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**T type Ca^{2+} channels may play an essential role in uterine contractility**

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**Abstract:**

**Introduction:** Increased intracellular calcium via myometrial voltage-gated calcium channels (VGCCs) plays a critical role in uterine contraction. L-type VGCCs have been thought to be the major pathway of intrauterine Ca^{2+} mobilization. However, T-type VGCCs have been detected in myometrium and fluctuate with pregnancy and labor. Spontaneous rhythmic contractions and oxytocin-induced contractions are abolished by the T-type VGCC blocker mibefradil. We recently saw a patient with life-long history of non-contractile uterus. Whole exome sequencing of myometrial DNA from hysterectomy specimen identified a single amino acid change in the T-type Ca^{2+} channel protein Cav3.1. Sequencing of blood DNA showed the mutation was germ line. To better understand the role of Cav 3.1 in uterine contractility we developed a tissue specific knockdown mouse model.

**Methods:** AMHR2cre C57BL/6J mice were bred with C57BL/6J mice containing a loxP flanked Cav3.1 gene. Immunohistochemical staining was performed to localize Cav3.1 expression. At 14-16 weeks of age, mice were euthanized with CO_{2} followed by extirpation of uterine horns. One horn was snap frozen in liquid nitrogen for quantitation of Cav3.1 mRNA by qPCR and protein by Western blot. For uterine contractility, strips of freshly isolated uterine muscle were suspended in a tissue organ bath and tension measurements were recorded under baseline conditions, oxytocin, or KCl. Labchart Pro was used to
calculate area under the curve (AUC) for 10 minute periods under each condition, and each AUC was normalized as a percentage of AUC observed during KCl.

**Results:** Successive breeding resulted in mice of varying genotypes (AMHR/Cav3.1). Immunohistochemical staining showed higher levels of Cav3.1 in myometrium of wild type (WT) compared to heterozygous (HET) mice. Quantitative PCR showed an approximate 10-fold decrease in Cav3.1 in uterus between WT and HET mice. Western blot analysis verified expression of Cav 3.1 protein in the mouse uterus and showed decreased expression in agreement with lower transcript levels. Contractile-force was higher across the range of oxytocin concentrations when comparing WT to HET mice (Figure 1).

**Conclusion:** In a cre/lox system, expression of the T-type calcium channel Cav 3.1 was decreased in the mouse uterus resulting in a blunted contractility response to oxytocin. These results suggest a role for this T-type voltage gated calcium channel and a possible target for new tocolytic drug development.

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