***Trying to keep calm in troubled times: The role of K channels in uterine physiology.***

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***Abstract***

Much research has been undertaken to define what determines the inherent contractility of the myometrial smooth muscle and what dictates the switch from relaxed to contractile phenotype. Whilst a lot has been deciphered the fundamental mechanisms that initiate and maintain the switch in uterine physiology are ill defined. Uterine physiology is indubitably complex so this article focuses on one piece of the puzzle: the role of certain potassium channels in determining myometrial contractility and how altered activity may contribute to the hyper-contractile uterus at term.

***Introduction.*** During gestation the uterus adopts a relaxed phenotype whilst the fetus implants and develops. But at term the uterus becomes a more aggressive creature as the myometrial smooth muscle actively propels the fetus towards the waiting world. When the delicate processes that dictate phenotype become aberrant the consequences can be dire for both fetus and mother. Pre-term delivery due to presumptuous appearance of a more contractile uterus increases the risk of neonatal morbidity and mortality. Alternatively, ineffective contractions during delivery impairs cervical dilation, increases the risk of the fetus suffering hypoxia and raises the likelihood of postpartum hemorrhage. Whilst the uterus is a multi-cellular organ it is the contractile activity of the smooth muscle cells in the myometrium that are ultimately central to the two faced character of the uterus.

Contraction of uterine smooth muscle has been well characterized and the reader is directed to reviews by Shmygol et al (2007) and Wray (2015) for more detail. In brief, smooth muscle cells contract through increased interaction of actin and myosin that is mediated by a calcium-dependent activation of myosin light chain kinase and the subsequent phosphorylation of the regulatory myosin light chain. The net rise in intracellular calcium ([Ca2+]) is mediated by a combination of Ca2+ influx through plasmalemmal ion channels and Ca2+ release from intracellular organelles balanced by removal mechanisms. However, the major precipitating factor is the increased open probability of voltage-dependent calcium channels, Cav2.1 and to a lesser extent Cav3.1 (Shmygol et al., 2007; Wray, 2015). As pregnancy reaches term increasingly forceful contractions of the uterus develop linked to long term hypoxia-induced changes in uterine signaling (Alotaibi et al., 2015).

As Figure 1 illustrates the myometrial smooth muscle is spontaneously active, which is modulated by various receptor agonists or mechanical influences. Force increases without any external influence at a regular interval that is maintained for a limited period of time before returning to baseline levels. This activity is due to cyclical membrane depolarization from a resting potential driving Ca2+ channel opening and Ca2+ influx although the nature of the membrane depolarization can adopt various guises from single action potential, long duration bursts or trains of action potentials (see Fig 11 in Tong et al., 2011 or Fig 9 in Testrow et al., 2018). The precise mechanisms determining the spontaneous activity have not been defined unequivocally but may originate from specialized interstitial cells (platelet-derived growth factor receptor alpha positive or C-kit positive cells) identified in mouse and monkey reproductive tracts (Duquette et al., 2005; Allix et al., 2008, Gravina et al., 2014; Peri et al., 2015) and may involve the efflux of chloride through ANO-1 encoded Ca2+-activated chloride channels (Bernstein et al., 2014, Hyuga et al., 2018) or cation influx through TRP encoded cation channels (Dalrymple et al., 2007). Fast voltage-gated sodium currents analogous to those detected in heart, nerve and skeletal muscle have also been detected in certain myometrial smooth muscle cells electrophysiologically although the molecular identity is unknown (Testrow et al., 2018).

***Keeping it calm.***

As the myometrial smooth muscle is prone to be excitable, rhythmical contraction is reliant upon each depolarizing event being curtailed effectively and a relatively negative membrane potential being maintained. Potassium (K+) channels provide this calming influence by allowing K+ efflux, which generates membrane hyperpolarization sufficient to oppose depolarizing surges in membrane potential and to repolarize depolarisation. Depending upon the biophysical properties of the channels concerned (ie mechanism of activation, voltage dependence, rate of closure) specific K+ channels can provide a background K+ leak that contributes to the resting membrane potential and to action potential repolarization. As such, K+ channels determine the frequency of myometrial contractions as well as the amplitude and breadth of individual contractile events. Since K+ channels have such an important role in the contractile activity of the myometrium and myometrial smooth muscle depolarizes considerably as pregnancy progresses (Parkington et al., 1999) it has been postulated that alterations in K+ channel contribution either transcriptionally or through attenuated activity in the membrane underpins the shift in uterine phenotype at late pregnancy.

Expression of several K+ channel genes have been detected in rodent and human myometrium (see Greenwood & Tribe, 2011 for summary) but functional effects of K+ channel modulators have not been recorded for all genes expressed. This article will focus on specific K+ channels where pharmacological modulators or molecular manipulations have functional impact and whose functional impact is dependent upon gestational stage. In the interest of brevity this review will not comment upon the large conductance Ca2+-activated K+ channel encoded by KCNMA1 (KCa1.1, Alexander et al., 2011) that is robustly expressed in the myometrium. The function of this protein in the myometrium is complicated and there is evidence that it operates as a protein integrator hub more than a determinant of membrane potential. A recent review by Lorca et al (2014) provides a good summary of the various regulators of KCa1.1.

*Led by the heart - Kv channels.*

In many ways the uterus acts like a heart, contracting with a regular periodicity and characterized by action potentials superimposed on a depolarizing pacemaker potential. In the heart repolarization of the atrial and ventricular action potential is mediated by ion channels encoded by the genes KCNQ1 (Kv7.1) and KCNH2 (a.k.a *ether-a-go-go related* gene or ERG1). So are these channels present in the myometrium and do they have functional impact?

Kv7 channels are K+ channels encoded by 5 KCNQ genes (KCNQ1-5) that are activated by membrane depolarization with a threshold about -40 mV. Each functional channel is comprised of 4 Kv7 proteins and channel contribution can be modified by auxillary proteins encoded by KCNE genes as well as various intracellular signals including local phosphatidyl inositol bisphosphate concentration, calmodulin, βγ G proteins, and kinases (Barrese et al., 2018). These channels have established roles in neuronal membrane potential stabilization and cardiac action potential repolarization as well as arterial smooth muscle (Barrese et al., 2018). Expression of all KCNQ genes has been detected in murine myometrium and KCNQ1-4 in humans (McCallum et al., 2009, 2011). KCNQ expression reduces considerably in early murine pregnancy but is robust at late pregnancy (McCallum et al., 2011). Consistent with the working model shown in figure 1, blockers of Kv7 channels such as linopirdine or XE991 increase the frequency of contraction in murine and human myometrium but has negligible effect on the duration or amplitude of individual contraction events (McCallum et al., 2011). Kv7.1 specific blockers do not mirror these effects. Retigabine and flupirtine, which stabilize open Kv7.2-7.5 channels reduce the incidence of contraction discharge with a considerably greater effect in myometrium from late pregnant versus non pregnant mice or laboring versus non-laboring humans (McCallum et al., 2011). As gene expression is not different at late pregnancy these observations suggest that post-transcriptional regulation of Kv7 channels is altered at term. These changes would not contribute to a more contractile myometrium befitting the physiological need but may offer additional advantages to the laboring fetus. Additionally, Kv7 activators such as retigabine offer potential in treatment for pre-term labor and future work should exploit this axis.

There are 3 *ether-a-go-go related* genes, ERG1-3 (KCNH2, KCNH6 and KCNH7, Alexander et al., 2011). The expression products (Kv11.1 – 11.3, Alexander et al., 2011) form tetrameric voltage-dependent K+ channels with distinctive biophysical characteristics due to a dominant C-type inactivation (Smith et al). Kv11.1 is a key determinants of the late repolarizing phase of the cardiac action potential and defective channels underlie many hereditary arrhythmias. ERG1 expression has been detected in several phasically active smooth muscles including jejunum, portal vein and epididymis (refs) where Kv11.1 blockers heightened contractility. Non-pregnant mouse myometrium also expresses ERG1 but little ERG2 or 3 and the level of gene expression as well as protein abundance does not change throughout pregnancy (Greenwood et al., 2009). Similar to other phasic smooth muscles blockade of ERG channels by 3 structurally disparate agents enhanced the amplitude and duration of spontaneous contractions of the non-pregnant myometrium and application of oxytocin in the presence of ERG channel blockers resulted in a tonic contraction that persisted for many minutes (Greenwood et al., 2009). ERG channel blockade had a considerably greater effect on force development than Kv7-specific blockers or other non-selective Kv inhibitors. ERG expression and functional effects of ERG blockers on membrane potential was subsequently detected in myometrium samples from non-laboring and laboring human patients (Parkington et al., 2014). In the human non-laboring myometrium the ERG channel blocker dofetilide changed the myometrial action potential from a bursting profile to a tonic plateau-type waveform that was recreated in computer modeling simulations using the biophysical; parameters of ERG channels (Tong et al., 2014 ).

Strikingly, ERG blockers nor ERG enhancers had no effect on myometrial contractility in mice at late pregnancy, which was associated with the loss of K+ currents with distinctive kinetics characteristic of ERG channels that were present in smooth muscle cells from non-pregnant mice (Greenwood et al., 2009). Interestingly neither KCNH2 transcripts nor Kv11.1 levels changed throughout mouse gestation but the expression of a ~100 amino acid auxillary protein KCNE2 increased markedly as pregnancy developed and the expression product of this gene represses ERG channel abundance and activity. Identical, to the work done on mouse myometrium the functional effect of ERG channel blockers was lost in laboring myometrium, which was associated with the loss of ERG channel currents (Parkington et al., 2014). Like the mouse this was associated with an increased expression of KCNE2 whilst KCNH2 expression remained the same as non-laboring myometrium. This study not only confirmed the observations of the early work undertaken in mouse myometrium but showed that KCNE2 expression did not increase in the myometrium of obese women who failed to progress to vaginal delivery and ERG blockers were still effective in the myometrial tissues from obese women at labor (Parkington et al., 2014). These remarkable findings show that myometrial smooth muscle loses an anti-contractile mechanism through the energetically efficient expression of a small modifying protein. This area of physiology remains to be exploited further.

*Background makes a difference*.

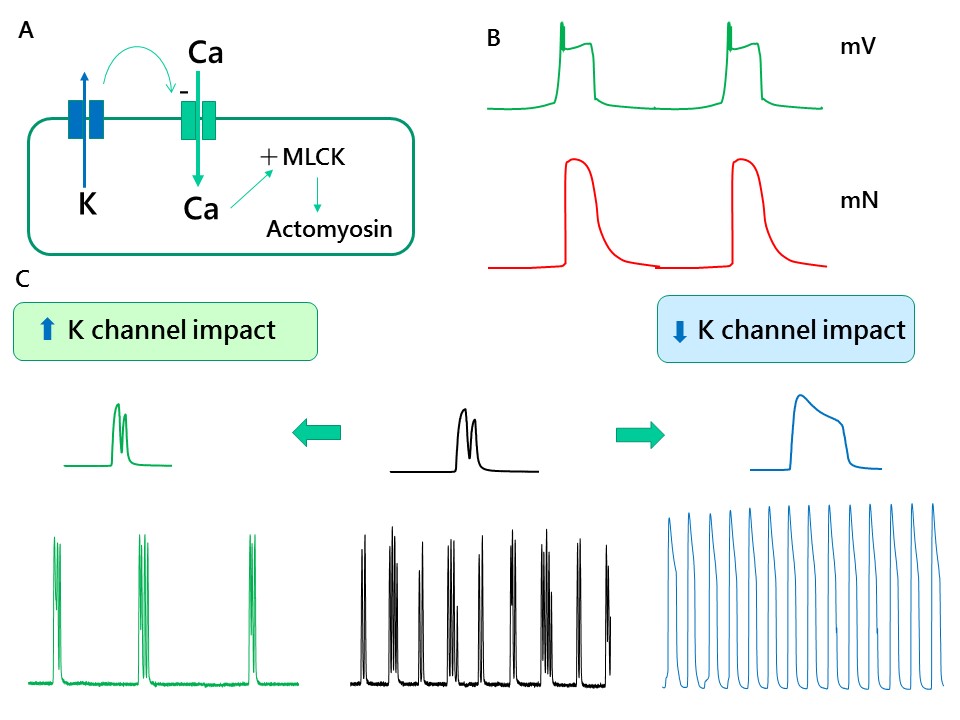
Smooth muscle cells need a stable resting membrane potential upon which depolarizing surges can be superimposed to allow long term function. Whilst Kv7 and ERG channels have been implicated in neuronal stabilization their voltage-dependent properties are not ideal for this role. Unlike Kv channels there are two families of K+ channel that are suited to a role as background contributors namely inward rectifiers (Kir) formed of tetramers of proteins with two transmembrane segments and K2P channels that are dimers of proteins with 4 transmembrane domains each with 2 pore forming regions (Niemeyer et al., 2016). K2P channels are active across a wide voltage range and exhibit some pH sensitivity. Of the different K2P isoforms human and mouse myometrium express mainly TASK1 and TREK-1 (Bai et al., 2005, Buxton et al., 2010, Monaghan et al., 2011). TREK-1 channel activity is increased by membrane distention (Monaghan et al., 2011) and enhanced by NO independent of cGMP, arachidonic acid and intracellular acidification. TREK-1 transcripts and protein decreases considerably in myometrium from laboring humans and this was correlated with a decrease in arachidonic acid activated currents in the freshly isolated myometrial smooth muscle cells (Heyman et al., 2013). Unlike most K2P channels TREK-1 channels have little activity at rest but become increasingly active either after stimulation with arachidonic acid or by mechanical stretch. Moreover, expression of TREK 1 gene is enhanced by stretch. Yin et al (2018) reported that spontaneous and oxytocin-induced activity in human myometrium was less in mothers with twins compared with singletons where resting tension is considerably different (Turton et al., 2013) or if the myometrium was set to 4 x higher basal tension. This diminished contractility was offset by application of L-methionine, which inhibits TREK-1. TREK-1 transcript level increased significantly with the greater stretch. Thus, physiological stretch as the fetus develops increases TREK-1 expression that contributes to maintaining relative quiescence (Yin et al., 2018). Interestingly, in the myometrium of mothers who went into preterm labor 5 alternatively spliced variants of TREK-1 were detected (Cowles et al., 2015). Co-expression of any of these variants with wild type TREK-1 resulted in grossly impaired currents due predominantly to altered membrane trafficking (Cowles et al., 2015).

Inwardly rectifying K+ channels (Kir) pass K+ in easier than out (Hibino et al., 2010). There are 7 families separated into constitutively active, ATP regulated or Gβγ stimulated. Recently, expression of Kir 7.1 was identified in mouse myometrium and transcript levels exhibited a bell –shaped dependence on gestational stage. Kir7.1 was also detected in human myometrium and transcript levels were significantly lower in laboring uterus compared to non-laboring (McCloskey et al., 2014). Impressively, McCloskey and colleagues were able to use molecular interference to knockdown or augment expression of Kir7.1 in murine myometrial strips, which led to heightened or suppressed contractile activity respectively. Knockdown of Kir 7.1 also depolarized the membrane potential by ~ 30 mV. The effects of Kir7.1 knockdown were mirrored by two structurally different blockers of Kir7.1 (VU590 and MRT2000769) in murine and human myometrium.

A third candidate background K+ leak has been identified recently – the sodium-activated large conductance K channel (KNa encoded by Slo2.1/ 2.2, Ferreira et al., 2019). This channel has a similar structure to the large conductance Ca2+-activated K+ channel (KCa1.1 encoded by KCNMA1) but is insensitive to intracellular [Ca2+] and gated by intracellular [Na+]. In human myometrial cells the influx of Na+ through channels that provide a persistent Na+ leak current is sufficient to generate an opposing K+ conductance across a wide voltage range (Ferreira et al., 2019). Expression analysis and use of siRNA identified the SLO2.1 variant as the molecular correlate of the KNa and the activity of this channel was diminished considerably by oxytocin providing another mechanism to reduce myometrial quiescence at labor.

***Summing up.***

The collected data suggest that myometrial phenotype is determine by a collage of background Kir7.1, KNa and TREK-1 K+ channels supported by Kv7 and Kv11.1 channels. The functional impact of these channels diminishes as pregnancy nears an end but there is little insight into the mechanisms that determine the functional switch. It may be transcriptional (eg Kir 7.1), splice variant composition (TREK-1) or due to the increased expression of a suppressive auxillary subunit (Kv11.1). The latter case is the most intriguing concept and one that offers a tantalizing insight into a new tocolytic for the future.



***Figure 1. Representation of K channel impact on myometrial contraction.***

A, simplified model of smooth muscle contraction. Ca2+ influx through depolarization activated channels is major influence on myometrial contractile state. The hyperpolarization produced by K+ efflux through open channels limits Ca2+ channel opening and therefore opposes contraction. B representation of electro-mechanical coupling in the myometrial smooth muscle. Development of force is preceded by rapid membrane depolarization superimposed on a slower creep potential. Relaxation then progresses slower than membrane repolarization. C representation of the effect of altering K+ channel impact on myometrial contraction. Depending on the K channel concerned a decrease in channel activity is manifest as either prolongation of the contractile amplitude, which may summate to a tonic response, increased frequency of contraction or a combination of the two. Traces based upon murine myometrial data generated by the author.

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