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