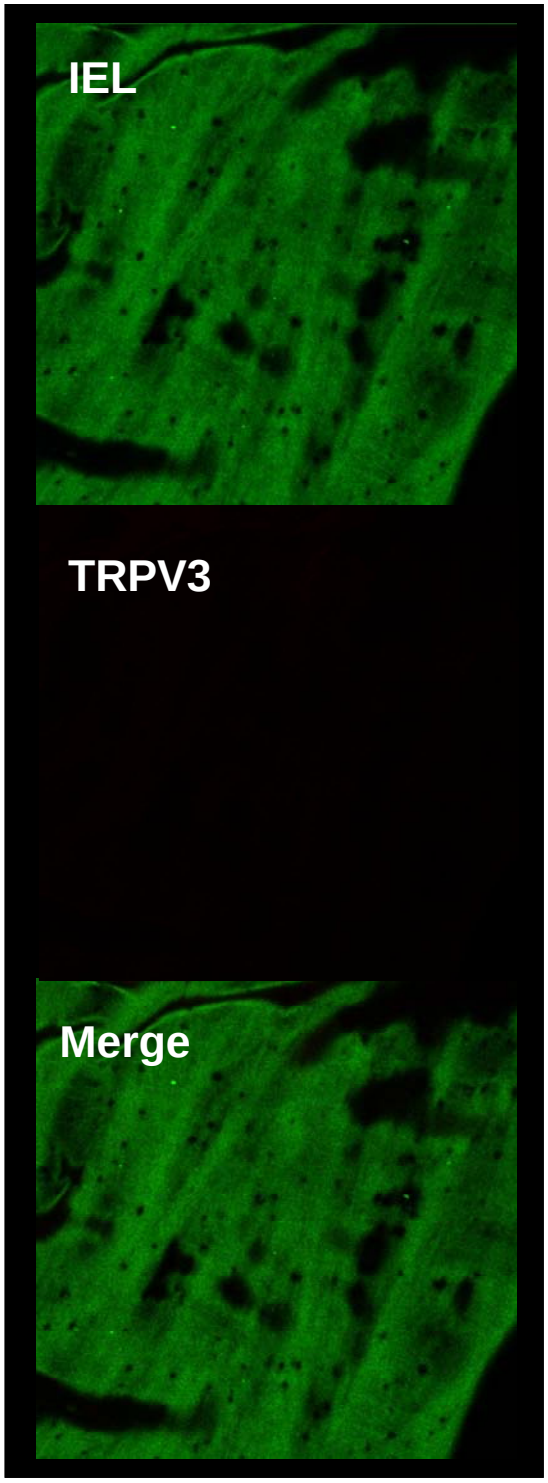
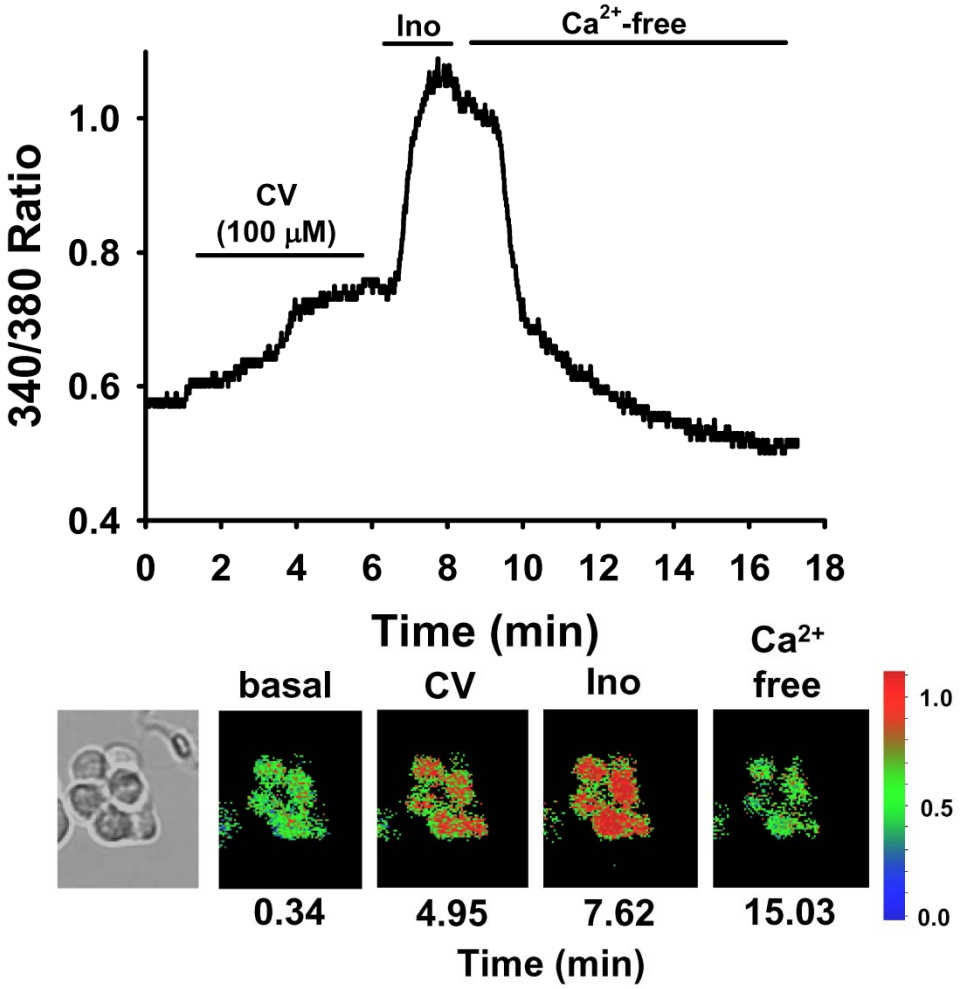
A Dietary Agonist of TRPV3 Elicits Endothelium-Dependent Vasodilation

Scott Earley, Albert L. Gonzales, and Zarine I. Garcia

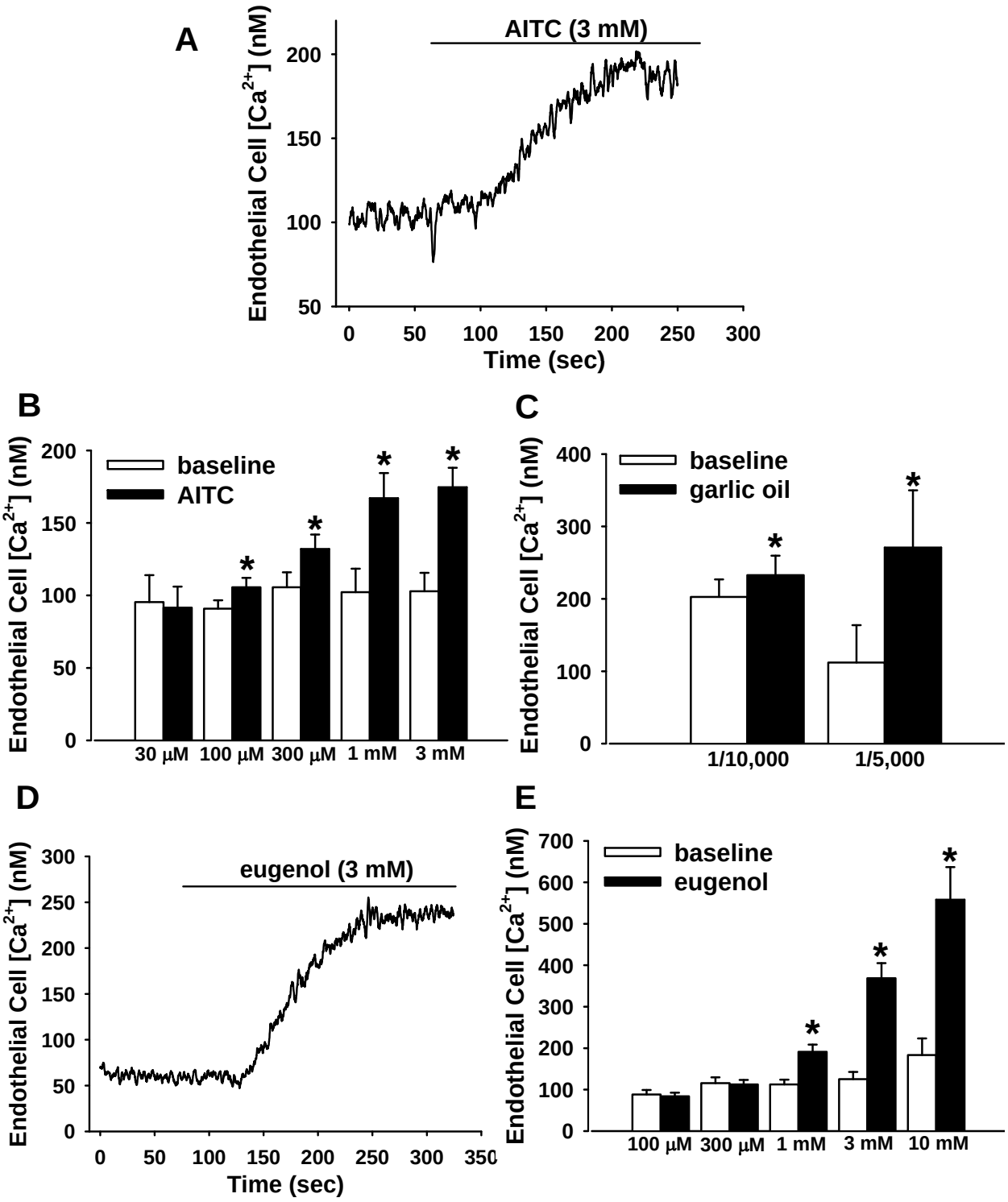
Vascular Physiology Research Group
Department of Biomedical Sciences
Colorado State University
Fort Collins, CO 80523-1680, USA



Earley et al. Supplemental Figure 1: Mechanical Disruption of the Endothelium Abolishes TRPV3 Immunostaining.

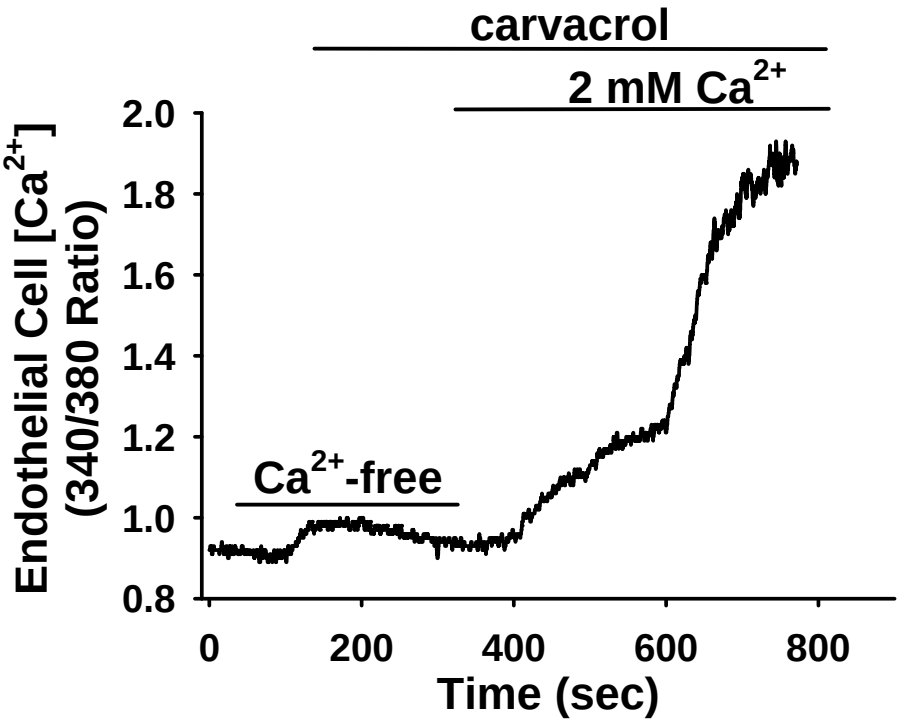


Earley et al. Supplemental Figure 2: Ca^{2+} Imaging of Cerebral Artery Endothelial Cells. *Top: Example recording. Bottom: Representative images taken at the indicated time points.* Rat cerebral arteries were harvested and enzymatically dispersed. Cells were loaded with the ratiometric Ca^{2+} indicator dye Fura-2AM (10 μM, 30 minutes) Endothelial cells were identified based on their characteristic cobblestone morphology, imaged, and individual cells were selected as regions of interest. Ca^{2+} levels were recorded under basal conditions and during agonist (CV, 100 μM) stimulation. After steady-state conditions were achieved, cells were exposed to the Ca^{2+} ionophore ionomycin (Iono) (1 μM) followed by Ca^{2+}-free bathing solution (3 mM EGTA).

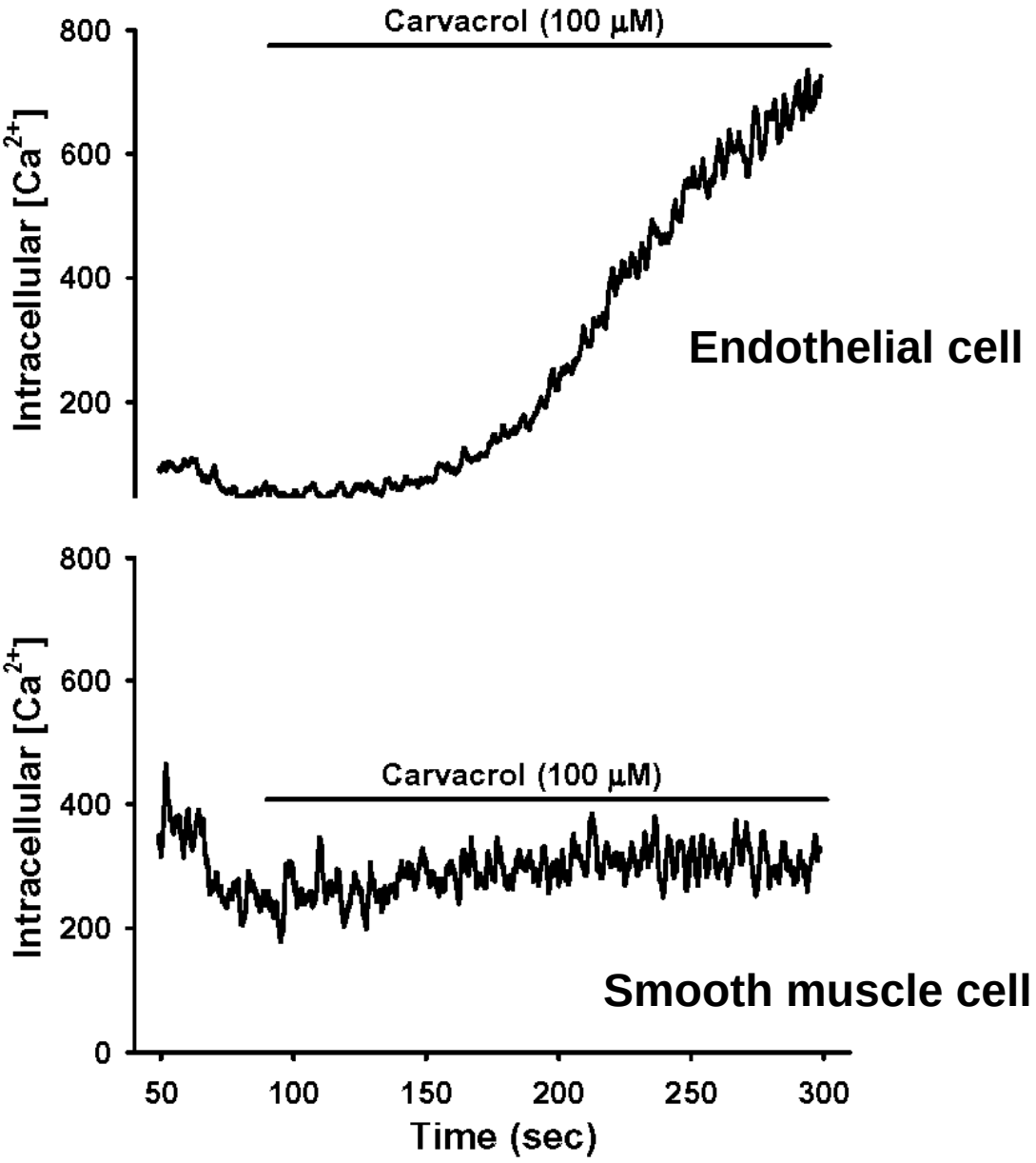


Earley et al. Supplemental Figure 3: Selective Agonists of TRPA1 and TRPV3 Promote Ca^{2+} Influx in Freshly Isolated Cerebral and Cerebellar Artery Endothelial Cells

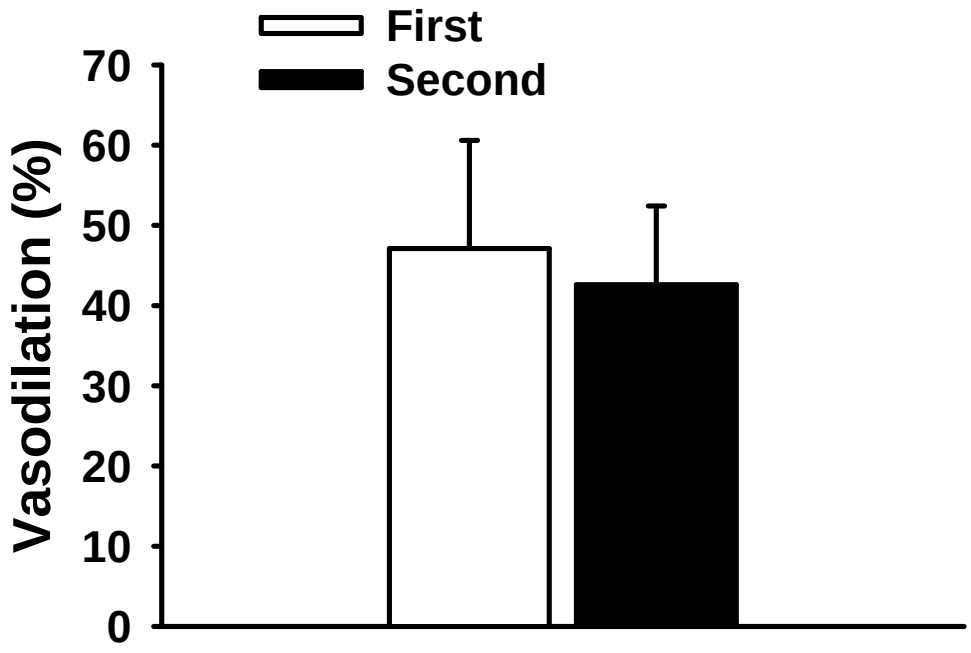
A: Representative recording of increases in endothelial cell [Ca^{2+}] in response to AITC (3 mM). B: Endothelial cell [Ca^{2+}] before and after administration of AITC. *P ≥ 0.05 vs. baseline. n = 6-24 cells per group C: Changes in endothelial cell [Ca^{2+}] in response to 1/10,000 and 1/5000 dilutions of garlic oil in PSS. *P ≥ 0.05 vs. baseline. n=3 (1/5000 dilution), n=6 (1/10,000 dilution). D: Representative recording of increases in endothelial cell [Ca^{2+}] in response to eugenol (3 mM). E: Endothelial cell [Ca^{2+}] before and after administration of eugenol. *P ≥ 0.05 vs. baseline. n = 3-17 cells per group.



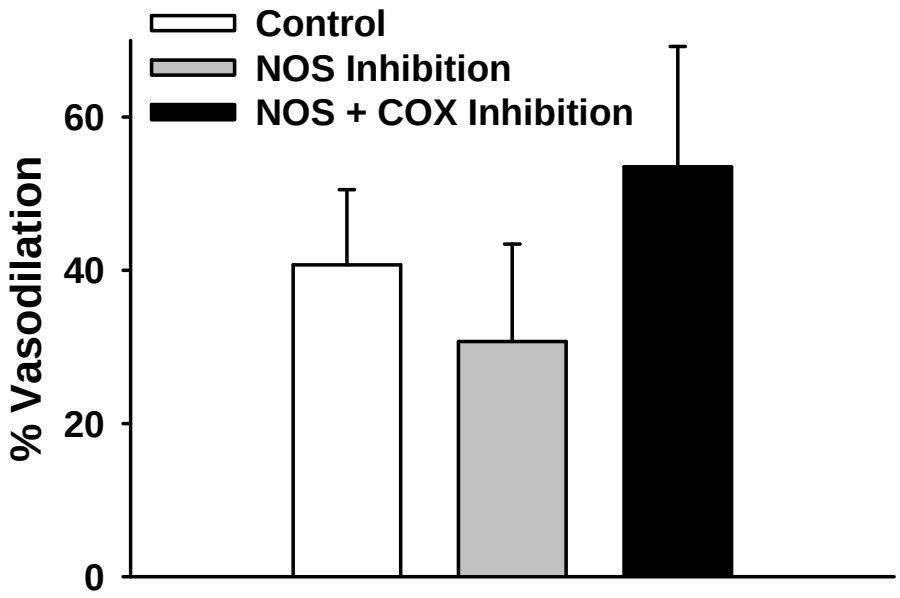
Earley et al. Supplemental Figure 4: Representative Recording Demonstrating the Effects of Removing Ca^{2+} From the Bathing Solution on Carvacrol-induced Increases in Endothelial Cell [Ca^{2+}]. Representative of 3 experiments



Earley et al. Supplemental Figure 5: Simultaneous Recordings of Changes in Intracellular Ca^{2+} in Response to Carvacrol in an Endothelial Cell (top) and a Vascular Smooth Muscle Cell (bottom).



Earley et al. Supplemental Figure 6: Reproducibility of Carvacrol-Induced Vasodilation. n=5. There are no significant differences.



Earley et al. Supplemental Figure 7: Effects of nitric oxide synthase (NOS) inhibition and combined NOS and cyclooxygenase (COX) inhibition on carvacrol-induced dilation. There were no significant differences. n= 5.