Uterine spontaneous contractions in the estrous cycle and the effect of mitochondrial inhibitors

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Uterine smooth muscle plays an essential role in parturition. During labour the smooth muscle of the uterus contracts forcefully and rhythmically in order to deliver the newborn. In non-pregnant animals the uterus also contracts, possibly in response to the menstrual period or estrous cycle. Despite its importance, the mechanism of uterine contractions is not well understood. The role of Ca^{2+} stores in this process is still controversial. A recent study in the guinea pig gallbladder has shown that mitochondrial Ca^{2+} plays an essential role in tone and motility of this tissue (Balemba *et al.,* 2008). Preliminary studies on the uterus have shown that mitochondria may indeed play a role in uterine pacemaking (Gravina *et al.*, 2007).

The aim of the present study is: i) to determine the effect of the estrous cycle (estrus *vs* metestrus) on the spontaneous contractions; and ii) to compare the effects of mitochondrial inhibitors on the spontaneous contractions during different stages of the estrous cycle. Swiss mice (6-10 weeks) were euthanized 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. Vaginal smears were performed with NaCl 0.9% in order to determine the phase of the estrous cycle (Marcondes *et al.,* 2002). Uteri were dissected out and each one provided 4 pieces of uterine horn. These preparations were set up in 3 ml organ baths containing Krebs solution, bubbled with carbogen at 37°C, under 0.5 g wt tension to record the force developed by the longitudinally-arranged layer. Tissues were allowed to equilibrate for 20 minutes. The force of contractions was recorded isometrically from a Grass FT.03 tension transducer connected to a MacLab4e system. Log concentration-response curves were constructed using 3 different mitochondrial inhibitors: carbonyl cyanide 3-chlorophenylhydrazone (CCCP, a mitochondrial uncoupler), 7-chloro-5-(2-chlorophenyl)-1,5-dihydro-4,1-benzothiazepin-2(3H)-one (CGP37157, a mitochondrial Ca^{2+}/Na^{2+} exchange blocker) and oligomycin (an ATP synthase inhibitor). Spontaneous contractions during estrus were significantly different from metestrus (0.20 ± 0.02 g wt/mg, $n = 5$ *vs* 0.29 ± 0.02 g wt/mg, $n = 10$; $p < 0.05$). Spontaneous contractions were inhibited by CCCP (pIC50 = 5.78 ± 0.10, n = 5-10) and CGP37157 (pIC50 = 4.29 ± 0.10, n = 4-9). In contrast, oligomycin did not affect contractions ($n = 4$). CCCP did not have a significantly different effect in estrus (pIC50 = 6.17; 95% CI 5.61-6.73, $n = 3$) when compared with metestrus (pIC50 = 5.66; 95% CI 5.46-5.87, $n = 3$). However, the effect of CGP37157 was significantly different ($P<0.05$) between estrus (pIC50 = 3.65; 95% CI 3.21-4.09, $n = 3$) and metestrus (pIC50 = 4.38; 95% CI 4.14-4.62, $n = 3$).

In conclusion, this study reinforces our previous finding that mitochondria may play a role in uterine pacemaking, not only as an ATP producer (as oligomycin, an ATP synthase inhibitor, did not inhibit contractions, showing that the effects of the other drugs were not due to lack of ATP). Moreover, this study shows different sensitivities to at least one of these inhibitors depending on the estrous cycle.

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