a pattern conserved in recombinant channels expressed in HEK293 cells. This clustering is dependent on the extent of channel phosphorylation. Our recent studies have shown that the clustered localization and gating properties of Kv2.1 are dynamically regulated by altered neuronal activity, ischemia, and by neuromodulatory stimuli via Ca2+/calcineurin-mediated dephosphorylation of the channel protein. These changes in Kv2.1 play a neuroprotective role. A number of studies have proposed the association of Kv2.1 with caveolar lipid rafts. We observed that Kv2.1 clusters in HEK293 cells do not overlap with caveolin1- or flotillin1/2-containing lipid rafts/microdomains on the cell surface. Moreover, caveolin1 staining/puncta were detected only in cultured rat astrocytes/glia, not in cultured rat hippocampal neurons. Caveolin1-RFP, overexpressed both in HEK293 cells stably expressing Kv2.1, and in cultured hippocampal neurons, exhibited distinct surface puncta that did not overlap with Kv2.1 clusters, and also did not alter the current density and voltage-dependent channel gating properties. Cyclodextrin-induced disruption of lipid rafts did not alter the clustered localization of Kv2.1 in HEK293 cells. Similarly, the staining pattern of Kv4.2 and Kv4.3 channels with or without the overexpression of KChIP2 did not overlap with caveolin1- and flotillin1/2-containing lipid rafts/microdomains in HEK293 and COS cells. These results suggest that in mammalian central neurons, somatodendritic Kv channels are not recruited to, and function independently of, lipid rafts/microdomains.

2885-Plat

Stim-regulated Assembly And Stoichiometry Of The CRAC Channel Subunit Orai

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Recent RNAi screens have identified Stim and Orai as critical components of the Ca^{2+} release-activated Ca^{2+} (CRAC) channel. Stim senses depletion of the Endoplasmic Reticulum (ER) Ca^{2+} store, translocates from the ER to junctions adjacent to the plasma membrane (PM), and activates Orai pore-forming channel subunits in the PM to open the CRAC channel. The Orai oligomerization interface was investigated by co-immunoprecipitation of N-and/or C-terminal Orai deletion mutants and expressed Orai N- and C-terminal fragments. The transmembrane core domain plays a predominant role in subunit assembly; a weaker interaction interface was identified at the N-terminal region. We analyzed the quaternary structure of the Orai subunit and showed by cross-linking, and by non-denaturing gel electrophoresis that Orai is predominantly a dimer under resting conditions with or without co-expression of Stim. Single-molecule imaging of GFP-tagged Orai expressed in *Xenopus* oocytes revealed predominantly two-step photo-bleaching, consistent with a dimeric basal state. In contrast, co-expression of GFP-tagged Orai with the C-terminus of Stim as a cytosolic protein to activate the Orai channel without inducing Ca^{2+} store depletion or clustering of Orai into punctae yielded predominantly four-step photobleaching, consistent with a tetrameric Orai stoichiometry of the active CRAC channel. Interaction of the Orai C-terminal coiled-coil domain (as shown by structure-disruptive mutations) with the C-terminus of Stim thus induces Orai dimers to dimerize, forming a tetramer that constitutes the Ca^{2+}-selective pore. This represents a novel mechanism in which assembly and activation of the functional ion channel are mediated by the same triggering molecule and may reveal a new channel gating mechanism. New data will be presented on the Stim-Orai stoichiometry and activation mechanism.

2886-Plat

Protein Histidine Phosphatase 1 Negatively Regulates CD4 T Cells by Inhibiting the K+ Channel KCa3.1

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The calcium activated K+ channel KCa3.1 plays an important role in T lymphocyte Ca2+ signaling by helping to maintain a negative membrane potential which provides an electrochemical gradient to drive Ca2+ influx. We previously showed that Nucleoside Diphosphate Kinase Beta (NDPK-B), a mammalian histidine kinase, is required for KCa3.1 channel activation in human CD4 T lymphocytes. We now show that the mammalian protein histidine phosphatase (PHP)-1 directly binds and inhibits KCa3.1 by dephosphorylating histidine 358 on KCa3.1. Overexpression of wild type, but not a phosphatase dead PHPT-1 inhibited KCa3.1 channel activity. Decreased expression of PHPT-1 by siRNA in human CD4 T cells resulted in an increase in KCa3.1 channel activity and increased Ca2+ influx and proliferation following T cell receptor (TCR) activation indicating that endogenous PHPT-1 functions to negatively regulate CD4 T cells. Our findings provide the first example of a mammalian histidine phosphatase negatively regulating TCR signaling and are one of the few examples of histidine phosphorylation/dephosphorylation influencing a biological process in mammals.

2887-Plat

Targeting The Voltage Sensor of Kv7 channels: Novel Strategies to Cure Hyperexcitability Disorders

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The pore and gate regions of ion channels have been often targeted with drugs acting as channel blockers or openers. In contrast, the voltage sensing domain (VSD) was practically not exploited for therapeutic purposes. We recently designed a series of novel diphenylamine carboxylate derivatives to generate powerful Kv7.2 channel openers and blockers. Openers like the compound NH29 with aromatic nitro groups, robustly increased Kv7.2 K^+ currents. In sensory DRG and hippocampal neurons, the opener depressed evoked spike discharges. NH29 dampened hippocampal glutamate and GABA release thereby inhibiting spontaneous EPSCs and IPSCs. *In vivo*, these openers exhibited anti-convulsant activity. To identify the target residues involved in the action of NH29 we designed various mutations of Kv7.2 channels and checked the potency of NH29 using the whole-cell patch-clamp technique in CHO cells. We found that the mutants S121A at S1-S2 loop and L197G, R198A, R201A, R207W and R214W at S4 helix are significantly less sensitive to the activating effect of NH29 compared to WT Kv7.2 channels. Interestingly, our results indicate that NH29 does not act at the same target site as the opener retigabine (W236) and zinc-pyrithione. Docking experiments suggest that the nitro functionality of NH29 acts as a H-bonding acceptor which interacts with the guanidinium group of arginine 207 of the S4 helix. Thus, the new Kv7.2 channel opener NH29 acts as a gating modifier which interacts with the externally accessible surface of the VSD. NH29 docks to the groove formed by the interface between S4 helix and S1-S2, thereby stabilizing the VSD in the activated conformation. Our research is expected to generate a new generation of gating-modifiers specifically targeted to the VSD of potassium channels for the treatment of hyperexcitability disorders.

2888-Plat

Regulation of the NALCN Sodium Leak Channel by Neuropeptides

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Several neurotransmitters act through G-protein coupled receptors (GPCR) to evoke a ''slow'' excitation of neurons. These include peptides, such as substance P (SP) and neurotensin (NT), as well as acetylcholine and noradrenaline. Unlike the fast (~ ms) ionotropic actions of small molecule neurotransmitters, the slow excitation is poorly understood at the molecular level, but can be mainly attributed to suppressing K^+ currents and/or activating a non-selective cation channel. Several K^+ channels, including members in the K_V7 subfamily, are inhibited by the neurotransmitters in a G protein-dependent fashion. The molecular identity of the cation channel has yet to be determined; similarly how the channel is activated and its relative contribution to neuronal excitability induced by the neuropeptides are unknown. We show that, in the hippocampal and ventral tegmental area neurons, SP and NT activate the voltage-independent Na^+-leak channel NALCN, a unique member in the 4×6TM channel family that also includes the voltage-gated Ca^{2+} (Ca_V) and Na^+ (Na_V) channels. The activation by SP through NK1R (a GPCR receptor for SP) is not blocked by the non-hydrolyzable GTP or GDP analogs. These findings identify NALCN as the cation channel activated by SP receptor, and suggest that a G protein-independent mechanism is involved in the coupling from receptor to channel.

2889-Plat

Role of Aromatic Residues for Local Anaesthetic Binding to Ion Channels

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Local anesthetic and antiepileptic drugs acting on Nav and hERG channels have been assumed to bind to aromatic residues in the internal vestibule; to 1764F and 1771Y in Nav (Liu et al., 1996) and to 652Y and 656F in hERG (Mitcheson et al., 2000). Despite a lack of such residues in Kv channels, some local anaesthetics (e.g. bupivacaine) bind to Kv channels with a considerable affinity. To explore the role of aromatic residues for local anaesthetic binding we investigated the effect of bupivacaine on mutated Shaker channels (P466F and V473Y; I470Y and P474F), expressed in Xenopus oocytes, with a voltage clamp technique.

The results suggest that aromatic residues do not increase the binding affinity of bupivacaine to Kv channels. Rather, the affinity decreases. The Kd value for the wildtype channel is 300 μM, for the V473F channel 550 μM and for the P474F channel 740 μM. Thus, aromatic residues seem not to be necessary for high-affinity local anaesthetic binding to voltage-gated ion channels. The specific role of aromatic residues in Nav and hERG channels seems thus related to specific structural constraints in these channels.

2890-Plat
A Regulator for Eag Family Channels
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Ether-a-go-go (Eag) family channels, which include hErg1, are voltage-gated K^+ channels that are important in cardiac and neural function. From the genetic sequence of this family of channels, we identify two probable ligand-binding sites based on their similarities with well-characterized ligand binding domains. The first putative ligand-binding domain is in the carboxy-terminal region and shares sequence similarity with the cyclic nucleotide-binding domain of cyclic nucleotide-gated (CNG) channels. Yet, this binding domain of Eag family channels lack a critical arginine required for cyclic nucleotide binding, and channel gating was not altered by cAMP or cGMP. The second potential ligand-binding site is a Per-Arnt-Sim (PAS) domain in the amino-terminal region. High conservation of these putative binding domains amongst all Eag family channels indicates their functional importance. We therefore categorize these channels as orphan receptors. We reasoned that a chemical screen of cellular metabolites will lead us to physiologically relevant channel-regulators. Using a novel, high through-put screen of the ''Fragments of Life'' chemical library of metabolites and metabolite-like compounds (deCODE Biostructures) and inside-out patch-clamp recording, we have identified regulators of Eag family channels. We identified six regulators that cluster into four chemical families. One indole, and one indole-like compound were increased Eag channel opening at hyperpolarizing potentials. Indoles are particularly interesting because of their structural similarity to purines, the core of the cyclic nucleotides that bind to and regulate CNG channels. In contrast to indoles, compounds from the flavonoid family strongly inhibited Eag current. These results indicate that metabolites regulate Eag family channels and may lead us to physiologic channel regulators.

Platform BC: Cardiac Electrophysiology

2891-Plat
The Vena Cava Is Pacing The Embryonic Heart
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The mechanism and location of the pacemaker in the embryonic heart is highly controversial due to the lack of physiological *in vivo* recordings. Here we report fluorescent macroscopic *in vivo* recordings of embryonic hearts (E12.5-E14.5) from mice with cardiac expression (α-MHC or Cx40 promotor) of the Ca^{2+} sensor GCaMP2.
Initial observations from the ventral surface showed regular uniform Ca^{2+} transients in the atrium, ventricle and a structure below/behind the right atrium that preceded every atrial activation. To better understand the origin of these Ca^{2+} transients we established a dorsal preparation leaving the heart and veins intact. Ca^{2+} transients activated in the region of the putative sinus node, propagated bidirectionally along the superior right and in a u-shaped pattern into the coronary sinus and left superior vena cava, and conducted faster (24 ± 4 mm/sec) than the atria (15 ± 2 mm/sec; n=5; $p<0.02$); we have termed this the ''streak''. In most hearts the streak fired before every atrial activation with a delay of 79 ± 10 ms (n=12); variations in this delay was not dependent on the heart rate (104 ± 15 bpm, n=12). Some hearts showed 2:1 coupling and in others only the streak fired at 178 ± 39 bpm (n=5). The streak contracted, could be electrically paced and spontaneous local field potentials were recorded as sharp spikes at the onset of Ca^{2+} transients. In some experiments we observed a diastolic Ca^{2+} increment just prior the field potential, in line with the proposed role of Ca^{2+} oscillations for the initiation of pacemaking in embryonic heart cells. The spontaneous activity of the streak prior to atrial activation, higher intrinsic frequency of this region, and diastolic Ca^{2+} release establish the pacemaker function of the streak. Thus, cardiomyocytes within/on the vena cava walls are involved in murine embryonic heart pacemaker activity. Values: mean ± SEM.

2892-Plat
Increased Vulnerability To Atrial Fibrillation Under Vagal Hyperinnervation Associated With Vasoactive Intestinal Polypeptide'S Release In Dog'S Atrium
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Increased vagal tone promotes atrial fibrillation (AF). Vasoactive intestine polypeptide (VIP, a neural transmitter co-released with acetylcholine) was shown to shorten atrial refractory periods. AF was reportedly associated with VIPoma, a rare tumor secreting excessive VIP. Yet, the effects of VIP on atrial electrophysiology remain unclear. **Methods:** Canine left atria were isolated with intact coronary perfusion. Programmed stimulation (300 ms drive cycle length followed by up to 4 extrastimuli) was used to assess AF induction. Action potential duration (APD) at 500 ms pacing was recorded with optical mapping system. Potassium currents (I_{Ks}) were recorded with patch-clamp techniques. Immunohistochemical staining for VIP receptors was performed on atrial tissue/myocytes. **Results:** AF was induced in 1 of 6 atria at baseline but 5 of 6 during 1 μM VIP perfusion ($p=0.021$) and 2 of 6 after 15 min washout. VIP shortened APD and increased inhomogeneity in a dose-dependent manner at 0, 0.1, 1.0, 10 μM and washout (n=13): APD_{75} was 134.61 ± 5.36, 116.31 ± 6.80, 117.50 ± 7.86, 100.71 ± 8.73 and 124.50 ± 4.84 ms; standard deviation of APD_{75} was 15.83 ± 1.55, 15.59 ± 2.33, 21.58 ± 3.57, 25.76 ± 1.16 and 18.67 ± 1.62 ms, respectively ($p<0.05$). VIP (1 μM) increased I_{Ks} current density (14.5 ± 9.5%, n = 9, $p<0.01$). Staining on isolated atrial myocytes revealed the expression of VIP receptor 1 and 2 was highly variable among cells and tissue staining showed spatial heterogeneity. **Conclusions:** VIP shortens APD and increases APD spatial inhomogeneity that could lead to increased AF vulnerability. Enhanced I_{Ks} and heterogeneity of receptor expression may contribute to these effects.

2893-Plat
Ablation of Protein Kinase A or Calmodulin Kinase II Phosphorylation Sites on Phospholamban Confer Arrhythmia Resistance in Sinoatrial Nodal Pacemaker Cells
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Phospholamban (PLN) is a negative regulator of the sarcoplasmic reticulum Ca^{2+} ATPase (SERCa). PLN is phosphorylated by Protein Kinase A (Ser16) or Calmodulin Kinase II (Thr17). These phosphorylations reduce the inhibitory effects of PLN on SERCa. Phosphorylation at PLN Ser16 and Thr17 are important for catecholamine effects on excitation-contraction coupling in ventricular myocytes, but their role in sinoatrial nodal (SAN) cells is unknown. We isolated SAN cells from wild type (WT) controls and from transgenic mice where Ser16Ala or Thr17Ala PLN mutants are expressed in lieu of WT PLN. We recorded spontaneous action potentials by perforated patch clamp at 35 ± 1°C. SAN cell automaticity rates ('beats'/min) were not significantly different between Ser16Ala, Thr17Ala or WT at baseline: WT (n=18) 269 ± 12; T17A (n=9) 264 ± 23; S16A (n=16) 256 ± 17. No early (EAD) or delayed (DAD) afterdepolarizations were observed at baseline. EADs and DADs were induced by isoproterenol (1-5 μM) in WT SAN cells (cells with afterdepolarizations/total cells tested: 10/13) but not in Ser16Ala (0/6) and Thr17Ala (0/5) SAN cells ($p<0.01$ in both cases compared to WT). These results suggest that catecholamine induced EADs and DADs require phosphorylation of PLN Ser16 or Thr17 and highlight the importance of the intracellular Ca^{2+} cycling machinery for determining SAN cell vulnerability to adverse effects of catecholamine stimulation.

2894-Plat
Arrhythmogenic potential of activated fibroblasts
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Many cardiovascular disorders including ischemic heart disease and heart failure are associated with extensive fibrosis. A critical event in the development of cardiac fibrosis is the transformation of fibroblasts into an active fibroblast phenotype or myofibroblast. Fibroblasts isolated from healthy hearts and grown under standard tissue culture conditions start expressing the myofibroblast marker α-SMA 24-48 hours after isolation. These cells have been referred to as myofibroblasts. However, there is evidence indicating *in vitro* phenotypic changes due to culture conditions do not fully replicate the *in vivo* activation process.