Symposium: Awards Symposium

1873-Symp
Cholesterol Nanodomains: their Effect on Monolayer Morphology and Dynamics
Kyuhan Kim1, Todd M. Squires1, Siyoung Q. Choi2,
Joseph A. Zasadzinski2.
1University of California, Santa Barbara, CA, USA, 2University of Minnesota, Minneapolis, MN, USA.
Small mole fractions of cholesterol segregate into 10 - 100 nm diameter nanodomains in dipalmitoylphosphatidylcholine (DPPC) monolayers. The nanodomains segregate to DPPC domain boundaries, reducing the line tension, D, which alters domain shapes. The nanodomains consist of a 6:1 lipid: cholesterol "complex" and the surface viscosity, ηs, decreases exponentially with the area fraction of the complex at a given surface pressure. ηs increases exponentially with surface pressure, independent of cholesterol content, as predicted by a free area model that relates to monolayer compressibility and collapse pressure. G′, the elastic modulus, decreases with a rise in overall cytosolic calcium [Ca2+]c, in the arterial smooth muscle cell (SMC). This rise leads to activation of large-conductance, Ca2+/ voltage-gated K+ (BK) channels. This generates outward currents that hyperpolarize the membrane and limit Ca2+ entry. Thus, BK channel activation opposes SMC contraction.

Several negatively charged endogenous lipids, such as PIP2, fatty and bile acids have been shown to directly activate SMC BK channels. The chemical structure of leukotrienes (LTs) includes a long hydrophobic backbone and a carboxylic end group, which is largely ionized at physiological pH=6.8-7.4. Thus, we hypothesized that LTs were BK channel activators.

We tested LT4, LTB4, LTC4, LTD4, and LTE4 on heterologously expressed BK channels conformed by cbv1 (AY330293) and r1 (FJ154955) submitins cloned from rat cerebral artery SMC. LTs were applied at 1 nM to the cytosolic side of inside-out (I/O) membrane patches from Xenopus oocytes; Vm=80 to −20 mV, [Ca2+]c=10 μM. LTB4 was the only LT that significantly increased BK channel activity (130% from pre-LT values), with a ≈ 330% increase in activity in response to 2 μM LTB4. LTB4-induced BK channel activation was also observed in I/O patches from freshly isolated cerebral artery SMCs. Therefore, we identified LTB4 as a potent and effective SMC BK channel activator that works independently of cell signaling, using pressureurized, de-endothelized cerebral arteries from rats, we blocked LTB4 production by 50 μM LTB4. This treatment reduced artery diameter by ≈ 5%, which is expected to cause up to 10-20% decrease in cerebral blood flow. This result suggests that LTB4 production within arterial SMC exerts a tonic effect on arterial tone, favoring SMC relaxation and opposing vasoconstriction.

1876-Plat
Leukotriene B4 Activation of Arterial Smooth Muscle BK Channels
Anna Bukiya, Alex Doppelfeld
University of Tennessee HSC, Memphis, TN, USA.
Blood circulation depends on the myogenic tone of small arteries. Tone is increased by a rise in overall cytosolic calcium [Ca2+]c, in the arterial smooth muscle cell (SMC). This rise leads to activation of large-conductance, Ca2+/voltage-gated K+ (BK) channels. This generates outward currents that hyperpolarize the membrane and limit Ca2+ entry. Thus, BK channel activation opposes SMC contraction.

Several negatively charged endogenous lipids, such as PIP2, fatty and bile acids have been shown to directly activate SMC BK channels. The chemical structure of leukotrienes (LTs) includes a long hydrophobic backbone and a carboxylic end group, which is largely ionized at physiological pH=6.8-7.4. Thus, we hypothesized that LTs were BK channel activators.

We tested LT4, LTB4, LTC4, LTD4, and LTE4 on heterologously expressed BK channels conformed by cbv1 (AY330293) and r1 (FJ154955) submitins cloned from rat cerebral artery SMC. LTs were applied at 1 nM to the cytosolic side of inside-out (I/O) membrane patches from Xenopus oocytes; Vm=80 to −20 mV, [Ca2+]c=10 μM. LTB4 was the only LT that significantly increased BK channel activity (130% from pre-LT values), with a ≈ 330% increase in activity in response to 2 μM LTB4. LTB4-induced BK channel activation was also observed in I/O patches from freshly isolated cerebral artery SMCs. Therefore, we identified LTB4 as a potent and effective SMC BK channel activator that works independently of cell signaling, using pressureurized, de-endothelized cerebral arteries from rats, we blocked LTB4 production by 50 μM LTB4. This treatment reduced artery diameter by ≈ 5%, which is expected to cause up to 10-20% decrease in cerebral blood flow. This result suggests that LTB4 production within arterial SMC exerts a tonic effect on arterial tone, favoring SMC relaxation and opposing vasoconstriction.

1877-Plat
Maxik Interaction with Gaba Transporter 3 and Heat Shock Protein 60 in the Mouse Brain
Harpreet Singh1, Lyra R.M. Hall1, Scarlett M. Chen1, Andrea L. Meredith2, Enrico Stefani1, Ligia Toro3,
1UCLA, Los Angeles, CA, USA, 2University of Maryland, Baltimore, MD, USA.
Large conductance voltage- and calcium-activated potassium (MaxiK) channels regulate Ca2+ signaling, transmitter release, and repolarization of the action potential in neurons. Analysis of MaxiK interactome from the mouse brain using liquid chromatography/mass spectrometry in tandem and knockout animals, as negative controls, revealed that MaxiK interacts with a variety of proteins localized to the mitochondria, plasma membrane, cytoplasm, Golgi apparatus, endoplasmic reticulum and other intracellular organelles. We examined whether proteins identified by mass spectrometry colocalize with MaxiK channel in cells. To this end, we have first chosen two proteins, namely gamma-aminobutyric acid (GABA) transporter (GAT)-3, and a heat shock protein showed that two members of the TMEM16 family, TMEM16A and B, encode for CaCCs. The TMEM16 family is comprised of 10 human homologues whose molecular packing. Understanding the effects of small mole fractions of cholesterol should resolve the role of cholesterol in human lung surfactants and may give clues as to how cholesterol influences raft formation in cell membranes.

Platform: Ca-activated Channels

1874-Plat
Tmem16F forms a Ca2+-Activated Cation Channel Required for Lipid Scrambling in Platelets during Blood Coagulation
Huangle Yang1, Andrew Kim1, Tovo David2, Daniel Palmer2, Taihao Jin1, Jason Tien1, Fen Huang1, Tong Cheng1, Shaun Coughlin2, Yuh Nung Jan1, Lily Y. Jan1.
1UCSF/HHMI, San Francisco, CA, USA, 2UCSF, San Francisco, CA, USA.
Collapse of membrane lipid asymmetry is a hallmark of blood coagulation. TMEM16F, a novel Small-conductance Ca2+/ voltage (SCCa) channel, was identified in platelets that were required for CaCCs. TMEM16A and B encode CaCCs. Simultaneous charge-neutralization of these residues eliminates Ca2+ dependence, activation of ion and lipid transport, suggesting that a single Ca2+ site regulates both.

Our results demonstrate for the first time that a member of the TMEM16 family is simultaneously a Ca2+-dependent ion channel and a Ca2+-dependent lipid scramblase. This suggests that other family members, such as TMEM16F, might also be dual function proteins thus resolving the confusion regarding their function.

1875-Plat
Ca2+-Dependent Ion and Lipid Transport Mediated by a Fungal TMEM16 Homologue
Mattia Malvezzi1, Madhavan Chalati1, Hiroiyuki Terashima2, Radmila Janjusevic1, Anant Menon1, Alessio Accardi1.
1Weill Cornell Medical College, New York, NY, USA, 2Osaka University, Osaka, Japan.
Ca2+-activated Cl- channels (CaCCs) play crucial roles in human physiology, from epithelial secretion to nociception and sensory transduction. Recent work