930-Pos Board B809

Ca^{2+}- and Thromboxane-Dependent Distribution of Functional MaxiK Channels in Cultured Astrocytes

Jimmy W. Ou, Yogesh Kumar, Abderrahmane Alioua, Enrico Stefani, Ligia Toro.
University of California at Los Angeles, Los Angeles, CA, USA.

Large-conductance, voltage- and Ca^{2+}-activated K^+ channels (MaxiK, BK) are broadly expressed ion channels typically observed as a plasma membrane protein in various cell types. In murine astrocyte primary cultures, which are more indicative of *in-vivo* reactive astrocytes rather than resting astrocytes, our previous results using high-resolution confocal microscopy have revealed the novel finding that MaxiK pore-forming α subunit (MaxiKα) is distributed intracellularly, colocalized along the microtubule network. This MaxiKα association with microtubules was further confirmed by *in vitro* His-tag pulldown assays, co-immunoprecipitation assays from brain lysates, and microtubule depolymerization experiments. Changes in intracellular Ca^{2+} elicited by general pharmacological agents, caffeine (20mM) or thapsigargin (1μM), resulted in increased MaxiKα labeling at the plasma membrane. More notably U46619, a stable analog of thromboxane A2 (TXA2) which triggers Ca^{2+}-release pathways and whose levels increase during cerebral hemorrhage/trauma, also elicits a similar increase in MaxiKα surface labeling. We now show using whole-cell patch clamp recordings that U46619 stimulated cells develop a ~3-fold increase in current amplitude. This data indicates that TXA2 stimulation results in the recruitment of additional, functional MaxiK channels to the surface membrane. These changes in MaxiKα plasma membrane distribution are effectively blocked by preincubating astrocytes with a cell permeable Ca^{2+}-chelator, BAPTA-AM, or by microtubule disruption prior to stimulation. While microtubules are largely absent in mature astrocytes, our immunohistochemistry results in brain slices show that cortical astrocytes in the developing newborn mouse brain (P1) have a robust expression of microtubules that significantly colocalize with MaxiKα. The results of this study provide the novel insight that suggests Ca^{2+} released from intracellular stores, may play a key role in regulating the traffic of intracellular, microtubule-associated MaxiKα stores to the plasma membrane of reactive astrocytes. Supported by NIH.

Voltage-gated Ca Channels I

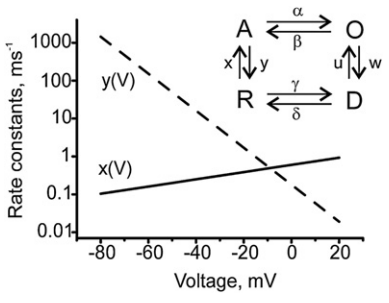
931-Pos Board B810

Membrane Voltage More Efficient In Closing Than Opening $Ca_V1.2$?

Stanislav Beyl[1], Philipp Kuegler[2], Michaela Kudrnac[1], Annette Hohaus[1], Steffen Hering[1], Eugen Timin[1].
[1]Pharmacology and Toxicology, Vienna, Austria, [2]Johann Radon Institute for Computational and Applied Mathematics, Linz, Austria.

Point mutations in a gating-related sequence stretch in the pore-lining segment IIS6 of $Ca_V1.2$ (779-782:LAIA motive) convert the high-voltage activated calcium channel into a low-voltage activated one. Here we analyze the changes in current activation and deactivation induced by these and glycine mutations in this region. Our model describes channel activation as voltage-dependent sensor movement and a voltage-independent pore opening and deactivation as voltage-dependent return of the sensor and subsequent pore closure.

An inverse problem approach enabled the estimation of current activation and deactivation rate constants from 16 mutants and wild type $Ca_V1.2$ with narrow confidence intervals. Current activation, deactivation and steady-state activation of wild type and 12 mutants could be fitted with identical voltage dependencies of the voltage sensing machinery (x(V),y(V)). Steeper voltage dependence of y(V) compared to x(V) (see Figure) suggest that a membrane hyperpolarisation more efficiently closes than a depolarization opens the channel. Mutations in IIS6 of $Ca_V1.2$ destabilizing the closed state simultaneously appear to stabilize the open state in all 16 mutants.



* This study was supported by a grant from FWF (Project P19614-B11).

932-Pos Board B811

Position Specificity of the Glycine Residues in IS6 of the L-type Cav1.2 Channel

Florian LeCoz, Alexandra Raybaud, Sebastien Wall-Lacelle, Yolaine Dodier, Lucie Parent.
Université de Montréal, Montreal, QC, Canada.

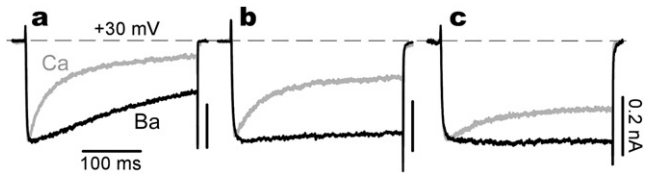
In the Timothy Syndrome (LQT8), mutations of the distal glycine residues in IS6 severely delayed the inactivation kinetics of Cav1.2 (Splawski et al., 2004, 2005). Our mutational analysis confirmed that Ala mutations of any glycine within IS6 significantly decreased the inactivation kinetics (Raybaud et al., 2006). To evaluate the position-specificity of these residues in IS6, we performed a glycine scan of this region between S423 and E437 (NLVLGVLSGE). We hypothesized that introduction of a glycine residue at neighboring positions within the LTQ8 mutants might rescue the Cav1.2 channel inactivation kinetics. Although S423G, F424G, and L434G yielded channels with slightly faster inactivation kinetics than the wild-type Cav1.2 channel, the double mutants G422A/S423G and G422A/F424G failed to rescue the channel normal inactivation kinetics. As G422 is located next to a bulky phenylalanine residue, we aimed to evaluate the role of steric hindrance in controlling channel inactivation kinetics. F421G, F421A, F421W, as well as the F421G/G422A double mutant have been characterized. Both F421A and F421G behaved like the wild-type channel whereas F421W did not yield functional currents. The double mutant F421G/G422F that switched the positions of the wild-type Phe and Gly residues inactivated like the G422A mutant. Furthermore, permutations such as F421A/G422F and F421A/G422A also yielded the G422A phenotype. Altogether, these experiments confirm the position-specificity of the 3 glycine residues in IS6 in modulating the inactivation kinetics of Cav1.2. In contrast, none of our mutations altered the voltage-dependence of activation suggesting that residues in IS6 do not play a determinant role in the activation properties of Cav1.2. *Supported by the Heart and Stroke of Canada and the Canadian Institutes of Health Research.*

933-Pos Board B812

Distinctive Inactivation Profiles of $Ca_V1.2$ Channels Encoding Different Timothy Syndrome Mutations in Various Alternative Splicing Backgrounds

Ivy E. Dick, Sarah A. Park, David T. Yue.
Johns Hopkins, Baltimore, MD, USA.

Timothy Syndrome is a $Ca_V1.2$ channelopathy, wherein mutations in the S6 segment of domain II affect channel inactivation. Interestingly, different mutations (G402S or G406R) within alternatively spliced exons (8a or 8) entail characteristic disease phenotypes, including autism, syndactyly, and long QT syndrome. Investigating Timothy Syndrome thereby promises mechanistic traction into these complex outcomes. The prevalence and distribution of channels bearing exon 8 versus 8a may explain some phenotypic variation. Here, we examine such variation at a more fundamental level, resolving intrinsic differences of inactivation among distinct mutant channels. Specifically, $Ca_V1.2$ inactivation comprises two separate mechanisms, voltage-dependent inactivation (VDI), and Ca^{2+}/calmodulin-mediated inactivation (CDI) (Barrett & Tsien PNAS 2008). Systematic mutagenesis of S6 domains in $Ca_V1.3$ suggest that VDI and CDI alterations can be dissociated, and changes in channel activation are likely (Tadross *et al*, this meeting). Our data here furnish remarkable examples of differing CDI/VDI effects (**a**, wild-type, with Ba^{2+} current decay showing VDI, and Ca^{2+} decay showing CDI; **b**, **c**, distinct CDI/VDI alterations). It would be interesting if distinctive disease phenotypes and therapeutics ultimately correspond to specified deficits of VDI, CDI, or both.



934-Pos Board B813

Silencing of Cav1.2 gene in Rat Neonatal Cardiomyocytes by Lentiviral delivered shRNA

Eddy Karnabi[1], Yongxia Qu[1], Natalia Grinkina[1], Raj Wadgaonkar[1], Yunkun Yue[2], Salvatore Mancarella[3], Mohamed Boutjdir[3].
[1]SUNY Downstate Medical Center, Brooklyn, NY, USA, [2]VA Medical Center, Brooklyn, NY, USA, [3]SUNY Downstate Medical Center/VA Medical Center, Brooklyn, NY, USA.

Background: Two types of L-type Ca Channels are expressed in the heart: Cav1.2 (α_{1C}) and Cav1.3 (α_{1D}). In contrast to α_{1C}, α_{1D} Ca channel is highly expressed in the sinoatrial node and atria, and is involved in the impulse generation and propagation through the AV node. Deletion of the α_{1C} gene results in embryonic lethality before E14.5 and there are no pharmacological or biophysical means to separate α_{1D} from α_{1C} Ca currents. The aim of this study