitation due to electrical activity is occurring. This can be done using Ca^{2+}-sensitive fluorescent indicators and confocal microscopy. The descriptions of the changes in [Ca^{2+}] closely mirror those for electrical activity. Thus, all these data are consistent with there being no anatomical or histological pacemaking structure in the uterus. A recent review concluded "… previous studies unanimously reveal a unique complexity as compared to other organs in the pattern of uterine electrical activity propagation" [70]. There is consensus that in rodents it is easier to get spontaneous contractions from cells closer to the oviducts than cervix and along the mesometrial border [71]. As far as we are aware, there is no mechanistic explanation for these findings.

Taking a lead from the gastrointestinal system, where a discrete network of cells, interstitial cells of Cajal (ICC), produces pacemaking, researchers have looked for similar-appearing cells around myometrial bundles. Such interstitial cells, called telocytes by some authors [72], and ICC-like cells by others [73, 74], have been identified in the uterus by a variety of techniques from confocal and electron microscopy to immunohistochemistry and molecular biology. Some have suggested that such cells are stem cells or transitional cells, or form some sort of support network, while others have suggested that they are pacemakers. In our

opinion, to make the claim that these interstitial cells are pacemakers they must be capable of depolarisation and spreading excitation. Claiming pacemakers based on histological similarities with ICC or using drugs such as imatinib to inhibit c-KIT signalling is not sufficiently rigorous. Our study in myometrium, where we reported ICC-like cells, passed from excitement to disappointment as we found that they did not depolarise when stimulated [73]. Indeed, it was easier to propose, given their ability to hyperpolarise, that they could form a network to keep the myometrium quiescent ahead of parturition. Similarly telocytes do not show any excitable properties but did display hyperpolarising currents [75]. A recent review of telocytes concluded that they are not pacemakers but perhaps participate in cell renewal and signalling [76]. More recently, another type of interstitial cell, platelet-derived growth factor receptor alpha-positive (PDGFR alpha$^+$), has been reported in the female reproductive tract, including myometrium [77]. These cells however are unlikely to be pacemakers, as they are not reported to express any of the necessary genes. They were able to form connections with myocytes through gap junctions. In bladder and GI tract, these cells may mediate inhibitory neurotransmission [78, 79]. Finally, Young [80] has postulated that long-distance signalling mechanism based on mechano-transduction aids the action potentials spreading in the pregnant myometrium. He refers to these as "mechanically sensitive electrogenic pacemakers, distributed throughout the (uterine) wall".

10.2 Excitation in Myometrial Cells

To understand how electrical activity arises in myometrial cells requires knowledge of the ion channels, pumps and currents in the myometrium, their densities, conductance, activation and inactivation, coupling to neighbouring cells, modulation and how they relate to the resting membrane potential and action potential. Figure 10.2 summarises the uterine myocyte plasma membrane and its ion channels, pumps, receptors and exchangers.

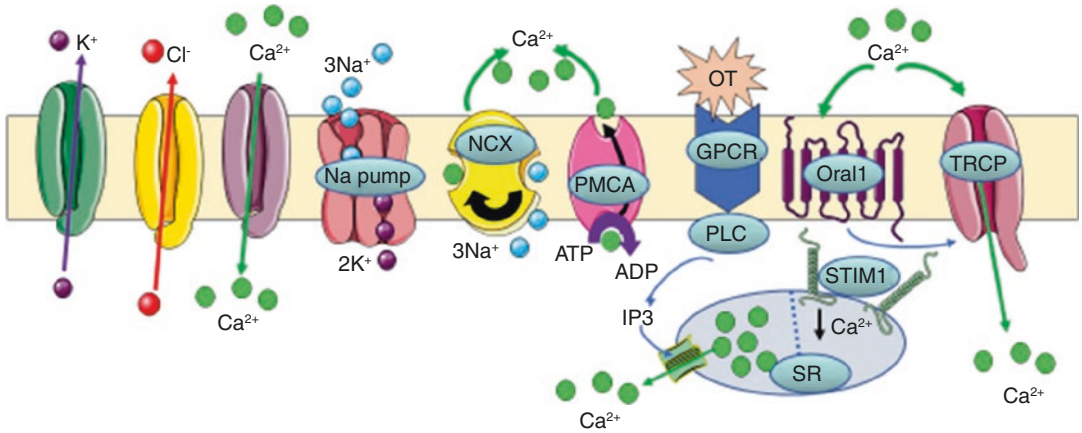

Fig. 10.2 Myometrial channels, pumps and exchangers. Calcium entry is controlled by L-type Ca^{2+} channels, and is essential for contraction. Cells express a wide variety of K^+ channels, gated by voltage, Ca^{2+}, ATP and stretch. Chloride channel opening leads to Cl^- efflux. Plasmalemmal calcium fluxes are balanced by two efflux pathways: PMCA (plasma membrane Ca-ATPase) and NCX (Na,Ca exchanger). The Na pump (Na,K ATPase) removes $3Na^+$ linked to the entry of $2K^+$. The SR (sarcoplasmic reticulum) takes up Ca^{2+} via SERCA (sarco-endoplasmic reticulum Ca-ATPase), and only releases Ca^{2+} via IP_3-gated channels. When SR Ca^{2+} is decreased STIM1 and Orai1 interact, forming a Ca^{2+}-selective pore, and stimulating the opening of non-specific cationic TRCP channels. By this store-operated mechanism producing an inward current, the SR can modulate plasma membrane excitability. Agonists, e.g. oxytocin (OT), bind to G-protein-coupled receptors (GPCR) and via phospholipase C (PLC) generate IP_3 as well as contribute to depolarisation and stimulate Ca^{2+} entry and enhance contractility

10.2.1 Ion Channels Present in Myometrium

10.2.1.1 Calcium Channels

A ubiquitous feature of contraction in muscle cells is the requirement for a rise in intracellular [Ca^{2+}]. This can be understood in smooth muscles by the need of myosin light-chain kinase (MLCK) to be activated by binding of Ca^{2+}-calmodulin. If Ca^{2+} is omitted from a physiological solution bathing a piece of contracting myometrium, contractions will rapidly fail, demonstrating the absolute necessity of external Ca^{2+} entry for the rhythmic contractile activity in the myometrium. Application of blockers of voltage-dependent L-type Ca^{2+} channels (also known as dihydropyridine receptors) abolishes myometrial contractions, even when Ca^{2+} is present in the external bathing solution [50].

L-type Ca^{2+} channels consist of a transmembrane pore-forming α_1-subunit and several auxiliary proteins (β and $\alpha_2\delta$) [81]. The $\alpha_2\delta$ subunit promotes the surface expression of the channel [82], while binding of the β-subunit prevents the ubiquitination and proteasomal degradation of the channel protein and promotes trafficking of the channel to the membrane [83]. In pregnant rat myometrial smooth muscle cells, depolarisation causes a nifedipine-sensitive inward Ca^{2+} current, down its electrochemical gradient [50]. The voltage threshold for activation of this Ca^{2+} current (I_{Ca}) is −50 to −40 mV. The mean maximal I_{Ca} density is calculated to be ~6.3 pA/pF when depolarisation is applied from a holding potential of −80 mV [84, 85]. These channels close when the membrane hyperpolarises. There is also a fast Ca^{2+}-sensitive inactivation which is a negative feedback mechanism preventing Ca^{2+} overload of the cells. Inactivation of the L-type Ca^{2+} channels leads to relaxation of the myometrial contraction.

Expression of L-type Ca^{2+} channel subunits varies during gestation in the rat. A fourfold increase in the number of dihydropyridine-binding sites, measured by saturation binding, was found by day 14 of gestation and maintained through parturition [86]. A gradual increase in α_1-subunit expression was also observed throughout gestation, though it decreased during labour [87] and was accompanied by a rapid increase in the β-subunit. Similar increases in α_1-subunit expression and L-type Ca^{2+} channel function are seen in guinea pig [88] and human [89] myometrium.

Another form of Ca^{2+} channel is the T-type. There is evidence for their expression (mRNA, protein) in myometrium [90, 91] and electrophysiology experiments have identified a nickel-sensitive T-type Ca^{2+} current [89]. However, there is debate concerning their physiological relevance and function in myometrium, as it is questioned whether the uterus will ever be hyperpolarised long enough to significantly activate T-type Ca^{2+} channels.

10.2.1.2 Potassium Channels

Myometrial smooth muscle cells express a wide range of potassium channels, including voltage-dependent channels (K_V), large-conductance voltage and calcium-dependent (BK) channels, small-conductance calcium-dependent (SK) channels and ATP-sensitive (K_{ATP}) channels. Under resting conditions, K^+ conductance is the main determinant of the resting membrane potential. As well as contributing to resting membrane potential, activation/inactivation of K^+ channels will influence the time course of the action potential and repolarisation. For these reasons, K^+ channel activity is usually associated with uterine quiescence and changes in channel expression linked to the onset of labour.

BK channels are expressed in all smooth muscles [92]. In blood vessels, they form part of an important negative feedback loop, where an increase in intracellular Ca^{2+} stimulates contraction, but also activates BK channels (via Ca^{2+} sparks) leading to hyperpolarisation and vasorelaxation. In uterine smooth muscle, BK channels are present with relatively high density and with their large conductance (~200 pS) might be expected to play a significant role. However, blockade of BK channels has little effect on either uterine contractility or calcium signalling [93, 94]. This may be because uterine smooth muscle cells do not produce Ca^{2+} sparks [48], meaning the BK channels are perhaps only minimally activated under physiological conditions. SK channels are also expressed in the myometrium throughout gestation. SK channel blockers

inhibited outward current and caused membrane depolarisation in myometrial cells and increased contractility in myometrial strips throughout gestation [94]. Therefore, the functional effect of SK channel inhibition is greater than BK channel inhibition. In a transgenic mouse model, overexpression of the SK3 channel delays labour [95].

K_{ATP} channels, the predominant isoform consisting of an inward rectifier K^+ channel (Kir6) and a sulfonylurea subunit (SUR2B), which provides the ATP sensitivity, also play an important role in regulation of myometrial quiescence in pregnancy and are downregulated ahead of labour [96]. K_{ATP} channels are inhibited by intracellular ATP and stimulated by MgADP and thus are involved in coupling metabolic state to cellular excitability. These myometrial channels may also be regulated by the endogenously produced gasotransmitter H_2S, leading to sulfhydration and myometrial relaxation [97], as demonstrated upon blockade of K_{ATP} channels with glibenclamide which attenuates the ability of H_2S to relax the myometrium.

Myometrial cells also express stretch-activated K^+ channels [98, 99] which are coded for by the two-pore family of K^+ channels (KCNK). Several groups have identified TREK-1 channels as being expressed in the myometrium and regulated during pregnancy and labour [99, 100]. In both mouse and human myometrial cells, TREK-1 channels were shown to be activated and produce an increased outward current in response to stretch [101]. TREK-1 gene expression is regulated during pregnancy, increasing towards term and falling at the time of labour [100]. In this way perhaps, myometrial quiescence is maintained as the foetus grows and the uterus distends.

Voltage-dependent potassium channels (K_V) are widely expressed in uterine smooth muscle. Depolarisation of the plasma membrane activates these channels and an efflux of K^+ ions from the cell leads to repolarisation to the resting membrane potential. Inhibition of these channels with a non-specific channel blocker, such as tetraethylammonium (TEA), increases uterine contractions clearly demonstrating a role in contractility. Different voltage-dependent K^+ channels have been identified as having a role in maintaining the membrane potential and therefore quiescence or controlling the transition from quiescence to labour. Two novel K_V channels, K_V7 and K_V11, encoded by the KCNQ and KCNH (ERG) gene families, respectively, appear to be important regulators of uterine contractility [102]. Parkington et al. [103] reported that ERG activity (K_V11) leads to inhibition of myometrial contraction, but during labour there is an increase in the expression of ERG's inhibitory β-subunit that causes a decrease in ERG activity and thus a stimulation of contraction. Similar findings were reported for $K_V10.1$ [104]. Interestingly, Parkington et al. also showed that the inhibitory β-subunit is expressed at a lower level in obese women, which results in elevated ERG activity and increased K^+ conductance and presumably poorer myometrial contractility. Indeed, we have shown in electrophysiological studies that cholesterol, which is often elevated in obese women, increases an outward K^+ current. The finding of an enhanced outward K^+ conductance in obese women may be a significant factor in the finding that obese women are more likely to require delivery by caesarean section.

KIR7.1 expression (KCNJ gene family), an inward rectifier channel, was identified in both mouse and human uterus to peak at mid-gestation and decline at term [105]. KIR7.1 current hyperpolarises uterine myocytes and knockdown of the channel leads to an increase in contractile force and duration. Another member of the K_V family that may have a role in uterine quiescence and transition to labour is $K_V4.3$ channels, whose expression is significantly reduced at term in mouse myometrium [106]. It is hoped that specific agonists of these channels may provide a new generation of more effective relaxants of uterine contractions, drugs referred to as tocolytics.

10.2.1.3 Chloride Channels

Work conducted in the 1980s revealed an unusual feature of smooth muscle cells; the intracellular Cl^- concentration is higher than in most cells [107]. Values reported of ~50 mM mean that there must be active mechanisms maintaining intracellular [Cl^-] above a passive distribution, that its membrane permeability is low and that the Cl^-

equilibrium potential is ~−20 mV [107]. The net effect is that if Cl^- channels are activated in the myometrium, their opening will lead to Cl^- leaving the myocytes. In electrical terms, this is equivalent to positive ions entering the cell, and hence depolarisation ensues. Thus, knowledge concerning the types of Cl^- channels in myometrium is important in understanding its excitability.

Ca^{2+}-Activated Cl^- Channels

In addition to Ca^{2+}-activated K^+ channels, the myometrium expresses Ca^{2+}-activated Cl^- channels (CACCs), which are sensitive to both voltage and Ca^{2+}. There has been much speculation over the molecular identity of these channels, with several candidates being proposed, but only two families of proteins (the bestrophin and the anoctamin (Ano1 or TMEM16) families) are able to replicate the classical properties of endogenous CaCC [108–110]. CaCC-mediated responses are stimulated upon activation of G protein-coupled receptors (GPCR) by agonists such as noradrenaline, endothelin-1, acetylcholine, ATP and angiotensin II. These agonists cause release of Ca^{2+} from the internal store via IP_3 receptors and it is this released Ca^{2+}, rather than external Ca^{2+}, that activates CaCCs in many tissues [111]. However, there are examples such as in portal vein and tracheal smooth muscle, where Ca^{2+} entry via L-type Ca^{2+} channels can activate a Cl^- current. Spontaneous release of Ca^{2+} from the internal store, Ca^{2+} sparks, can also activate CaCCs, resulting in spontaneous transient inward currents (STICS), in a similar way to BK channel activation by Ca^{2+} sparks producing STOCs.

CaCC expression has been identified in myometrium of human and rodent species [112–116]. In a study of myometrial isolated cells and muscle strips [117] we investigated the role of CaCCs in myometrium. A CaCC current was evident in 30% of freshly isolated rat myocytes. Blockade of the channels with niflumic acid significantly decreased the frequency of contraction in oxytocin-stimulated and spontaneously contracting strips of myometrium. Later studies have further demonstrated a role for CaCCs in myometrial contractility [115]. In addition, the demonstration that CaCCs are upregulated at term [114] suggests that they may play a role in increasing contractility of the myometrium at parturition.

10.2.1.4 Sodium Channels

Once calcium ions entering via L-type Ca^{2+} channels had been shown to be the dominant carrier of inward current in uterine myocytes, a role for Na^+ channels was understandably neglected. An early comprehensive electrophysiological investigation of membrane currents [118] reported fast sodium channels in rat myometrium. Increased Na^+ current expression in mid- and late pregnancy was reported in human myometrium [119]. Inoue and Sperelakis [85] suggested from their studies on rat that fast Na^+ channels play a role in spreading myometrial excitation and that this becomes more important as term approaches. A more recent study using expression and molecular biology approaches [120] found that veratridine, which increases Na^+ influx through voltage-gated Na^+ channels (VGSC), causes the rapid appearance of phasic contractions accompanied by changes in intracellular [Ca^{2+}]. Using RT-PCR, in the same study the authors detected the VGSC α-subunits Scn2a1, Scn3a, Scn5a and Scn8a in the cDNA from longitudinal myometrium. The mRNAs of the auxiliary β-subunits Scbn1b, Scbn2b and Scbn4b, and traces of Scn3b, were also present. The same group also showed that amiloride-sensitive Na^+ channels were expressed in the rat uterus and that "mRNA expression levels of the alpha, beta and gamma subunits are selectively and differentially regulated during pregnancy" [121].

It is tempting to speculate that fast Na^+ current channels, producing inward current, may be expressed in those myocytes acting as pacemakers within myometrial fibres.

10.2.1.5 Store-Operated Channels

As described earlier, elucidating the role of the sarcoplasmic reticulum in myometrium was not straightforward. Measurements of SR luminal Ca^{2+} showed a fall of Ca^{2+} as it was released into the cytoplasm when IP_3-producing agonists were applied to uterine myocytes [122]. SERCA was shown to bring about a slow refilling of luminal Ca^{2+}. The question then arises as to whether the

uterine myocytes possess a mechanism to refill the store with Ca^{2+} when SR luminal levels fall—in other words, is capacitative- or store-operated calcium entry (SOCE) functional in the uterus? Were there SOCE channels in the plasma membrane, or the more selective calcium release-activated channels (CRAC), or both?

The existence of a pathway between the internal Ca^{2+} store and plasma membrane, that replenishes the Ca^{2+} store, was first demonstrated in non-excitable cells. The store in these cells is referred to as the endoplasmic reticulum (ER). The existence of such a mechanism was postulated as a way of balancing ER Ca^{2+} release and plasma membrane Ca^{2+} entry, and thereby regulating Ca^{2+} signals in cells. Given the important and diverse cellular processes regulated by Ca^{2+}, such control is seen as essential in preventing aberrant signalling and pathologies [123]. The SOCE was eventually demonstrated by showing a steady-state rise in cytoplasmic Ca^{2+} or a small inwardly rectifying current, designated I_{CRAC} [124], upon lowering of ER Ca^{2+}. Both measurements were experimentally hard to obtain due to their small size and the molecular mechanisms involved proved elusive. This situation changed when, in 2005, STIM1 was identified as an ER transmembrane protein that could sense luminal Ca^{2+} [125, 126]. Interestingly, STIM had been identified about 10 years earlier and stands for stromal interacting molecule and is linked to cancer.

As luminal Ca^{2+} falls, Ca^{2+} dissociates from STIM and it undergoes conformational changes. The next step in unravelling the SOCE mechanism followed rapidly, with the plasma membrane channel subunit Orai1 being identified [127–129]. When low ER Ca^{2+} evokes the conformational change in STIM1, it interacts with Orai1 and a functional and selective Ca^{2+}-entry channel is activated. Overexpression of these components of SOCE greatly increased I_{CRAC}. Of note for smooth muscles, Ca^{2+} entry via Orai1 channels can also recruit members of the transient receptor potential channels (TRPCs) [130]. These non-specific cation channels in smooth muscle plasma membrane interact with STIM1 and open to produce cation entry, including Ca^{2+}. Cheng et al. showed that TRPC1 and ORAI1 are components of distinctly different channels, both of which are regulated by STIM1. It is the Orai-1-mediated Ca^{2+} entry that triggers trafficking and insertion of TRPC1 proteins into the plasma membrane where they are gated by STIM1. It is suggested that CRAC channels, which are highly selective for calcium, are restricted to non-excitable cells. The less selective and less well-characterised SOCE channels, and their still controversial relation to TRPCs, are more likely to occur in the uterus [131]. For a current account of STIM and Orai, see the review by Putney [123].

An interesting study in Orai1 knockout mice should be mentioned. The female mice are fertile and give birth to live pups, but they are not able to let down milk from their mammary glands. The explanation for this could be found in examination of their Ca^{2+} signals; Ca^{2+} oscillations induced by oxytocin in myoepithelial cells are substantially reduced, due to lack of Ca^{2+} entry and contraction failure [132]. Interestingly, these findings are similar to those obtained in oxytocin receptor knockout mice; parturition surprisingly occurs normally but milk let-down and contraction of the myoepithelial cells fail [133].

The evidence for a role and the importance of SOCE in smooth muscle has recently been reviewed by Feldman et al., and many key references can be found there [134]. It appears to us fair to conclude that its importance to contractility, as opposed to say proliferation and remodelling, is still debatable. Studies performed on cultured smooth muscle cells and lines have to be treated with extreme caution, due to their phenotypic change to a non-excitable state.

STIM and Orai isoforms have been identified in myometrial biopsies from women undergoing C-section operations [135]. The authors proposed that SOCE may play a role in Ca^{2+} signalling during pregnancy. These findings of STIM1 expression in human myometrium have been confirmed by Feldman et al., and extended to mouse myometrium [134]. In the same reference, an account is given of preliminary work showing a thinning of the myometrium in STIM1 null mice, along with a contractile impairment.

When it comes to directly identifying a role for SOCE in myometrial function, the only study to

date remains that of our group [56]. To understand the protocols necessary to demonstrate SOCE in myometrium, it has to be emphasised that in this excitable tissue, the major source of Ca^{2+} for contractions is entry through the L-type Ca^{2+} channels. This Ca^{2+} influx is necessary and sufficient for contraction. The Ca^{2+} that enters must later be removed by efflux mechanisms across the plasma membrane, as described in Sects. 10.3.4.1 and 10.3.4.2. Thus, a role for SOCE can only be to support agonist-induced contractions. In the study of Noble et al., mentioned above [54], depletion of the SR Ca^{2+} produced a prolongation of the bursts of action potentials and corresponding series of Ca^{2+} spikes, which increased contraction amplitude and duration. The rise of baseline Ca^{2+} and membrane depolarisation continued until all electrical and Ca^{2+} spikes and phasic contractions ceased, revealing a maintained, tonic force and a raised basal Ca^{2+}. We also showed that lanthanum, a blocker of SOCE, but not the L-type Ca^{2+} channel blocker nifedipine, abolished the maintained force and Ca^{2+}. The physiological relevance was emphasised by using an agonist, carbachol, which produces similar effects to depleting the SR upon SERCA blockade, depolarisation and elevation of force and basal Ca^{2+}. A brief, high concentration of carbachol, to cause SR Ca^{2+}depletion without eliciting receptor-operated channel opening, also produces these results. We therefore consider that in pregnant rat myometrium SR Ca^{2+} release is coupled to a marked Ca^{2+} entry via SOCE.

10.2.1.6 Sodium Pump: Na,K ATPase

The Na,K ATPase has long been known to maintain intracellular concentrations of Na^+ ($[Na^+]$) low as it moves $3Na^+$ out of the cell coupled to the entry of $2K^+$. For a review of its enzymatic activity and structure focussed on smooth muscle, see [136]. The resulting Na^+ pump current makes a small contribution to the negative membrane potential found in uterine and other cells [137]. The sodium gradient created can also be linked to the transport of other ions, notably H^+, Cl^- and Ca^{2+}, all of which are important to the excitability and function of the myometrium [138]. In smooth muscle, the Na,K ATPase and Na–Ca exchanger (NCX) colocalise [139]. Golovina et al. [140] showed that intracellular $[Na^+]$ in the sub-plasmalemmal spaces influences the activity of the NCX and the SR Ca^{2+} content. As discussed already, in the myometrium the SR has many close appositions to caveolae and the plasma membrane [141] and thus the presence of Na,K ATPase within these microdomains could affect contraction in the uterus.

The sodium pump is a P-type ATPase, composed of two α-subunits, two β-subunits and usually a third, FXYD, subunit, each of which has several isoforms [142, 143]. Developmental and tissue-specific differences in the expression of these isoforms allow the Na,K ATPase function to be tightly regulated. This is because the isoforms govern kinetics, membrane localisation, sensitivity to ions, and endogenous pump inhibitors. Not surprisingly therefore, it has been suggested that the Na,K ATPase may contribute not just to excitability in myometrium, but also to gestational changes in uterine activity [144]. It has also been reported that inhibitory prostaglandins work, in part, by stimulating the Na,K ATPase and thereby producing a hyperpolarisation [145].

Our group has studied the isoforms present in myometrium from human and animal tissues [144, 146–148] with respect to the expression of mRNA transcripts encoding Na,K ATPase and FXYD isoforms, their protein expression and tissue distribution, as well as their functional effects throughout gestation. In rats, we found that all three isoforms of the α- and β-subunits were expressed, along with FXYD1. Interestingly three of these isoforms, α2, α3 and β2, change their expression during pregnancy, suggesting that they are functionally regulated. In addition, sensitivity to the pump inhibitor, ouabain, changed during gestation; its effect of increasing frequency of contractions and the accompanying Ca^{2+} transients became larger as pregnancy advanced. In human myometrium we also found differences in α-isoform expression between non-pregnant and pregnant tissues. These findings extend and strengthen preliminary studies reporting isoform switching during pregnancy in rat and human myometrium [149, 150]. Furthermore, decreased α3 isoform expression correlates with reduced contractility in oestradiol-treated rats [151] sug-

gesting that changes in expression can have functional consequences in uterine smooth muscle.

In summary we are learning more about how subtleties in both isoform expression and distribution of Na^+ pumps can contribute to uterine function. They have been implicated in some complications of pregnancy, such as pre-eclampsia, a condition characterised by hypertension. Both defects in their activity and derangements mediated by circulating inhibitors can lead to an increase in $[Na^+]$ and therefore contribute to the disease [152].

10.2.2 Membrane Potentials and Action Potentials

The resting membrane potential of a cell is the voltage difference between the inside and outside of a cell, with typical values ranging from −40 to −80 mV. It is the balance of activity of the aforementioned ion channels that determines the resting membrane potential in a cell. The uterine smooth muscle contracts spontaneously. Contractions occur as a direct result of the generation of action potentials within the myometrial smooth muscle cells. The time course of action potentials measured in uterine smooth muscle varies greatly depending on the species, the part of the uterus examined and gestational status. Simple monotonic action potentials have been recorded in longitudinally oriented myometrium from rats and rabbits and circular muscle of sheep and guinea pig myometrium. However more complex action potentials with a sustained plateau of depolarisation have been observed in the circular myometrium of pregnant rats and mice and longitudinal myometrium of pregnant guinea pigs (for review see [153]).

When the membrane potential of uterine smooth muscle cells becomes sufficiently depolarised (threshold potential), voltage-dependent channels are activated, and the upstroke of the action potential resulting from the opening of voltage-dependent L-type Ca^{2+} channels and an inward Ca^{2+} current ensues. The threshold potential for these Ca^{2+} channels is around −40 mV and the inward Ca^{2+} current peaks within about 10 ms and then slowly reduces. Repolarisation is due to a variety of processes, such as the opening of voltage-dependent K^+ channels which generate a hyperpolarising outward K^+ current, and the voltage and Ca^{2+}-dependent inactivation of the L-type Ca^{2+} channels. In the more complex action potentials, an initial spike or series of spikes are followed by a sustained depolarisation (around −30 mV in rat myometrium). The duration of this plateau determines the duration of the resulting contraction.

As pregnancy progresses towards term it is thought that the resting membrane potential of the myometrial cells becomes more depolarised. While this certainly makes physiological sense, the evidence base is limited. This can be explained by the difficulty of making sharp electrode impalements into myometrial cells that are strongly contractile. Probably the best study to date was made in the third trimester of human pregnancy [154]. In this study, the membrane potential went from an average value of −70 mV at 29 weeks to −55 mV at parturition and was accompanied by a progressive increase in the frequency of myometrial contractions. This decrease in the level of negativity of the resting membrane potential would facilitate Ca^{2+} influx through voltage-operated Ca^{2+} channels. Reductions of potassium channels in the myocyte membranes in late pregnancy would also prolong myocyte action potentials and enhance contractility.

10.3 From Excitation to Contraction

10.3.1 Calcium Signalling

In myometrial smooth muscle cells, contraction is critically dependent on Ca^{2+} and removal of extracellular Ca^{2+} [50], abolishes contractions. The uterus exhibits a variety of Ca^{2+} signals; single transients, multiple transients on a raised basal Ca^{2+}, as shown in Fig. 10.3, as well as spikes and waves propagating through cells and bundles. These signals are dependent upon the frequency and duration of action potentials. As for the spread of electrical activity, the origins and rises of $[Ca^{2+}]$ within muscle bundles appear

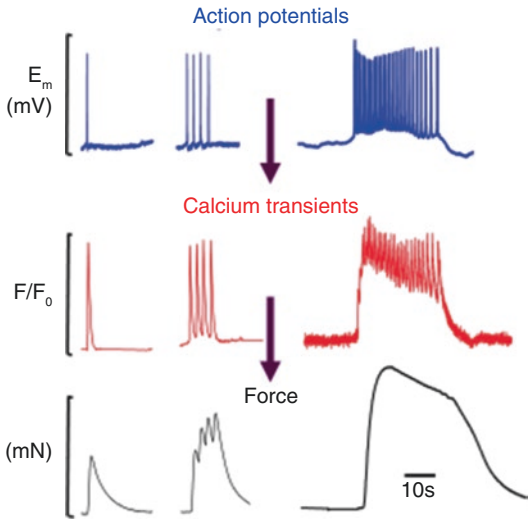

Fig. 10.3 Excitation-contraction coupling in myometrium. Electrical activity in the form of action potentials shapes the intracellular calcium signals and thereby modifies force in the uterus. Altering electrical activity is key to changing [Ca^{2+}] and hence force. E_M: potential measured with sucrose gap technique. Calcium was measured using the fluorescent indicator, Fluo4. Adapted from Burdyga et al. [155]

chaotic. Activity may originate in one area and then switch to another. Calcium waves might propagate to the end of the myocytes or terminate ahead of the cell border. We found that the amplitude of force generated by a single Ca^{2+} spike was around a third of the maximal and was dependent on the number of bundles recruited within the strip. If Ca^{2+} spikes appeared in bursts, they generated longer lasting fused contractions, the amplitudes of which were dependent on the number and the frequency of the spikes [155].

10.3.2 Pathway to Contraction

Figure 10.4 illustrates a schematic representing a uterine myocyte and the pathways leading to contraction, and how they might be inhibited, for example to prevent preterm birth.

Once in the cell, Ca^{2+} binds calmodulin ($4Ca^{2+}$ for every calmodulin molecule) and activates MLCK. MLCK phosphorylates serine 19 on the regulatory light chains of myosin, which in turn triggers interaction of phosphorylated myosin with actin myofilaments. The resulting cross-bridge cycling generates contraction and is an active process that requires ATP hydrolysis. Inhibition of MLCK with wortmannin also abolishes contractions. Relaxation occurs when Ca^{2+} channels are inactivated, and K^+ currents cause the membrane potential to return towards resting levels so that Ca^{2+} concentrations fall and myosin light-chain phosphatase (MLCP) dephosphorylates the myosin light chains. The process of excitation-contraction coupling in the uterus has been comprehensively reviewed [138, 156].

10.3.3 Effects of Agonists and Sensitisation

Binding of hormones such as oxytocin to its receptor in the cell membrane will lead to an increase in intracellular [Ca^{2+}]. The oxytocin receptor is a GPCR, which couples to phospholipase C via $G\alpha_{q/11}$. This in turn controls the hydrolysis of phosphoinositide-bis-phosphate (PIP2) into IP_3 and diacylglycerol (DAG). IP_3 releases Ca^{2+} from the internal store (SR) and triggers the aforementioned activation of MLCK and generation of contraction (see Fig. 10.4 and recent review [157]).

Agonists can also initiate other intracellular pathways and these signals may influence force generation in other ways, such as changing enzyme and channel activity. The phenomenon of Ca^{2+} sensitisation is observed in other smooth muscles, whereby the activity of MLCK and MLCP can be regulated in such a way that the contractions generated are altered without any change in [Ca^{2+}] [158]. However, there is little direct evidence for Ca^{2+} sensitisation occurring in myometrium [159]. Crichton et al. [160] described Ca^{2+} sensitisation in chemically skinned myometrial preparations, but the relevance to in vivo situations is unclear. Certainly, inhibiting the major sensitisation pathways (Rho-Rho kinase) in the uterus has little effect on [Ca^{2+}] or force [161]. It is our considered opinion, in the light of little to no direct evidence of sensitisation in the myometrium, and direct evidence against it, that it is not a feature of the normal physiologi-

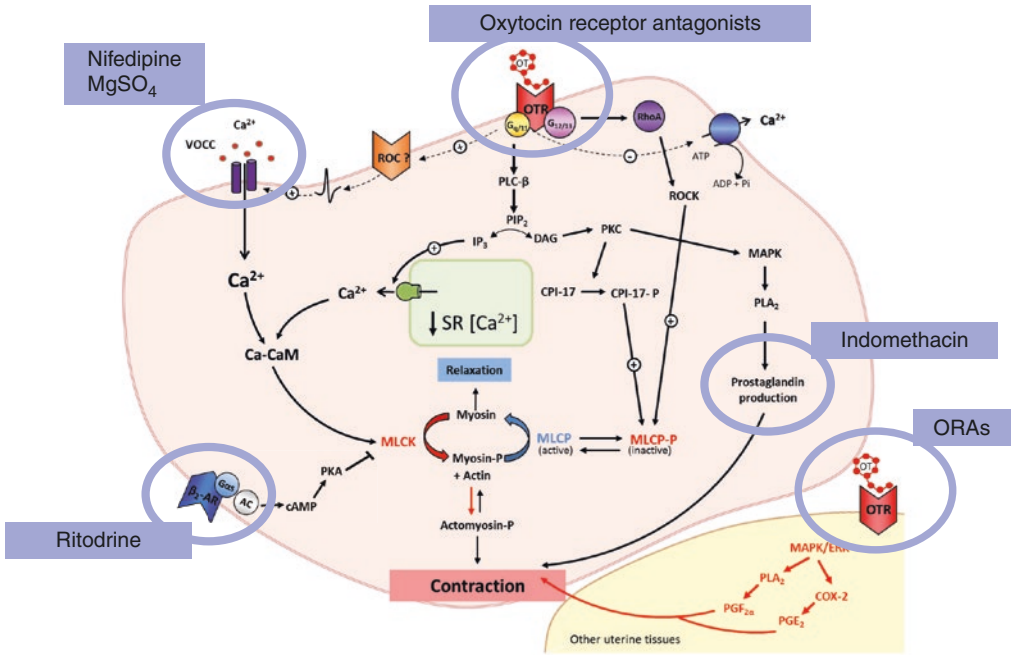

Fig. 10.4 Schematic of uterine force production pathways, and mechanisms of decreasing force used to help prevent preterm delivery, tocolysis. *VOCC* voltage-operated Ca^{2+} channel, *PLC-β* phospholipase C-β, PIP_2 phosphatidylinositol 4,5-bisphosphate, IP_3 inositol 1,4,5-trisphosphate, *DAG* diacylglycerol, *PKC* protein kinases type C, *Ca-CAM* Ca-calmodulin complex, *MLCK* myosin light-chain kinase, *MLCP* myosin light-chain phosphatase, *MAPK* mitogen-activated protein kinase, PLA_2 phospholipase A_2, *ROCK* RhoA-associated protein kinase. Adapted from Arrowsmith and Wray [157]

cal activity of the myometrium. As always, future work may lead to tempering of this view.

10.3.4 Calcium Balance and Efflux Mechanisms

Figure 10.3 demonstrates the close relationship between the intracellular Ca^{2+} transient and force. However, for labour it is important that contractions do not become tonic, as this produces hypoxia in the myometrium and can cause foetal asphyxia (see Sect. 10.4.5.1 and Fig. 10.5). It therefore follows that the mechanisms that terminate both action potentials and associated Ca^{2+} rises are as important as those that initiate them. The Ca^{2+} that entered the uterine myocyte must be removed to ensure flux balance. There are two mechanism that remove Ca^{2+} from myocytes, a plasma membrane Ca-ATPase (PMCA) and a Na/Ca exchanger (NCX). Both of these have been reviewed elsewhere [162, 163], and so we only present a brief overview.

10.3.4.1 Plasma Membrane Ca-ATPase (PMCA)

There is considerable similarity between PMCA and SERCA; both are P-type Ca-ATPases, and both play a crucial role in maintaining Ca^{2+} homeostasis and signalling. By analogy to SERCA uptake of SR-released Ca^{2+} after agonist stimulation, PMCA expels Ca^{2+} that enters the cell during excitation. Furthermore, there is structural homology between the two transporters; they both have transmembrane-spanning regions, have similar ATP-phosphorylated intermediaries, counter transport H^+ and are regulated by second messengers. They both also exist in several splice variant isoforms, which impart some tissue specificity. PMCA is regulated by calmodulin. Four isoforms of PMCA [1–4] have been identified with 80–90% amino acid sequence

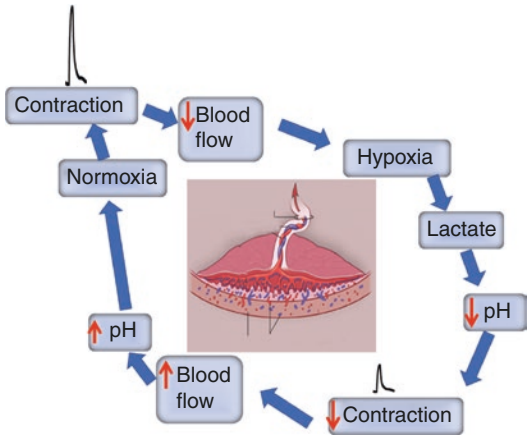

Fig. 10.5 The relation between contractions and blood flow in the uterus. Myometrial contractions are powerful, especially in labour. This leads to compressions of the uterine blood vessels (see central insert showing uterus and placenta and their closeness). The resultant hypoxia increases lactate which acidifies the myocytes. The fall in pH decreases calcium entry and force falls. This relieves the compression on vessels and the uterine environment is restored, and another effective contraction occurs. The feedback cycle will be protective as it prevents hypoxic damage to foetus (and myometrium)

homology and ten membrane-spanning regions. PMCA 1, 2b and 4 have been reported in myometrium [164]. In isolated uterine myocytes, 70% of Ca^{2+} efflux occurs via PMCA during the decay of a calcium transient, with the remaining 30% removed by NCX [165]. Inhibition of both mechanisms prevents all Ca^{2+}calcium efflux. In a study of intact rat uterus, 35% of SR-released Ca^{2+} was extruded by the NCX, and 65% by the PMCA [166]. Therefore, in the uterus, we can conclude that whatever the source of Ca^{2+} influx, PMCA plays the predominant role in Ca^{2+} efflux. We also found that the coupling between SR Ca^{2+} release and the NCX changes with gestation, altering its relative contribution to Ca^{2+} efflux.

10.3.4.2 Na/Ca Exchanger (NCX)

In the myometrium, the Na/Ca exchanger is a membrane-spanning antiporter. It has 938 amino acids and 9 transmembrane domains and utilises the Na^+ gradient provided by the sodium pump to extrude Ca^{2+}. During each cycle, one Ca^{2+} ion is exchanged for $3Na^+$ ions and thus it is electrogenic. The exchanger reaction may occur in either direction, as determined by the relative [Na^+] inside and outside of the uterine myocyte. Under most physiological conditions, the exchanger runs in its Ca^{2+} efflux mode. However, with the presence of plasma membrane microdomains in which NCX lies adjacent to SR, and couples to isoforms of the Na,K ATPase, it is possible that the NCX can bring Ca^{2+} into myocytes [167]. Even though PMCA extrudes more Ca^{2+} than NCX in uterine myocytes, both efflux mechanisms may well function in a compartmentalised fashion. It is suggested that the PMCA regulates the low-resting [Ca^{2+}] in the bulk cytosol while the NCX regulates the small microdomains between the plasma membrane and the SR [167].

10.4 Metabolism in Uterine Smooth Muscle

During labour the myometrium performs a series of sustained and powerful contractions to expel the young. These events are underpinned by growth, biochemical and metabolic changes. Throughout pregnancy there is enormous uterine growth, largely as a result of hypertrophy of the myometrium. Pronounced biochemical changes also occur during this period. These include increases in contractile proteins to support parturition but also changes in glycolytic and oxidative enzymes [168]. Metabolically, the uterus lays down reserves of glycogen and free fatty acid droplets in preparation for the hypoxic conditions it will experience in labour. After parturition these changes are rapidly reversed in a process known as involution. In women the uterus is back to near its pre-pregnancy size in about 6 weeks, a process that occurs due to changes in endocrine levels and removal of mechanical stretch [169]. We will briefly overview growth of the uterus in pregnancy, describe the uterine metabolites involved in contraction and then discuss metabolism and relation between contractions and blood flow. This latter part will include recent data examining the effects of hypoxia and pH in the myometrium.

10.4.1 Uterine Growth in Pregnancy

The human uterus is traditionally described as being a pear-shaped organ situated within the pelvis, ballooning up to the size of a large water melon with pregnancy. By week 20 it reaches the umbilicus and by week 30 it reaches the epigastric region.

In the early days and weeks of pregnancy the uterus does not need to grow. It needs to change to accept the foetus and help make the placenta, as it can easily accommodate the tiny embryo. The uterus contains a volume of about 10–25 mL, and this is ample for the embryo which is only about 5 mm long at 6 weeks. By weeks 12–14, the uterine wall has started to thicken from around 10 to 15 cm. By week 22, the baby will weigh 15 ounces, be the size of a dinner plate and contained in a uterus that is around three times thicker than it was at the start of pregnancy and extending 22 cm from the top of the pubic bone. Over the next 10 weeks the uterus increases its capacity to match the foetus and becomes so stretched it starts to thin. The uterus is so stretched at the end of term that it is thinner (about 5 mm) than at the start of pregnancy. From start to finish of pregnancy, the length of the uterus has increased from around 7 to 20 cm, its weight has increased more than ten times, going from about 70 g to more than 1000 g and its capacity from about 25 to 5000 mL. This adaptive growth of the uterus starts with some hyperplasia of myocytes in early pregnancy, stimulated by the endocrine changes of pregnancy and growth factors. From mid-pregnancy onwards, the myometrial hypertrophic growth is stimulated by wall stretch, with the endocrine environment being necessary in a permissive manner. Experiments of distending one of the two horns of a rodent uterus in a non-pregnant animal reveal that significant growth will occur. Likewise, there is little growth in a uterine horn of a pregnant rodent if there are no foetuses present; the side with foetuses grows normally. The uterine myocytes can increase fivefold in size, mainly due to an increased length but also thickness [170]. Progesterone supports this growth and also helps in dampening the contractile power of the myometrium. There are also changes in the extracellular matrix, with increased integrin expression bridging between the matrix and the hypertrophying myocytes. As with other smooth muscle cells, the uterine myocytes can synthesise some matrix elements, e.g. collagen. It is during the third trimester that the well-recognised contractile phenotype of the uterine myocyte comes to the fore, being packed with contractile proteins. A good account of these changes and the relation between endocrine and mechanical stimuli on the myocytes is provided in the review by Shynlova et al. [171]. Other investigators have pointed to the concurrent increase or appearance of contraction-associated proteins. A cassette of genes is assumed to be switched on at this time leading to increased gap junctions and Na^+ channels, as well as oxytocin and prostaglandin F receptors. In this way contractions can be coordinated and strengthened for labour.

10.4.2 Contractile Proteins and Kinetics

It has been noted that there is a change in the actin isoform close to term in rats, with a switch from α- to γ-actin [172]. As shown by Word et al. [173], the content of myosin and actin per milligram of protein or per tissue cross-sectional area is similar between myometrium of non-pregnant and pregnant women. In other words, despite the significant increase in myocyte size with pregnancy in women, the amount of contractile proteins per cellular cross-sectional area is similar to that found in myometrium obtained from non-pregnant women. In addition, myosin light-chain kinase and phosphatase activities are similar in the two tissues. The content of two thin filament-associated proteins was also examined in this study; caldesmon content was significantly increased in myometrium of pregnant women, whereas calponin was not different. In an earlier study, Sparrow et al. [174] examined the kinetic properties of the myocytes and myosin composition in rats. They concluded that the length-force relationship was of similar shape in the non-gravid and gravid skinned tissues. The energetic

tension cost (ATP turnover/active stress) in skinned fibres was also similar. The mechanical and metabolic characteristics of the gravid and non-gravid rat uterus do not suggest an obvious difference in the intrinsic properties of the myosin, although significant functional alterations in the tissue appear during pregnancy. This corresponds to the lack of a difference in the pattern of the heavy chains. Taken together, at least to a first approximation, the above data surprisingly suggest that there is little other than scale, to differentiate the intrinsic contractile proteins and contractions between non-pregnant and pregnant myometrium.

10.4.3 Metabolites and Contraction

As with other muscle types, the myometrium uses ATP during contraction. ATP is required both for cross-bridge cycling and phosphorylation of myosin light chains. The uterine myocytes also contain a store of phosphocreatine (PCr) which buffers the supply of ATP. In common with other smooth muscles, the store of PCr is low (~5 mM) compared to striated muscles (~30–50 mM). In the myometrium, these values were determined in intact muscle strips using ^{31}P NMR spectroscopy [175]. Some changes occur with pregnancy; PCr concentration ([PCr]) was one- to fourfold greater in late-pregnant than in non-pregnant rat uterus and recovered over several days during involution of the uterus.

When considering the low reserves of high-energy phosphates in the myometrium (and all smooth muscles) it is worth remembering that smooth muscles contract economically; contraction and relaxation are slow compared with striated muscles. This is partly because smooth muscle relies on Ca^{2+}-regulated phosphorylation of myosin rather than the Ca^{2+} troponin system, and partly due to the slower release of inorganic phosphate. This slow-release step means that there is a slow rate of cross-bridge cycling and longer lasting cross-bridge attachment periods and force production per ATP hydrolysed in smooth muscles. The oxygen consumption of smooth muscle is low compared to other muscle types. Consequently, metabolism can keep up with contraction during normal smooth muscle activity and no large increases in oxygen consumption occur. As with all muscles, good control of Ca^{2+} and pH is important for uterine smooth muscle function, and both regulation mechanisms make energetic demands requiring ATP. Further details and references can be found in [176].

10.4.4 Metabolism

There are some major differences in metabolism in smooth muscle compared to other cell types. One of the most striking is the degree of metabolic compartmentalisation. Oxidative phosphorylation directly supports contractile activity, while ionic regulation, especially Na,K ATPase, is supported by the ATP generated from anaerobic metabolism [177, 178]. There is direct interaction between the glycolytic enzymes and subunits of the Na,K ATPase and H-ATPase. The compartmentalisation of metabolism in smooth muscle was further supported by the demonstration that the enzymes for glycolysis are membrane bound, anchored by F-actin [179].

The myometrium produces lactate even with sufficient oxygen supplies for oxidative phosphorylation, i.e. oxygen is not limiting metabolism [180]. Lactic acid is produced by glycolysis and dissociates into lactate and protons at physiological pH. Lactate is produced during normoxic conditions in myometrium and transported out of the myocyte by a family of proton-linked monocarboxylate transporters [181]. Lactate efflux also increases severalfold under hypoxic conditions [180]. In a recent study on rat myometrium the effects of lactate on contractions, intracellular Ca^{2+} and pH were investigated [182]. We found that lactate in the physiological range decreases myometrial contractility because of its inhibition of Ca^{2+} transients. This inhibition in turn was a consequence of lactate acidifying the myometrial cytoplasm. The accumulation of extracellular lactate by reducing myometrial contractions is suggested to play a role in poor labours, discussed further in Sect. 10.5.

10.4.5 Blood Flow, pH and Myometrial Contractions

The myometrium contracts strongly in labour. Repetitive and intense contractions of labour are required to dilate the cervix, so that the foetal head and then body can pass through the birth canal. It is a feature of labour that the contractions increase in amplitude and frequency as they progress. Not always appreciated is that these powerful uterine contractions will compress and occlude the vessels coursing within the myometrium. In all species studied, clear dips in blood flow can be monitored during these contractions, as they compress the myometrial blood vessels [183]. From this it follows that hypoxia and ischemia are a normal occurrence in labour. If, however, the uterus contracts hypertonically and becomes more tonic than phasic, then the clamp on the uterine vessels will produce asphyxia in the foetus and ultimately in utero death. This relation is shown in Fig. 10.5.

10.4.5.1 Protective Feedback Mechanism

Phasic uterine contractions lead to cyclic changes in high-energy phosphates and lactate and pH. During uterine contractions, ATP concentration ([ATP]) would be expected to fall during the periods of hypoxia and ischemia and recover during the reperfusion that occurs between contractions [183]. In addition, because of the increased lactate and hypoxic conditions during force production, intracellular pH falls and is restored to normal values during the intervals between contractions [183]. The fall in [ATP] during normal labour may not be sufficient to limit contractions but the fall of pH will be. In single-uterine myocytes, intracellular acidification significantly decreased Ca^{2+} inward currents [184]. When examined in myometrial strips we found that intracellular acidification caused membrane hyperpolarisation [185], which was associated with failure of action potential firing and cessation of contraction. Thus, at the peak of uterine contractions the myocytes become progressively more acid and the contractions will start to reduce in strength. This, along with the changes in excitability and inactivation of the L-type Ca^{2+} current, results in the phasic contraction returning to baseline (see Fig. 10.5). The ensuing rest period, equivalent to diastole in the heart, should ensure that the metabolic changes produced by hypoxia and ischaemia are reversed, and [ATP], [PCr] and pH are restored. The environmental conditions, including normoxia, are then conducive to firing of action potential and myocytes responding with strong inward Ca^{2+} current and producing coordinated activity and the next, strong uterine contractions.

Thus, there is a negative feedback mechanism intrinsic to uterine myocytes. By acting to limit contraction strength and duration, this metabolic feedback loop will help prevent ischaemic damage to the myometrial muscle bundles. This is because the ischaemic period is curtailed, and blood flow and metabolites can be restored during the intercontraction intervals. There is also a huge importance of this feedback loop to the foetus. Because of the direct link between uterus and foetus via the placenta, myometrial hypoxia and ischaemia will be transmitted to the foetus, as indicated by insert in Fig. 10.5. During normal labour the peak of the uterine contractions can be detected by changes in foetal heart rate that accompany it; the hypoxia detected by the foetal cardiovascular system increases its heart rate. In other words, the cycle of hypoxia and its stimulation of foetal heart rate are a normal part of human labour. The intrinsic mechanism that limits contractile activity, and uterine damage, also protects the foetus. It copes with transient hypoxia by increasing its heart rate but does less well if the hypoxia and ischaemia become prolonged. The foetal heart rate pattern changes and large decelerations are a warning sign of foetal distress. It is for this reason that foetal heart rate is recorded in many labours so that warning signs can be detected and acted on, e.g. by recommending an emergency C-section. It is hard in such in vivo situations to dissect if the root cause of the problem is with the uterine vessels or myometrial cells.

10.4.5.2 Hypoxia-Induced Force Increase (HIFI) in Myometrium

Finally, we would like to draw attention to the possibility of hypoxic conditioning in the uterus, and describe another potential benefit to transient ischaemia and hypoxia in the uterus [176]. Hypoxic preconditioning is a protective effect arising from brief, intermittent hypoxic or ischemic episodes, on subsequent more severe hypoxic episodes. First discovered in cardiac muscle, the effect has now been documented in other organs. We found a novel response in uterus of hypoxic-induced force increase (HIFI), which we suggested helps maintain contractions during labour [186].

We know, as discussed above, that many changes occur towards the transition to labour. A conundrum however of labour is that contractions become progressively stronger as the myometrium experiences the repetitive metabolic stress of hypoxia, i.e. transient decreases of oxygenation, pH and ATP, all of which if sustained can decrease contractile activity. Oxytocin is often cited as driving contractions in labour, but oxytocin receptor knockout mice deliver normally. This led us to search for other, possibly intrinsic or metabolic, drivers of contraction.

Many genes concerned with metabolism and contraction are regulated by hypoxia, and changes in genes have been identified in a transcriptomic study of poorly labouring women [187]. There was, however, no evidence showing how such changes could be important to successful labours, and no mechanism linking hypoxia to an increase in contractions. Given a literature on hypoxic preconditioning and that decreases in oxygenation are part of normal labour, we wanted to consider if the effects of brief, repetitive periods of hypoxia could differ from the chronic changes used in most experimental protocols. We found a novel mechanism whereby brief but repetitive hypoxic episodes stimulated the contractile activity, HIFI. In other words, cycles of brief hypoxia initiate and maintain the progressive augmentation of contractility needed for labour. The underlying mechanism involves adenosine and prostaglandin and a rise in intracellular Ca^{2+} [186]. Of note was that HIFI is present in animal and human uterus, but only close to parturition. We speculate that aberrations in this powerful mechanism could underlie contractions being triggered too early (preterm labour) or, if HIFI is deficient, weak contractions, and thus poor and unsuccessful (dysfunctional) labours.

10.5 Uterine Activity and Parturition

Although this is a scientific chapter, it would be wrong to not, albeit briefly, address uterine contractility with respect to childbirth, and mention some of the problems that can arise due to aberrant uterine contractility. Examples would be contractions starting too early in gestation leading to preterm delivery; dysfunctional labours where contractions are too weak or uncoordinated to deliver the neonate or prevent postpartum haemorrhage, or contractions that are too strong and tonic-like that they lead to foetal hypoxia and stillbirths. Here we describe the progression to labour, followed by an overview of preterm birth and dysfunctional labour.

10.5.1 Progression to Labour

Labour is viewed as the culmination of changes that have been progressing over many months—it is no longer considered that there is one key event that flips a switch to labour. These ongoing changes include the changes in ion channels which in turn will affect the shape and frequency of action potentials. The increase in both gap junctions and their conduction helps ensure that changes in Ca^{2+} and excitability are rapidly transmitted to the muscle bundles and contractions can be coordinated. There is a change in endocrine environment, notably increased oxytocin release and receptors, increased prostaglandin production and reduced efficacy of progesterone due to isoform switching [188]. Coupling between other mediators of contraction, both intra- and extracellular, also increases [189]. As noted earlier there are also increases in myocyte size, myofilament content and iso-

forms, changes in actin polymerisation close to term, and along with increased agonist drive, all contribute to the strong contractions necessary for parturition.

During labour, the resultant coordinated contractions of the myocytes raise intrauterine pressure to dilate the cervix. As noted by Smith et al., "the emergent behaviour of the uterus has parallels in the behaviour of crowds at soccer matches that sing together without a conductor. This contrasts with the behaviour of the heart where sequential contractions are regulated by a pacemaker in a similar way to the actions of a conductor and an orchestra" [190].

10.5.2 Preterm Labour

Preterm labour is classified clinically as one that commences before 37 weeks of gestation. Post-term labours are those that go beyond 42 weeks. It is, however, the preterm births that give rise to the most concern. Preterm births remain the biggest cause of neonatal deaths and handicap. Preterm birth syndrome remains the most important clinical and research challenge facing pregnant women, their families and health professionals. The global burden of preterm birth is 15 million babies, and rates are rising [191, 192].

From a scientific perspective, the key challenges related to preterm birth research lie in its multiple aetiologies, long preclinical stage and complex gene-environment interactions.

The aetiology of premature labour is largely unknown. Risk factors are infection, a short/"incompetent" cervix as well as carrying more than one foetus. Almost half of all women pregnant with twins will deliver prematurely [193]. It is still not clear whether this increased risk comes from mechanical factors, such as uterine distension and pressure on the cervix or altered hormonal levels, as both oestrogens and progesterone are increased and maternal corticotrophin-releasing hormone (CRH) may be elevated [194]. Unfortunately, progress in better understanding of underlying pathologies, phenotyping and more targeted preventative therapies remains slow [195].

10.5.3 Dysfunctional Labour

While most term labours proceed successfully to a normal vaginal delivery and delivery of a healthy baby, unfortunately around 10% will not, due to weak uterine contractions arising from the failure of increasing strength and frequency of contractions that are the hallmark of labour. Contractions that start out well become weak and uncoordinated. This halts the dilation of the cervix and the labour slows or arrests. These labours are termed dysfunctional or dystocic. The condition is particularly a feature of women labouring for the first time. There is little evidence for a genetic cause [138].

The first insight into the physiological cause of dysfunctional labour came from our group [196]. This work involved obtaining a sample of myometrial capillary blood at the time of the first incision into the myometrium at C-section. This blood was immediately analysed for pH, lactate and other standard haematic variables. The data obtained was then matched to the blinded clinical classification of the reason for the C-section, provided by two consultant obstetricians. The results were compelling: women who had a C-section for reasons other than a diagnosis of dystocia had a similar range of myometrial capillary blood pH (around 7.3), but those labouring dysfunctionally had a significantly lower pH near 7.1. Furthermore, these women also had about double the lactate in their myometrial capillary blood. These findings can be understood in terms of a breakdown in the feedback cycle depicted in Fig. 10.5. If uterine blood flow is not restored adequately between contractions, so that lactate and pH are not restored to normal values, then contractions will become progressively impaired—i.e. the labour becomes dysfunctional. We have recently reported the outcome of a small randomised con-

trol trial using oral bicarbonate ingestion to help neutralise the low pH found in dysfunctional labours [197]. The results were extremely encouraging, with a significant reduction in the number of operative deliveries required in women who had had a diagnosis of dysfunctional labour. These results, if replicated in larger studies, could have a major impact in helping women worldwide suffering difficult births.

10.6 Conclusions

In this chapter we have summarised knowledge focussed on how the uterus contracts. We have journeyed from molecules to intracellular organelles, through in vitro and in vivo experiments and to women in labour. We have attempted to show areas where understanding has progressed. We have overviewed the discussion in a balanced manner, but undoubtedly our personal opinions emerge at times. Real progress has been made in understanding the role of the SR in this organ, and more details of K^+ channels and their nuanced roles are emerging. There have been some novel mechanisms uncovered, such as hypoxia-induced force increase (HIFI). There has also been a small clinical trial that has successfully bridged the gap from our basic science understanding around blood flow, contractions and acidity, to using bicarbonate to improve labour outcomes in women destined to have an emergency C-section because of dysfunctional labours. Despite this, true insights into key questions, such as what channels or myocytes determine pacemaking in the myometrium, remain beyond our grasp. We still must tell our students that one of the fundamental questions about this myogenic smooth muscle, which initiates excitation, remains unanswered. Our treatments to prevent preterm delivery are failing as the science has not translated to the clinic. We have now access to fantastic methods, from imaging to genomic, proteomic and metabolomic, as well as better computational techniques to handle and share large data. As a community we need to come together more to test our questions and ideas, so that the best protocols, techniques and collaborations can be made. Real progress will be measured by how quickly some of the missing data and questions arising from this chapter can be provided and the next chapter written.

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