2176-Pos Site 4 of the selectivity filter controls the increase in conductance by external K+ in Hyperpolarization Cyclic Nucleotide-gated (HCN) channels

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HCN channels encode for the hyperpolarization-activated current, I_f, which is responsible for generating rhythmic activity in neurons and the heart. The whole cell conductance of HCN channels is enhanced by increasing concentrations of $^{[K+]}o$ in the physiological range, but the biophysical and molecular mechanisms underlying this increase are not known. The selectivity filter of HCN channels contains CXGYG instead of TXGYG motif found in most K+-selective channels. Thus, the HCN selectivity filter likely contains four binding sites, as for K+ selective channels, but the fourth intracellular binding site is formed by cysteine rather than threonine. We converted the cysteine to threonine of mouse HCN2 (HCN2 C400T), expressed both wildtype and mutant channels in CHO cells and measured I_f using the whole cell patch clamp technique. We found that, in the mutant channel, increasing $[K+]_o$ no longer enhanced whole cell conductance. However, lowering $[K+]_i$ restored the large increase in whole cell conductance in high $[K+]_o$ solutions for the mutant channel. These data suggest that the occupancy of the fourth intracellular binding site by potassium inhibits the increase in conductance produced by increases in $[K+]_o$.

*This work was supported by the Canadian Institutes of Health Research, the Michael Smith Foundation for Health Research and the Heart and Stroke Foundation of Canada.

2177-Pos Knowledge-based Structural Models of Hyperpolarization-activated Cyclic Nucleotide-gated Channels

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Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels are cation selective channels having a wide range of physiological functions, but especially important in regulating cardiac and neuronal pacemaker activity. HCN channels share common structural features with the voltage-activated sodium and potassium channels: six transmembrane segments organized in a "Voltage-Sensing" and "Pore-Forming" domain. However, their behavior is significantly different: HCN channels are opened by membrane hyperpolarization, not depolarization. In addition, activation of HCN channels is modulated by the binding of cyclic adenosine monophosphate (cAMP) to a cytoplasmic cyclic nucleotide binding domain (CNBD). The mechanism of activation is still unclear for HCN channels, despite extensive electrophysiological and biochemical data on the transmembrane domain and x-ray structure of CNBD. Our goal is to further the understanding of HCN functional mechanism by building accurate structural models containing both the transmembrane and cytoplasmic domains. This is a challenging task for several reasons: low resolution of available templates (voltage-gated potassium channels KvAP and Kv1.2), low level of target-template sequence similarity, longer loops between transmembrane segments. Consequently, in building the models we used additional constraints from published experimental work, prediction of secondary structure and transmembrane segments and profiles of multiple sequence alignments. Although our models are consistent with a helical screw activation mechanism of HCN channels, previously proposed for other voltage-activated channels, we suggest alternative pathways for modulation by intracellular factors.

Channel Regulation & Modulation

2178-Pos Regulation Of The Voltage-gated K^+ Channels KCNQ2/3 And KCNQ3/5 By The Serum- And Glucocorticoid-regulated Kinase, SGK-1

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KCNQ2/3 and KCNQ3/5 K^+ channels regulate neuronal excitability. We have shown previously that KCNQ2/3/5-channels are regulated by the ubiquitin ligase Nedd4-2 (1). SGK-1 is a serum/glucocorticoid-induced kinase which plays an important role in the regulation of epithelial ion transport. The consensus phosphorylation motif for protein recognition for SGK-1 is R-X-R-X-X-(S/T). The KCNQ3-subunit contains such a motif in its N-terminus. In this study we investigated SGK-1 regulation of KCNQ-channels and if the mechanism phosphorylates the KCNQ3 subunit directly or Nedd4-2. Capped RNA transcripts were synthesized for the different plasmids and *Xenopus* oocytes were injected with cRNAs. The membrane currents were recorded using the two-electrode voltage clamp technique. Cell surface expression levels were determined using hemagglutinin (HA)-tagged KCNQ2 RNA (2). SGK-1 significantly up-regulated the K^+ current amplitude of KCNQ2/3 ~1.4 times and KCNQ3/5 ~1.8 times, whereas the kinase dead SGK-1 mutant did not alter the current amplitude. The cell surface levels of KCNQ2-HA/3 were also increased by SGK-1. Deletion of the KCNQ3 C-terminal in the presence of SGK-1 did not affect the amplitude of KCNQ2/3/5-mediated currents. Co- injection of Nedd4-2 and SGK-1 with either KCNQ2/3 or KCNQ3/5 did not significantly alter the current amplitude. Only the Nedd4-2-mutant [S448A] exhibited a significant down-regulation of the KCNQ2/3/5 current amplitudes. In addition, mutation of the SGK-1 binding motif (RXRXXS) on the KCNQ3 subunit [S20A] appears to abolish the effect of SGK-1 on the KCNQ2/3 current amplitude. Taken together, these results demonstrate two potential mechanisms for the regulation of KCNQ2/3 and KCNQ3/5 channels by SGK-1. One by

direct phosphorylation of KCNQ channels and the other by regulating the activity of the ubiquitin ligase Nedd4-2.

References

1. Ekberg et al. (2007) *J. Biol. Chem. 282*:12135–12142.
2. Zerangue et al. (1999) *Neuron. 22*:537–548.

2179-Pos Pkc And Alpha-adrenergic Regulation Of Herg Channel Processing

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HERG potassium channel dysfunction is implicated in acquired as well as hereditary cardiac arrhythmias. Altered PCK signaling has been implicated as contributory to a variety of chronic cardiovascular disease states. Acute PKC activation (seconds to minutes) has been reported to have suppressive effects on HERG channel gating and amplitude. We have begun to examine the effects on HERG from more chronic stimulation of PKC. Activation of PKC with low nanomolar concentrations of β-phorbol 12-myristate,13-acetate (PMA) enhanced the channel protein abundance 4-5-fold in stably transfected HEK cells. The effect was time- and dose-dependent with changes apparent between 2–4 hours and was prevented by treatment with PKC inhibitors. HERG current density was also augmented in a comparable time course. The PKC-dependent augmentation of channel protein was markedly reduced when HERG harboring alanine substitutions in 17 Prosite-recognized PKC consensus sites was examined, suggesting that direct phosphorylation of the channel is involved. When a variant HERG containing alanine substitutions in the 4 PKA consensus sites was examined PKC was able to augment channel protein. The effect appears to be specific to HERG K channels in that PKC did not significantly alter Kv1.5 or KvLQT1 abundance. Stimulation of α1-adrenergic receptor also increased the HERG protein in a PKC-dependent fashion. Metabolic labeling indicated that the enhancement of HERG protein was due to a combination of increased translation rates and decreased channel degradation. Thus, α-adrenergic signaling through PKC must be considered as a regulator of HERG channels both chronically as well as acutely.

2180-Pos Extracellular Tryptophane (w454) And Proline (p455) Residues Involved In The Regulation Of Enac By Serine-proteases And Sodium Self-inhibition

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The epithelial sodium channel (ENaC) is a key component of the transepithelial Na+ transport. It is composed of three homologous subunits (α, β and γ). The channel has a large extracellular domain of as yet unclear function; a number of extracellular factors have been shown to modulate ENaC activity, including extracellular Na+ itself (a phenomenon named self-inhibition) and serine proteases (Channel Activating Proteases: CAPs, prostasin, furin and elastase). The molecular mechanism of these regulations is still an open question. It has been shown that the α and γ (but not β) ENaC subunits are direct substrates for furin and are cleaved at specific sites in their extracellular domains. Using site-specific mutagenesis of channel subunits and two-electrode voltage-clamp technique (TEVC), we determined that the WPS domain, a conserved region of the terminal extracellular loop of ENaC, is involved in the regulation of this channel by proteases and extracellular Na+. Substitution by alaline of the residues αW-454 or αP-455 abolishes the effect of 5 μg/ml trypsin on the amiloride sensitive current in *Xenopus* oocyte expressing ENaC. Substitution of α W-454 by R, E, S or K has a similar effect. Substitution W454R in β subunit decreases the response to trypsine of 54%. However, this mutation in γ subunit had no effect. In addition, this substitution in α subunit, but not in γ abolishes the self inhibition. Our results suggest that trypsin activates ENaC by relieving Na+ self-inhibition.

2181-Pos ACTH and cAMP Inhibit bTREK-1 K+ Channels Through A PKA Independent Mechanism

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Bovine adrenal zona fasciculate (AZF) cells express the two-pore/four transmembrane segment (2P/4TMS) bTREK-1 K^+ channels. These channels set the resting membrane potential and link adrenocorticotropic hormone (ACTH) receptor activation to membrane depolarization and cortisol secretion. Whole cell patch clamp recording showed that protein kinase A (PKA) inhibitors, PKI (6–22) amide and H-89 failed to reduce the inhibition of bTREK-1 by both ACTH and forskolin. An ACTH derivative, *O*-nitrophenyl sulfenyl-ACTH inhibited bTREK-1 at concentrations which produced little or no activation of PKA. EPAC2 (a guanine nucleotide exchange factor activated by cAMP) is highly expressed in AZF cells and can be selectively activated by 8-CPT-2′-O-Me-cAMP. When patch clamp recordings were made with pipette solutions that contained 8-CPT-2′-O-Me-cAMP, bTREK-1 was inhibited with an IC_{50} of 0.63 micromolar, a concentration which produced no activation of PKA in these cells. The inhibition of bTREK-1 by 8-CPT-2′-O-Me-cAMP required ATP hydrolysis and was unaffected by PKA inhibitors at concentrations that completely blocked PKA activation. 8-CPT-2′-O-Me-cAMP failed to inhibit bTREK-1 channels expressed in HEK 293 cells which do not express EPAC2. These results indicate that ACTH inhibits bTREK-1 K^+ channels through cAMP by a PKA independent pathway which may involve activation of EPAC2.

2182-Pos AKAP150, A Switch To Convert Mechano-, pH- And Arachidonic Acid-sensitive TREK K^+ Channels Into Open Leak Channels

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TREK channels are unique among two-pore-domain K^+ channels. They are activated by polyunsaturated fatty acids (PUFAs) including arachidonic acid (AA), phospholipids, mechanical stretch and intracellular acidification. They are inhibited by neurotransmitters and hormones. TREK-1 Knock-Out mice have impaired PUFA-mediated neuroprotection to ischemia, reduced sensitivity to volatile anaesthetics, altered perception of pain and a depression-resistant phenotype. Here, we show that the A-Kinase Anchoring Protein AKAP150 is a constituent of native TREK-1 channels. Its binding to a key regulatory domain of TREK-1 transforms low activity outwardly-rectifying currents into robust leak conductances insensitive to AA, stretch and acidification. Inhibition of the TREK-1/AKAP150 complex by Gs-coupled receptors such as serotonin 5HT4sR and noradrenaline β2AR is as extensive as for TREK-1 alone, but is faster. Inhibition of TREK-1/AKAP150 by Gq-coupled receptors such as serotonin 5HT2bR and glutamate mGluR5 is much reduced when compared to TREK-1 alone. The association of AKAP150 with TREK channels integrates them into a postsynaptic scaffold where both G protein-coupled membrane receptors (as demonstrated here for β2AR) and TREK-1 dock simultaneously.

2183-Pos Positive and Negative Modulation of SK3 Channels by CyPPA and NS8593 are Mediated by Different Regions

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The pores of small conductance Ca^{2+}-activated K^+ channels (SK1–3) are specifically blocked from the outside by apamin and the amino acids responsible for binding have been described (Ishii et al. JBC, 1997; Nolting et al., JBC, 2006). The three SK channels exhibits identical Ca^{2+}-sensitivity mediated by calmodulin (CaM) constitutively bound to a CaM binding domain (CaMBD) in the C-terminal. Positive gating modifiers of SK channels shift the Ca^{2+}-sensitivity towards lower $[Ca^{2+}]_i$ and we recently reported the first negative gating modifier, NS8593, that in anology shifts the Ca^{2+}-sensitivity towards higher $[Ca^{2+}]_i$. This compound, unlike the previously described blockers, did not prevent apamin binding (Strøbæk et al., Mol. Pharmacol., 2006). In line with this, we here show that SK3 channels containing point mutations (Q492A, D494A) that make the channels insensitive to apamin (100 nM), are still sensitive to NS8593 (K_d = 256±54 nM compared to 116±61 nM for transiently transfected wt SK3), when expressed in HEK293 cells and recorded in the whole-cell configuration. NS8593 inhibits all SK channel subtypes, but not the related IK channel ($K_d > 10$ μM). In order to define regions important for the negative modulation by NS8593, chimeric SK3/IK channels were constructed. We initially focused on the C-terminal that is important for the effect of the positive modulators 1- EBIO (IK/SK activator) and CyPPA (SK3/SK2 activator). However, a chimeric construct, SK3-$IK_{C\text{-}term}$ was still sensitive to NS8593 ($K_d = 350 \pm 20$ nM). This chimera was sensitive to the IK/SK activator NS309 (30 nM), whereas it was insensitive to 100 nM CyPPA. In conclusion, the effect of the negative gating modulator NS8593 is not dependent on the calmodulin binding region in the C-terminal, as is the case for the positive modulators.

2184-Pos Comparison of a Small Molecule ASIC3 Modulator to the Peptide FMRFamide

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The acid-sensing ion channel subtype 3 (ASIC3) provides a major detector of extracellular acidification in mammalian sensory neurons. Following activation by protons, ASIC3 desensitizes <2s. The invertebrate neuropeptide FMRFamide slows ASIC3 desensitization and results in a steady state current that lasts for many seconds. We present data that shows a non-peptide small molecule (Cmpd1, MW ~350kD) has similar modulatory activity on both rat and human ASIC3 isoforms. At 50 μM, Cmpd1 and FMRFamide produced a sustained acid-evoked current that was 40% and 28% of the peak current for hASIC3, respectively. The non-selective ASIC antagonist amiloride blocked peak and sustained components of Cmpd1- and FMRFamide-modulated ASIC3 current to a similar extent (85% and 87% inhibition at 30μM, respectively). Modulation by Cmpd1 was unaffected by changes in voltage potential. As observed for FMRFamide, modulation by Cmpd1 depended on the pH at which it was applied and only occurred when it was applied at pH7.3. The sustained current was unaffected by removal of Cmpd1 under acidic conditions. Reversal of modulation by Cmpd1 required a return to neutral pH. The actions of Cmpd1 on ASIC3 were rapid; full modulation occurred with <1s preincubation. The modulatory effect of Cmpd1 was selective for ASIC3; it did not promote a sustained current on ASIC1. Instead Cmpd1, but not FMRFamide, was a potent antagonist of ASIC1 current (290 nM). This study suggests a mechanistic link between the actions of FMRFamide and Cmpd1. Further, Cmpd1 may have stimulatory or inhibitory effects on excitability to acid, depending on the relative expression of ASIC1 and ASIC3 isoforms in individual sensory neurons.

2185-Pos Effects Of An Insecticidal Extract Of Fischerecha Sp On Neuroblastoma Cells

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Prokariotic cyanobacteria have become a major source of bioactive and toxic metabolites. Recently, it has been published that the biofilm-forming cyanobacterium Fischerella sp. exerts insecticidal activity against Chironomus sp. This effect was attributed to a fraction of four indole derivatives. Basing on the insecticidal activity, we tested the toxic extracts on excitable mammmalian cells, using a human neuroblastoma cell line (BE(2)-M17) and different fluorescent dyes: AlamarBlue to monitor cytotoxicity, Fura-2 to evaluate variations in cytosolic calcium levels, and Bis-oxonol in order to detect changes on membrane potential. Data showed that the extract did not induce cellular death in neuroblastoma cells, even when incubated 72h. With regard to the ions flow, any signs of depolarization nor calcium influx were observed on the cells. Neverthless, results indicate that the indole derivatives could be acting as neurotoxins modulating ion channels and suggesting that its toxicity may be related to ionic-dependent disturbances of the excitable membranes.

2186-Pos Expression And Regulation Of M-type K^+ Channels In Visceral Sensory Neurons

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Board B301

Nodose Ganglia (NG) neurons are critical in relaying information from peripheral sensory receptors to the CNS. Both myelinated (A-type) and unmyelinated (C-type) NG neurons were found to express M-type (Kv7) channels. Under perforated-patch clamp, M-current densities (at −60 mV) of A-type and C-type neurons were 1.2 ± 0.2 (n=7) and 0.95 ± 0.11 pA/pF (n=12), with time constants of deactivation of 65 ± 12 and 51 ± 8 ms, respectively. Immunostaining revealed most NG neurons to express angiotensin II (AngII) AT_1 and purinergic P_2Y_1 or P_2Y_6 receptors. AngII (500 nM), ADP-β-s (10 μM) and UTP (10 μM) suppressed M-current amplitude (I_M) in subsets of neurons. Of 16 cells, AngII suppressed I_M in 7 neurons by 42 ± 9% and ADP-β s in 6 neurons by 29 ± 8%. UTP suppressed I_M in 6 of 9 cells by 43 ± 9%. To investigate involvement of PIP_2 hydrolysis, we transfected neurons with the PLCδ-PH-EGFP probe and monitored agonist-induced translocations from membrane to cytoplasm. AngII increased cytosolic fluorescence by 46 ± 30% in 4 of 17 neurons, ADP-β-s by 18 ± 6% in 2 of 13 neurons, and UTP by 36 ± 13% in 7 of 14 neurons. Ca^{2+} imaging was performed to test whether stimulation of AT_1 or P_2Y receptors in NG neurons cause $Ca^{2+}{}_i$ rises. We used Ca^{2+}-free external solution to prevent Ca^{2+} influx through voltage-gated Ca^{2+} channels. Of 40 cells challenged with AngII and UTP, 9 and 12 neurons responded with substantial $[Ca^{2+}]i$ increases, with 340/380 ratio increases of 0.15 ± 0.01 and 0.10 ± 0.02, respectively. ADP-β-s caused no $Ca^{2+}i$ rises in 28 neurons studied. Thus, AT_1 and P_2Y_6 receptors modulate I_M in NG neurons and induce IP_3- mediated $[Ca^{2+}]_i$ signals, but the P_2Y_1-receptor action cannot involve $Ca^{2+}{}_i$ signals.

2187-Pos Receptor Chaperones At The Focal Contact Between ER And Mitochondrion: Sigma-1 Receptors Regulate Ca^{2+} Signaling And Cell Survival

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The endoplasmic reticulum (ER) and mitochondria are apposed at close contacts referred to as mitochondrion-associated ER membrane (MAM). The ER directly supplies Ca^{2+} into mitochondria *via* 1,4,5-trisphosphate receptors (IP3R). Mitochondrial Ca^{2+} subsequently activates key enzymes of the TCA cycle, thus regulating bioenergetics and cellular survival. IP3Rs degrade through multiple pathways. Newly synthesized IP3Rs, whose folding processes involve a transient association with calnexin, undergo relatively rapid degradation. On the other hand, channel-forming tetramers of mature IP3Rs have considerably long half-life. However, IP3 binding to the IP3 channel leads to a rapid ubiquitination and degradation of IP3Rs *via* the ER-associated degradation (ERAD). ER lumenal Ca^{2+} has been implicated in ERAD of IP3Rs. However, exactly how the ER lumenal Ca^{2+} regulates the stability of IP3Rs and roles of ER chaperones in this regard has never been examined. We find that the ER protein sigma-1 receptor (Sig-1R), which is implicated in neuroprotection, carcinogenesis, and neuroplasticity, is a novel Ca^{2+}-sensitive and ligand-operated receptor chaperone stabilizing IP3Rs at MAM. Normally, Sig-1Rs form a complex at MAM with another chaperone, BiP. Upon ER Ca^{2+} depletion or *via* ligand stimulation, Sig-1Rs dissociate from BiP, switching from dormancy into active chaperones thus chaperoning IP3Rs at MAM. Sig-1Rs prevent ubiquitination and degradation of IP3Rs, leading to a prolonged Ca^{2+} signaling into mitochondria. The dissociation of Sig-1Rs form BiP is regulated by synthetic compounds in an agonist-antagonist fashion. Increasing Sig-1Rs in cells counteracts ER stress response whereas decreasing them enhances apoptosis. Thus, Sig-1R chaperones, by sensing ER Ca^{2+} concentrations, regulate ER-mitochondrial interorganellar Ca^{2+} signaling and cell survival. Since Sig-1Rs ligands prevent neurodegeneration induced by stroke and beta-amyloid, ligand-operated receptor chaperones may open a new avenue for therapeutic opportunities.

(Supported by IRP/NIDA/NIH/DHHS).

2188-Pos Toxic Beta Amyloid Peptide Rapidly Suppresses Kv1.1 Channel Activity

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Board B303

Amyloid Beta (Aβ 1-42) is a 42 amino acid peptide that has been implicated in the pathogenesis of Alzheimer's Disease (AD). Previous studies indicate that Aβ 1-42 affects diverse potassium currents and may represent a very early step in promoting neurotoxicity. Kv1.1 is a voltage dependent K^+ channel responsible for repolarization of action potentials in many mammalian neurons, including those affected by AD. Here, we demonstrate that Aβ 1-42 suppresses Kv1.1 currents within 15 min of exposure. We expressed homomeric α-subunit Kv1.1 channels in *Xenopus* oocytes and used two-electrode voltage clamp to monitor macroscopic current and oocyte membrane capacitance. Exposure to a 1 μM bath application of Aβ 1-42 produced an approximate 50% decrease in Kv1.1 current after 30 min, with no change in voltage dependency nor with any indication of use-dependent pore-block. Solvent and 1μM Aβ 40-1 control peptide experiments produced little change (≤10%) in Kv1.1 current over the same time. Intracellular ionophoresis of Aβ 1-42 also produced a strong (~60%) decrease in Kv1.1 current. We have previously studied calcium-mediated suppression of Kv1.1 currents in oocytes by a pathway involving tyrosine phosphorylation, PKC-, and calcineurin-activation. Suppression of Kv1.1 activity through this pathway appears to depend largely upon endocytosis of Kv1.1 channels, which is accompanied by robust increases in capacitance. However, no significant changes in capacitance were observed in the present experiments despite Aβ 1-42's clear reductions in Kv1.1 macroscopic current. Suppression of Kv1.1 by Aβ 1-42 was also evidently not dependent on Ca^{2+}-influx since bath application in the presence of Ca^{2+}-free media still produced strong suppression of K^+ currents (>50%). However, suppression was partially (~50%) dependent upon intracellular Ca^{2+}-, as shown by partial abrogation by BAPTA-AM. Our results suggest that Aβ 1-42 suppresses Kv1.1 activity by interfering with voltage-dependent activation.

2189-Pos Reduced Calmodulin Expression Accelerates Transient Outward Potassium Current Inactivation In Diabetic Rat Heart

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In myocytes isolated from diabetic heart, the reduction in the amplitude of the transient outward potassium current (I_{to}) as well as the acceleration of its inactivation contribute to the action potential duration (APD) lengthening. Whereas the reduction in amplitude is attributable to a reduced support of trophic factors, the mechanism underlying the acceleration of inactivation remains unknown. Because of reversible phosphorylation by Ca^{2+}/Calmodulin-dependent protein kinase II (CaMKII) slows down the inactivation kinetics of native I_{to}, in this work we explored the hypothesis that acceleration in I_{to} current inactivation found in diabetic cells is caused by a defective regulation by CaMKII.

Methods: We used patch-clamp and immunoblotting techniques in enzymatically-isolated myocytes from healthy and streptozotocine-induced diabetic rat hearts.

Results: In healthy myocytes, calmodulin or CaMKII inhibition accelerates I_{to} current inactivation. However, in diabetic myocytes I_{to} inactivation is already accelerated, and it does not respond to calmodulin or CaMKII inhibition. Calmodulin protein abundance is severely reduced in diabetic myocytes compared to healthy cells. Incubation of diabetic myocytes with insulin restores calmodulin levels to normal values. Insulin treatment also restores I_{to} current inactivation to normal values as well as the responsiveness to regulation by calmodulin.

Conclusion: diabetes-induced acceleration of I_{to} current inactivation kinetics is due to a reduced effect of CaMKII on I_{to} channels as a result of a diabetes-induced decrease in calmodulin levels.

2190-Pos β_1 (*KCNMB1*) Subunits Distinctively Amplify PIP_2 Potentiation Of BK Channel Activity

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Board B305

PIP_2 can directly interact with proteins and, thus, regulate their activity (Suh and Hille, 2005; Hilgemann, 2007). PIP_2 and related phosphoinositides (PPI) increase BK channel activity (NPo) in cerebral artery myocytes independently of phosphoinositide metabolism (Liu et al., 2006). This activation involves both positive charge and the inositol moiety of PPI. Furthermore, PPI activation of BK channels display stereoselectivity. Collectively, these findings suggest a BK protein recognition site for PPIs (Vatihianthan et al., 2007). Cerebral artery myocyte BK channels consist of pore-forming (cbv1) and accessory (β_1) subunits. Current results show that PIP_2 *directly* increases cbv1 NPo (590±15 % of control) after channel expression in *Xenopus* oocytes,. Alanine mutagenesis of the positively charged cluster RKK in the cbv1 S6-S7 linker, a region that fulfills major criteria for a PIP_2 binding site (Dantsker & Logothetis, 2007), significantly blunts PIP_2 action. In addition, PIP_2 activation of cbv1 is drastically amplified by co-expression of β_1 (NPo in PIP_2=2,029±286% of control) but not β_4 subunits (NPo in PIP_2=461±18% of control). Consistently, PIP_2 causes a robust activation of native BK in vascular myocytes, which highly express β_1, but not in skeletal myocytes, where β_1 is barely detected. PIP_2 activation of vascular myocyte BK channels is negligible in solutions containing zero nominal Ca^{2+} and 5 mM EGTA. Furthermore, PIP_2 action increases with increased $Ca^{2+}{}_i$, reaching a maximum at

10 μM $Ca^{2+}{}_i$. PIP_2 causes a drastic increase in channel apparent Ca^{2+} sensitivity with substantial changes in both open and closed time distributions. Voltage-dependence of gating and unitary conductance, however, remain unmodified. Collectively, our data suggest that PIP_2 action on BK channels results from an interaction between the PPI and the channel-forming subunit, resulting in potentiation of Ca^{2+}-driven gating, which is distinctly amplified by β_1 subunits.

2191-Pos HIV-1 Vpu Interaction with Host TASK Channel

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Oligomerization between HIV-1 accessory protein Vpu and host background potassium channel TASK destroys their individual normal functions. On one hand, TASK-1 antagonizes Vpu-mediated virus release, and may provide an immediate protection to neighboring uninfected cells. On the other hand, its interaction with both Vpu and βTrCP could accelerate self-degradation and result in destabilized membrane potentials. Conceivably, TASK's "protective" effect is likely determined by the level of its cellular expression. We recently found that the TASK-1 mutants that exhibit larger potassium conductance pose stronger inhibitory effects on spread of viruses than wild-type TASK channel. For activated $CD4^+$ T lymphocytes that exhibit a low level of background conductance, their TASK-1 may be rapidly degraded when wrestling with Vpu during HIV-1 infection. Our results suggest that the mechanism through which Vpu promotes viral particle budding involves destruction of background K+ channels such as TASK-1.

2192-Pos Coincident Detection of Ischemic Conditions through the Interaction of Two Ion Channels: an ASIC and P2X Receptor

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Board B307

Acid sensing ion channel 3 (ASIC3) is highly expressed on sensory neurons innervating cardiac and skeletal muscle. Ischemia results in the release of lactic acid as well as other metabolites from working muscle. ASIC3 is ideally suited to sense this lactic acid, making acid an attractive mediator of the pain associated with muscle ischemia. Acid, however, is not sufficient to trigger ischemic pain or to excite ischemia sensitive sensory nerve fibers. We have proposed that other metabolites in addition to acid are necessary for sensing ischemia. Here we report that ATP acts to sensitize ASIC3-like currents in sensory neurons, making ASIC3 more sensitive to the small pH changes that occur during ischemia (from pH 7.4 to about 7.0). Surprisingly, we have found that ATP modulation of ASIC3 is not mediated by the G-protein coupled P2Y family of receptors, but rather by the ionotropic family of P2X receptors. Other channel-channel interactions have been reported previously, however the modulation of ASIC3 by ATP-activated P2X receptors is unique because modulation occurs slowly, on the order of seconds, and does not reverse with time, even long after the removal of ATP. The ATP-dependent modulation of ASIC3 may be reconstituted in cell lines by the co-expression of ASIC3 with either P2X 2, 4, or 5. We demonstrate that the mechanism of modulation does not involve the most common signaling pathways; protein kinases, phosphatases, G-proteins, or calcium. Fluorescent resonant energy transfer experiments indicate that the two channels are closely associated, suggesting a direct channel-channel interaction. Therefore, through its interaction with a P2X receptor, ASIC3 is able to differentiate ischemic acidosis from systemic acidosis through the coincident appearance of ATP, lactate, and protons that occurs only during ischemia.

2193-Pos A Bottom-up Approach to the Electrophysiology of the Beta-cell

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Insulin secretion and misfunction of pancreatic beta-cell is intimitely related to intracellular calcium dynamics and to the electropyhsiology of the beta-cell. It is difficult to disentangle the exact role of different membrane molecules to electrical bursts and calcium signalling which both are essential to the exocytosis of insulin-loaded granules.

With the help of a mathematical bottom-up approach directly based on experimental data the electrophysiology of the beta-cell is reconsidered. The model is based on the characteristics of single transmembrane protein properties which allows to use a far better and more precise data-basis for validation of the model as compared to models based on whole cell currents. The latter experience a strong variability between different and seemingly equivalent cells. The single-protein properties are complemented by protein surface densities to generate typical whole cell currents.

With this model architecture, which separates single protein currents from the protein expression, it becomes possible to predict the behaviour of the cell upon various modulations of protein expression or within knock-out experiments. The quality of the predictions is ensured by the well and precisely determined single-membrane conductivity measurements. A novel picture of the function of various currents in beta-cell bursts is drawn [1]. In particular, a novel role is attributed to the large-conductance calcium gated potassium channel. It is also found that, depending on the species, the role of voltage-gated sodium channels has to be revisited.

References

[1]. M. Meyer-Hermann, The electrophysiology of the beta-cell based on single transmembrane protein characteristics, Biophys. J. *93* (2007) 2952–2968.

2194-Pos Involvement Of NCS-1 In K^+ Channel Modulation By Amyloid β Peptide

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Neuronal calcium sensor-1 (NCS-1) plays an important role in ion channel function by increasing surface expression and regulating the activation and inactivation of channel properties. NCS-1 shows similar homology to calsenilin, a member of the NCS superfamily which plays an important role in Alzheimer disease (AD) and Kv4 channel trafficking. Our group has shown that the AD associated peptide, amyloid β (Aβ), modulates K^+ channel function, by a mechanism which augments the association of Kv4.2 with calsenilin. Overexpressing NCS-1, causes an increase in Kv4.2 and Kv4.3 subunit activity. In this study we examine the effects of NCS-1 on K^+ channel function in the presence of physiological concentrations Aβ.

Dissociated cultures of cerebellar granule neurones were prepared from 6–8 day old rats. Whole-cell patch clamp measurements of K^+ channel currents were carried out using quasi-physiological intra- and extracellular solutions. Stock Aβ was solubilised in DMSO before dilution in culture media to a concentration of 10nM and applied to cultures for 24 hours. NCS-1 activity was inhibited by adding antibodies raised against NCS-1 into the intracellular pipette solution. Protein expression levels for NCS-1 in the presence of Aβ were assayed using immunoblotting.

Rat recombinant $A\beta_{1\text{-}40}$ with the intracellular KChIP2 antibody as control caused a significant increase of 41±2% (n=10 control, 10 Aβ-treated, $p<0.05$) in the peak K^+ channel current density/voltage relationship. When the antibody against NCS-1 was added to the pipette solution, there was a significant block of 48.8±0.8% of the Aβ-induced current. Immunoblotting revealed a 25.9±9.3% increase in NCS-1 protein expression after 24 hour pre-incubation with Aβ.

These data suggest that the Aβ-induced increase in K^+ channel current density is due to a novel mechanism involving NCS-1 altering ion channel trafficking.

2195-Pos The *KCNE4* Product MiRP3 May Modulate Cardiac I_{to} Currents

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Board B310

MinK-related peptides (MiRPs) are single-span membrane proteins that assemble with voltage-gated K^+ channels to establish channel behavior in native cells. MiRP3 is widely expressed, with transcript levels in the heart equal to MinK (a subunit that forms I_{Ks} channels with KCNQ1). MiRP3 fully inhibits KCNQ1 current even in the presence of MinK and may interact with a variety of cardiac ion channels. Yeast two-hybrid screening of human cardiac cDNA library and co-immunoprecipitation to verify, MiRP3 was found to interact with the cytoskeletal protein filamin. Filamin is known to direct membrane location of Kv4.2 potassium-channel subunits that underlie I_{to} currents. We therefore examined biophysical and biochemical effects of MiRP3 on Kv4.2. Whole-cell patch-clamp recordings after heterologous expression of human MiRP3 and Kv4.2 in tsA201 cells revealed that MiRP3 modulates several properties of Kv4.2, including slowing activation and inactivation, speeding recovery from inactivation, and causing a rightward (depolarized) shift of steady-state inactivation. With pulses to 40mV, the current density was unaffected by MiRP3 (5.9±0.9 *vs.* 6.5±1.1nA/pF for MiRP3 *vs.* empty vector, mean±SEM, n=9–13 per group). Conversely, the Tau_{fast} of inactivation was 26.6±1.9 *vs.* 14.2±0.7ms and the Tau_{slow} was 146.7±16.6 *vs.* 93.3±7.7ms without a change in the fractional component of Tau_{fast}. At 40mV, time-to-peak was 5.8±0.6 *vs.* 2.8±0.2ms and $V_{1/2}$max for activation was −3.3±2.5 *vs.* −22.7±1.4mV. Moreover, steady-state inactivation occurred with a voltage midpoint of −55.2±1.9 *vs.* −66.8±1.2mV and the time constant for recovery from inactivation at −100mV was 57.8±5.1 *vs.* 74.5±4.9ms with a notable "overshoot" of recovery to 12.2±2.2 *vs.* 0.0±0.6% above baseline current. Co-immunoprecipitation of hKv4.2 and hMiRP3 showed their specific interaction as well as their stable assembly with KChIP2.1, another known subunit in I_{to} channels. We are evaluating the properties of the ternary complex and evidence for its occurrence *in vivo*.

2196-Pos Identification Of Protein-protein Interactions Between KCNE1 And The Activation Gate Of KCNQ1

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Board B311

KCNQ1 forms a homotetrameric, voltage-gated potassium channel that is found in a variety of tissues including heart muscle and epithelial cells. To generate the diversity of potassium currents required by these different tissues, KCNQ1 subunits assemble with KCNE type I transmembrane peptides to afford membrane-embedded complexes with varied gating kinetics. In cardiomyocytes, KCNQ1/KCNE1 (Q1/E1) complexes produce the I_{Ks} current, which contributes to the termination of action potentials and is essential for normal heart rhythmicity. Accordingly, mutations in both E1 and Q1 give rise to Long QT syndrome, a congenital arrythmicity that can cause torsade de pointes, fibrillation and sudden death. The slow gating kinetics of I_{Ks} are proposed to arise from interactions between Q1 and the cytoplasmic C-terminal domain of E1 (Rocheleau et al. 2006 *Journal of General Physiology* **128**, 721–729; Tapper & George 2000 *Journal of General Physiology* **116**, 379–389). We used oxidant-mediated cysteine cross-linking to further resolve these protein-protein interactions to faces and discrete amino acid pairings. We found within this membrane-abutting C-

terminal region of E1 residues that are associated with residues in the Q1 S4-S5 linker and the S6 gate; many of these contacts correspond well with Long QT mutations in both Q1 and E1. Complexes containing the cysteine pairings are functional and are sensitive to oxidation in whole-cell perforated patch clamp experiments. Our results demonstrate that Q1 and E1 interact on their cytoplasmic exposed surfaces, and suggest that Long QT mutations in this area act through disruption of these protein-protein interactions.

2197-Pos Early and Late Activation of the Voltage-Gated Proton Channel during Lactic Acidosis through pH-Dependent and -Independent Mechanisms

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Voltage-gated proton (H^+) channels are highly selective to protons and play a crucial role in compensating charge and pH imbalances. The activation thresholds and the driving forces for H^+ efflux through the channel are determined primarily by the pH gradient across the plasma membrane ($\Delta pH = pH_o - pH_i$). Thus the effects of pH_i and pH_o on the current magnitude are reciprocal. Lactic acidosis is a clinically important metabolic condition accompanying various tissue disorders, in which the extracellular pH and the intracellular pH often change in parallel. In this study, we investigated the responses of the H^+ channel in microglia to lactate-induced pH disturbances using the perforated-patch recordings. Na-lactate (pH 6.8) acidified the cells and activated the H^+ channel within 5 min. This early activation was correlated with increases in the ΔpH and was dose-dependent over a concentration range of 10–150 mM. At 10 mM, the change in ΔpH was only slight, but the H^+ currents continued to increase over an hour after the cell acidosis was stabilized. Prolonged exposure to lactate (10–20 mM, >1 hr) increased the amplitude by 2~3 fold. The late activation was not explained by increased ΔpH, but by changes in the property of the channel *per se*. Pretreatment with staurosporine and chelerythrine, inhibitors for protein kinase C, prevented the late activation. These results suggest that the H^+ channel could be activated greatly during long-lasting lactic acidosis through both ΔpH-dependent and -independent mechanisms.

2198-Pos Activation of Proton Channels in Basophils differs from other Granulocytes

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The activation of proton channels by phorbol ester (PMA), or physiological agonists like arachidonic acid and fMLP, in both eosinophils (Eos) and neutrophils (Neut) has been extensively studied by perforated patch recordings. Each agonist activated both proton channels and electron current. Electron current reflects the translocation of electrons by the NADPH oxidase. It has been shown that proton channels compensate charge of this translocation.

Stimulation of Eos and Neut with PMA changes the gating behavior of the proton channel. The enhanced gating mode represents the activated proton channel. It exhibits a distinct increase in proton current amplitude, faster activation kinetics, slower kinetics of tail current decay and a shift in threshold of activation (−40 mV).

We investigated in perforated patch the activation of basophils by PMA and anti-IgE to determine whether the proton channel is activated. NADPH oxidase is active in all granulocytes except basophils (de Boer and Roos 1986). PMA increased the activation kinetics and current amplitude in basophils, similar to its effects in Eos and Neut. In stark contrast, tail current kinetics was unchanged, and the observed shift in threshold was only −20 mV. Evidently the lack of NADPH oxidase in basophils influenced the activation of proton channels. The pattern of activation is comparable to that in gp91phox knock out cells (PLB-985 / CGD). The results speak against NADPH oxidase being the voltage gated proton channel, but are consistent with cross-talk between these molecules.

Anti-IgE as a more physiological agonist, was also able to activate the proton channel. This activation was less profound and more transient than the activation with PMA.

Supported by the NIH-HLBI (HL61437) and Philip Morris

2199-Pos Positive Modulation by the SK2/SK3-selective Compound, CyPPA, is Mediated via the C-terminal Tail

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SK channel activity is regulated by $[Ca^{2+}]_i$ via calmodulin (CaM) constitutively bound to a CaM binding domain (CaMBD) in the C-terminal. The Ca^{2+}-sensitivity of SK channels can be shifted towards lower $[Ca^{2+}]_i$ by pharmacological agents. 1-EBIO and NS309 activate IK and, with lower potency, SK channels. Recently, we have discovered a chemically unrelated compound, CyPPA, which is selective for SK3 and SK2 channels (Hougaard et al., Br. J. Pharmacol., 2007).

An IK/SK2 chimer study has shown that the positive modulator 1-EBIO exerted its effect via the C-terminal region (Pedarzani et al., JBC, 2001). Chimeric proteins were made between the CyPPA-sensitive SK3 and the -insensitive SK1 channels in order to reveal regions important for the selective positive modulation. The effect of 100 nM CyPPA on the wild types (SK3: 171 ± 21% baseline, n = 8; SK1: 107 % ± 13, n = 8) and chimeric proteins was measured using whole-cell patch clamp experiments. Insertion of an increasing

portion of SK1 from the C-terminal showed that chimeras being SK1 distal to SK3-656 had unaltered CyPPA-sensitivity (157 ± 19 %, n = 3), whereas chimeras being SK1 from SK3-643 had reduced sensitivity (102 ± 5%, n = 4; but responded to 10 μM CyPPA). Extending the SK1 part further into the CaMBD (from SK3-565) reduced the sensitivity further rendering it nearly insensitive to 10 μM CyPPA. All chimeras were activated to the same extend by 30 nM NS309, indicating that the basic Ca^{2+}-sensitivity was conserved. Reduced CyPPA-sensitivity was also obtained on chimeras with restricted insertions of SK1 (SK3-565 to SK3-642 (the CaMBD) and SK3-643 to SK3-656). In conclusion, amino acids within and just distal to the CaMBD are important for positive modulation of SK3 by CyPPA.

2200-Pos Effects Of pH And Temperature On Background K^+ Channels In Mouse B Lymphocytes

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K^+ channels like Kv1.3 and SK4 play critical roles in lymphocytes. In mouse B lymphocytes, we have newly found background (voltage-independent) K^+ channels with large conductance (LK_{bg}, ~300 pS) and medium conductance (MK_{bg}, ~100 pS). LK_{bg} is negatively regulated by PIP_2 in a membrane-delimited manner (Nam et al., 2004, *J Biol Chem*). Although the molecular identities are still unclear, the large conductance and activation of LK_{bg} by arachidonic acid are similar with TREK-2, a subgroup of two-pore domain K^+ channels. Now, we studied pH and thermo-sensitivity of LK_{bg} and MK_{bg} in WEHI-231, a murine B cell line. LK_{bg} was activated by decreasing intracellular pH (6.8 – 6.0) while not by extracellular pH changes. In contrast, MK_{bg} was inhibited by intracellular acidification. By warming bath solution from 25 to 35 °C, the activity of LK_{bg} was slowly increased by 10 to 20 fold in the cell-attached and inside-out patches. However, the whole-cell K^+ current and the LK_{bg} activity of pre-warmed WEHI-231 were only weakly increased, suggesting that the microenvironment for LK_{bg} might be changed by warmed temperature in a membrane-delimited way. MK_{bg} activity was not temperature-sensitive. Neither LK_{bg} nor MK_{bg} were affected by halothane. These results suggest that, in spite of some similarities, LK_{bg} and MK_{bg} are entirely different subgroups of background-type K^+ channels in B lymphocytes. The pH and temperature sensitivity might play a role in the inflammatory conditions and immune reactions.

2201-Pos Corticotropin-Releasing Factor Regulates Somatodendritic Dopamine Release by Inhibiting Voltage-dependent Calcium Channels

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Corticotrophin-releasing factor (CRF) is the main regulator of the body's stress axis and its signal is translated through an interaction with G-protein coupled CRF receptors (CRF-R1 and CRF-R2). Even though it is known that CRF plays a key role in the modulation of drug addiction by acting on dopamine neurons, the mechanism of CRF action is not clear yet. We have already shown that the application of CRF-related peptide, urocortin, reversibly inhibited T-type Ca^{2+} channels via the activation of CRF-R1 from MN9D cells, a model of dopamine neurons (Kim et al., 20007). Here, we tested whether CRF show the same effect on dopamine neurons isolated from rat substantia nigra. Urocortin (100 nM) acutely inhibited not only low-threshold T-type Ca^{2+} current, but also high-threshold Ca^{2+} current by 50.0 ± 4.7% via the activation of CRF-R1, similar to MN9D cells. Since Ca^{2+} influx through Ca^{2+} channels plays a pivotal role as the final signal for rapid stimulus-evoked release of neurotransmitters and hormones, we tested the effect of CRF on dopamine release. Amperometry method using carbon fiber was used to measure somatodendritic dopamine release from acutely isolated single dopamine neurons. Application of 300 μM glutamate increased the number of secretion events by 100% (from 2.5 ± 0.6/ 10 s to 5.0 ± 0.5/ 10 s). The enhanced secretion events induced by glutamate was significantly reduced by the application of urocortin (from 6.4 ± 1.0/ 10 s to 3.7 ± 0.7/ 10s). These results may suggest that the CRF act on dopamine neurons to regulate the dopamine release via inhibition of voltage-dependent Ca^{2+} channels.

2202-Pos Hypoxia/Reoxygenation Regulates T-type Calcium Channels in Cardiomyocytes

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Board B317

As part of ischemia/reperfusion injury, hypoxia/reoxygenation is one of the factors that contribute to the onset of the hypertrophic phenotype. Although they carry a small calcium current, T-type calcium channels are associated with hypertrophy, albeit with an unknown role. Previously, the regulation of these channels under oxidative stress was shown in neuronal cells, but not clearly in cardiomyocytes. Therefore, we investigated the regulation of the two cardiovascular T-type calcium channel alpha-subunits, Cav3.1 and Cav3.2, under the hypoxia/reoxygenation conditions in rat neonatal ventricular myocytes (NRVM). NRVM subjected to 24 hours of anoxia and 24 hours of reoxygenation were harvested for qRT-PCR analysis of Cav3.1, Cav3.2 and VEGF mRNA levels. Hypoxia downregulated Cav3.1 mRNA levels 3.7 fold and increased Cav3.2 mRNA levels 1.9 fold (n=6), while VEGF, a known hypoxia sensitive gene, was increased 5.9 fold. Under reoxygenation conditions (n=4), Cav3.1 mRNA levels were increased 2 fold when compared to normal conditions and 6.9 fold when compared to hypoxic cells. Levels of VEGF mRNA returned to normal, while Cav3.2 remained increased by 2 fold compared to normal conditions. Patch clamp recordings of T-type calcium current and Ni sensitivity were performed to establish if the current amplitudes

mirror the mRNA changes. Preliminary results show increased T-type current, of which 70% was insensitive to 50μM $NiCl_2$. Because Cav3.1 is highly regulated in these conditions, the hypoxia/reoxygenation effect on the Cav3.1 gene promoter was confirmed by transfecting promoter-reporter constructs into HEK 293 cells and measuring luciferase activity under the same conditions. We show that T-type calcium channels are regulated in hypoxia/reoxygenation conditions, and we propose that T-type calcium channels contribute to intracellular calcium accumulation and the severity of injury in cardiomyocytes.

Biophysics of Ion Permeation

2203-Pos The Path Of Ions In Ompf Pore: An Analysis Using X-ray Crystallography

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Board B318

OmpF porins are proteins found in the outer membrane of Escherichia coli which form wide aqueous pores that are slightly cation-selective. They serve to facilitate the translocations of hydrophilic solutes with molecular mass up to 600 Da across the outer membrane. Because they are well-characterized, both structurally and functionally, OmpF and other porins represent ideal systems for addressing questions about the fundamental principles underlying ion flow in molecular pores. Previously, PD, BD and MD simulations hadshown that there exist two well-separated pathways for cations and anions inside OmpF. Thus, suggesting that the charge specificity of OmpF porin does not arise from a few local interactions in the constriction zone, but rather from a number of residue distributed over a large fraction of the aqueous pore.

In this study, we use x-ray crystallography to observe the path taken by rubidium ions inside OmpF. X-ray diffraction data on OmpF were collected from crystals equilibrated with solutions containing rubidium chloride and snap frozen (100 degrees K). Difference electron density maps [F(rubidium) - F(native)], at 3.0 Angstrom resolution as well as rubidium anomalous scattering maps suggest that the cations prefer to be located along the walls of the OmpF pore, following a screw axis path. Also observed are densities of water molecule not seen in previous structures. Taken together the xray structure and previous PD, BD and MD studies add to our understanding of the factors that determine the path taken by ions inside the porins.

2204-Pos Interaction Of Ampicillin With The OmpF Channel Studied By Site Directed Mutagenesis

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We use site directed mutagenesis to study molecular mechanism of β-lactam antibiotics translocation through the single trimers of outer membrane protein F (OmpF). Using high resolution conductance recordings and noise analysis, we investigate ampicillin interaction with the following mutants: *D113A, R82A*R132A, R168A, R82A, R42A, E29A, E117A*R132A, E29A*R168A, E117A, D113A*R42A, E62A*R82A*, and *E71A*R132A*. For all mutants we observed a measurable current noise increase in the presence of antibiotics in the membrane bathing solutions. However, we could distinguish at least two different modes of ampicillin-channel interaction. The first group of mutants binds ampicillin in a way similar to the wild type exhibiting clear 1/3 blockages of the current through the single trimeric pore. The second group demonstrates no 1/3 blockages but induces a pronounced high-frequency spectral component suggesting that the characteristic time of the interaction was too short to be resolved.

Comparing the results for the first and second groups of mutants we discuss the possible involvement of certain amino acid residues in the ampicillin transport through the channel. We examine two different scenarios of antibiotic interaction to the mutants of the second group. One of them is that we deal with extremely fast ampicillin translocation through the pore, while the other is that the high-frequency component of the spectra is caused by antibiotic binding somewhere close to the opening of the channel without translocation through the pore. To discriminate between these scenarios, we compare channel blockage parameters under conditions when antibiotic was asymmetrically added only to *cis* or only to *trans* side of the membrane.

2205-Pos

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WITHDRAWN

2206-Pos The Effects Of High Frequency Electromagnetic Field On The Behavior Of Single OmpF Channel In Planar Bilayer

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The Effects of high frequency electromagnetic waves, 800 MHz, on the single-channel activity of OmpF porin channel reconstituted in lipid bilayer membrane were studied here. Ion channels as one the major components of biological membranes involved in the appropriate function of cell can be considered of the major target sites for