Oxytocin depolarizes mitochondrial membrane in freshly isolated myometrial cells

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Oxytocin is a peptide hormone involved in reproduction, including stimulation of uterine contractions and lactation. Even though oxytocin agonists and antagonists have been used for the induction and prevention of labour, the mechanism of action on uterine smooth muscle is not fully understood. It is known that oxytocin increases intracellular Ca^{2+}, stimulating Ca^{2+} entry and Ca^{2+} release from the sarcoplasmic reticulum (SR). The SR also interacts closely with mitochondria and we have previously shown that mitochondria play an important role in uterine activity. The aim of this study is to investigate whether and if so how mitochondria respond to oxytocin. Female Swiss mice (6-10 weeks) were euthanased by overexposure to the inhalation anaesthetic isoflurane (5-10%) followed by exsanguination, a procedure approved by the Animal Care and Ethics Committee at the University of Newcastle. Uteri were dissected out and the endometrium removed by fine dissection. Isolated cells were obtained by placing myometrial strips into an enzymatic solution containing 0.1% collagenase, 0.03% elastase and 0.3% hyaluronidase for 50 minutes, followed by trituration. To evaluate changes in mitochondrial membrane potential, myometrial cells were loaded with 5μg/ml 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolocarbocyanide iodide (JC-1) for 1 hour and then were perfused for 30 minutes with Hepes buffer prior to experiments. JC-1 J-aggregates (complexes formed in high mitochondrial membrane potential) were visualized with excitation/emission of 488/585 nm, while JC-1 monomers were detected at 488/522 nm. Application of 0.1nM and 1nM oxytocin, perfused for 11 minutes, decreased mitochondrial membrane potential (Ψm) to 92 ± 2% (n=5 cells) and 73 ± 4% (n=9 cells, different from control, $p<0.001$) of control respectively. As a positive control, 1 μM carbonylcyanide 3-chlorophenylhydrazone (CCCP) was perfused and caused a large decrease in Ψm, this being 58 ± 9% of control ($p<0.001$). To understand the mechanisms involved in this mitochondrial depolarization, oxytocin 1nM was applied in combination with 2-APB (an inhibitor of IP3 receptors and store-operated Ca^{2+} entry), in Ca^{2+} free (0.5mM EGTA) solution, or with 2 mM nifedipine (L-type Ca^{2+} channel blocker). When decreasing intracellular Ca^{2+}, either by inhibiting Ca^{2+} entry or SR Ca^{2+} release, the effect of oxytocin on mitochondrial depolarization was diminished. In the presence of 2-APB, the mitochondrial depolarization evoked by 1nM oxytocin was 88 ± 3% of control (n=8 cells), in Ca^{2+} free (EGTA) solution it was 91 ± 2% of control (n=8 cells) and with nifedipine it was 87 ± 5% of control. These results were not significantly different from control ($p>0.05$), suggesting that Ca^{2+} has a substantial role in mediating the oxytocin-induced change in Ψm. It is concluded that oxytocin depolarizes the mitochondrial membrane and this mechanism is in part dependent on intracellular $[Ca^{2+}]$.