Based on FEBS Lett 445:131; 459:367 and subsequent structure-activity analyses and mechanistic models of KATP channels (reviewed in J Mol Cell Cardiol 39:79), Babenko predicted three possible mechanisms of KATP hyperactivation by mutations in ABCC8 and started testing the hypothesis (DK077827 R01 project ABCC8/KCNJ11 Mechanisms and Diabetes). Following the analysis described in N Engl J Med 355:456 and J Biol Chem 283:8778, we established that the majority of CD mutations which map to the canonical ABC exporter core increase the open channel probability (Po) in intact cells by enhancing the MgATP/ADP-dependent stimulatory action of SUR1 on the channel, without affecting its maximal Po in the absence of nucleotides (Pomax) or its Mg-independent sensitivity to inhibitory ATP, IC50(ATP). This is called the A-type mechanism of KATP hyperactivation. The majority of CD mutations which map to the non-canonical, TMD0-L0 "gatekeeper" (J Biol Chem 278:41577) domain of SUR1 increase Pomax and apparent IC50 (ATP). Single-channel kinetics analysis reveals that this effect is due to reciprocal changes in the rates of channel transitions to and from its long-lived closed state with the lowest apparent Kd(ATP) for the inhibitory nucleotide. This establishes the second, or B-type, mechanism of KATP hyperactivity. Although our search for the third, Kd(ATP)-increasing type mutations in ABCC8 continues, we are in the position to conclude that A/B-mechanisms cause the majority of CD-KATP cases and our refined Sav1866/MsbA / KcsA/Kir3.1/BacKir/Chimera-based models of KATP channels help correctly predict the principal effect of the majority of diabetogenic mutations in ABCC8/KCNJ11 on KATP gating.

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Expression of ATP-Sensitive Potassium (K_{ATP}) Channel Subunits in Mammalian Liver

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ATP-sensitive potassium (K_{ATP}) channels are expressed in multiple tissues, where they provide a link between cellular metabolism and membrane excitability. They consist of two subunits: the inward rectifier potassium channel Kir6, which forms the pore, and the sulfonylurea receptor SUR, an ATP-Binding Cassette (ABC) transporter that functions as the regulatory subunit. Differential expression of Kir6 and SUR isotypes leads to formation of K_{ATP} channels with distinct electrophysiological and pharmacological properties, and a number of tissue-specific subunit combinations have been described. Although K_{ATP} channels in the liver remain largely unexplored, there is some evidence that K_{ATP} channels are expressed in hepatocytes. However, a rigorous characterization of the subunit composition of hepatocytic K_{ATP} channels is still lacking. In order to address this, we performed end-point RT-PCR for K_{ATP} channel subunit mRNA in extracts from mouse and rat liver, as well as from the human-derived hepatocyte cell line HepG2. Interestingly, a range of expression patterns was observed. In mouse liver, Kir6.1 and SUR2 were detected, while no signal was seen for Kir6.2 and SUR1 (n=3-5). In rat liver, signal was detected for all major subunits: Kir6.1, Kir6.2, SUR1, SUR2A, and SUR2B (n=3). In HepG2 cells, signal was detected for Kir6.1, Kir6.2, and SUR2B, but not for SUR1 or SUR2A (n=3). While detection of Kir6.1 and SUR2/SUR2B in mouse and rat liver may reflect vascular smooth muscle channels, the presence of these subunits in HepG2 cells indicates that they may in fact represent K_{ATP} channels expressed in hepatocytes. Additionally, expression of Kir6.2 in both rat liver and HepG2 cells is suggestive of a Kir6.2-containing hepatocytic K_{ATP} channel. Ultimately, correlation with electrophysiological and pharmacological experiments will be necessary to elucidate the subunit composition of hepatocytic K_{ATP} channels, as well as their physiological role.

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Effects of ATP-Dependent Potassium Channel Activator Flocalin Include Sodium and Calcium Channels Inhibition in Cardiomyocytes

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ATP-dependen potassium (K_{ATP}) channels are cellular metabolic sensors linking potassium transport to changes in concentration of nucleotide phosphates, lipids and glycolysis metabolites. Cardiac K_{ATP} channels mainly open under hypoxic and ischemic conditions and take part in decreasing intracellular calcium concentration and cardiac excitability.

Fluorine-containing analogue of pinacidil is an effective, nontoxic and stable hypotensive preparation that opens K_{ATP} channels (with EC_{50}=8.1 ± 0.4 μM on heterologously expressed cardiac channels). At submicromolar concentrations flocalin decreases cardiac excitability by hyperpolarizing plasma membrane, shortening action potential (AP) duration and decreasing rate of spontaneous contractions.

Here using patch clamp method we found that flocalin blocks sodium current of neonatal cardiomyocytes with IC_{50}=17.0 ± 1.6 μM and maximal block of 70 ± 18% and shifts stationary inactivation curve by −5.8 ± 1.8 mV. High-voltage actived calcium current is also inhibited with IC_{50}=21.7 ± 2.1 μM and maximal block of 52 ± 16% not changing inactivation properties and voltage dependency. Flocalin significantly changed intracellular calcium signal. 5 μM flocalin reduced amount of Ca^{2+} released during contraction, amplitude of $[Ca^{2+}]_i$ transients and rate by 2-3-fold and decreased resting calcium level. Described effects explain decrease of AP amplitude and upstroke velocity observed earlier. Inhibition of sodium currents was in part due to hyperpolarizing shift of inactivation curve. Block of sodium and calcium channels in addition to K_{ATP} opening accounts for complex effect of flocalin on cardiac activity reduction.

Ligand-gated Channels II

2345-Pos Board B331

TPC2 is a Novel NAADP-Sensitive Intracellular Ca^{2+}-Release Channel with Unique Gating Characteristics

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We have recently demonstrated that human TPC2 is an ion-channel equipped with the specialised gating and conduction properties required to fulfil the role of the lysosomal NAADP-sensitive Ca^{2+}-release channel (Pitt et al., 2010 *J.Biol.Chem.* In press). We now examine the mechanisms that underlie NAADP activation of TPC2 channels after reconstitution of purified, recombinant TPC2 into artificial bilayers. We find that TPC2 channels gate in three basic modes: 1. The constitutively-active state, 2. The NAADP-activated state and 3. The NAADP-inactivated state. In the constitutively-active state, TPC2 can open, albeit with very low Po, in the apparent absence of activating ligands. Reducing cytosolic or luminal $[Ca^{2+}]$ from 10μM to <1nM does not abolish channel openings. The NAADP-activated state is observed at low [NAADP] (<1μM) and in this state, the affinity and efficacy of NAADP are crucially dependent on luminal pH and $[Ca^{2+}]$ whereas cytosolic Ca^{2+} ($\leq$ 10 μM) does not increase Po. A unique characteristic of the NAADP-activated state is episodic gating where multiple channels open and close simultaneously in a synchronized manner. The NAADP-inactivated state is observed at high [NAADP], typically $\geq$1mM. In this state, constitutive channel openings are abolished and Po becomes zero indicating the presence of high affinity activation and lower affinity inactivation sites. An important finding of this study is that, unlike other major classes of Ca^{2+}-release channel (RyR and IP_3R), activation of TPC2 is not heavily dependent on a rise in cytosolic $[Ca^{2+}]$. We predict that the unique, episodic coupled gating behaviour of TPC2 plays an important role in enabling TPC2 to provide a flexible Ca^{2+}-release system capable of wide variations in the magnitude and time-dependence of Ca^{2+} fluxes from lysosomal stores.

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The K276E Startle Disease Mutation in the Glycine Receptor: Effects on Channel Gating

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Glycine receptors mediate inhibitory synaptic currents that are essential for motor control. Loss-of-function mutations in human glycine receptors cause hyperekplexia, a rare inherited disease associated with an exaggerated startle response. We have studied a human disease mutation in the M2-M3 loop of the glycine receptor alpha1 subunit (K276E) using direct fitting of mechanisms to single-channel recordings with the program HJCFIT. Whole-cell recordings from HEK 293 cells suggested that mutant receptors, both homomeric alpha1 and heteromeric alpha1beta, were much less sensitive to glycine than their wild-type counterparts. Single-channel recordings showed that homomeric alpha1 K276E receptors are barely active, even at 200 mM glycine. In contrast, heteromeric channels show brief bursts of openings at 300 μM glycine (~EC95 for wild-type) and reach a maximum one-channel open probability of about 45% at 100 mM glycine (cf. 96% for wild-type). Distributions of apparent open times contained more than one component at high concentrations, and so mechanisms that allowed only one open state in the fully liganded receptor were extremely poor at describing the observed single-channel behaviour. Instead, mechanisms in which opening can also occur from more than one fully liganded intermediate state (e.g. 'primed' models) gave a much better description of the data. Brief pulses of glycine (0.5-2 ms, 30 mM) applied to outside-out patches activated currents with a slower rise time (1.3 ms) than wild-type channels (0.2 ms), and a much faster decay. These features of the macroscopic currents were well predicted by the mechanisms obtained from fitting single-channel data. Our results show that the mutation K276E impairs the gating of the receptor, demonstrate an unforeseen role for the beta subunit in promoting channel opening, and give further evidence of reaction intermediates in the activation pathway.