Physiologic and Physical Interactions between the TRPV4 Channel and Oxytocin Receptor Modulate Human Myometrial Contractility

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Preterm birth and preterm labor remain significant public health concerns, affecting millions of infants worldwide. Previously, we demonstrated that transient receptor potential vanilloid-4 (TRPV4) channel activity modulates myometrial contractility in murine models. The present work addressed the hypothesis that *direct interaction between the TRPV4 channel and the oxytocin receptor (OXTR) regulates calcium signaling and contractility in the human myometrium*.

After obtaining informed consent, myometrial tissue was collected from pregnant non-laboring women at term gestation (≥37 weeks) undergoing cesarean section (n=7). Myometrial biopsies were obtained from the upper margin of the lower uterine segment incision following delivery of the placenta. Myometrial tissue was used for histological analyses or enzymatically dispersed for the isolation and culture of human uterine smooth muscle cells (HuSMCs). Spatial protein expression and co-localization of TRPV4 and OXTR were assessed by immunofluorescence. Proximity ligation assays (PLA) were performed to visualize protein-protein interactions in situ by detecting the formation of fluorescent signals when the TRPV4 and OXYR were <40 nm apart. To determine whether TRPV4 channel activation mediated contraction via OXYR, dynamic calcium (Ca^{2+}) imaging was performed to measure changes in intracellular calcium concentration ($[Ca^{2+}]_i$). HuSMCs were loaded with the Ca^{2+}-sensitive fluorophore, fura-2, and then stimulated with GSK1016790A (1μM), a TRPV4 agonist, or oxytocin (1μM) in the presence or absence of extracellular Ca^{2+}. Contractility was evaluated by collagen gel contraction assay, HuSMCs were treated with oxytocin (1μM) in the presence or absence of GSK1016790A, and the cross-sectional diameter of each gel was measured. Specific pharmacological antagonists of TRPV4, RN-9893 (500nM) and GSK2728745 (1nM), were used in the presence or absence of oxytocin, with $[Ca^{2+}]_i$ and myometrial contractility as outcome measures.

Co-localization of TRPV4 and OXTR was demonstrated via immunofluorescence. PLA showed proximity between TRPV4 and OXTR, supporting the notion of their physical association. In HuSMC treatment with either GSK1016790A or oxytocin increased $[Ca^{2+}]_i$ ($p< 0.0001$ vs baseline). The absence of extracellular calcium attenuated the GSK1016790A and oxytocin-induced increases in $[Ca^{2+}]_i$. In contractility assays, oxytocin treatment decreased the cross-

sectional diameter of collagen gels loaded with HuSMCs, by 27±5% ($p< 0.05$ vs control), consistent with contraction. Co-treatment with GSK1016790A potentiated the oxytocin-induced contraction by 10±6% ($p< 0.05$ vs oxytocin). Moreover, pharmacological antagonists of TRPV4, RN-9893 and GSK2728745, markedly attenuated oxytocin-induced increases in $[Ca^{2+}]_i$ by 81% ($p<0.05$ vs. oxytocin) and 75% ($p<0.05$ vs. oxytocin), respectively.

We conclude that HuSMCs express both the TRPV4 channel and the OXTR. Activation of either the TRPV4 channel or OXTR increases HuSMCs $[Ca^{2+}]_i$ and contractility, effects contingent on entry of extracellular calcium. Moreover, the physiologic effects of activation of either the TRPV4 or the OXTR were potentiated by activation, and attenuated by blockade, of the other. The physiologic interaction between TRPV4 and OXTR was supported by evidence of a physical interaction between the channels. These findings underscore the importance of the TRPV4 channel and interactions with the OXTR in regulating myometrial contractility. Targeting TRPV4-OXTR interactions presents a promising avenue for therapeutic interventions aimed at managing preterm labor and reducing the incidence of preterm birth.