The role of myometrial smooth muscle cell TRPV4 in modulating inflammation and uterine contractility.

Zahidee Rodriguez1, Lihua Ying1, Xiaoyuan Han2, Cristina M. Alvira1, David N. Cornfield1.
1Department of Pediatrics, 2Department of Urology, Stanford University School of Medicine, Stanford, CA.

Abstract

BACKGROUND: Prematurity is a major cause of global neonatal mortality and morbidity. Currently there are no effective treatments to treat or prevent preterm birth. Accumulating evidence suggests that myometrial inflammation is an important inciting event in both preterm and term labor, yet the mechanism by which myometrial inflammatory response is not known. We recently identified the transient receptor potential vanilloid 4 (TRPV4) channel as a key modulator of myometrial calcium entry and uterine tone. Further, in other cell types TRPV4 serves to promote inflammation, whether TRPV4 modulates myometrial inflammation has not been studied.

OBJECTIVE: To determine whether TRPV4 promotes lipopolysaccharide (LPS)-induced inflammation in mSMC.

METHODS: Human myometrial smooth muscle cells (mSMC) were treated with either phosphate buffered saline (PBS) or HC-067047 (HC, 10 μM) for 30 min prior to stimulation with LPS (100 ng/ml) for 24 h. The gene expression of IL-1β, IL-6, COX-2 and MCP-1 was determined by quantitative RT-PCR (Yang et al. Sci Sci Transl Med, 2015).

RESULTS: Concentration of mSMC with LPS increased the gene expression of IL-6, COX-2, and MCP-1 (p<0.05), but not IL-1β. Pharmacologic blockade of TRPV4 with HC-067047 decreased the LPS-induced increases in IL-6 (p<0.05), COX-2 (p<0.01), and MCP-1 (p<0.01), decreasing expression to levels that were no different than those found in mSMC treated with PBS alone.

CONCLUSION: Pharmacologic blockade of TRPV4 channels prevents LPS-induced cytokine expression in mSMC, suggesting a pro-inflammatory function for TRPV4 in the myometrium.

Background

• Myometrium is the most significant cause of neonatal morbidity and mortality across the globe.
• Nonetheless, therapeutic strategies to halt preterm labor are lacking.
• Infection and inflammation are among the most significant causes of preterm labor.
• Although the contractile state of mSMC is dependent on the entry of calcium into the cytosol, voltage-dependent L-type calcium channel blockers as monotherapy only transiently prolongs pregnancy.

Method & Materials

• TRPV4 channel activity modulates LPS-induced mSMC inflammation.

In vitro:
1. mSMC were pretreated with phosphate buffered saline (PBS) or LPS (100 ng/ml) for 1, 4, or 18 hours. IL-1β and IL-6 gene expression was determined by quantitative RT-PCR (qPCR).
2. mSMC were pretreated with PBS or HC-067047 (10 μM) for 30 min prior to stimulation with LPS (100 ng/ml) for 24 h. IL-6, IL-8, COX-2 and MCP-1 expression were determined by qPCR.

Hypothesis

TRPV4 channel activity modulates LPS-induced mSMC inflammation.

• Might calcium enter the mSMC as an alternative route?
• The TRPV4 channel is widely expressed and is activated by temperature, stretch, swelling, and hypertonicity.
• TRPV4 channels can also play a proinflammatory role.

In vivo:
1. C57BL/6J mice were used as wildtype (WT) mice and treated with intraperitoneal (IP) PBS or LPS (2 mg/kg) on day 15 of timed pregnancy. Uterine gene expression of IL-1β, IL-6, COX-2 and MCP-1 was determined by qPCR at 4 h.
2. WT mice and TRPV4−/− mice were treated with IP PBS or LPS (3 mg/kg) on day 15 of timed pregnancy. Mice were anesthetized and euthanized, uterine horns collected, cut into 3 mm x 2 mm segments and suspended in modified Krebs buffer at 37°C. Muscle strips equilibrated at 1.25 times tension for 45 minutes and dose-response to oxytocin was performed (1 to 1000 μM). Area under the curve was normalized to baseline spontaneous contractions and to WT PBS control.

Methods & Materials

Pharmacologic blockade of TRPV4 suppresses LPS-induced mSMC inflammation.

In vivo murine model:
1. Human mSMC in an alternative route?
2. The TRPV4 channel is widely expressed and is activated by temperature, stretch, swelling, and hypertonicity.
3. TRPV4 channels can also play a proinflammatory role.

Methods: To determine whether TRPV4 promotes lipopolysaccharide (LPS)-induced inflammation in mSMC.

Conclusions

Pharmacologic blockade of TRPV4 suppresses LPS-induced inflammation in mSMC.