PRESENTING AUTHOR'S NAME & RESEARCH TITLE

Scott D. Barnett, PhD

Leveraging Stretch-Activated Channels in the Uterus to Develop Novel Therapeutic Approaches to Halt Preterm Labor

PURPOSE/BACKGROUND

Approximately 10-12% of US births are preterm, which is defined as birth before 37 weeks of gestation. There are currently no FDA-approved drugs that can delay birth beyond 48-hours, despite 70+ years of active development. One of the reasons for this failure is that multiple contractile pathways converge upon the onset of labor to ensure productive and forceful contractions. As a result, traditional "one drug, one target" approaches have been unsuccessful at halting labor. Because the uterus must endure increasing tension as the pregnancy progresses, it is logical that stretch-activated channels play a critical role in maintaining quiescence. Here we explore the combined effects of small molecule activation of the mechanosensitive ion channels, Piezo-1, a Ca^{2+} modulator, and TREK-1, a K^{+} modulator, on uterine contractile dynamics. *Hypothesis*: Mechanosensitive signaling in pregnant human myometrium (muscle of the uterus) regulates quiescence, and this effect can be bolstered through the co-administration of small molecule agonists, which will synergistically decrease the intensity of contractions.

MATERIALS & METHODS

Membrane Potential Assay: 1° Isolated myocytes (n=3) were seeded into a 96 well plate (7000 cells/well) and exposed to either yoda1 (3 μM) or ML335 (30 μM) or both simultaneously. Changes in membrane potential ($\Delta\Psi$) were recorded using FluoVolt™ (491nm/516nm, ex/em) over 30 minutes and the change in fluorescent signaling from baseline was recorded. *Organ Bath:* Human myometrial strips (n=3-5 term non-laboring) were isometrically stretched in an organ bath then sequentially challenged with KCl (60 mM) and oxytocin (8 nM). Tissues were treated with either the Piezo-1 agonist, Yoda-1 (0.1-30 μM, accumulative - 15 min intervals), the TREK-1 agonist, ML335 (1-300 μM, accumulative - 15 min intervals), or a combination thereof.

RESULTS

In pregnant human uterine smooth muscle cells, the addition of the Piezo-1 agonist, yoda1 (3 μM) or the TREK-1 agonist, ML335 (30 μM), decreased the resting membrane potential by 26% (±1.3) and 13% (±2.6), respectively. When co-administered (3 μM/30 μM), the myocyte membrane potential fell by 55% (±6.3), suggesting that the dual-activation of these pathways results in synergistic quiescent signaling. In 'term non-laboring' human myometrial tissue (n=3), Yoda1 and ML335 decreased area under the curve (AUC) in a dose-dependent manner. When co-administered at their EC_{50} values (Yoda1 3 μM, ML335 30 μM) AUC fell to 19% (±16.8) of baseline, greatly diminishing contractile activity.

DISCUSSION/CONCLUSION

These data reveal that co-activation of the mechanosensitive channels Piezo-1 and TREK-1 by small molecule agonists impart a synergistic effect on membrane potential and contractile activity. The negative inotropic effects of these drugs in human myometrium are particularly compelling because under our experimental conditions the tissue is pre-stretched (activated), revealing that Yoda1 and ML335 further enhance channel activity beyond stretch alone. As such, these drugs represent a novel therapeutic approach to treating preterm labor, a result enhanced by their co-administration.