Dynamic regulation of $\beta 1$ subunit trafficking controls vascular contractility

M. Dennis Leo^a, John P. Bannister^a, Damodaran Narayanan^a, Anitha Nair^a, Jordan E. Grubbs^a, Kyle S. Gabrick^b, Frederick A. Boop^b, and Jonathan H. Jaggar^{a,1}

Departments of ^aPhysiology and ^bNeurosurgery, University of Tennessee Health Science Center, Memphis, TN 38163

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Ion channels composed of pore-forming and auxiliary subunits control physiological functions in virtually all cell types. A conventional view is that channels assemble with their auxiliary subunits before anterograde plasma membrane trafficking of the protein complex. Whether the multisubunit composition of surface channels is fixed following protein synthesis or flexible and open to acute and, potentially, rapid modulation to control activity and cellular excitability is unclear. Arterial smooth muscle cells (myocytes) express large-conductance Ca^{2+} -activated potassium (BK) channel α and auxiliary $\beta 1$ subunits that are functionally significant modulators of arterial contractility. Here, we show that native BK α subunits are primarily (~95%) plasma membrane-localized in human and rat arterial myocytes. In contrast, only a small fraction (~10%) of total $\beta 1$ subunits are located at the cell surface. Immunofluorescence resonance energy transfer microscopy demonstrated that intracellular $\beta 1$ subunits are stored within Rab11A-positive recycling endosomes. Nitric oxide (NO), acting via cGMP-dependent protein kinase, and cAMP-dependent pathways stimulated rapid (≤ 1 min) anterograde trafficking of $\beta 1$ subunit-containing recycling endosomes, which increased surface $\beta 1$ almost threefold. These $\beta 1$ subunits associated with surface-resident BK α proteins, elevating channel Ca^{2+} sensitivity and activity. Our data also show that rapid $\beta 1$ subunit anterograde trafficking is the primary mechanism by which NO activates myocyte BK channels and induces vasodilation. In summary, we show that rapid $\beta 1$ subunit surface trafficking controls functional BK channel activity in arterial myocytes and vascular contractility. Conceivably, regulated auxiliary subunit trafficking may control ion channel activity in a wide variety of cell types.

artery | protein trafficking

Ion channels control physiological functions in virtually all cell types (1). Current (I) generated by an ion channel population is a product of the number of channels (N), open probability (P_o), and single-channel current (i), such that $I = N \cdot P_o \cdot i$. Previous studies have focused on identifying physiological and pathological mechanisms that regulate the P_o of plasma membrane-resident ion channels. In contrast, mechanisms that control the number of functional plasma membrane ion channels (N) and their regulatory auxiliary subunits in native cell types are unclear. A conventional view is that ion channels assemble with their auxiliary subunits before anterograde trafficking and plasma membrane insertion of the protein complex. Auxiliary subunits can modulate ion channel surface expression and activity, but it is unclear whether the multisubunit composition of surface channels is fixed following protein synthesis or flexible and open to acute and rapid modulation (2–4). Some recombinant ion channel proteins, including large-conductance calcium (Ca^{2+})-activated potassium (BK) α subunits, can traffic to the plasma membrane in the absence of their regulatory auxiliary subunits (5–9). This stimulated us to investigate the hypothesis that physiological stimuli rapidly modulate surface levels of auxiliary subunits to control functional ion channel activity. Such a mechanism would fine tune ion channel activity to control cell functionality. To test this hypothesis, we studied trafficking and surface

expression of native BK channel subunits in smooth muscle cells (myocytes) of small arteries that regulate regional organ blood flow and systemic blood pressure (10).

BK channels are expressed in a wide variety of mammalian cell types and regulate functions including arterial contractility, neurotransmission, and endocrine secretion (11). BK channels are heterotetramers of pore-forming α subunits that can also contain auxiliary β subunits, of which four are known ($\beta 1$ to $\beta 4$) (12, 13). β subunits are expressed in a tissue-specific manner and modulate BK channel Ca^{2+} sensitivity and gating properties to customize cellular functionality (14). Arterial myocytes express $\beta 1$ subunits, which elevate BK channel Ca^{2+} sensitivity into a concentration range that permits functional coupling to local micromolar intracellular Ca^{2+} transients termed Ca^{2+} sparks (15). $\beta 1$ subunits are essential for BK channels to control arterial myocyte membrane potential and contractility and to modulate systemic blood pressure (16, 17). Ca^{2+} spark to BK channel coupling is weak in $\beta 1$ subunit knockout mice, leading to membrane depolarization, vasoconstriction, and systemic hypertension (14, 15). Pathological alterations in BK channel $\beta 1$ subunit expression and function are also associated with cardiovascular diseases, including atherosclerosis, stroke, and hypertension (18). Whether physiological signaling mechanisms can control the α -to- β subunit ratio in BK channels to modulate channel activity and myocyte contractility is unclear.

Results

$\beta 1$ Subunits Are Primarily Intracellular and Can Be Stimulated to Rapidly Traffic to the Plasma Membrane in Arterial Myocytes. Arterial surface biotinylation was used to measure the cellular distribution

Significance

Plasma membrane ion channels composed of pore-forming and auxiliary subunits regulate physiological functions in virtually all cell types. A conventional view is that ion channels assemble with their auxiliary subunits prior to surface trafficking of the multiprotein complex. Arterial myocytes express large-conductance Ca^{2+} -activated potassium (BK) channel α and auxiliary $\beta 1$ subunits that modulate contractility and blood pressure and flow. The data here show that although most BK α subunits are plasma membrane-resident, $\beta 1$ subunits are primarily intracellular in arterial myocytes. Nitric oxide, an important vasodilator, stimulates rapid surface trafficking of $\beta 1$ subunits, which associate with, and activate, BK channels, leading to vasodilation. Thus, we show that rapid auxiliary subunit trafficking is a unique mechanism to control functional surface ion channel activity.

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¹To whom correspondence should be addressed. E-mail: jagggar@uthsc.edu.

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Fig. 3. Rab11A knockdown inhibits SNP-induced $\beta 1$ subunit plasma membrane trafficking in arterial myocytes. (A) Representative Western blots illustrating plasma membrane (M) and intracellular (I) BK α and $\beta 1$ protein in cerebral arteries transfected with control or rab11A shV and modulation by SNP (10 μ M). (B) Mean data for surface BK α and $\beta 1$ ($n = 6$ for each). * $P < 0.05$ vs. control. (C) Representative Western blots of $\beta 1$ in control arteries and those expressing constitutively active (CA) or dominant-negative (DN) rab11A and regulation by SNP ($n = 6$ for each). (D) Mean data ($n = 6$ for each). * $P < 0.05$ vs. empty vector.

myocyte contractility. This study raises the possibility that regulated and rapid trafficking of auxiliary subunits may be a common mechanism to control the activity of many different ion channels and the physiological functions of a multitude of cell types.

In the absence of exogenous stimuli, a very small proportion of total $\beta 1$ is surface-localized in arterial myocytes. L-NNA, brefeldin, nocodazole, Rab11A knockdown, and Rab11A mutants alone did not alter basal surface $\beta 1$ levels, suggesting that endothelial cell-mediated NO release and a sGC-, PKG-, and Rab11A-independent mechanism controls basal surface expression of $\beta 1$ protein. Carbachol, iloprost, and PKG and PKA (based on data with forskolin) activation increased surface $\beta 1$ protein. An elevation in surface $\beta 1$ may have occurred because of stimulation of anterograde trafficking and/or inhibition of subunit internalization. Data with brefeldin, nocodazole, Rab11A knockdown, and Rab11A mutants indicate that stimulated anterograde trafficking is the primary mechanism elevating surface $\beta 1$. These experiments also suggest that $\beta 1$ -containing Rab11A-positive recycling endosomes directly bud from the ER–Golgi complex in arterial myocytes. shRNA reduced Rab11A protein by half, which abolished SNP-induced $\beta 1$ surface trafficking but did not ablate the FRET signal between Rab11A and $\beta 1$. These data suggest that partial Rab11A suppression does not prevent the formation of some $\beta 1$ -containing, Rab11A-positive recycling endosomes but blocks PKG-mediated stimulation of anterograde trafficking. BK channel activation was the primary mechanism mediating NO-induced vasodilation. Iberitoxin and Rab11A knockdown did not abolish NO-induced vasodilation, supporting evidence that BK channel-independent mechanisms, including the activation of other K $^{+}$ channel types and a reduction in contractile apparatus Ca $^{2+}$ sensitivity also contribute (29). Downstream targets of PKG/PKA activation that stimulate recycling endosome-mediated $\beta 1$ subunit trafficking were not determined here. PKG and PKA phosphorylate a wide variety of proteins in arterial myocytes (30). Phosphorylation targets may include Rab11A or other regulatory protein(s) that stimulate this

ante-rograde trafficking pathway. In support of this conclusion, NO stimulated $\beta 1$ trafficking more than the constitutively active Rab11A mutant (Rab11AQ70L), suggesting that PKG acts via proteins other than Rab11A, which amplify $\beta 1$ anterograde trafficking. Given the number of potential targets for these kinases, regulatory proteins involved were not identified here. In the absence of exogenous stimuli, Con A elevated surface $\beta 1$, suggesting that $\beta 1$ recycles. To test the hypothesis that PKA and PKG also elevate surface $\beta 1$ by inhibiting retrograde trafficking would require identification of the pathway that internalizes this protein in arterial myocytes. Given that the Rab family alone contains more than 50 members, such an investigation was beyond the scope of the current study. Future studies should aim to identify the $\beta 1$ subunit internalization pathway.

Previous studies have investigated the composition of BK channels formed from recombinant BK α and $\beta 1$ subunits overexpressed in immortalized cells (31). These experiments suggested that a recombinant BK channel tetramer can contain one to four $\beta 1$ subunits and that BK α can associate with $\beta 1$ in a 1:1 ratio (17, 32).

Fig. 4. Stimulated $\beta 1$ subunit surface expression activates BK $_{Ca}$ channels. (A) Representative traces of BK $_{Ca}$ channel activity recorded at 10 μ M $[Ca^{2+}]_i$ in patches pulled from control myocytes or myocytes exposed to either SNP (10 μ M, 10 min) or SNP (10 μ M, 10 min) plus brefeldin (10 μ M, 1 h). (B) Mean data ($n = 6$ for each). Minimal and maximal P_o values for control, SNP, and brefeldin plus SNP were 0.03, 0.01, and 0.05 and 0.75, 0.77, and 0.75, respectively. (C) Mean BK $_{Ca}$ channel P_o in control and lithocholate (150 mM) from untreated and SNP-treated myocytes ($n = 15$ paired experiments for each group). * $P < 0.05$ vs. control untreated myocytes; # $P < 0.05$ vs. untreated myocytes plus lithocholate; $\delta P < 0.05$ vs. control SNP-treated myocytes.

Fig. 5. SNP-induced vasodilation occurs due to Rab11A-mediated BK channel activation. (A) Original recordings from the same artery illustrating dilation to SNP (10 μ M) in control (red trace) and after treatment with brefeldin A (10 μ M, 1 h; blue trace). (B) Mean data (experimental number, $n = 6-8$ for each). $*P < 0.05$ compared with control; $\#P < 0.05$ vs. brefeldin; $\delta P < 0.05$ vs. iberitoxin. (C) Original recordings illustrating dilation to SNP (10 μ M) in arteries transfected with control shV (red) and rab11A shV (blue). (D) Mean data ($n = 6$ for each). $*P < 0.05$ vs. control shV. (E) Dilation to lithocholate (45 μ M) in control (green) and in the presence of SNP (red). (F) Mean data ($n = 6$ for each). $*P < 0.05$ vs. untreated control; $\#P < 0.05$ compared with SNP.

These studies also indicated that the $\alpha\beta 1$ -tetramer ratio can shift BK channel voltage dependence and Ca^{2+} dependence (31). One focus of our study was to measure whether stimulated anterograde $\beta 1$ subunit trafficking shifts BK channel properties consistent with an increase in channel-associated $\beta 1$, leading to a functional response. The data indicate that native BK channel subunit composition is not rigid, but dynamic, and can be rapidly modulated by physiological vasoregulatory stimuli to control activity in arterial myocytes. In myocytes, BK channels are activated by Ca^{2+} sparks, with $\beta 1$ subunit knockout attenuating coupling to these local Ca^{2+} transients (15, 33, 34). Our data indicate that only a small fraction of total $\beta 1$ is plasma membrane-localized under basal conditions. It is likely that BK channels within the vicinity of Ca^{2+} sparks contain $\beta 1$ subunits, although the subunit ratio of these coupled channels is unclear and remains to be determined. Conceivably, other intracellular signals or Rabs may promote basal $\beta 1$ trafficking, which then provides a baseline level of BK channel coupling to Ca^{2+} sparks. SNP and forskolin elevate both Ca^{2+} spark frequency and transient BK current frequency and amplitude in arterial myocytes, inducing vasodilation (35). This kinase-mediated vasodilation is dependent upon BK channel activation, which we show is blocked by inhibition of $\beta 1$ subunit trafficking. Thus, $\beta 1$ subunit anterograde trafficking is likely to be a major contributor to

the NO-induced, PKG-stimulated increase in transient BK current frequency and amplitude.

Four BK channel β subunit isoforms exist that exhibit tissue-specific expression in cells including those in the brain, kidney, immune system, and adrenal gland (17). Our findings suggest that the composition of BK channels containing other β subunit isoforms may also be dynamic and subject to rapid and precise regulation to control activity and physiological functions. In addition, many other ion channel pore-forming subunits can traffic to the plasma membrane independently of their auxiliary subunits, including K_V (6), K_{ATP} (7), GABA (8), and epithelial sodium channel (9). The data here raise the possibility that the subunit composition of many ion channels may be dynamically regulated to alter activity and function in a wide variety of cell types. In summary, our study describes a unique mechanism that controls ion channel activity. We show that regulated and rapid recycling endosome-mediated $\beta 1$ subunit anterograde trafficking controls BK channel subunit composition and activity in arterial myocytes and vascular contractility.

Materials and Methods

Expanded information can be found in [SI Materials and Methods](#).

Tissue Preparation. Animal protocols were reviewed and approved by the Animal Care and Use Committee at the University of Tennessee Health Science Center. Adult male Sprague-Dawley rats (7–9 wk of age) were used for all experiments on rodents. Small (<200- μ m-diameter) cerebral and mesenteric arteries were dissected and cleaned of connective tissue. Myocytes were isolated as previously described (36). A human brain sample, from which small (<200- μ m-diameter) cerebral arteries were obtained, was obtained after institutional review board approval, written informed consent, and in accordance with the guidelines of the *Declaration of Helsinki*.

Surface Biotinylation of Intact Arteries. Biotinylation of intact arteries was performed as previously described (2). Arteries were incubated in a mixture each of EZ-Link Sulfo-NHS-LC-Biotin and Maleimide-PEG2-Biotin reagents (Thermo Fisher Scientific) for 1 h. Arteries were washed with 100 mM glycine in PBS for 15 min to remove any unbound biotin. Arteries were then washed in PBS, placed in ice-cold lysis buffer, and homogenized in lysis buffer. Cellular debris was removed by centrifugation. Total protein was then determined to allow normalization for avidin pull down of biotinylated surface proteins. Following pull-down, the supernatant comprised of the non-biotinylated (intracellular) protein fraction. Biotinylated surface proteins were eluted from the avidin beads.

Western Blotting. Surface and intracellular proteins were analyzed using Western blotting [7.5% (wt/vol) SDS polyacrylamide gels] and probed with either mouse monoclonal anti-BK α (1:500; NeuroMab, University of California), rabbit polyclonal anti-BK $\beta 1$ (1:500; Abcam), or mouse monoclonal anti-rab11A (1:500; Abcam) antibodies. Blots were cut at ~50 kDa to allow simultaneous probing for both BK α and $\beta 1$ subunits. Proteins were visualized using SuperSignal West Pico Chemiluminescent Substrate (Thermo Fisher Scientific). Band intensities were analyzed using Quantity One software (Bio-Rad).

Confocal Imaging and Immunofluorescence Resonance Energy Transfer Microscopy. Fixed, permeabilized myocytes were incubated with the same anti-BK α , anti- $\beta 1$, or anti-rab11A antibodies used for Western blotting experiments or ER-Tracker Green (Life Technologies). Alexa 488- or 546-conjugated secondary antibodies were used. Fluorescence images were acquired using a laser-scanning confocal microscope (LSM5 Pascal; Carl Zeiss). Percentage of weighted colocalization was calculated using Pascal system-embedded software program. For N-FRET analysis, images were background-subtracted and N-FRET was calculated on a pixel-by-pixel basis for the entire image and in regions of interest using the Xia method (37) and Zeiss LSM FRET Macro tool Version 2.5, as previously described (38).

Co-IP. For each experiment, lysate was harvested from cerebral arteries pooled from six rats using ice-cold radioimmunoprecipitation buffer. Co-IP was performed using the Catch and Release V2.0 Coimmunoprecipitation kit (Millipore) according to the manufacturer's instructions. Briefly, arterial lysate was incubated with control mouse IgG or BK α mouse monoclonal antibody, antibody-affinity ligand, and capture resin in the column provided. The column was centrifuged and washed, and bound proteins were

released using denaturing buffer. Boiled eluate was run on a SDS/PAGE gel, and protein samples were analyzed by Western blotting using mouse monoclonal anti-BK α (NeuroMab) or rabbit polyclonal anti-BK β 1 (Abcam) and horseradish peroxidase-conjugated secondary antibodies.

Rab11A shRNA and Mutant Constructs. A commercial Rab11A shRNA kit was obtained from OriGene Technologies. Rab11A dominant-negative (Rab11AQ70L) and constitutively active (Rab11AS25N) mutants were generated from rat rab11A cDNA obtained from a clone library (GenScript).

Transfection of Intact Cerebral Arteries. shRNA, mutant construct, or empty vectors were transfected into arteries using either reverse permeabilization or electroporation (CUY21Vivo-SQ electroporator; Bex), as previously described (39).

Patch-Clamp Electrophysiology. Single BK channel currents were recorded in inside-out patches from isolated cerebral artery myocytes. The pipette and bath solution both contained: 130 mM KCl, 10 mM Hepes (4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid), 1 mM MgCl₂, 5 mM EGTA, 1.6 mM HEDTA (N-(2-Hydroxyethyl)ethylenediamine-N,N',N'-triacetic acid, pH 7.2 with KOH). Free Ca²⁺ concentration was adjusted to between 1 and 300 μ M by the addition of CaCl₂, with free Mg²⁺ maintained at 1 mM by altering MgCl₂. Free Ca²⁺ concentration in solutions was measured using Ca²⁺-sensitive (no.

476041; Corning) and reference (no. 476370; Corning) electrodes. Inside-out patch experiments were performed at a membrane voltage of -40 mV. BK currents were filtered at 1 kHz and digitized at 5 kHz. Analysis was performed offline using Clampfit 9.2 (MDS Analytical Technologies). Ca²⁺-sensitivity data were fit with an unconstrained single Boltzmann function.

Pressurized Artery Diameter Measurements. Experiments were performed using endothelium-denuded rat cerebral arteries cannulated at each end in a perfusion chamber (Living Systems Instrumentation). Arterial diameter was measured at 1 Hz using a CCD camera. Myogenic tone (percentage) was calculated as follows: $100 \times (1 - D_{\text{active}}/D_{\text{passive}})$, where D_{active} is active arterial diameter, and D_{passive} is the diameter determined in the presence of Ca²⁺-free physiological saline solution supplemented with 5 mmol/L EGTA.

Statistical Analysis. Values are expressed as means $\pm$ SEM. Student t test was used for comparing paired and unpaired data from two populations, and ANOVA with Student–Newman–Keuls post hoc test used for multiple group comparisons. $P < 0.05$ was considered significant.

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- Niemeyer BA, et al. (2001) Ion channels in health and disease. 83rd Boehringer Ingelheim Fonds International Titisee Conference. *EMBO Rep* 2(7):568–573.
- Bannister JP, et al. (2009) Smooth muscle cell $\alpha_2\delta$ -1 subunits are essential for vaso-regulation by Ca_v1.2 channels. *Circ Res* 105(10):948–955.
- Mathie A, Rees KA, El Hachmane MF, Veale EL (2010) Trafficking of neuronal two pore domain potassium channels. *Curr Neuropharmacol* 8(3):276–286.
- Vandenberghe W, Nicoll RA, Bredt DS (2005) Stargazin is an AMPA receptor auxiliary subunit. *Proc Natl Acad Sci USA* 102(2):485–490.
- Chiu YH, Alvarez-Baron C, Kim EY, Dryer SE (2010) Dominant-negative regulation of cell surface expression by a pentapeptide motif at the extreme COOH terminus of an Slo1 calcium-activated potassium channel splice variant. *Mol Pharmacol* 77(4):497–507.
- Deutsch E, et al. (2012) Kv2.1 cell surface clusters are insertion platforms for ion channel delivery to the plasma membrane. *Mol Biol Cell* 23(15):2917–2929.
- Makhina EN, Nichols CG (1998) Independent trafficking of K_{ATP} channel subunits to the plasma membrane. *J Biol Chem* 273(6):3369–3374.
- Vithlani M, Terunuma M, Moss SJ (2011) The dynamic modulation of GABA(A) receptor trafficking and its role in regulating the plasticity of inhibitory synapses. *Physiol Rev* 91(3):1009–1022.
- Wesch D, et al. (2012) Differential N termini in epithelial Na⁺ channel δ -subunit isoforms modulate channel trafficking to the membrane. *Am J Physiol Cell Physiol* 302(6):C868–C879.
- Mulvany MJ, Aalkjaer C (1990) Structure and function of small arteries. *Physiol Rev* 70(4):921–961.
- Cui J, Yang H, Lee US (2009) Molecular mechanisms of BK channel activation. *Cell Mol Life Sci* 66(5):852–875.
- Lee US, Cui J (2010) BK channel activation: Structural and functional insights. *Trends Neurosci* 33(9):415–423.
- Lu R, et al. (2006) MaxiK channel partners: Physiological impact. *J Physiol* 570(Pt 1):65–72.
- Orio P, Rojas P, Ferreira G, Latorre R (2002) New disguises for an old channel: MaxiK channel beta-subunits. *News Physiol Sci* 17:156–161.
- Brenner R, et al. (2000) Vasoregulation by the β 1 subunit of the calcium-activated potassium channel. *Nature* 407(6806):870–876.
- Patterson AJ, Henrie-Olson J, Brenner R (2002) Vasoregulation at the molecular level: A role for the beta1 subunit of the calcium-activated potassium (BK) channel. *Trends Cardiovasc Med* 12(2):78–82.
- Wu RS, Marx SO (2010) The BK potassium channel in the vascular smooth muscle and kidney: α - and β -subunits. *Kidney Int* 78(10):963–974.
- Carvalho-de-Souza JL, Varanda WA, Tostes RC, Chignalia AZ (2013) BK channels in cardiovascular diseases and aging. *Aging Dis* 4(1):38–49.
- Berney C, Danuser G (2003) FRET or no FRET: A quantitative comparison. *Biophys J* 84(6):3992–4010.
- Chardin P, McCormick F (1999) Brefeldin A: The advantage of being uncompetitive. *Cell* 97(2):153–155.
- Lapierre LA, et al. (2003) Rab11b resides in a vesicular compartment distinct from Rab11a in parietal cells and other epithelial cells. *Exp Cell Res* 290(2):322–331.
- Zerial M, McBride H (2001) Rab proteins as membrane organizers. *Nat Rev Mol Cell Biol* 2(2):107–117.
- Stenmark H (2009) Rab GTPases as coordinators of vesicle traffic. *Nat Rev Mol Cell Biol* 10(8):513–525.
- Maxfield FR, McGraw TE (2004) Endocytic recycling. *Nat Rev Mol Cell Biol* 5(2):121–132.
- Sönnichsen B, De Renzis S, Nielsen E, Rietdorf J, Zerial M (2000) Distinct membrane domains on endosomes in the recycling pathway visualized by multicolor imaging of Rab4, Rab5, and Rab11. *J Cell Biol* 149(4):901–914.
- Butterworth MB, et al. (2012) Rab11b regulates the trafficking and recycling of the epithelial sodium channel (ENaC). *Am J Physiol Renal Physiol* 302(5):F581–F590.
- Cox DH, Aldrich RW (2000) Role of the beta1 subunit in large-conductance Ca²⁺-activated K⁺ channel gating energetics. Mechanisms of enhanced Ca²⁺ sensitivity. *J Gen Physiol* 116(3):411–432.
- Bukiya AN, Vaithianathan T, Toro L, Dopico AM (2009) Channel beta2-4 subunits fail to substitute for beta1 in sensitizing BK channels to lithocholate. *Biochem Biophys Res Commun* 390(3):995–1000.
- Morgado M, Cairaño E, Santos-Silva AJ, Verde I (2012) Cyclic nucleotide-dependent relaxation pathways in vascular smooth muscle. *Cell Mol Life Sci* 69(2):247–266.
- Francis SH, Busch JL, Corbin JD, Sibley D (2010) cGMP-dependent protein kinases and cGMP phosphodiesterases in nitric oxide and cGMP action. *Pharmacol Rev* 62(3):525–563.
- Wang YW, Ding JP, Xia XM, Lingle CJ (2002) Consequences of the stoichiometry of Slo1 alpha and auxiliary beta subunits on functional properties of large-conductance Ca²⁺-activated K⁺ channels. *J Neurosci* 22(5):1550–1561.
- Knaus HG, Garcia-Calvo M, Kaczorowski GJ, Garcia ML (1994) Subunit composition of the high conductance calcium-activated potassium channel from smooth muscle, a representative of the mSlo and slowpoke family of potassium channels. *J Biol Chem* 269(6):3921–3924.
- Nelson MT, et al. (1995) Relaxation of arterial smooth muscle by calcium sparks. *Science* 270(5236):633–637.
- Jaggard JH, Porter VA, Lederer WJ, Nelson MT (2000) Calcium sparks in smooth muscle. *Am J Physiol Cell Physiol* 278(2):C235–C256.
- Porter VA, et al. (1998) Frequency modulation of Ca²⁺ sparks is involved in regulation of arterial diameter by cyclic nucleotides. *Am J Physiol* 274(5 Pt 1):C1346–C1355.
- Jaggard JH (2001) Intravascular pressure regulates local and global Ca²⁺ signaling in cerebral artery smooth muscle cells. *Am J Physiol Cell Physiol* 281(2):C439–C448.
- Xia Z, Liu Y (2001) Reliable and global measurement of fluorescence resonance energy transfer using fluorescence microscopes. *Biophys J* 81(4):2395–2402.
- Zhao G, et al. (2010) Type 1 IP3 receptors activate BK_{Ca} channels via local molecular coupling in arterial smooth muscle cells. *J Gen Physiol* 136(3):283–291.
- Narayanan D, et al. (2013) Smooth muscle cell transient receptor potential polycystin-2 (TRPP2) channels contribute to the myogenic response in cerebral arteries. *J Physiol* 591(Pt 2):5031–5046.