K\textsubscript{ATP} channels are hetero-octamers composed of two subunits; a pore forming Kir6 and a sulfonylurea receptor (SUR). SUR subunits regulate K\textsubscript{ATP} channel gating allosterically, in response to nucleotides and pharmacological agents.

We have previously shown that residue D232 of Kir6.2 is central to interaction with the nucleotide-binding fold 2 of SUR2A (Rubaiy et al, Biophysical J. 2011: 100; P432a). The aim of this study was to assess whether E332, the corresponding residue in Kir6.1, plays an equally important role in Kir6.1/SUR2A complexes. Wild type Kir6.1/SUR2A channels expressed in HEK293 cells required activation by potassium channel opener pinacidil (EC\textsubscript{50} = 43.90 ± 1.28 μM), in the presence of UDP (10 mM) before passing current. Introduction of the single point mutation E332K into full length Kir6.1 caused constitutive opening of Kir6.1/SUR2A channels in the absence of pinacidil. Reinstatement of putative inter-subunit salt bridges by expressing Kir6.1-E332K with charge reversal mutants SUR2A-Q1336E or SUR2A-K1322D restored regulated opening. Channels containing the Kir6.1E332K mutant were also insensitive to block by high concentrations of glibenclamide (100 μM). However, co-expression of Kir6.1-E332K with SUR2A-K1322D restored glibenclamide sensitivity to wild type levels (EC\textsubscript{50} = 9.12 ± 1.12 nM, p < 0.15 versus wild type Kir6.1/SUR2A channel (IC\textsubscript{50} = 6.14 ± 1.13 nM). Together, these data suggest a key functional role for inter-subunit salt bridges involving Kir6.1-E332K. Constitutive channel opening on mutation of this residue suggests that Kir6.1E332 and salt bridge(s) formed between it and the SUR subunits are crucial for stabilizing closed states of Kir6.1-containing K\textsubscript{ATP} channels.

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A Cytoplasmic Inter-Subunit Salt Bridge, Kir6.1R347E/SUR2A1318, Contributes to Allosteric Information Transmission in Kir6.1/SUR2A4 Channel Complexes

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K\textsubscript{ATP} channels are hetero-octamers composed of pore forming Kir (Kir6.1 or Kir6.2) and a regulatory sulfonylurea receptor (SUR1, SUR2A or SUR2B) subunits. Previous studies have revealed a salt bridge between Kir6.2K338 and SUR2A1318 that is involved in inter-subunit transmission of allosteric information (Rubaiy et al, Biophysical J. 2011: 100; P432a). The aim of this study was to assess whether Kir6.1R347, which corresponds to Kir6.2K338, plays a similar role in Kir6.1-containing channels.

Whole-cell patch clamp recording was employed to assess channel sensitivity to pinacidil and glibenclamide following mutation of single residues or reinstatement of the proposed salt bridge by paired charge reversals in full length Kir6.1/SUR2A channel subunits after heterologous expression in HEK293 cells. A single point mutation, Kir6.1R347E expressed with wild type SUR2A was sufficient (p < 0.002) for induction of sensitivity to activation by pinacidil of Kir6.1347E/SUR2A2WT channels (EC\textsubscript{50} = 0.71 ± 1.21 μM) versus wild type Kir6.1/SUR2A channels (EC\textsubscript{50} = 43.90 ± 1.28 μM). Reinstatement of the cytoplasmic electrostatic interaction in the Kir6.1347E/SUR2A1318 subunit combination reversed the sensitivity to pinacidil to near wild type (EC\textsubscript{50} = 23.5 ± 1.3 μM, p < 0.028). Furthermore, glibenclamide sensitivity was reduced significantly in the Kir6.1347E/SUR2A2WT channel (IC\textsubscript{50} = 241 ± 1.09 nM, p < 0.015) and restored in Kir6.1347E/SUR2A1318 (IC\textsubscript{50} = 13.75 ± 1.11 nM, p < 0.080) versus wild type Kir6.1/SUR2A channel (IC\textsubscript{50} = 6.14 ± 1.13 nM). These data indicate that, like Kir6.2K338, Kir6.1R347 makes a crucial contribution to allosteric information transmission from SUR2A to the channel pore through inter-subunit salt bridge formation with SUR2A1318.

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Use of Resonance-Wavelength Grating Optical Biosensors to Detect Channel-Protein Interaction in Slack K\textsubscript{ATP} Channels

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Slack protein is fancy (MMPSI), a rare epileptic encephalopathy of infancy that combines pharmacoresistant seizures with severe developmental delay. Slack protein is known to interact with a variety of cytoplasmic signaling molecules. Using resonance wavelength grating optical biosensors (the SRU Biosciences BIND system), we have detected that pharmacological activation of Slack channels by bithionol produces a sustained decrease in mass distribution close to the plasma membrane, and that phosphorylation of Slack channels mimics this decrease in mass. The very C-terminal domain of Slack has been previously shown necessary for channel-protein interactions, and deletion of this region abolished the observed signal. To determine which proteins or signaling molecules are translocating from the plasma membrane upon channel activation, an RNAi screen against probable channel binding partners was performed, and the Protein Phosphatase 1 (PP1) targeting protein Phactr1 was found to be necessary for this decrease in mass. We hypothesize that activation of Slack by either bithionol or phosphorylation leads to the dissociation of Phactr1 with PP1 from the channel complex, allowing the Slack channel to remain in its phosphorylated and active state. Activation of PKC does not result in a decrease in mass in the human MMPSI mutants, possibly linking channel excitability to downstream signaling mechanisms which may result in developmental delay.

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Biogenesis of the Sodium-Activated Potassium Channel Slack-B is Controlled by the Dephosphorylation of N-Terminal Serines


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Slack-B channels are expressed predominantly in brainstem regions and the olfactory bulb. Using liquid chromatography tandem mass spectrometry, we determined that two Ser residues (S34 and S44) close to the N-terminus of Slack-B are basally phosphorylated. To test the biological role of these sites we performed site-directed mutagenesis, creating mutations that mimic either phosphorylation (S->E) or dephosphorylation (S->A) at each or both site. We found that correlated with a 20-fold increase in whole-cell currents, levels of the nonphosphorylated mutant channel are greatly increased in cRNA injected mouse oocytes as compared to wild type (WT) Slack. Next, comparing the rate of protein accumulation in oocytes injected with equal amounts of WT or mutant-encoding cRNA, we confirmed that the initial rate of protein accumulation is significantly increased for the nonphosphorylated channel. Additionally, the time constant for protein accumulation was much faster for nonphosphorylated channel, becoming saturated within two days. In contrast, levels of the WT channel accumulated with linear kinetics. We observed similar changes in Slack-B channel levels in HEK293 cells transiently transfected with bicistronic vectors carrying DsRed (transfected cell indicator) and WT or mutant Slack-BI. To rule out the possibility that changes in current result from effects of the mutations on electrophysiologic parameters, we performed both macroscopic and single channel evaluation of each of the mutants. We found no significant differences between the WT and mutant Slack-BI records. Taken together, the results indicate that dephosphorylation of these sites in nascent peptide chains is a required step for permitting the translation of Slack mRNA to go to completion during channel synthesis. Our findings also suggest that regulation of the phosphorylation state of S34 and S44 may allow neurons to alter channel abundance rapidly in response to stimulation.

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Phospholipase C is Required for G\textsubscript{q}Q Protein-Coupled Receptor Mediated Task Channel Inhibition

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TWIK-related acid sensitive K\textsuperscript{+} (TASK) channels belong to the K2P channel family and contribute significantly to the background conductance in various cell types, e.g. to IK\textsubscript{SO} in cerebellar granule cells. It is known that stimulation of G\textsubscript{q} protein coupled receptors (G\textsubscript{q}PCRs) causes strong and reversible inhibition of TASK channels. Yet, the underlying signaling cascade is still controversial: Both, the inhibition of TASK by G\textsubscript{q}PCRs and activation of PLC\textsubscript{y} by G\textsubscript{q}PCRs has been shown necessary for channel-protein interactions, and deletion of the PLC\textsubscript{y} gene: Both, the inhibition of TASK by G\textsubscript{q}PCRs and activation of PLC\textsubscript{y} by G\textsubscript{q}PCRs has been shown necessary for channel-protein interactions, and deletion of the PLC\textsubscript{y} gene. While the PLC inhibitor U73122 blocks the inhibition of TASK channels by G\textsubscript{q}PCRs, we found that correlated with a 20-fold increase in whole-cell currents, levels of the nonphosphorylated mutant channel are greatly increased in cRNA injected mouse oocytes as compared to wild type (WT) Slack. Next, comparing the rate of protein accumulation in oocytes injected with equal amounts of WT or mutant-encoding cRNA, we confirmed that the initial rate of protein accumulation is significantly increased for the nonphosphorylated channel. Additionally, the time constant for protein accumulation was much faster for nonphosphorylated channel, becoming saturated within two days. In contrast, levels of the WT channel accumulated with linear kinetics. We observed similar changes in Slack-B channel levels in HEK293 cells transiently transfected with bicistronic vectors carrying DsRed (transfected cell indicator) and WT or mutant Slack-BI. To rule out the possibility that changes in current result from effects of the mutations on electrophysiologic parameters, we performed both macroscopic and single channel evaluation of each of the mutants. We found no significant differences between the WT and mutant Slack-BI records. Taken together, the results indicate that dephosphorylation of these sites in nascent peptide chains is a required step for permitting the translation of Slack mRNA to go to completion during channel synthesis. Our findings also suggest that regulation of the phosphorylation state of S34 and S44 may allow neurons to alter channel abundance rapidly in response to stimulation.