SUPPLEMENTAL MATERIALS

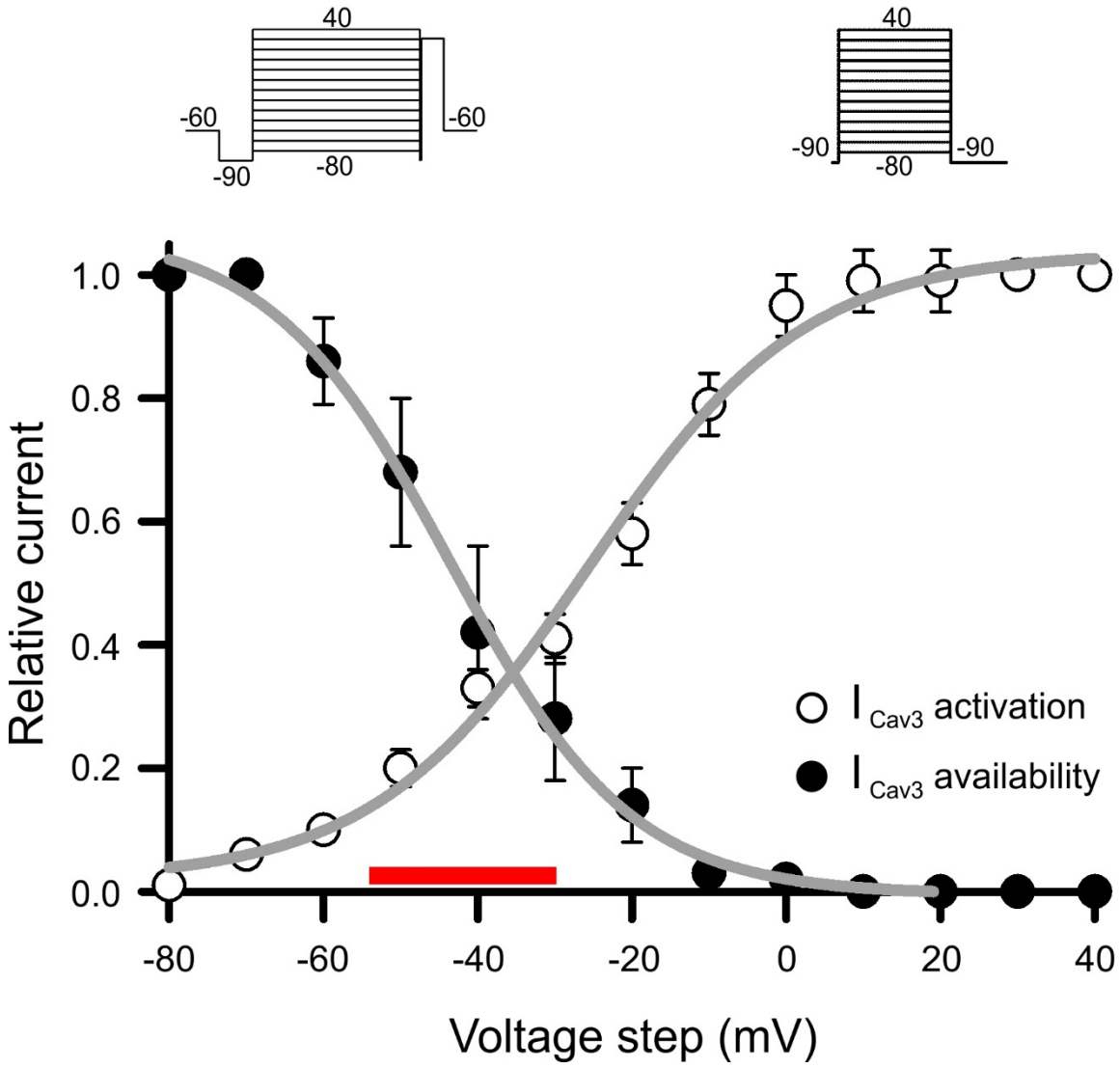
Genetic ablation of $Ca_V3.2$ channels enhances the arterial myogenic response by modulating the RyR-BK_{Ca} axis

Osama F. Harraz[1,2]; Suzanne E. Brett[1]; Anil Zechariah[1]; Monica Romero[3]; Jose L. Puglisi[4]; Sean M. Wilson[3]; Donald G. Welsh[1,5,#]

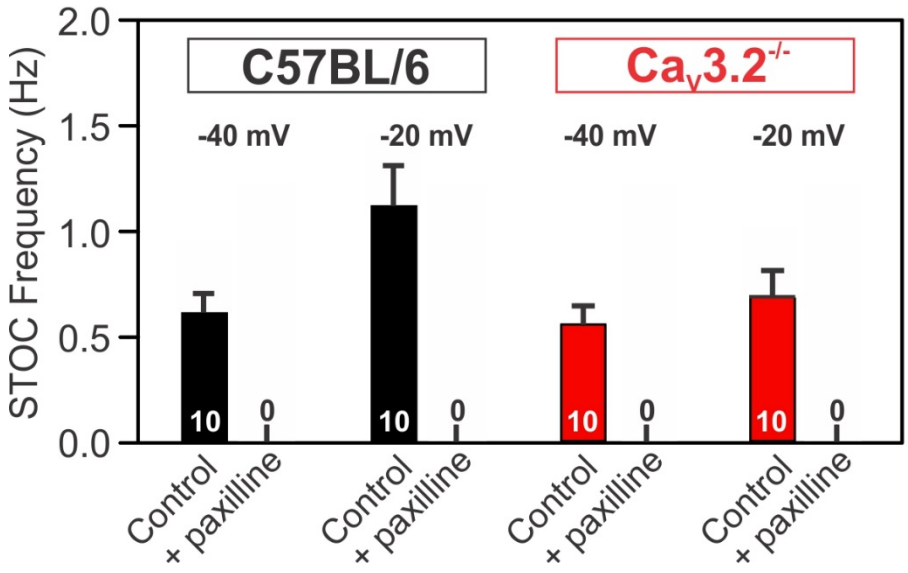
[1] Dept. of Physiology and Pharmacology, Hotchkiss Brain and Libin Cardiovascular Institutes, University of Calgary, AB, Canada
[2] Dept. of Pharmacology and Toxicology, Alexandria University, Egypt
[3] Division of Pharmacology, Loma Linda University, CA, USA
[4] Dept. of Pharmacology, University of California Davis, CA, USA
[5] Dept. of Physiology and Pharmacology, University of Western Ontario, ON, Canada

Running Title: $Ca_V3.2$ counterbalances myogenic constriction

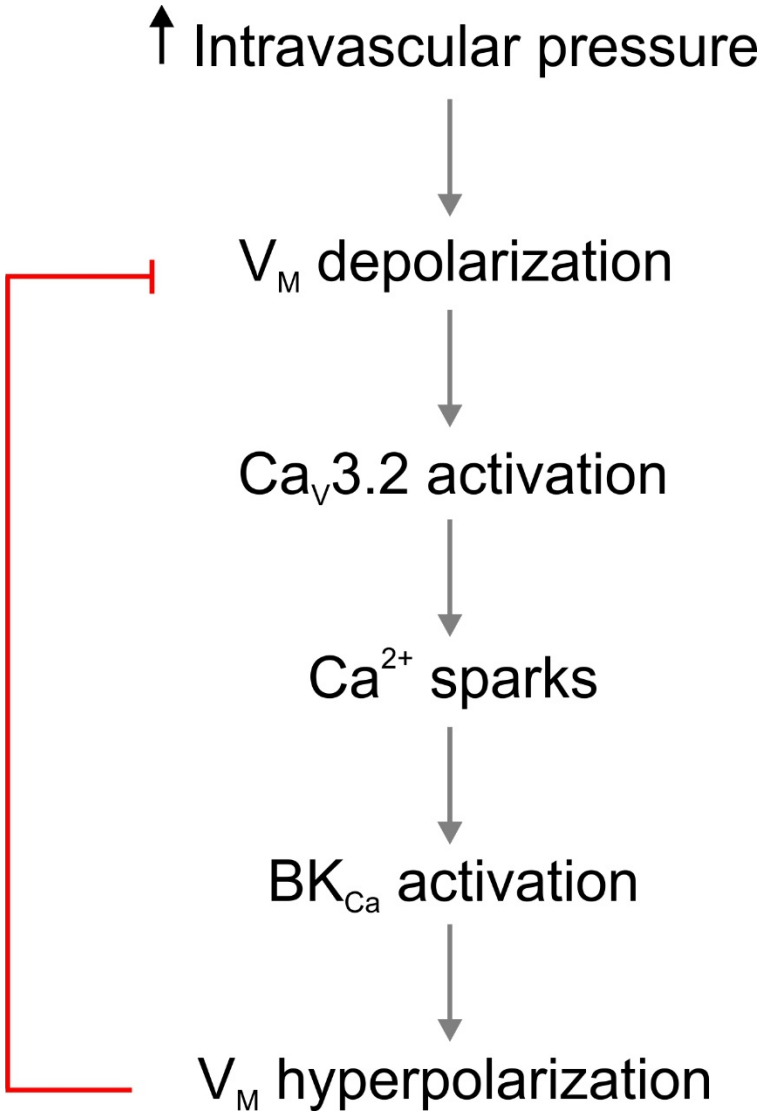
[#] **Corresponding Author:**
Donald G. Welsh, PhD
GAA-14 Health Research Innovation Center
University of Calgary, 3280 Hospital Dr NW
Calgary, Alberta, Canada, T2N4N1
E-mail: dwelsh@ucalgary.ca
Tel: +1-403-210-3819



Supplementary Figure I: Voltage-dependence of activation and steady-state inactivation of T-type current. In C57BL/6 mesenteric arterial smooth muscle cells, voltage dependence of activation and steady-state inactivation (availability) of T-type current was assessed. Barium (10 mM) was employed as the charge carrier and recordings were made in the presence of an L-type blocker (nifedipine, 200 nM). Upper insets depict protocols employed to assess availability (left) and activation (right). The red bar denotes physiological potentials that overlaps with the window current. Note that in physiological Ca^{2+} curves are expected to shift rightward by ~10-20 mV and window currents are expected to remain overlapping with physiological potentials.



Supplementary Figure II: Paxilline abolishes STOCs. Spontaneous transient outward K^+ currents were fully abolished in wild-type (C57BL/6) or knockout $Ca_V3.2^{-/-}$ smooth muscle cells by paxilline. This BK_{Ca} inhibitor was equally effective at holding potentials -40 or -20 mV (*n*=10 cells each).



Supplementary Figure III: A diagram highlighting the proposed role of $Ca_V3.2$ channels in vascular smooth muscle. A stimulus, such as an increase in intravascular pressure, elicits membrane potential (V_M) depolarization of the smooth muscle cells. This electrical stimulus triggers the activation of the voltage-gated Ca^{2+} channel, $Ca_V3.2$. Ensuing Ca^{2+} influx through $Ca_V3.2$ pores activates RyR on the sarcoplasmic reticulum to release Ca^{2+} sparks. The latter release events then activate BK_{Ca} to generate hyperpolarizing K^+ currents. This hyperpolarizing stimulus feedbacks upon membrane depolarization (*red*) and Ca^{2+} influx responsible for smooth muscle contraction.