Inhibitors of mitochondrial function disrupt uterine pacemaking

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Natural birth occurs through rhythmic contractions of the smooth muscle of the uterus. However, there is surprisingly little understanding of the mechanism of the pacemaker clock that both initiates and times the remarkable regularity of uterine contractions of labour. We aim to investigate whether store pacemaking, a mechanism where the release-refill cycle of entrained intracellular stores acts as the pacemaker clock, has a role in uterine pacemaking. This mechanism was first shown in lymphatic smooth muscle and functions through entrainment of stores through coupled oscillator-based interactions, with sub-plasmalemmal Ca^{2+} release interacting with channels in the cell membrane to activate a pacemaker current (see van Helden & Imtiaz, 2003). However, uterine pacemaking may be different, in that agents that inhibit the SERCA pump and hence presumably block store function do not inhibit spontaneous uterine contractions. The present study is a first stage investigation of mechanisms that may contribute to pacemaking, focusing on the role of mitochondria.

Experiments were on single bundle strips (diameter = 100-300 μm; length = 2-4 mm) of longitudinal smooth muscle dissected from the uterus of freshly killed young adult mice (age 5-8 weeks). Mice were euthanased by overexposure to the inhalation anaesthetic isoflurane (5-10% in air), a procedure approved by the Animal Care and Ethics Committee at the University of Newcastle. The strips were secured in a 1-3 ml chamber and superfused at 1-5 ml/min with oxygenated (95% O_2, 5% CO_2) physiological saline of composition (mM): NaCl (120), KCl (5), $CaCl_2$ (2.5), $MgCl_2$ (2), $NaHCO_3$ (25), NaH_2PO_4 (1), glucose (10), with a pH of 7.3 at 35ºC. In most cases pacemaking was measured using video recording of spontaneous contractions. Some recordings were also made using intracellular microelectrodes (resistance 100-150 MΩ filled with 1 M KCl), though for this tissues generally needed to be immobilised by Wortmannin, an agent that blocks myosin light chain kinase and inhibits uterine contractions without marked effects on pacemaker function. A third procedure was to monitor pacemaking through Ca^{2+} imaging using a high speed Nipkow spinning disk system. In this case strips were pre-loaded with 5-10 μM Oregon Green/AM for 1 h at room temperature. Strips were either spontaneously active or could be induced to exhibit pacemaker activity by application of oxytocin (3 nM). In the case of the latter, oxytocin was applied throughout (*i.e.*, before and during the test procedures). Two classes of agents that inhibit aspects of mitochondrial function have been tested so far. The first was the mitochondrial uncoupler CCCP (1 μM), which is a protonophore that decreases the transmembrane potential across the inner mitochondrial membrane. The second class of inhibitor was the respiratory chain (complex I) inhibitor rotenone (3 μM). Both agents initially enhanced and then markedly decreased or abolished uterine pacemaking. These effects were unlikely to be due to depletion of ATP as pacemaking persisted in the presence of oligomycin (5 μM), an agent known to block the F1/F0 ATP synthase. These data are enticing as they indicate that mitochondria have a seminal role in store pacemaking. How this is achieved needs to be determined. The data do not exclude a role for store pacemaking, as it has been demonstrated in intestinal wall that inhibition of mitochondrial function disrupts store pacemaking (Ward *et al.*, 2000).

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