a neutralization mutant D818N and a charge reversal D818R mutant within this site dramatically decreased Na^+ sensitivity. Thus, D818 is engaged in an important electrostatic interaction with Na^+. Similarly, the H823N mutant within this site also greatly decreased Na^+ sensitivity of Slack channels. Simulations of the Slack RCK2 domain based on the crystallized structure of a prokaryotic RCK domain structure (Jiang et al., 2001, Neuron 29:593) provided a model of the Na^+ coordination site in Slack channels. Moreover, simulations of the Na^+ coordination site in Slo2.2 channels predicted a 5~7 fold selectivity for Na^+ over Li^+ that were confirmed by electrophysiological data. Our results suggest that the Slack channel shares a similar Na^+ regulatory mechanism with Kir channels but with important differences, such as an intricate coupling mechanism to Cl^- co-regulation and possibly additional Na^+ sensitive sites.

2756-Pos

Oxidation of K^+ Channels Leads to Progressive Decline in Sensory Function during Ageing

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Reactive oxygen species (ROS) play an important role in the progressive neuronal function loss that is part of both the normal ageing process and neurodegenerative disease. A central question is whether voltage-gated K^+ (Kv) channels, which are key regulators of neuronal excitability, are physiological targets of ROS and whether these interactions have a role in the mechanisms underlying age-related neurodegeneration. Here, we show that oxidation of K^+ channel KVS-1 during ageing causes sensory function loss in *Caenorhabditis elegans,* and that protection of this channel from oxidation preserves neuronal function.- Thus, chemotaxis to biotin and lysine, a function controlled by KVS-1, was significantly impaired (70%) in normal or wild-type young worms exposed to chloramine-T (CHT) or hydrogen peroxide (H_2O_2), but only moderately affected (35%) in worms harboring an oxidation-reduction (redox)-resistant KVS-1 mutant (C113S). In ageing C113S worms, the effects of free radical accumulation were significantly attenuated (40% loss-of-function) compared to wild-type (75%). Electrophysiological analyses showed that both ROS accumulation during ageing, and acute exposure to oxidizing agents, acted primarily to modify native KVS-1 channels expressed in the ASER neuron (which mediates chemotaxis) and as a consequence altered the excitability of neurons harboring wild-type but not C113S KVS-1. Together, these findings establish a pivotal role for ROS-mediated oxidation of voltage-gated K^+ channels in sensorial decline during ageing.

2757-Pos

Regulation of the Cardiac I_{ks} Channel Complex by Ubiquitylation and De-Ubiquitylation

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KCNQ1 and its β-subunit KCNE1 form the delayed rectifier potassium current I_{Ks}, playing an important role in repolarisation of the cardiac tissue and in water and salt transport across epithelial tissues. In the heart I_{Ks} is partly responsible for terminating the cardiac action potential. Malfunctions in this channel can result in arrhythmias leading to cardiac arrest.

In heart physiology proper function and regulation of I_{Ks} current is essential. It has been reported that one of the mechanisms controlling the membrane density of KCNQ1 channels is mediated by ubiquitylation. I_{Ks} was shown to be down-regulated by Nedd4/Nedd4-like ubiquitin-protein ligases and this interaction was dependent on the PY-motif on the C-terminal of KCNQ1. Recently it was also discovered that epithelial sodium channel ENaC is regulated by the reverse process - de-ubiquitylation, mediated by an enzyme USP2 (ubiquitin-specific protease 2), which is one of the best described de-ubiquitylases. Therefore the aim of the work was to investigate whether a similar mechanism is valid for KCNQ1/E1 channel complex.

The effect of USP2-mediated de-ubiquitylation on I_{Ks} channel was investigated using electrophysiology and biochemistry. We observed that when KCNQ1/E1 was co-expressed with USP2-45 or USP2-69 isoform and Nedd4-2 in oocytes, USP2 counteracted the Nedd4-2-specific down-regulation of I_{Ks}. It resulted in a rescue of the current amplitude, which was then comparable to the one of I_{Ks} expressed alone. Biochemical studies of transfected HEK293 cells confirmed this observation as both total and surface expressed KCNQ1 protein was more abundant when co-expressed with USP2-45/-69 and Nedd4-2 as compared to Nedd4-2 alone. Co-immunoprecipitation assay suggested that USP2 can bind to KCNQ1 independently of the PY-motif and the presence of Nedd4-2.

These results point towards an interplay between ubiquitylating enzymes and de-ubiquitylases acting on I_{Ks} channel complex *in vitro*.

2758-Pos

Mechanism for Strict Regulation of Certain K+ Channels by Small, Fast Changes in Cell Volume

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A number of physiological processes, such as salt and water transport, neuronal activity, migration and apoptosis, involve changes in cell volume. The response of the cells to such challenges often is a regulatory volume decrease (RVD), a mechanism which involves activation of K^+ channels. However, so far it has not been entirely clear which types of K^+ channels should be considered sensitive to cell volume changes and, in particular, the mechanism for regulation has been obscure. To address this issue, we have co-expressed a number of K^+ channels with aquaporins in *Xenopus laevis* oocytes and subsequently induced changes in cell voume by exposure to hypo- or hypertonic media. In all cases, the results are very clear; some K^+ channels (e.g. KCNQ1 and 4, Kir4.1/5.1, Ca^{2+}-activated IK and SK) are strictly regulated by small, fast changes in cell volume (approx. 5 %), whereas others are not (e.g. KCNQ2/3, Slo1 (BK) and Slo2.2 (slack)). Most recently, we have shown that the high-conductance slick channel (Slo 2.1) is dramatically stimulated (to 196 % of control) by cell swelling and inhibited (to 44 % of control) by a decrease in cell volume. Our results show that the mechanism responsible for the strict regulation of certain K^+ channels by small, fast changes in cell volume, in some cases, involve the cytoskeleton.In contrast, cellular release of ATP is not involved, and the regulation is not mediated by membrane stretch. Our recent finding, that the high conductance slick channel is highly cell volume sensitive, will allow for further investigations at a single channel level.

2759-Pos

Fluorinated General Anesthetics Modulate Kv1.3 Potassium Channels and Interact With β-Amyloid Peptide: Is there a Link?

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There is growing evidence that, in some cases, commonly used general anesthetics may cause long-term molecular changes reminiscent of those observed in the Alzheimer's diseased brain. We investigated the effects of the anesthetic sevoflurane on the voltage-gated potassium channel Kv1.3. In the central nervous system, Kv1.3 channels are present in olfactory regions and in the dentate gyrus of the hippocampus, areas implicated in AD pathology. The expression of Kv1.3 is also up-regulated in activated microglia, suggesting its possible role in microglial response to β-amyloid peptide. Using whole-cell patch clamp recording from L929 cells stably expressing Kv1.3, we found that sevoflurane modulates biophysical properties of the Kv1.3 channel. At clinically relevant concentrations, sevoflurane biphasically alters peak current amplitude, irreversibly facilitating the current at lower voltages (EC50 ~ 1/2 MAC) and reversibly inhibiting it at higher voltages (IC50 ~ 1 MAC). The kinetics of the Kv1.3 current were also changed in a voltage- and dose-dependent manner. The time constants of both current activation and the slow C-type inactivation were significanly decreased, whereas current deactivation was slower at low voltages but faster at higher voltages in the presence of sevoflurane. Sevoflurane slightly increased the voltage sensitivity of Kv1.3 conductance at a clinically relevant dose. The effects of sevoflurane resemble the previously-reported effects of the exogenous β-amyloid oligomers on the same channel. Using ^{19}F NMR, we found that, in the test tube, sevoflurane interacts with β-amyloid peptide and forms stable complexes. Furthermore, dot blot immunochemistry revealed that sevoflurane appears to facilitate the rate of cytotoxic β-amyloid oligomer formation.Thus, modulation of Kv1.3 channels by sevoflurane and its interaction with β-amyloid peptide might both enhance the progression of Alzheimer's disease. *Supported by the Hillblom Foundation and NIH 1P01AG032131.*

2760-Pos

Modulation of Plant Slow Vacuolar (sv) Channel by Flavonoid Naringenin

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The Slow Vacuolar (SV) channel is one of the most extensively studied channel present in plant vacuoles. Features of the SV channel are the slow activation, outward rectification at elevated cytoplasmic Ca^{2+} concentration and selectivity for both monovalent and divalent cations. It's well known that SV currents recorded in a typical patch-clamp experiment require unphysiologically high cytosolic and low vacuolar calcium concentrations for full activation. We aim at identifying endogenous plant substances which

may modify the voltage activation threshold of this channel towards more physiological conditions. Flavonoid Naringenin (Nar) is present in all plant species where it plays a central role in the flavonoid biosynthetic pathway. Nar is stored in the vacuoles in glycosylated form. To confirm the presence of non-glycosylated Nar in the cytoplasm we isolated the gene encoding for Arabidopsis glycosyltransferase (AtGT) which glycosilates Nar. AtGT gene was cloned in fusion with yellow fluorescent protein (YFP) and was used for localization studies. When Naringenin was added to cytosolic bath solution, we recorded a dose-dependent reversible decrease in SV channel activity described by a half block concentration of 0.44 mM. Investigating Nar effects on the voltage dependence of the channel, we observed that the activation threshold of the SV channel is shifted towards more positive voltages and that Nar does not affect the single channel conductance. Investigating the effects of Nar at varying pH, we observed an increase in current inhibition with the decrease of the pH. When Naringin, the glycosylated form of Nar, was applied at the cytosolic or at the vacuolar side it did not modify the channel activity. We are currently investigating the role of the phospholipid composition of the membrane in this modulation.

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2761-Pos

Effects of Cannabinoids on Ion Channels of Pancreatic Beta Cells

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The function of the beta cell of the pancreatic islet is to sense minute changes in blood glucose levels and secrete insulin to maintain euglycemia. Though this secretion is governed chiefly by glucose, other chemical factors such as insulin itself and the incretin hormones also modulate this function. Peliminary evidence in our lab indicates that endocannabinoids are important negative regulators of insulin secretion. Beta cells are electrically excitable and undergo depolarization upon glucose stimulated insulin secretion resulting from closure of the K(ATP) channels. As a consequence, the calcium channels open, intracellular levels of calcium rise and exocytosis of the insulin secretory granules occurs. In addition to the K(ATP) channels and the calcium channels, beta cells also possess an array of ion channels that generate the complex electrical waveforms and participate to varying degrees in the regulation of the membrane potential.

Using rodent beta cells we investigated the effects of cannabinoids on some of these ion channels. Thus, 10 μM 2-arachidonylglycerol (2-AG) decreased the amplitude of the delayed rectifier current by about 40%. Simple washing did not reverse this blockade, and the addition of the cannabinoid receptor 1 (CB1) antagonist AM 251 (1 μ) also had no effect. However, the blockade was completely reversed by washing with lipid free bovine serum albumin. Moreover, the CB1 agonist, WIN-55,212-2(1 μM), an indole derivative structurally unrelated to the lipid 2-AG, had no antagonistic effect on the delayed rectifier. Taken together, these results suggest that the block was independent of the CB1 receptor and was mediated instead via the plasma membrane. Cannabinoid blockade of both sodium and HVA calcium currents were similar to the effects on the delayed rectifier.

2762-Pos

Effects of Small Molecule Kv1.3 and K(Ca)3.1 Inhibitors on T_{EM}-Cell Proliferation

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The homotetrameric forms of the voltage- or calcium-activated Kv1.3 and K(Ca)3.1 channels are the predominant potassium channels in T-lymphocytes and play an important role in membrane potential regulation of these cells. Since several years Kv1.3 has been suggested as a promising target for addressing autoimmune diseases because Kv1.3^{high}/K(Ca)3.1^{low}phenotype T-lymphocyte subsets (activated T_{EM} cells) are thought to play an important role in the mediation of the pathologic effects.

We developed several small-molecule compounds with Kv1.3 or K(Ca)3.1 inhibitory properties in the nanomolar range determined by manual patch-clamp experiments. To further test the efficacy of the compounds on a cellular level we tried to suppress T_{EM} subtype specific anti-CD3 induced proliferation of freshly isolated PBM, T, and T_{EM} cells (fraction of purified T and T_{EM} cells characterized by FACS detection of CD45RO and CCR7 antigens). Inhibition of proliferation was either essayed by exclusive application of 4SC or reference Kv1.3 inhibitors or by co-application in combination with a K(Ca)3.1 inhibitor. In all cases inhibition of Kv1.3 potassium channels alone did not or only weakly abolish cell proliferation. However, co-inhibition of Kv1.3 and K(Ca)3.1 channels widely suppressed proliferation to various degrees. Furthermore we observed that the degree of block seemed to be strongly dependent on the donor and/or the individual's immunological status. This potentially T-cell subset unspecific inhibition has recently been suggested as a novel strategy in preventing kidney allograft rejection (*Transplant. Proc.* (2009) **41**:2601-2606).

2763-Pos

Effects of PKC on Closed-State Inactivation in Kv4.3 Isoforms

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Kv4.3 is expressed as two isoforms, a short form and a long form, that has a 19 aa insertion downstream from S6 in the C terminus which has a putative PKC phosphorylation site at T504. To understand the role of PKC on modulation of closed-state inactivation (CSI), we expressed channels mutated in putative PKC phosphorylation sites and compared their response to PKC activation with PMA to the responses of WT Kv4.3 isoforms. PMA had similar effects on Kv4.3-S and Kv4.3-L open-state inactivation. However, PMA induced opposite effects on CSI in the two channel splice variants: the magnitude of CSI in Kv4.3-S was reduced, while there was an increase in CSI in Kv4.3-L, an effect was abolished by mutation of the long form T504 to alanine. To understand the structural basis of the reduction of CSI in Kv4.3-S, we constructed several mutants of putative PKC phosphorylation sites in the N terminus. Of these, the largest effect on PKC modulation of CSI occurred in the T53A mutant in both Kv4.3-S and Kv4.3-L; Kv4.3-S mutants lacking threonine at position 53 showed no or minimal response to PKC. These data show that isoform-specific modulation of CSI by PKC in Kv4.3 involves complex interactions of the cytoplasmic N and C termini of the channels.

2764-Pos

Ca^{2+}-Dependent PKC Facilitates Voltage-Dependent Activation of IKs Through Phosphorylation of An Isoform Specific Site on the KCNE1 Subunit

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Protein kinase C (PKC) regulates heart inotropy and chronotropy in both physiological and pathophysiological states. In human heart, at least 6 PKC isofoms are expressed, with the Ca^{2+} dependent isoforms (classic PKCs, cPKCs) being the most abundant. However, little is known about the effect of cPKC on heart rhythm and cardiac ion channel regulation. The slow delayed rectifier current (IKs) is one of the main currents responsible for cardiomyocyte repolarization. In this study, we investigated the regulation of human IKs regulation by cPKC.Human IKs channel (KCNQ1 and KCNE1) and α_1-adrenergic receptor were co-expressed in HEK293T cells. IKs was measured by conventional whole-cell and perforated patch-clamp techniques. The selective α_1-adrenergic agonist phenylephrine (30 μM) activated IKs by both shifting the voltage dependence of activation ($V_{1/2}$) to the left, ~ −20 mV, and increasing in the maximal conductance (Gmax), ~ 175%. Pretreatment with cell-permeable cPKC inhibitory peptide selectively blocked the agonist-induced voltage shift, but not the increase in Gmax. Application of a cell-permeable cPKC activator peptide mimicked the agonist-induced leftward shift in $V_{1/2}$, and showed no increase in Gmax. A mutation in a putative PKC phosphorylation site in the auxiliary subunit, KCNE1(S102A), abolished the cPKC-mediated voltage shift. Expression of the phosphorylation-mimicking mutant, KCNE1(S102E), produced channels that had a leftward shift in $V_{1/2}$ compared to KCNE1(S102A). Our data indicate that cPKC phosphorylation of KCNE1(S102) facilitates voltage-dependent activation of IKs . In addition, we showed that a mutation associated with Long QT type1 at the S4-S5 linker of KCNQ1 and associated with high cardiac risk, also abolished cPKC activation of this channel. Our results suggest that KCNE1(S102) phosphorylation is transduced through the KCNQ1(S4-S5) linker to modulate channel voltage sensing and thereby facilitate channel opening.

2765-Pos

Ancillary Subunits Regulate PKC Mediated Effects on Closed-State Inactivation of Kv4.3

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Kv4 channels are expressed with a variety of ancillary subunits in vivo. The most prominent of these proteins are the KChIPs, a family of cytoplasmic proteins that modulate channel gating and act as chaperones. We have previously shown that heterologously expressed Kv4.3 is regulated by PKC. After induction of PKC by PMA, current expression level is reduced. PKC also influences closed-state inactivation (CSI) in an isoform dependent manner; CSI is decreased upon PKC induction in Kv4.3-S, and increased in PKC-L. To understand the role of PKC on modulation of Kv4-based currents, we expressed channels in the presence of three KChIP2 isoforms and compared their responses to PKC. Two KChIP2 isoforms, KChIP2a and 2b, negated PKC influence on channel gating, kinetics, and expression levels in both Kv4.3-S and