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The physiological mechanism of uterine contraction with emphasis on calcium ion

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***Abstract*— Uterine contractions are important in many reproductive functions including the transport of sperms and embryo, menstruation, pregnancy and parturition. Improper or irregular uterine activity may underlie the common pathological disorders such as infertility, improper implantation, preterm labor, and weak uterine contraction during labor. In addition, successful labor is controlled by the coordinated activity and harmony between the uterine smooth muscle cells. If however, this activity becomes too weak or strong, normal labor may not be progressed which could lead to fetal morbidity and mortality. Uterine contraction is generated by shortening of uterine smooth muscle cells. Calcium ion is the key regulatory factor for this contraction to occur and its influx into the cell is initiated predominantly by depolarization of myometrial cell membrane. The transient increase in intracellular calcium concentration initiates cycles of myometrial contraction and relaxation. Basically, uterine contraction depends heavily on intracellular calcium concentration and any alteration of this concentration could affect the strength of uterine activity. This review presents an overview of the physiology of myometrial contraction and the role of calcium ions during contraction and relaxation.**

***Keywords* — calcium, calcium channel, contraction, myometrium, uterus.**

INTRODUCTION

The uterus is a hollow muscular organ situated deep within the female pelvic cavity. The smooth muscle contained within it (myometrium) is able to produce regular spontaneous contractions without any hormonal or nervous input (Wray, 2007). Much progress has been made over the last two decade to understand the molecular and cellular mechanism of uterine contractions and to investigate how uterine smooth muscles are modulated by several agonists. The myometrium is quiescent throughout the early pregnancy to allow fetus to grow and it changes dramatically during labor to a very strong active organ to expel the fetus and placenta. However, the exact mechanism of the sudden change of this activity from a quiescent state is not known. It is to be expected that some labor chances, however can go wrong with devastating consequences. Uterine contractions initiated too early throughout pregnancy could lead to premature delivery of the baby and resulting in fetal morbidity and mortality. However, uterine contractions with such a very strong intensity during labor could result in fetal hypoxia and compromise the normal delivery of the baby. Furthermore, irregular and very weak uterine contractions during labor could lead to failure to progress or unplanned cesarean section. It is anticipated that these aberrant uterine contractions may occur in some women as the mechanisms modulating the uterine activity are not fully described. In this review, I will briefly consider the normal physiological contraction and relaxation of the uterus and I will emphasize on the role of calcium as a key regulator of uterine activity.

Uterine contractions in non-pregnant and pregnant state

The non-pregnant uterus is not a quiescent organ as some may thought and it can produce contractions to facilitate the journey of sperms to the fallopian tubes and to help expel the shed inner lining of the uterus (endometrium) during menstruation. Recently, special attention has been paid to the physiology and the mechanism of uterine contractility in the non-pregnant state (Meirzon et al., 2011, Şimşek et al., 2014, Lychkova et al.,

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2014, Novakovic et al., 2013). It appears that uterine contractile patterns differ in the non-pregnant than in pregnant state. It has been shown that the non-pregnant uterus produces "waves-like activity" throughout the menstrual cycle involving the sub-endometrial layer (Van Gestel et al., 2003). On the other hand, throughout the early gestation the uterine contractions are normally of irregular pattern with weak intensity to maintain the conceptus but at the time of labor these contractions must be transformed to a very strong and regular pattern to expel the fetus and placenta. However, throughout the menstruation period, the non-pregnant uterus could produce irregular and uncoordinated contractions and it could produce labor-like contractions to expel the endometrial shedding (Ijland et al., 1996). Like any visceral smooth muscle cells, the contraction of uterine smooth muscle is phasic in nature, showing cycles of discrete intermittent contractions of varying amplitude, duration, and frequency. These contractions are relatively short lasting and fast with relaxation periods between them. Example of *in vitro* human uterine contractions is shown in figure 1.

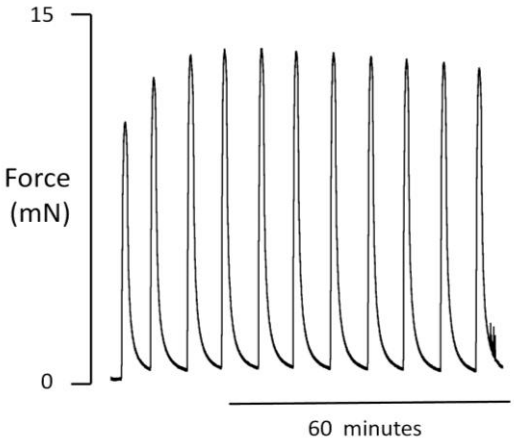

Figure 1: An Example of spontaneous human uterine contractions.
The uterus is able to produce spontaneous regular contractions without any neural or hormonal stimuli if placed in proper condition and these contractions are phasic in nature; showing maintenance of resting tone with repeated cycles of contraction and relaxation.

It is to be noted that the uterine smooth muscle *in vitro* contracts in a remarkably similar pattern to *in vivo* and its requirements for membrane depolarization and calcium influx. The free intracellular calcium concentration is a key regulatory factor for uterine contraction. However, Contraction is fundamentally controlled and triggered by a transient increase in intracellular calcium ($[Ca^{2+}]_i$), which is initiated and controlled by uterine action potential (Matthew et al., 2004), and it can also be modulated by several factors and agonists that can affect their amplitude, duration, and frequency (Wray, 2007).

Intracellular calcium store in myometrium

Intracellular calcium is normally stored in a special intracellular organelle known as sarcoplasmic reticulum (SR) that is located either very close to the myometrial surface membrane or towards the center of the cell (Broderick and Broderick, 1990). The main physiological function of myometrial SR is to actively take up the cytosolic calcium against Ca^{2+} gradient and store it until needed. Calcium ions can be released from the SR via two main channels present on the SR membrane. The first mechanism is via IP_3 channels that are mainly activated by IP_3 second messenger and the second mechanism is via ryanodine receptors (RyRs) which are activated mainly by Ca^{2+} itself leading to a phenomenon known as Ca^{2+} induced Ca^{2+} release (CICR). Both these channels have been demonstrated on the membrane of myometrial SR (Awad et al., 1997, Mironneau et al., 2002, Young and Mathur, 1999). Three isoforms of RyRs have been cloned and identified (RyR1, RyR2, and RyR3). It is suggested that there is no or little role for CICR in myometrium (Taggart and Wray, 1998, Holda et al., 1996) although it was confirmed that some of RyRs isoforms are expressed in myometrium (Martin et al., 1999, Mironneau et al., 2002). Therefore, it is of interest and importance to further investigate the role of CICR in myometrium during different gestational states.

Electrophysiology of myometrium (excitation-contraction coupling)

The sequence of events, between the generation of action potential and the initiation of muscle contraction, is known as excitation-contraction coupling (ECC); and it is the central component of a healthy functioning uterus. The basic process for the excitation-contraction mechanism resides mainly within

the uterine smooth muscle itself, and it is apparent that the resting membrane potential of uterine smooth muscle cells falls between -35 to -80 mV (see (Sanborn, 2000) for review). The spontaneous electrical activity of uterine myocytes is characterized by cycles of depolarization and repolarization that occur within uterine plasma membrane and is known as action potential. As uterine smooth muscle is spontaneously active, changes in membrane potential are necessary for the contraction to occur. Contraction is primarily dependent on the generation of action potential, a transient rise in intracellular calcium, and the presence of contractile elements and a conducting system between uterine cells (Wrayzx et al., 2003). However part of these values can be determined by species type and also may depend on gestational state of the myometrium. When there is no or minimal change of membrane potential, the membrane can be considered in a resting potential or even if there is a minimal movement of ions across the plasma membrane. Similar to most other excitable tissues, the excitability of uterine smooth muscle is largely determined by the movement of sodium (Na^+), calcium (Ca^{2+}) and chloride (Cl^-) ions into the cytoplasm and the movement of potassium (K^+) ions into the extracellular space. The former three ions are concentrated outside the myometrium whereas the latter are concentrated inside the myometrial cytoplasm. However, the plasma membrane is normally more permeable to K^+ ion, which moves it down its concentration and electrochemical gradients (i.e. from extracellular to intracellular space); hence electrical potential inside the myocytes is created (Jain et al., 2000).

The excitation-contraction coupling in myometrium can occur via two main mechanisms; electrochemical or pharmacomechanical coupling. In electrochemical coupling, the primary drive for the rise in intracellular calcium concentration $[Ca^{2+}]_i$ is the depolarization of plasma membrane. Basically, changing the ionic permeability of uterine cell membrane leads to action potential generation, which therefore depolarizes the cell membrane and opens the (voltage gated calcium channel (VGCC)/L-type calcium channel), resulting in a significant calcium influx into the cell and binding of calcium to Calmodulin (CaM). Calcium-CaM complex then activates the myosin light chain kinase (MLCK) which would then phosphorylate the serine 19 on the regulatory light chain of myosin (MLC_{20}), enabling acto-myosin crossbridge cycling and interaction, hydrolysis of Mg-ATP, and production of contraction (Taggart, 2001). For uterine relaxation to occur, another cytoplasmic enzyme; myosin light chain phosphatase (MLCP) must dephosphorylate the phosphorylated myosin (Figure 2).

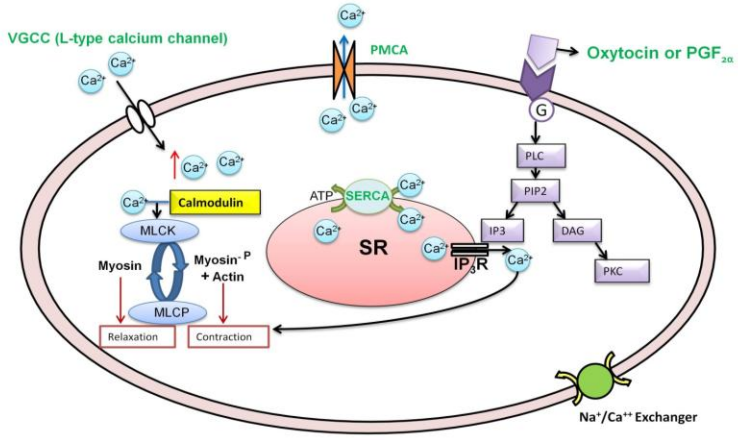

Figure 2: Schematic diagram showing calcium entry and initiation of contraction in uterine smooth muscle. Depolarization of plasma membrane opens the VGCC (L-type Ca^{2+} Channel) resulting in Ca^{2+} influx into the cell. Calcium then complexes with calmodulin protein and activates Myosin light chain kinase (MLCK) which then phosphorylates light chain of myosin (P). Phosphorylated myosin binds with actin and initiate cross bridge cycling leading to uterine contraction. On the other hand, relaxation is brought about by dephosphorylation of light chain of myosin by myosin light chain phosphatase (MLCP) and calcium extrusion outside the cell via an active transport of calcium across the plasma membranes Ca^{2+}-ATPase (PMCA) and/or sequestration into the SR by SERCA pumps and/or by Na+/ Ca2+ exchanger. Oxytocin and other uterine stimulants augment contraction by binding to their specific receptor on the cell membrane and cause small monomeric G-proteins to bind GTP and activate PLC. This would subsequently cleave phosphatidylinositol biphosphate (PIP2) at the cell membrane and generates inositol triphosphate (IP3) and diacylglycerol (DAG) second messengers. IP3 then binds to its specific receptor at the surface of SR and thereby increasing [Ca2+]i. DAG activates PKC.

During pharmacomechanical coupling, the increased $[Ca^{2+}]_i$ is brought about by receptor-agonist binding rather than membrane depolarization (although changes in membrane

potential may occur). When Agonists such as oxytocin or prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) bind to their specific receptor on plasma membrane, they can cause the small monomeric G-proteins to bind GTP and activate phospholipase C (PLC). This subsequently cleaves phosphatidylinositol biphosphate (PIP_2) at the cell membrane and yields inositol triphosphate (IP_3) and diacylglycerol (DAG) second messengers. IP_3 then binds to its specific receptor at the surface of sarcoplasmic reticulum (SR) and thereby increasing $[Ca^{2+}]_i$. DAG activates protein kinase C (PKC) (Figure 2). All of these would further augment the uterine contractions.

Do myometrial pacemakers exist?

The concept of a "pacemaker" in myometrial smooth muscles has been investigated for several years. The uterine myocyte is a myogenic being able to contract spontaneously and generate slow wave, simple, and complex action potential (Khan et al., 2001). The ionic nature of spontaneous generation of action potential and how it is triggered in the myometrium is still not fully understood. In other smooth muscle cells such as gastrointestinal tract and urinary bladder, the specialized interstitial cells of Cajal (ICC) or ICC-like cells (ILC) act as pacemakers to generate rhythmical activity in these smooth muscle cells (Johnston et al., 2010, Zheng et al., 2014). In myometrial cells there is evidence for the presence of ILC, but whether they act as pacemakers is not clear (Duquette et al., 2005, Cretoiu et al., 2011). Moreover, it is likely that any individual myometrial cell can display pacemaker activity, however it is not anatomically fixed or confined to specific specialized myometrial cells as in other types of smooth muscle cell and it is not clear why some uterine cells should become pacemaker. Therefore, further research is needed to elucidate if pacemaker cells are exist in uterine smooth muscles.

Calcium sensitization

As mentioned previously, the force of uterine contraction can be augmented by the action of some agonists such as oxytocin and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Shmygol et al., 2006) by promoting the action potential and increasing the intracellular calcium concentration. Furthermore, agonists can also initiate other intracellular pathway and signals and involve other mechanisms that augment and maintain the force of contraction. The activity of MLCK and MLCP phosphorylation/dephosphorylation can be modulated by agonists binding to their specific receptors on myometrial membrane leading to changes in the level of contractile apparatus without changes in the $[Ca^{2+}]_i$. Therefore, the relationship between the contractile filaments and the $[Ca^{2+}]_i$ is referred to as calcium sensitization. Studies have demonstrated that the major mechanism controlling the calcium sensitization may due to the inhibition of MLCP in the myometrium following the stimulation of G protein coupled receptors (GPCRs) (Arthur et al., 2007). There are several mediators of MLCP inhibitory pathway in smooth muscles including the small monomeric G protein RhoA and its downstream effectors Rho-associated kinase (ROK) and the 17-kDa PKC-potentiated inhibitory protein (CPI-17) (Arthur et al., 2007). Studies have shown that blocking the RhoA pathways could abolish the calcium sensitization in myometrium, suggesting the importance of calcium sensitization to maintain and augment the uterine activity (Woodcock et al., 2004, Kupittayanant et al., 2001).

Uterine contraction and the regulation of intracellular calcium concentration $[Ca^{2+}]_i$

A transient rise in $[Ca^{2+}]_i$ is the major trigger for smooth muscle contraction including the uterus (Shmygol et al., 2007). The myometrial contraction is always preceded by a transient increase in $[Ca^{2+}]_i$. In figure 3, we show an example of simultaneous recording of myometrial contractions preceded by changes in $[Ca^{2+}]_i$ by using a fluorescent calcium indicator, Indo-1 acetoxymethyl ester (Indo-1AM, Molecular Probes, Oregon, USA). The concentration of intracellular calcium is relatively very low (50-100nM) compared to the extracellular concentration (2 mM) and this is critically regulated by intracellular calcium mechanisms. However, contraction of smooth muscle cells including the myometrium depends mainly on the increase of $[Ca^{2+}]_i$ and indeed this can occur via

calcium influx pathways from extracellular space into the cell and/or calcium release from sarcoplasmic reticulum (SR). Calcium can enter the cell via different membrane gates including Voltage-Gated Calcium Channels (VGCCs) in particular L-type calcium channel, store-operated calcium channels (SOCCs or capacitative Ca^{2+} entry), and/or via receptor-operated calcium channels (ROCCs). For detailed reviews on the structure and function of these channels in smooth muscle the reader is referred to these references (McFadzean and Gibson, 2002, Albert and Large, 2003).

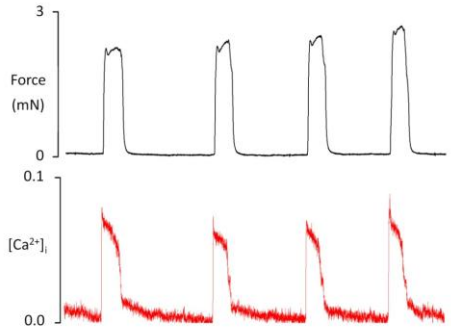

Figure 1: An original recording of simultaneous measurement of force of contraction and intracellular calcium in rat myometrium.
The top black trace is the changes in myometrial force of contraction and the bottom red trace is the changes in the intracellular calcium. Note that each uterine contraction is always preceded by an increase in intracellular calcium transient indicating the importance of calcium ion for the initiation of contraction.

Relaxation of the myometrium - Ca^{2+} Extrusion mechanism

Uterine contraction is decreased or terminated by a fall in $[Ca^{2+}]_i$ which gradually dissociates from CaM and eventually decreasing the activation of MLCK. Calcium homeostasis is critical and is maintained by calcium pumps which move the calcium against its concentration gradients across the cell or SR membranes. The mechanisms responsible for the removal of calcium are through specific proteins spanning the plasma membrane; these are plasma membrane Ca^{2+}-ATPase (PMCA), Na^+/Ca^{2+} exchanger (NCX), and SERCA.

The PMCA which was identified and investigated biochemically in the myometrium (Carrera et al., 2000), transports calcium from the cytoplasm to the extracellular space at the expense of ATP hydrolysis. NCX allows one calcium ion to leave the cells in exchange with three sodium ions. In addition, SERCA pumps calcium ions from the cytoplasm into the sarcoplasmic reticulum using ATP hydrolysis and its role in sequestering the calcium into the SR has been investigated in pregnant rat myometrium (Shmigol et al., 1999, Taggart and Wray, 1998). An additional mechanism is by mitochondria which could play an essential role in removing the calcium from the cytoplasm in smooth muscle cells (Kamishima et al., 2000) and there is no clear evidence that calcium flux via mitochondrial membrane may contribute to excitation-contraction coupling and its role is very minor in calcium movement. However, further studies are needed to elucidate the involvement of mitochondria in uptaking and removing $[Ca^{2+}]_i$ in smooth muscle cells including the myometrium.

In summary, although major advances in understanding the molecular physiology of myometrium have been achieved, there is a pressing need to understand human uterine contractile activity and the role of other channels and receptors such as chloride, sodium, ryanodine and the role of nucleotides such as adenosine, adenosine diphosphate (ADP), and ATP in human myometrium. The role of CICR is still unclear in myometrium and needs further elucidation. There is also a need to fully understand the role of mitochondria in calcium homeostasis. These investigations and the development with the action of agonists and antagonists on uterine smooth muscle will add more to our understanding of uterine physiology and lead to more successful approaches in diagnosing and managing the reproductive disorders such as preterm labor, dysmenorrhea, prolonged labor, and weak uterine contractions (dystocia). It is apparent that understanding the normal physiology of uterine contractions and relaxation at the molecular and cellular level would help clinicians and healthcare providers to modulate unnecessary uterine activity if problems arise throughout

pregnancy and to plan a suitable therapeutic target according to each problem.

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