

Inhibition of TRPC1/TRPC3 channels by PKG contributes to NO-mediated vasorelaxation

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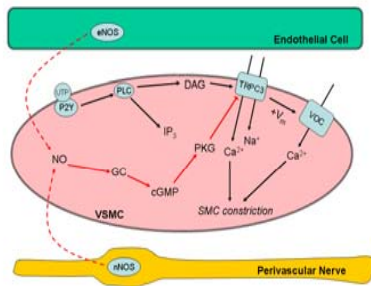
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Introduction

TRPC3 is a member of the canonical transient receptor potential (TRPC) family. TRPC3 has been shown to play a variety of roles in receptor-mediated vasoconstriction and in maintenance of vascular tone. Recently, it was reported that nitric oxide (NO)/cGMP/protein kinase G (PKG) leads to inhibition of TRPC3 channels in HEK 293 cells. We sought to determine if the same mechanism of regulation occurs in vascular smooth muscle cells (SMCs) of rat carotid artery (CA) through TRPC3 channels and if this mechanism contributes to NO-mediated vasorelaxation.

Hypotheses

- 1) NO-mediated inhibition of TRPC3 channels occurs in native smooth muscle cells.
- 2) Inhibition of TRPC3 channels contributes to NO-mediated vasorelaxation in the intact artery.



Summary cartoon of TRPC1/TRPC3 inhibition by NO signaling pathway

Methods

- TRPC3 mRNA and protein expression in carotid artery (CA) was analyzed by RT-PCR, Western blot and immunohistochemistry.
- TRPC1/TRPC3 protein interaction was evaluated by co-immunoprecipitation.
- TRPC1/TRPC3 currents in freshly isolated CA SMCs were elicited by UTP and measured by whole cell patch clamp.
- NO-mediated vasorelaxation in isolated CA segments was evaluated by wire myograph studies.

Results

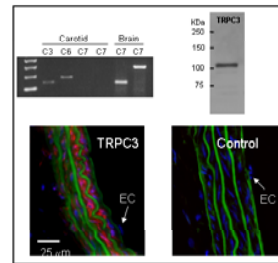


Fig1. TRPC3 mRNA and protein in CA

TRPC3 message and protein were detected in rat carotid artery by RT-PCR and Western blot. TRPC3 immunofluorescence (red) was clear throughout the smooth muscle layers, but was not detectable in the endothelial cells (EC).

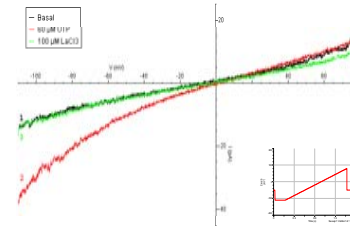


Fig2. UTP-activated whole cell current (I_{UTP}) in SMCs of CA

60 μ M UTP activated a non-selective current in freshly isolated SMC of rat carotid artery, which was inhibited by 100 μ M $LaCl_3$. Insert: Patch clamp protocol

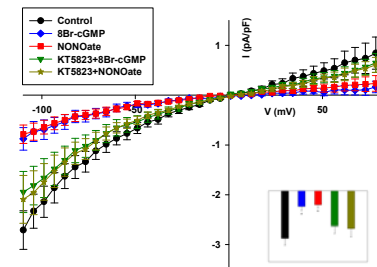


Fig3. Inhibition of I_{UTP} by NO/cGMP/PKG

I_{UTP} was inhibited by a NO-donor (MAHMA NONoate, 10 μ M) and cyclic GMP analog (8Br-cGMP, 100 μ M). Inhibition of PKG by KT5823 reversed the inhibitory effects. Insert: I_{UTP} at -110 mV. * $P < 0.05$.

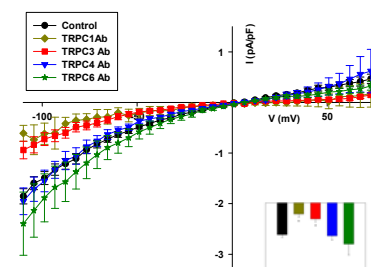


Fig4. I_{UTP} was carried by TRPC1/TRPC3

I_{UTP} was blocked by inhibiting antibodies: TRPC1 and TRPC3 antibodies, but not TRPC4 and TRPC6 antibodies. Insert: I_{UTP} at -110 mV. * $P < 0.05$.

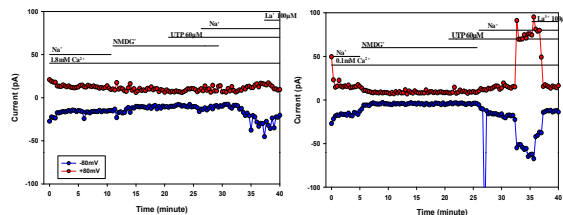


Fig5. Partial inhibition of I_{UTP} by extracellular Ca^{2+}

I_{UTP} was inhibited by replacing Na^+ with $NMDG^+$. I_{UTP} in the low extracellular Ca^{2+} was larger than in the physiological Ca^{2+} .

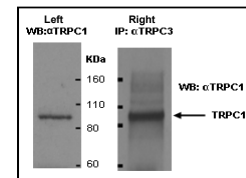


Fig6. TRPC1/TRPC3 heterotetramer in CA

TRPC1 protein was detected directly by Western blot (Left) or after co-immunoprecipitation (Right) with TRPC3.

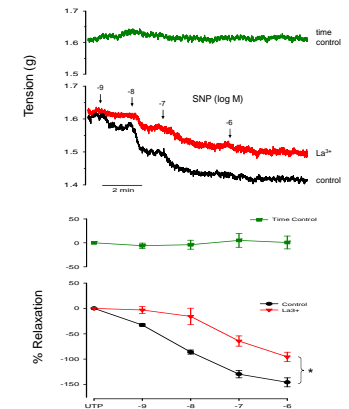


Fig7. La^{3+} -sensitive component of the NO-mediated relaxation

Intact carotid artery segments were preconstricted with 100 μ M UTP. NO-mediated relaxation to SNP was significantly inhibited by 100 μ M La^{3+} .

Summary and Conclusions

- A UTP-activated whole cell current (I_{UTP}) was significantly inhibited by an NO-donor as well as a cyclic GMP analog.
- Inhibition of PKG reversed the inhibitory effect of the NO-donor as well as the cyclic GMP analog.
- The specific involvement of TRPC1 and TRPC3 as effectors of I_{UTP} was confirmed by using inhibiting antibodies, TRPC1 and TRPC3.
- TRPC1 and TRPC3 co-immunoprecipitated from rat CA protein isolates.
- I_{UTP} was attenuated by extracellular Ca^{2+} , a characteristic of TRPC1-containing channels.
- Vasorelaxation to NO was significantly reduced in the presence of La^{3+} , a known inhibitor of TRPC channels.
- **Conclusions:** These studies show for the first time that vascular smooth muscle cells exhibit TRPC1/TRPC3 channel currents that are inhibited by the NO signaling pathway. Furthermore, these studies suggest that TRPC1/TRPC3 is part of a feedback system that is functionally involved in NO-mediated vasorelaxation.

Acknowledgements

This work was supported by NIH grant. RO1 HL088435 (PI: Marrelli SP) and AHA BGIA 0665100Y (PI: Marrelli SP)