We previously found that both ET-1 and phenylephrine (PE) induced similar HDAC5 phosphorylation and nuclear export in adult cardiac myocytes (which contributes to hypertrophic signaling). However, ET-1 requires IP_3 receptor (IP_3R) activation (at the nucleus) and CaM-CaMKII activation to mediate this full effect, while PE does not. That is, IP_3R inhibition or CaMKII block do not prevent PE-induced HDAC5 nuclear export, despite the fact that both agonists can activate IP_3 production. Here we test whether the apparent IP_3-independence of PE signaling is due to a failure of IP_3 elevation in the nucleus (i.e. IP_3 produced at the plasma membrane may be degraded before reaching the nucleus). Using a nuclear targeted FRET-based IP_3 sensor (Fire-1-Nuc) we assessed changes in nuclear [IP_3] upon ET-1 and PE application in adult rabbit ventricular myocytes. Both ET-1 and PE induce rapid and robust elevation of nuclear [IP_3] reaching an early peak in <1 min. While the ET-1-induced a slightly larger peak nuclear [IP_3], the PE-induced rise is more sustained (lasting more than 10 min). These results demonstrate that a PE induces a strong rise in nuclear [IP_3], and does not support the hypothesis that PE fails to induce a nuclear IP_3 signal (compared to ET-1). We cannot rule out the possibility that the kinetic differences in nuclear [IP_3] between these agonists contribute to different downstream signaling. Another explanation is that PE-induced nuclear IP_3 is less effective than ET-1-induced IP_3 in driving activation of nuclear CaM and CaMKII to phosphorylate HDAC5. That is, an IP_3-independent effect of one of these agonists could prevent or promote the ability of IP_3 to signal in the nucleus.

1609-Pos

Phosphorylation Dependent Nuclear Transport of Human Dutpase

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The nuclear isoform of human dUTPase plays an important role in maintaining genomic integrity. Its expression is strictly cell cycle regulated and is known to be a phosphoprotein in vivo. However, the role of this phosphorylation remained unknown.

Here we show regulation of the nuclear transport of human dUTPase via phosphorylation of a serine residue on its nuclear localisation signal. We found that hyperphosphorylation mimicking mutants (glutamic acid) are localized solely in the cytoplasm while hypophosphorylation mimicking mutants (glutamine) localize in the nucleus as the endogenously regulated protein. Our video microscopy studies have also shed light on the nuclear import dynamics of the wild type dUTPase and that of the mutants. These results showed that the phosphorylated wild type form may re-enter the nucleus (after cell division) only after a considerable delay of several hours while mutants that cannot be phosphorylated re-accumulate within the nucleus much faster. The delay observed with the wild type enzyme may indicate that either dephosphorylation or de novo protein synthesis is required. To reveal the mechanism by which cells accumulate sufficient amount of dUTPase in their nucleus after cell division, we are currently conducting protein transfection based experiments.

We are also trying to characterize the interaction of the human dUTPase with its possible partner in nuclear trafficking, importin-alpha. Based on Native-PAGE and ThermoFluor experiments, we detected a relatively high affinity complex of dUTPase with importin-alpha. Complex formation was also observed in the case of the hypophosphorylation mimicking mutant (S11Q), but not with the hyperphosporylation mimicking mutant (S11E). We also conduct crystallographic studies of the complex using various dUTPase NLS peptides.

Voltage-gated Na Channels II

1610-Pos

Stable Expression of Brain Sodium Channels in Human Cells by Multiplexed Transposon-Mediated Gene Transfer

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Generation of cultured human cells stably expressing one or more recombinant gene sequences is a widely used approach in biomedical research, biotechnology and drug development. Conventional methods are not efficient and have severe limitations especially when engineering cells to co-express multiple transgenes or multi-protein complexes. We harnessed the highly efficient, non-viral and plasmid-based *piggyBac* transposon system to enable concurrent genomic integration of multiple independent transposons harboring distinct protein-coding DNA sequences. Flow cytometry of cell clones derived from a single multiplexed transfection demonstrated ~60% (three transposons) or ~30% (four transposons) co-expression of all delivered transgenes despite selection of a single marker transposon. We validated multiplexed *piggyBac* transposon delivery by co-expressing large transgenes encoding a multi-subunit neuronal voltage-gated sodium channel (SCN1A) containing a pore-forming subunit and two accessory subunits while using two additional genes for selection. Previously unobtainable robust sodium current was demonstrated through 38 passages, suitable for use on an automated high-throughput electrophysiology platform. Co-transfection of three large (up to 10.8 kb) *piggyBac* transposons generated a heterozygous SCN1A stable cell line expressing two separate alleles of the pore-forming subunit and two accessory subunits (total of four sodium channel subunits) with robust functional expression. We concluded that the *piggyBac* transposon system can be used to perform multiplexed stable gene transfer in cultured human cells and this technology may be valuable for applications requiring concurrent expression of multi-protein complexes.

1611-Pos

The Functional Effect of R1648H, a Sodium Channel Mutation that Causes Generalized Epilepsy with Febrile Seizures Plus in Splice Variants of SCN1A

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SCN1A, the gene that encodes the alpha subunit of the voltage-gated sodium channel Nav1.1, is alternatively spliced at exon 5. SCN1A contains two copies of exon 5, denoted 5N and 5A (for 'Neonatal' and 'Adult' according to their developmental expression). There are 3 amino acid substitutions between the splice variants, all within the D1:S3/S4 extracellular linker. It is unknown how exons 5N and 5A alter channel function. Because patients with Generalized Epilepsy with Febrile Seizures plus (GEFS+) frequently exhibit age-dependent changes in seizure frequency and severity, we have asked whether the GEFS+-associated SCN1A mutation R1648H differentially affects Nav1.1-5N and Nav1.1-5A.

We examined brain tissue obtained from patients undergoing epilepsy surgery to examine the relative proportion of SCN1A transcripts containing exons 5A and 5N. A significantly greater proportion of Nav1.1 mRNA in epilepsy tissue contain exon 5N than in control brain tissue. We expressed either splice variant of SCN1A in HEK293 cells, and recorded whole-cell currents with a CsCl-based pipette solution. Nav1.1-5N demonstrated a leftward shift of both activation (Nav1.1-5N: V50= $-18.3 \pm$-0.6 mV ; Nav1.1-5A: $-15.3 \pm$-0.5 mV; $P<0.05$) and inactivation (Nav1.1-5N: V50= $-60.0 \pm$-1.0 mV; Nav1.1-5A: $-54.0 \pm$-1.1 mV). The GEFS+ mutation R1648H, did not affect activation or current density for either variant. The mutation also failed to increase the size of the persistent current evoked by prolonged depolarising steps. Instead, a hyperpolarizing shift in inactivation was observed when the mutation was expressed in Nav1.1-5A but not Nav1.1-5N channels (mutant: V50 inactivation = $-60.9 \pm$-1.0 mV; wild-type: $-54 \pm$-1.1 mV). This suggests that R1648H leads to a net loss of function in adult neurons. This effect may lead to an impairment of recruitment of GABAergic interneurons that preferentially express Nav1.1.

1612-Pos

Traumatic Brain Injury and Axonal Sodium Loading: Modeling the Impact of Left-Shifted Nav Channel Operation at Blebbed Nodes of Ranvier

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Traumatic brain injury like stretch immediately (<2 min) and irreversibly causes a TTX-sensitive axonal [Ca2+] increase that, in situ, underlies an untreatable pathology, diffuse axonal injury. Nav1.6-expressing mammalian cells, we showed, immediately (<2 min) exhibit TTX-sensitive Na+-leak following traumatic stretch (Wang et al 2009 Am J Physiol 297: in press). In situ, even mild axonal stretch injury can trigger adverse positive feedback so that leaks progress irreversibly to lethality. Though clinical trials are underway using Nav channel blockers that might reduce the severity of this outcome, molecular understanding of Nav channel damage has been lacking. Recently, however, we showed that activation and steady-state inactivation of recombinant Nav1.6 channels both irreversibly left-shift (up to -20 mV) in traumatized membrane (Wang et al 2009) as if their voltage sensors are responding to the increased bilayer disorder of traumatized (blebbed) membrane. In axonal membrane traumatized to various extents, this should smear out the window current range leftward between the normal range toward the resting potential range,

effectively degrading a well-confined window conductance into a TTX-sensitive ''Na+-leak''.
To assess whether minor membrane trauma could lead to Na+ (and hence Ca2+) loading of axons, we model partially left-shifted Nav operation in a free-running human node of Ranvier. Included are Kv and Nav conductances (linear and electrodiffusion driving forces, respectively) and a Na/K pump, with [ion]s (and associated ENa and EK) in realistic-sized intra- and extracellular compartments changing due to net ion fluxes. Left-shifting a fraction of the Navs immediately triggers a damped action potential burst then a voltage plateau dominated by window current. If resting conductances are large enough, pumping restores the system for several minutes, then more bursting starts and more ENa rundown occurs.

1613-Pos
Modulation of Nav 1.5 Variants by Src Tyrosine Kinase
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Cardiac sodium channel (Na_v 1.5) splice variant Q1077 deleted (hH1c1) and Q1077 present (hH1c3) mutants are present in 45% and 25 % of human population. In previous studies, Src tyrosine kinase (Fyn) showed opposite effects on cardiac and neuronal sodium channel inactivation. Half maximum inactivation of cardiac sodium channels was shifted to more positive potentials, whereas in neuronal channels it was shifted into the hyperpolarizing direction, despite having conserved Y1495, which is the site of phosphorylation in both channels. Activation was not affected.
In our study we found that Fynhas a different action on the cardiac sodium channel variants hH1c1 and hH1c3. Experiments were performed by means of the patch clamp technique in the whole cell mode. Fyn was transiently expressed with CD8 in stably expressed HEK293 cells embodying hH1c1 and hH1c3 clones. In hH1c1, Fyn shifted the activation (V_{mid} -51.6 $\pm$ 1.5 to -63.9 ± 1.0) and inactivation curves (V_{mid} -64.4 $\pm$ 0.7 to -72.5 ± 0.4) to more negative potentials, which could be reversed by the kinase inhibitor PP2 (activation: V_{mid} -63.9 ± 1.0 to -52.0 ± 1.9, and inactivation: V_{mid} -72.5 ± 0.4 to -63.7 ± 0.7). In contrast, in hH1c3 Fyn shifted both activation (V_{mid} -86.2 $\pm$ 2.3 to -65.8 ± 0.5) and inactivation (V_{mid} -85.1 ± 0.7 to -63.4 ± 0.3) curves to more positive potentials. PP2 reversed the shift of both, activation (V_{mid} -63.4 ± 0.3 to -88.2 ± 1.0)and inactivation (V_{mid} -63.4 ± 0.3 to -87.2 ± 1.7).
Above result proclaims that hH1c1 and hH1c3 encoding for Nav 1.5 are differently regulated by Fyn. These data will be pertinent in understanding the role of Q1077, which is present in the transport associated region that plays a pivotal role in regulating Fyn function.

1614-Pos
Calmodulin Regulation of the Neuronal Voltage-Dependent Sodium Channel
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Calmodulin (CaM) is an essential eukaryotic calcium sensor comprised of two homologous domains (N, C). Ca^{2+} binding to CaM changes its conformation and determines how CaM recognizes and regulates target proteins such as the neuronal voltage-dependent sodium channel ($Na_v1.2$) which is essential for the generation and propagation of action potentials. $Na_v1.2$ is a multimer with one pore-forming α-subunit and one or more β-subunits. CaM binds to an IQ-motif (IQxxxBGxxxB, B=K,R) of $Na_v1.2$ that is near the C-terminus of the α-subunit. Prior thermodynamic studies showed that this IQ peptide ($Na_v1.2_{IQp}$, KRKQEEVSAIVIQRAYRRYLLKQKVKK) selectively lowers the Ca^{2+}-binding affinity sites in the C-domain of CaM, without affecting the N-domain (Theoharis et al, *Biochemistry* 2008). This selective decrease correlates with $Na_v1.2_{IQp}$ having a higher affinity for apo CaM than for calcium-saturated CaM. Structural studies of complexes of CaM bound to target peptides or proteins demonstrated that the 4-helix bundle of the CaM C-domain adopts an ''open'' conformation when Ca^{2+}-saturated. There is only one high-resolution structure (2IX7) of apo CaM bound to an IQ motif; it shows the C-domain having a ''semi-open'' conformation. To understand the Ca^{2+}-dependent conformational switching CaM when regulating $Na_v1.2$, we applied heteronuclear NMR methods. Amide exchange, hnNOE, and chemical shift perturbation experiments revealed residue-specific changes consistent with a ''semi-open'' conformation of the apo C-domain of CaM when bound to $Na_v1.2_{IQp}$. NMR experiments are complete and analysis is underway to determine the solution structure of the apo C-domain of CaM bound to $Nav1.2_{IQp}$. Understanding the interface between CaM and the IQ-motif of the channel will result in a more complete model of how CaM regulates $Na_v1.2$ function at low physiological [Ca^{2+}] in neuronal tissues. NIH GM57001

1615-Pos
Electrophysiological Characteristics of Neonatal Nav1.5 Expressed in a Highly Invasive Human Breast Cancer Cell Line: Sensitivity to pH and Divalent Cations
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Electrophysiological recordings from human carcinoma cell lines have shown consistently that strongly metastatic cells express functional voltage-gated sodium channels (VGSCs). The predominant VGSC in metastatic breast cancer, in vitro and in vivo, is the 'neonatal' splice form of Nav1.5. In this developmentally regulated D1:S3 splice variant of Nav1.5, there are 31 nucleotide differences between the 5'-exon ('neonatal') and the 3'-exon ('adult') forms, resulting in 7 amino acid differences in D1:S3-S3/S4 linker. In particular, a conserved negative aspartate residue in the 'adult' is replaced with a positive lysine. 'Neonatal' and 'adult' Nav1.5 α-subunit splice variants were stably transfected into EBNA-293 cells and their electrophysiological properties were investigated by whole-cell patch-clamp recording. Compared with the 'adult' isoform, the 'neonatal' channel exhibited (1) depolarized threshold of activation and voltage at which current peaked; (2) much slower kinetics of activation and inactivation; (3) ~50% greater transient charge (Na^+) influx; (4) a slower recovery from inactivation; and (5) larger persistent Na^+ currents. Mutating the lysine in the 'neonatal' channel back to aspartate resulted in the electrophysiological parameters studied reverting strongly back towards the 'adult', i.e. the lysine residue was primarily responsible for the electrophysiological differences. The charge difference between the two Nav1.5 isoforms was 'challenged' by H^+ and Cd^{2+}. The main differential effect occurred at pH 5.25-5.75 in which the activation parameters of 'neonatal' Nav1.5 were affected significantly less. The biophysical characteristics of 'neonatal' Nav1.5 observed could have significant developmental and pathophysiological consequences. In particular, the prolonged Na^+ influx can alter intracellular Ca^{2+} and/or pH homeostasis, at least in microdomains, and channel activation remains relatively efficient under extreme acidosis.

1616-Pos
Sodium Channel Variants Associated with Atrial Fibrillation Exhibit Abnormal Fast and Slow Inactivation
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Mutations and rare genetic variants in *SCN5A*, the gene encoding the cardiac voltage-gated sodium channel $Na_V1.5$, have been associated with inherited predisposition to ventricular arrhythmia. More recently, *SCN5A* variants have been identified in families segregating atrial fibrillation. We evaluated the biophysical properties of seven novel *SCN5A* variants associated with atrial fibrillation identified by our previous genetic study to elucidate potential molecular mechanisms underlying this common arrhythmia. Functional properties of E428K, H445D, N470K, E655K, T1131I, R1826C and V1951M were assessed by whole-cell patch clamp recording of recombinant mutant channels heterologously expressed with the human β1 subunit in tsA201 cells. One variant (R1826C) did not exhibit substantial differences in biophysical properties of activation or fast inactivation, and another variant (E655K) only exhibited minor differences in recovery from inactivation as compared with wildtype (WT) channels. However, two mutants (H445D, T1131I) exhibited significant shifts in the voltage-dependence of activation toward more negative potentials ($p < 0.005$), and four other mutant channels (E428K, H445D, N470K, V1951M) exhibited significant shifts in the voltage-dependence of steady-state inactivation toward more positive potentials as compared with WT channels. Further, H445D and V1951M exhibited more rapid onset, impaired recovery and enhancement of slow inactivation evoked by 1000 ms depolarizing prepulses as compared with WT channels. For the five variants with either hyperpolarized activation voltage-dependence or depolarized steady-state inactivation, we predict increased window current as defined by the overlap of these two curves. Increased window current and enhanced slow inactivation of some variants is further predicted to alter excitability and/or conduction in myocardial tissue and is a plausible mechanism by which *SCN5A* variants may increase vulnerability to atrial fibrillation.