The sheep as a model of studying pregnant uterine smooth muscle activity

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Inappropriate uterine contractile activity during pregnancy can have adverse effects for the mother and offspring. Preterm birth predisposes the offspring to adverse outcomes, *e.g.* neurodevelopmental delay, cardiovascular disability, throughout life. Post-dates birth increases the risk of neonatal sudden death, and failure to progress in labour commonly results in caesarean delivery, which increases susceptibility to problems in future labours for the woman and immune deficiency in the offspring during childhood. Rodents are commonly used models in studies of the details of physiological and disease processes. However, the electrical activity in rodent uterine smooth muscle differs from that observed in human uterine muscle. While the ovary controls the progress into labour in rodents, the placenta assumes that control in women from the end of the first trimester. Furthermore, rodent offspring are very immature and organ systems incomplete at birth, suggesting the possibility of an “early” (preterm?) labour process. Here we investigated the suitability of the sheep as a model in which to interrogate uterine contractility at term before and during the progress into labour.

In this initial study, ewes at term were placed under deep general anesthesia (20mg/kg thiopental intravenous initially, maintained by 2% isoflurane) and myometrium was removed surgically. Membrane potential was recorded in strips, using sharp intracellular microelectrodes, simultaneously with contraction. Following collagenase treatment, ionic currents were recorded from isolated myocytes using patch-clamp electrophysiology. The main uterine blood supply divides the uterine wall into inner and outer muscle layers. The orientation of the smooth muscle cells and contractility can differ between these two regions. On most occasions, it is difficult to determine whether uterine muscle obtained from women undergoing caesarean delivery is inner or outer. Also, muscle tissue from the main contractile region, the fundus, is rarely available from human caesarean section, as the cut is usually made in the lower segment. Evidence has suggested that contractility in the lower segment may not represent events in the fundus. Thus, in this study activity in the upper and lower layers and in the inner and outer layers were compared. Experimental procedures were approved by the Animal Ethics Committee of Monash University and were conducted in accordance with the regulations set out by NHMRC.

Both inner and outer layers of the uterus from all ewes had spontaneous contractions. Contractions were small and irregular in the inner layer in tissues from both the upper and lower uterine regions and were initiated by action potentials of brief duration (<500ms). Contractile behavior of the outer layers was strong and regular, and underpinned by action potentials of long duration (>1min). To test if $K_V7.4$ channels have an influence on resting membrane potential or action potential in the sheep uterus we administered Ml213 to activate these channels. Inner and outer layers showed similar behaviour of contraction in all experiments. Ml213 induced dose-dependent hyperpolarization (by a maximum of 5mV) and markedly reduced the frequency of contractions. XE-991, which blocks K_V7 channels, caused depolarization (~11mV) and increased contraction frequency in inner and outer muscle layers from both fundus and lower segment. K_V11 channels have a significant role in determining action potential and contraction duration in human uterus. To test the importance of this ion channel in sheep myometrium we used dofetilide to block this channel. Dofetilide increased the rate of contraction in inner-cervical and inner-fundus to 150% of control contraction and the level of contraction in outer-upper and outer-lower to 300% of control contraction. Analysis of variance revealed that the difference between layers was very strong, $P<0.0001$, and, while differences between upper fundus *versus* lower segment were present, the effect was weak, $P=0.025$. Single channel potassium currents in isolated myometrial cells confirmed the presence of K_V7 and K_V11 in sheep myometrium at term.

Here we confirmed that sheep myometrium at term possesses functional K_V7 and K_V11 channels similar to observations in human myometrium. Of interest was the very significant difference between the extent of responses in the inner *versus* the outer muscle layers. In contrast, and different from previous reports in rodent, there was very little difference between responses in the fundus, *versus* those immediately above the cervix.