mutants, while K_4 (force generation step) was unchanged. V95A showed significantly lower K_2 (cross-bridge detachment step: 0.93 ± 0.06) than WT (1.37 ± 0.13). D175N and V95A showed significantly lower K_1 (ATP association constant, 0.91 ± 0.13 and 0.86 ± 0.16, respectively) than E180G (1.84 ± 0.33) and WT (1.60 ± 0.35). However, the cross-bridge distribution was not significantly different among 4 Tms, indicating that force/cross-bridge in E180G is larger than WT, but it is unchanged in V95A and D175N. In conclusion, all three mutants showed significant deviations in force/cross-bridge, pCa_{50}, cooperativity or cross-bridge kinetics; in particular, E180G had the largest effect. Because E180G and D175N are located in the Tm-Troponin (Tn) interaction region and result in the net charge increase, and E180G causes the largest hydropathy change, we infer that both electrostatic and hydrophobic interactions between Tm and Tn play vital roles in maintaining normal muscle functions.

37-Plat

UNC-45 Knock-Down in Drosophila Heart Targets Myosin Accumulation and Yields Severe Myofibrillar Disarray and Cardiac Dysfunction

Girish C. Melkani[1,2], Karen Ocorr[2], Rolf Bodmer[2], Sanford I. Bernstein[1].
[1]Department of Biology, Molecular Biology and SDSU Heart Institute, San Diego State University, San Diego, CA, USA, [2]Development and Aging Program, Burnham Institute for Medical Research, La Jolla, CA, USA.

UNC-45 belongs to the UCS (UNC-45, CRO1, and She4p) domain protein family, whose members interact with various myosins and are required for myosin function. The in vivo RNAi approach using the Gal4/UAS system permits us to knock down Drosophila unc-45 (dunc-45) transcripts in a tissue-specific manner. DUNC-45 knock-down with muscle-specific driver 24bGal4 leads to embryonic and larval lethality. However, DUNC-45 knock-down in the heart allows survival to adulthood, but lifespan is dramatically reduced, with ~90% dead within three weeks. Optical heartbeat analysis of semi-intact hearts from various age adults was carried out using high-speed video and movement analysis algorithms. Analysis of several cardiac parameters (e.g., diastolic and systolic diameters, heart period and arrythmicity index) showed severely compromised cardiac function. For example, ~80% of 1, 2 and 3 week old knock-down hearts do not show contraction or relaxation of normally highly-contractile regions (primarily in abdominal segment three). The remainder of the heart shows a greatly reduced and irregular beating pattern. Another striking response to DUNC-45 knock-down was dilation of the third segment of the heart. For example, the average diastolic diameter for one week old control fly heart is 76 μm. However, in the one-week-old knock-down heart the average diastolic diameter is 128 μm. Cardiac arrhythmias were observed in one-week-old flies, whereas arrhythmias were only observed after three weeks of age in wild type flies. Immunofluorescent images of relaxed hearts using myosin antibody showed irregular heart muscle patterning and missing myofibrils in DUNC-45 knock-down adults. Heart defects of the DUNC-45 knock-down heart were rescued to some extent by transgenic over-expression of DUNC-45. As UNC-45 is essential for accumulation of myosin, its knock-down in the heart results in myofibrillar disarray and reduced contraction.

Platform C: Voltage-gated Na Channels

38-Plat

The Nachbac Pore: Creation and Characterisation of a KcsA-Like Sodium Channel

Andrew M. Powl[1], Maurits R.R. de Planque[2], Hywel Morgan[2], B.A. Wallace[1].
[1]Birkbeck College, University of London, London, United Kingdom, [2]Southampton University, Southampton, United Kingdom.

Voltage-gated sodium channels (VGSC) are integral membrane proteins responsible for the transient flux of sodium ions across cell membranes in response to changes in membrane potential. In humans as well as lower eukaryotes they are essential for homeostasis and normal functioning, and mutations in them are associated with a range of disease states. Although potassium channels, which are members of the same large family of voltage-gated channels have been well characterized, much less known about the structural features of sodium channels. For potassium ion channels, an important advance in understanding resulted from the determination of the three dimensional structure of the bacterial potassium channel KcsA, a simplified channel composed only of two transmembrane segments per subunit present in the tetrameric structure. In 2001, Ren *et al* found that bacteria also possess simplified versions of sodium channels, although in this case the individual subunits of all the homologues that have been identified thus far possess six transmembrane segments, which include both a pore-forming subdomain (S5-S6) and a voltage-sensing subdomain (S1-S4). Here we report on the creation of a smaller KcsA-like pore-only version of a sodium channel from the *B. halodurans* VGSC (pNaChBac), engineered to contain S5-S6 plus the C-terminal region of the NaChBac channel. The NaChBac pore has been expressed and purified from *E. coli*membranes, solubilised in detergent micelles, reconstituted into lipid vesicles and characterized for its secondary structure and thermal stability, as well as its electrophysiological properties from single-channel recordings, providing new insight into features of sodium channel structure and function.

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39-Plat

A Central Role For Mitochondria in the Regulation of Cardiac Sodium Current

Man Liu[1], Georgia Gaconnet[1], Barry London[2], Samuel C. Dudley[3].
[1]Cardiology, University of Illinois at Chicago, Chicago, IL, USA, [2]The Cardiovascular Institute, University of Pittsburgh, Pittsburgh, PA, USA, [3]Cardiology, University of Illinois at Chicago, the Jesse Brown VAMC Chicago, Chicago, IL, USA.

Background: A mutant glycerol-3-phosphate dehydrogenase 1-like, A280V (A280V GPD1-L) reduces cardiac Na^+ current (I_{Na}) and causes Brugada Syndrome. Recent data suggest that this effect is dependent on alterations in NADH, reactive oxygen species (ROS), and PKC activation. Since NADH and PKC can activate ROS production from mitochondria, we investigated the role of this organelle in mediating the effects of mutant GPD1-L and NADH on I_{Na}.
Methods: HEK cells stably expressing the cardiac Na^+ channel were used, and effects on I_{Na} were assessed by whole-cell patch clamp recording.
Results: A280V GPD1-L caused a 2.48 ± 0.17-fold increase of intracellular NADH level (n=3; P<0.001). Cytosolic NADH application (100 μM) or co-transfection with A280V GPD1-L resulted in significant decrease of I_{Na} ($52 \pm 9\%$ or $81 \pm 4\%$, respectively; P<0.01), which was reversed by 5-50 μM chelerythrine, 5 μM superoxide dismutase (SOD), 5-10 μM mitoTEMPO (a specific inhibitor to block mitochondrial superoxide generation), 1-5 μM rotenone (a complex I inhibitor), and 40-80 μM 4′-chlorodiazepam (an inhibitor of mitochondrial benzodiazepine receptor). The decreased I_{Na} induced by 30 nM PMA ($60 \pm 7\%$, P<0.01) was prevented by SOD. Antimycin A (a complex III inhibitor known to produce ROS) at 20 μM decreased I_{Na} ($51 \pm 4\%$, P<0.01). L-NAME (an inhibitor for uncoupled NOS), cyclosporin A (an inhibitor for mitochondrial permeability transition pore), and KN-93 (an inhibitor of CAMKII) had no effect on NADH reducing Na^+ current.
Conclusions: A280V GPD1-L appears to regulate $Na_v1.5$ by altering the oxidized to reduced NAD(H) balance, which then activates mitochondrial ROS production through a PKC-dependent signaling mechanism. This ROS production leads to reduced I_{Na}. This signaling cascade may help explain the link between altered metabolism, conduction block, and arrhythmic risk.

40-Plat

NaV-Mediated Sodium Currents Are Necessary For Vertebrate Appendage Regeneration

Kelly Ai-Sun Tseng, Michael Levin.
Tufts University, Medford, MA, USA.

Mammals have a limited ability to regenerate tissues. In contrast, amphibians such as frogs can restore lost developmental structures, including the lens and tail. A detailed understanding of natural regeneration is important for developing therapies for organ repair. Recently, ion transport has been implicated as a regulator of regeneration. While voltage-gated sodium channels play a well-known and important role in propagating action potentials in excitable cells, we have identified a novel role in regeneration for the ion transport function mediated by the voltage-gated sodium channel, NaV1.2. After Xenopus tadpole tail amputation, a regeneration bud (containing progenitors required for regenerative growth) is formed within 1 day at the injury site, and a new tail is re-grown by 7 days. NaV1.2 is expressed early in the bud, and its function is required for regeneration. Inhibition of its activity causes regenerative failure by greatly reducing expression of downstream genes that drive tail outgrowth and patterning, leading to decreased proliferation and altered axonal patterning in the regeneration bud. Significantly, NaV1.2 is not expressed under non-regenerative conditions, suggesting that its activity is a determinant of regenerative ability. Most importantly, pharmacological induction of a brief, transient sodium current into the regeneration bud after tail amputation is sufficient to restore full regeneration of the tail during the refractory period (an endogenous developmental period when regeneration is blocked). Our study demonstrates that sodium transport is a critical mechanism for controlling regeneration, and suggests that short-term modulations of ion transport could represent an exciting new approach to tissue repair in mammals.