3438-Pos Board B299

LQT2 Nonsense Mutation Escapes NMD by the Reinitiation of Translation to Generate N-Terminally Truncated hERG Channels

Matthew R. Stump, Qiuming Gong, Jonathan D. Packer, Zhengfeng Zhou.
Oregon Health & Science University, Portland, OR, USA.

Mutations in the human ether-a-go-go-related gene (hERG) result in long QT syndrome type 2 (LQT2). hERG mRNA transcripts that contain premature termination codon (PTC) mutations are known to be rapidly degraded by nonsense-mediated mRNA decay (NMD). We have identified a LQT2 nonsense mutation Q81X which escapes NMD-mediated degradation through the reinitiation of translation to generate an N-terminally truncated channel with altered gating properties. RNA analysis of full-length wild-type or Q81X hERG minigenes revealed equivalent mRNA levels, in contrast with transcripts containing the P141fs+2X frameshift mutation which undergo NMD. Western blot analysis revealed that Q81X channels are expressed at the cell surface but have a lower molecular weight than wild-type channels. Functional analysis of the Q81X channels revealed reduced current levels and accelerated deactivation kinetics compared with wild-type channels. These results are consistent with the disruption of the hERG N-terminus, including the Per, Arnt, and Sim domain, which is known to play an important role in regulating hERG channel deactivation. Site-specific mutagenesis showed that translation of the Q81X transcript is reinitiated at Met124 following premature termination. Furthermore, we found that Q81X co-assembles with wild-type hERG channels resulting in the dominant-negative acceleration of deactivation in heteromeric channels. Our findings indicate that the reinitiation of translation may be an important pathogenic mechanism in patients with early nonsense and frameshift LQT2 mutations.

3439-Pos Board B300

Regulation of Airway Smooth Muscle Contraction by K_V7 (M-Type) K^+ Channels

Alexey I. Evseev, Iurii Semenov, Robert Brenner, Mark S. Shapiro.
University of Texas Health Science Center at San Antonio, San Antonio, TX, USA.

BK-type Ca^{2+}-activated K^+ channels have established roles in regulating voltage-dependent Ca^{2+} influx and contraction of airway smooth muscle (ASM). Recently, Kv7 (M-type) voltage-dependent K^+ channels have been uncovered as a regulator of membrane voltage of smooth muscle cell types. Here, we investigate the role of Kv7 channels in rodent trachea. We isolated Kv7 current in acutely dissociated rat ASM cells under perforated-patch voltage clamp with the BK-channel blocker, paxilline (1 μM), using 2s depolarizations from a holding potential of −60 mV. Kv7 current was quantified as the sustained current component over the last 400 ms of the step, which was almost completely blocked by the specific Kv7 blocker, XE991 (10 μM). The voltage-dependence of activation of this sustained current ($V_{0.5}$ = −28 ± 1 mV) was similar to the XE991-sensitive component ($V_{0.5}$ = −31 ± 2 mV), typical of M-type currents. The M-channel opener, flupirtine (10 μM), increased current amplitudes by two-fold and shifted the $V_{0.5}$ to −36 ± 1 mV for the total sustained current and to −40 ± 2 mV for the flupirtine-sensitive current. In contraction experiments on mouse trachea, we found little effect of XE991 on carbachol-evoked (0.5 μM) trachea constriction, but flupertine (30 μM) caused a relaxation of trachea by 21 ± 2%. The flupertine effect is likely via opposing voltage-dependent Ca^{2+} influx, since no effect of flupertine occurred in a low K^+/hyperpolarizing bathing solution (1mM K^+). Interestingly, although XE991 failed to affect wild type trachea, it does enhance contraction of trachea from BK channel β1 KO mice at low carbachol concentrations (*i.e.*, 2.3 fold increased contraction in 0.1 μM carbachol) These results indicate that Kv7 channels in rodent trachea may play a secondary role to BK channels in regulating airway constriction. Nevertheless, Kv7 channel agonists may be useful in moderating airway constriction in asthma.

3440-Pos Board B301

Pore Helix-S6 Interactions are Critical in Governing KCNQ3 Amplitudes

Frank S. Choveau, Sonya M. Bierbower, Mark S. Shapiro.
UTHSCSA, San Antonio, TX, USA.

Two mechanisms have been postulated as underlying the small KCNQ3 homomeric currents, compared with other KCNQ homomers, or KCNQ2/3 heteromers. The first involves differential channel expression, governed by the assembly domain of the distal C-terminus (Schwake et al., 2006. J. Neuroscience), centered on the "D helix," whose KCNQ4 crystal structure revealed a "coiled-coil" suggested to be much more favorable for tetramerization of KCNQ2/3 heteromers and KCNQ4 homomers than for KCNQ3 homomers (Howard et al., 2007. Neuron). The second suggests similar expression of KCNQ channels, but a KCNQ3 pore that is particularly unstable, leading to most KCNQ3 homomers being dormant, whereas mutation of an intracellular pore-helix residue, A315, to a hydrophilic T/S boosted currents by >25-fold (Zaika et al., 2008. Biophys J; Exteberria et al., 2004. J. Neuroscience). Pore instability is thought to underlie "C-type" inactivation of K+ channels, including KcsA, for which disruption of the interaction between F103 (S6) and the pore helix (T74, T75) stabilized the pore (Cuello et al., 2010. Nature). We found mutations at the analogous position in KCNQ3 (F344) dramatically decreased KCNQ3 currents, and TIRF showed negligible effects on membrane expression. Homology modeling of wild-type and mutant KCNQ3 suggest the decrease of the current in F344 mutants is due to a disruption of the interaction between F344 (S6) and the A315 residue. Finally, native PAGE revealed H2O2-enhanced oligomerization of KCNQ4 subunits at C643 at the end of the D-helix. However, H2O2-mediated enhancement of KCNQ4, previously showed to localize to a cysteine triplet in the S2-S3 linker (Gamper et al., 2006. EMBO J), was identical in the C643A mutant. Our results suggest a secondary role of the C-terminus, compared to pore-helix-S6 interactions, governing KCNQ3 amplitudes, and that variable surface expression among KCNQ channels plays only a minor role.

3441-Pos Board B302

Selective Formation of Oligomeric Kv7.5 (KCNQ5)/KCNE1 and Kv7.5 (KCNQ5)/KCNE3 Channels. Differential Targeting to Membrane Surface Microdomains

Laura Sole[1], Meritxell Roura-Ferrer[1,2], Anna Oliveras[1], Albert Vallejo[1], Sara Roig[1], Alvaro Villarroel[3], Nuria Comes[1], Antonio Felipe[1].
[1]University of Barcelona, Barcelona, Spain, [2]CSIC-UPV/EHU, Universidad del País Vasco, Leioa, Spain, [3]CSIC-UPV/EHU, Universidad del Pas Vasc, Leioa, Spain.

Kv7 (KCNQ) proteins form a family of voltage-gated potassium channels that is comprised of five members, Kv7.1-Kv7.5. While Kv7.1 is crucial in the heart, the Kv7.2-Kv7.5 channels contribute to the M-current in the nervous system. In addition, Kv7.5 is expressed in muscles, where its physiological role is currently under evaluation. Kv7 associations with KCNE accessory subunits (KCNE1-5) enhance channel diversity. KCNE peptides control the surface expression, voltage-dependence, kinetics of gating, unitary conductance, ion selectivity and pharmacology of several channels. KCNE subunits have been primarily studied in the heart; however, their activity in the brain and in many other tissues is being increasingly recognized. Here, we found that Kv7.5 and KCNE subunits are present in myoblasts. Therefore, oligomeric associations may underlie some Kv7.5 functional diversity in skeletal muscle. Expression in Xenopus oocytes and HEK-293 cells demonstrates that KCNE1 and KCNE3, but none of the other KCNE subunits, associate to Kv7.5. While KCNE1 slows activation and suppresses inward rectification, KCNE3 inhibits Kv7.5 currents. Furthermore, KCNE1 increases Kv7.5 currents in HEK cells. The membrane targeting is also affected. Biochemical isolation of lipid rafts demonstrates that Kv7.5 barely locates in rafts. KCNE1 and KCNE3 show differential targeting. While KCNE3 targets to rafts, KCNE1 does not. Association of Kv7.5 with KCNE3 impairs KCNE3 targeting to rafts and this is further supported by FRAP analysis. Our results have physiological relevance since Kv7.5 is abundant in skeletal and smooth muscle and its association with KCNE peptides may fine-tune cellular responses.

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