observed a significant weight loss during the two days of MMI exposure, depending on initial weight. Plasma TSH levels at 24 and 48 h after MMI were significantly reduced ($p<0.05$) as compared to controls, and T_3 levels were increased ($p<0.001$). Upper limb strength was evaluated using the hanging wire task at 24 and 48 h after MMI exposure. All treated animals showed reduced muscle strength compared to controls. Miniature endplate current frequency was also reduced in MMI treated animals, and diaphragm preparations showed reduced fluorescent alpha-bungarotoxin labeling compared to controls. A preliminary analysis of the muscle proteome in control and MMI treated animals suggests the involvement of alpha-actin in the THs effects. Together, these results strongly suggest THs may acutely regulate mammalian neuromuscular function. Similar rapid THs effects may contribute to the muscular weakness of thyroid dysfunction before and during initial treatment with MMI.

Peptide & Toxin Ion Channels

3063-Pos High Membrane Cholesterol Levels Characterize Cells Identified By Their Aβ Binding Affinity

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Board B366

Abundant evidence establishes that the Alzheimer's disease (AD) Aβ peptide is a cell-selective cytotoxic agent. We have recently demonstrated that neuronal cells, when analyzed on the basis of their capacity to bind Aβ, can be separated into two main subpopulations: one that distinguishably bind Aβ, and one that does not bind Aβ. The selective cell membrane binding of Aβ is established by distinctive characteristics of subpopulations of cells which are maintained even after multiple cell divisions. Because the level of cholesterol in the cell membrane has been considered an influential factor associated with Aβ toxicity, we investigated the level of membrane cholesterol in subpopulations of cells with differential Aβ membrane binding. We also studied the effect of changing the levels of membrane cholesterol on the membrane affinity for Aβ membrane. Using flow-cytometry and cell sorting we analyzed Aβ binding and the membrane content of cholesterol in PC12 cells and ex-vivo hippocampal neurons. Fluorescent Aβ and filipin were used to detect membrane bound Aβ and membrane cholesterol, respectively. Membrane cholesterol was either enriched or depleted by growing cells on cholesterol-enriched media or by interfering with the biosynthesis of cholesterol. We found that populations of cells that exhibited Aβ binding affinities also show higher membrane cholesterol levels, compared to cells that did not bind Aβ. The same direct relation was also observed when membranes were artificially enrich or depleted of cholesterol. This membrane characteristic was confirmed after cell sorting based on Aβ binding affinities, and was found to be maintained after several days in cultures and multiple cell divisions. We conclude that the level of cholesterol in the membrane is one of distinctive the membrane factors influencing the membrane binding affinity for Aβ.

3064-Pos G_{M1} and PS Enrichment of Cell Membranes Assists Aβ Membrane Binding and Ion Channel Formation

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Board B367

Aβ binding to cell membranes and Aβ aggregation to structures that form membrane ion channels has been considered crucial steps in the cytotoxicity of Aβ. Studies suggest that the seeding process to Aβ aggregation occurs in lipid raft mediated by clusters of monosialoganglioside G_{M1}, and that phosphatidylserine (PS) is a surface membrane receptor for Aβ. Aβ membrane binding is followed by intracellular calcium increases produced by the insertion and consequent formation of Aβ ion channels. This study examine whether the formation of aggregate structures corresponding to the cytotoxic Aβ ion channels correlates with G_{M1} and PS enrichment of cell membranes. Fluorescence microscopy and spectrophotometer evaluation showed that the GM1 and PS content of the cell membrane can be gradually increased by exogenous timely exposure of cells to G_{M1} and to PS. Membrane G_{M1} was recognized with fluorescence-labeled cholera toxin, and PS with annexin V-FITC. The binding of Aβ was tracked by Aβ42-AMCA. The formation and identification of Aβ channels was tracked by measuring the increase in intracellular calcium and by blocking the channels with a specific Aβ channel blocker. The increase in the intracellular calcium concentration and the reduction of viability observed after cells are exposed to Aβ is significantly enhanced in cells enriched with G_{M1} and PS. Both, Aβ-induced effects are totally prevented when cells are cultured in the presence of a specific Aβ channel blocker. PS enrichment is notoriously more effective than G_{M1} enrichment in favoring Aβ membrane binding. Our results strongly suggest the role of PS as a Aβ membrane receptor, and that the Aβ aggregation in the cell membrane lipid rafts mediated by clusters of G_{M1} additionally promote the formation of structures with the capacity to form of Aβ channels.

3065-Pos Characterization of Amyloid-Beta Membrane Association and Permeabilization: Dependence on membrane charge, curvature, and phase

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Board B368

Much evidence has demonstrated involvement of the peptide amyloid-β (Aβ) in instigating neuronal degeneration in Alzheimer's Disease. Whereas it was previously thought that Aβ becomes cytotoxic only as insoluble fibrillar aggregates, recent studies suggest that soluble intermediate sized oligomeric species cause cell death through membrane permeabilization. Here we characterize the steps leading to the formation of ion conducting channels,

and examine the dependence of Aβ membrane binding and permeabilization on membrane charge, curvature, and composition using liposomes. Fluorescence studies of Aβ binding to anionic membranes both in the gel phase and in the liquid crystalline phase showed insertion into the bilayer upon binding. Circular dichroism revealed a two-step transition in Aβ structure from an unstructured conformation to mostly β-sheet structure upon binding anionic liposomes at intermediate lipid:peptide (L:P) ratios, and to α-helical structure at high L:P ratios. Furthermore, while at intermediate L:P ratios higher order oligomerization (up to hexamer) was observed, at high L:P only peptide trimers were present. Neutral membranes did not induce any of these effects, pointing to the importance of charge in promoting Aβ aggregation and structural transitions. Similar observations were made for liposomes composed of either lipids in the gel or liquid crystalline phase. However, channels were formed only in membranes in the liquid crystalline phase as assessed by dye leakage from these liposomes. Thus binding and oligomerization are highly dependent on the lipid head group, whereas permeabilization also depends on the lipid tail group (lipid phase). Furthermore, kinetics-wise, permeabilization does not reach a maximum until much later after binding has reached equilibrium. These findings suggest membrane binding and permeabilization are two distinct events.

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3066-Pos Characterization of Aggregation of Amyloid-β Peptides and Cytotoxicity on Neuroblastoma SH-SY5Y Cells

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Board B369

Alzheimer's disease (AD), an ultimately fatal neurodegenerative disorder, is characterized by the presence of plaques containing fibrillar aggregates of amyloid-beta (Aβ) peptides. These peptides, with 39–43 amino acids, are the major components of plaques formed in the brain of patients with AD. Among Aβ peptides, Aβ (1–40) and Aβ (1–42) are pathologically most important. Both peptides aggregate rapidly in aqueous solution to form Aβ oligomers as well as Aβ fibrils. An increasing number of reports indicate that Aβ peptides particularly in their oligomeric state are cytotoxic to nerve cells and play a role in pathogenesis of AD. One of the challenges of the characterization of the cytotoxicity has been the variable aggregation states of these peptides from commercial sources. The formation of Aβ oligomers is influenced by duration of incubation, concentration of Aβ, temperature, pH, ionic strength, and solvent history. One approach to study the mechanism of Aβ is to examine the cytotoxicity of Aβ on neuroblastoma cells. For obtaining repeatable results from this assay, it is important to characterize the aggregation state of Aβ that yields consistent toxicity to cells. Here we show our results of characterizing Aβ oligomerization with various techniques and the corresponding cytotoxicity of Aβ oligomers to a human neuroblastoma cell line SH-SY5Y.

3067-Pos The Effects of Insulin on Islet Amyloid Polypeptide Induced Membrane Permeability

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Board B370

The formation of extra- and intra-cellular fibrils are characteristic of amyloid diseases including type II diabetes, Alzheimer's disease, Parkinson's disease, etc. Despite the commonality of amyloid fibrils in these disease states, recent evidence suggests that oligomeric forms of the amyloidogenic proteins, consisting of a few monomers may be the cause of cellular toxicity, rather than fibrillar amyloid species. Interestingly, the peptide involved in type II diabetes, islet amyloid polypeptide (IAPP), is present at milli-molar concentrations in secretory granules prior to secretion. At milli-molar concentrations, IAPP rapidly fibrillizes *in vitro*, yet within secretory granules of healthy individuals IAPP does not fibrillize. In addition to IAPP, insulin is also present within the secretory granules prior to secretion and has previously been shown to inhibit IAPP fibril formation *in vitro*. Using circular dichroism we show that at equimolar concentrations, insulin slows the structural transition of IAPP to beta-sheet structure in the presence of POPG/POPC membranes. The structural change is induced specifically by the addition of negatively charged liposomes. We further present evidence that insulin has the ability to block IAPP mediated membrane permeabilization and dye leakage from liposomes. Thus, insulin may play a protective role by preventing toxic oligomeric species of IAPP from forming in the beta-cell membranes in the pancreas. We also report progress on using cross linking and single molecule spectroscopy experiments to examine the interaction of insulin and IAPP.

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3068-Pos Membrane dipole potential modulates syringomycin E channel formation

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Syringomycin E (SRE) is a member of a family of cyclic lipodepsipeptides produced by certain strains of the plant bacterium Pseudomonas syringae pv. syringae. SRE and its analogs are potent fungicides. Their toxicity depends on channel forming activity in the plasma membrane of fungal cells. We studied the channel forming activities of SRE in model planar lipid bilayers in the presence of agents that modify the membrane dipole potential such as phloretin and RH 421. A reduction of the membrane dipole potential induced by addition of phloretin in the membrane bathing

solution led to an increase in the steady-state SRE channel number. An increase of the membrane dipole potential by the addition of RH 421 resulted in the opposite effect. These changes in the properties of SRE channel formation are related to the gating charge of SRE channels, water vs lipid partitioning of SRE, and the chemical component that contribute to channel formation work.

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3069-Pos Persistent Gramicidin A Channel Gating

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For over three decades it has been thought that gating of gA channels is well-explained by a dimerization model, namely that monomers float as helices in the leaflets, combine to form conducting channels, and then dissociate to terminate the conducting state. However, there is limited structural evidence for this model, and new vesicle-delivery data contradicts it. Lipid vesicles containing nystatin and ergosterol and a few gA dimers each were fused into a planar lipid bilayer. A few vesicle fusions, marked by nystatin-ergosterol-complex current spikes (Woodbury, 1999), were followed by a few characteristic gA "channels" that opened and closed repetitively long after stirring was halted and fusion events ceased (up to 80 minutes), indicating permanent gA dimerization. High resolution 13C solid state NMR shows that the 13C1-Val1 gA in hydrated phospholipid bilayers exhibits two conformational states with exchange times on the seconds time scale. Both the 13C1-Val1 states are hydrogen-bonded with the amide 15N-Ala5 across the monomer-monomer junction, indicating that both conformations are gA dimeric channel structures. SSNMR also shows that one of 13C1-Val1 states is slightly affected by the presence of K+ cation in the bath, suggesting that one of the conformations involves ion complexation. Preliminary conformational search MD fails to reveal bistable peptide librational conformations with no ion in the channel, and work is underway to extend the simulations. The channel persistence results are consistent with the high barrier to dimer dissociation computed using normal modes tracking (Miloshevsky and Jordan, 2006) and FRET evidence of non-conducting dimers (e. g. Lu, 2005).

3070-Pos Is The Physical State Of The Lipid Bilayer Responsible For The Selectivity Of Polyene Channels?

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Board B373

Amphotericin B (AmB) and Nystatin (Nys) are well know pore forming polyene antibiotics with preferred toxicity against fungi. Both have been studied since 1950 pursuing the basis of the selective toxicity they show. Five decades of research have led to two different hypothesis, one proposing that the selectivity mechanism is mainly due to the preferred interaction between the polyenes and ergosterol (the main sterol found in fungi) than that with phospholipids or other sterols in the bilayer, leading to a more stable open channel structure. The second one proposes that the average physical state of the bilayer -that can be modified by its composition, temperature or pressure- is the one responsible for the selectivity. Here, we present detailed results on the activity of polyenes along the phase diagram of 1,2-Dipalmitoyl-sn-Glycero-3-Phosphocholine (DPPC), 1-Palmitoyl-2-Oleoyl-sn-Glycero-3-Phosphocholine (POPC), 1,2-Dioleoyl-sn-Glycero-3-Phosphocholine (DOPC) with different molar fractions of cholesterol, ergosterol and polyene at different temperatures. These results show that the polyene channels are sensitive to subtle changes in the membrane environment.

3071-Pos Theoretical Studies of the Aggregation of Amphotericin-B in Vacuo, in DMSO and in Water

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The mechanism of aggregation of AmB molecules is relevant to elucidate how they assemble to be inserted in different biological membranes. The AmB dimer was studied by means of molecular dynamics. To validate the force-field used in this study, a comparison was made to quantum calculations in vacuo and to experimental data of AmB in DMSO; then the study was extended to aqueous solution at temperatures ranging from 298 K to 323 K. The results obtained from these latter simulations showed AmB to have a tendency to aggregate spontaneously in anti-parallel dimers, due to the alignment of the dipoles. Further simulations were carried with 4, 6 and 8 AmB molecules, that resulted in different aggregates: in water, two separate dimers were obtained with 4 AmB molecules, whereas the six- and eight-membered aggregates kept stable; in DMSO even the four-membered aggregate was found to be stable. A discussion is made of how different aggregates could be inserted in the membrane.

3072-Pos Biomolecular Engineering by Rational Combinatorial Chemistry: Small, Soluble Pore-forming beta-Strand Peptides that are Broad-Spectrum Antibiotics

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Board B375

Cationic antimicrobial peptides (AMPs) are present in many organisms and can kill a broad range of microorganisms by membrane permeabilization. Consequently short cationic peptides may serve as a new generation of antibiotics, especially in light of the alarming increase in microbial resistance to the conventional antibiotics. Membrane pore-forming peptides and known AMPs share a similar mechanism of action in lipid bilayer *in vitro*. In an attempt to explore the structure-function relationship of pore-forming peptides and AMPs, and also in an effort to develop a novel strategy to screen for new peptide antibiotics, we used combinatorial chemistry and high-throughput screening. We selected potent pore-forming peptides from a combinatorial library of 16,000 putative short, cationic amphipathic beta- strand peptides of 9–15 residues using our liposome-based leakage assay. We selected 10 water-soluble potent pore-forming peptides, less than 0.1% of library population. These pore-forming peptides are soluble in buffer but bind strongly to lipid vesicles and induce rapid transient leakage of encapsulated contents. All of the peptides are beta-sheets in membranes. Also we have found that these peptides release the contents of liposomes in all-or-none leakage mechanism. These pore-forming peptides have potent, broad-spectrum antimicrobial activity ranging from 2 to 4 μM minimum sterilization concentrations (MSC). But they have little lytic activity against erythrocytes or living human cells. There is a direct correlation among 1) the MSC of the peptides 2) their ability to disrupt the cytoplasmic membrane of the bacterial cells and 3) their ability to release the contents of liposomes. The selected pore-forming peptides have classical antimicrobial activity: They target the fundamental differences between microbial and mammalian cell membranes. The *in vitro* liposome leakage assay is a powerful, reliable and high-throughput screen for peptides of broad-spectrum antimicrobial activity.

3073-Pos A Combinatorial Peptide Library and High-Throughput Screen Designed to Reveal Peptide Translocation into Protease-Containing Lipid Vesicles

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Lipid vesicles provide a model system for studying membrane perturbations and interactions between lipids and peptides. We have developed a system for monitoring the non-pore, translocation of peptides across lipid bilayers which can be used to screen a peptide library in a high-throughput microplate format. The translocation is monitored by the fluorescence redshift of the fluorophore 7-amido-4-methylcoumarin (AMC) upon cleavage from peptide by protease entrapped in unilamellar phospholipid vesicles. For the screen we prepared LUVs that contain entrapped alpha-1-chymotrypsin along with terbium (III). The aromatic terbium chelator dipicolinic acid (DPA) and protease inhibitors are added to the external buffer. Pore-formation is discernable within minutes by the luminescence of the Tb3+/DPA complex. A redshift in the AMC substrate without an increase in Tb3+ fluorescence is indicative of peptide translocation. This system allows for the differentiation of peptides crossing into the core in concert with pore-formation from peptides entering the vesicle through translocation. We discuss the design of a rational combinatorial library for peptides that can translocate across bilayers. The peptide library was synthesized as a one-bead, one-peptide library on a custom resin with the alpha-1-chymotrypsin substrate, N-Glu-Phe(AMC)-C attached to each library member at the carboxy terminus.

3074-Pos Mitochondria Permeabilization and Aggregation by New Anticancer Polyarginine Modified KLA Peptides, 7R-KLA and 7r-kla

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Board B377

Polycation KLA peptides were originally designed as antimicrobial substances (Javadpour et al., J. Med. Chem., 1996, *39*:3107) and also demonstrated anticancer properties. Recently, the anticancer activity of one KLA peptide (KLAKLAKKLAKLAK) was strongly increased by the fusion of its D- and L-forms with polyarginine cell penetrating vector (peptides 7r-kla and 7R-KLA, respectively) aimed to increase the delivery of kla or KLA mitochondria-damaging fragments into tumor cells (Law et al., Mol. Cancer Ther., 2006, *5*:1944). In this work we show that polyarginine modification enhanced direct permeabilizing activity of KLA peptides on rat liver mitochondria. Added to energized mitochondria at a concentration of 3.6 uM, 7r-kla and 7R-KLA decreased the inner membrane potential and light dispersion, increased state 4 respiration and the rate of rotenone-insensitive oxidation of external NADH depended on the outer membrane permeabilization (or damage) to cytochrome c. Similar effects were observed in the presence of 0.36 uM BTM-P1, a new peptide derived from the Cry11Bb protoxin of *Bacillus Thuringiensis* subsp. *medellin* (Lemeshko et al., J. Biol. Chem., 2005, *280*:15579; Segura et al., Biochem. Biophys. Res. Commun., 2007, *353*:908). In the de-energized mitochondria, 7r-kla, but not BTM-P1, strongly decreased the oxidation of external NADH as result of the observed aggregation of mitochondria. In addition, 7r-kla and 7R-KLA significantly decreased ATP synthesis that seems to be caused by both potential-dependent membrane permeabilization and the aggregation of energized mitochondria. Thus, the anticancer activity of polycation peptides might depend not only on the permeabilization of mitochondrial membranes but also on the aggregation of mitochondria.

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3075-Pos Pore Forming Properties Of Viroporins And Antimicrobial Peptides

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The biophysical characteristics and the pore formation dynamics of synthetic, naturally occurring peptides forming membrane-spanning channels were investigated by using isolated rod outer segments (OS) of low vertebrates recorded in whole-cell configuration; the peptides were applied to (and removed from) the OS in <50 ms. The OS membrane resistance was >5 G once the OS endogenous conductances were blocked. A peptide spanning residues 35–55 of picornavirus 2B, a non-structural protein required for effective viral replication, produced voltage independent currents in symmetric K^+ (120 mM) and Ca^{2+} (1 mM) at 100 nM concentration. Current activated with a delay of 5 s to a steady state amplitude of ~1 nA within 25 s (10–90%). No measurable single channel activity was detected at lower 2B concentrations, while a synthetic potassium channel protein encoded by a modified chlorella virus PCBV-1 (inserted in phosphatidylcholine vescicles and applied to the OS) gave single channel events of ~300 pS in the same ionic conditions. Permeabilization produced by both peptides was irreversible, at difference with the synthetic 19-aminoacid long alamethicin F50/5 and its analog [L-Glu(OMe)^{7,18,19}] that produced inward rectifying currents of ~1 nA (~1 μM, −20 mV) that decayed to 0 upon peptide removal within <2 s. Current was carried equally well by monovalent and divalent cations, but activation kinetics accelerated by two-fold for a 10-fold increase of external Ca^{2+}. Both F50/5 and [L-Glu(OMe)^{7,18,19}] produced single channel events of different sizes at concentrations <250 nM: the most probable ones were ~50 pS for F50/5 and ~500 pS for [L-Glu(OMe)^{7,18,19}]. The large variability of pore-forming properties of all these simple peptides makes them an ideal tool to understand the biophysical properties of channel and transporter proteins and they represent a potential lead for the development of bioactive compounds

3076-Pos Two Cry1Ab Bacterial Toxin Structures Are Able To Insert Into Liposomes And Lipid Bilayer With Differential Activity

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Board B379

Bacillus thuringiesis is a bacteria that produces insecticidal Cry toxins against pests. Cry toxins are pore-forming toxins inducing cell death by converting into an oligomeric pre-pore form capable of inserting into the membrane with channel activity, causing osmotic lysis of midgut cells. However, electrophysiological characterization until today continue feeble.

Two types of Cry1A toxins receptors have been characterized in several lepidopteran species. Cadherin-like proteins, known as BtR receptors, and GPI-anchored proteins, as aminopetidase-N or alkaline phosphatase. Both receptors are important and participate in a sequential manner in toxic pore formation. After proteolitical activation of Cry1A protoxin by insect midgut proteases, the activated toxin binds to BtR receptor. This interaction facilitates additional cleavage of N-terminal end of the toxin, resulting in the formation of a pre-pore oligomeric.

In order to determine processes in proteolitical activation and ability of Cry1Ab to insert in model membranes, we used two different structures on pore activity tests. One was a monomeric structure of 60 kD and the other was an oligomeric structure 250 kD. These structures show different insertion kinetic on leak of calcein from liposomes experiments. Interestingly, only the oligomeric structure showed a very fast insertion into the membrane. In order to determine differential activity of monomeric and oligomeric structures inserting into the membrane, we have been working in obtain electrophysiological properties of both structures in black lipid bilayer. Our results demonstrate that the oligomeric structure show high open probability. Whereas, the monomeric structure shows instability and differential gaiting when compared to oligomeric toxin.

3077-Pos Determining the Molecular Basis of Protein Translocation Driven Through the Anthrax Toxin Channel by a Proton Gradient

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The toxin produced by *Bacillus anthracis* has proved to be a promising model system to study protein translocation across cell membranes. The toxin is composed of a translocase channel, called protective antigen (PA), which allows its two substrate proteins, lethal and edema factors (LF and EF), to translocate across a host cell's endosomal membrane, disrupting the cellular homeostasis. It has been shown that PA translocase incorporated into planar phospholipid bilayer membranes forms a channel capable of transporting LF, EF and other small proteins. Protein translocation through the channel is driven by a positive membrane potential ($+V_m$) or a

positive proton gradient ($pH_{trans-cis}>0$), with S-shaped kinetics on a time scale of seconds. We proposed a Brownian ratchet mechanism in which acidic residues can enter the channel only after being protonated in the endosome (low pH), because the channel excludes negatively charged segments of the polypeptide. After they reach the cytosol (neutral pH), these segments are then rapidly deprotonated and cannot back-diffuse into the channel, biasing the protein diffusion within the channel in a single direction. Put simply, the channel would work as a proton/protein symporter. Here we attempt to determine the molecular basis of the ($pH_{trans-cis}$)-driven protein transport, analyzing in planar bilayers the blocking dwell times within the PA channel lumen of engineered synthetic peptides, using single channel recordings. We are currently studying the engineered peptides $K_5(KELKESKESKEL)_3K$-amide (2-Glu) and $K_5(KSLKSSKSSKSL)_3K$-amide (2-Ser). If the basis for ($pH_{trans-cis}$)-driven transport is the titration of the aspartates and glutamates in the polypeptide chain of the substrate protein, then we expect that 2-Ser translocation should be driven by a $+V_m$ but not by a ($pH_{trans-cis}$), since it has no titratable carboxyls, whereas 2-Glu should be driven by both.

3078-Pos Blocking of an Ion Channel by a Highly Charged Drug: Modeling the Effects of Applied Voltage, Electrolyte Concentration, and Drug Concentration

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We present a simple physical model to estimate the blocked pore probability of an ion channel that can be blocked by a highly charged drug in solution. The model is inspired by recent experimental work on the blocking of the PA63 channel, involved in the anthrax toxin infection, by a highly charged drug [Karginov et al. PNAS *102*, 15075 (2005)]. The drug binding to the pore is highly specific but the strong dependence of blocking on the applied voltage and electrolyte concentration suggests that long range electrostatic interactions are important. Since basic electrostatic concepts rather than detailed molecular models are considered, the microscopic details of the channel blocking are ignored, although the model captures most of the qualitative characteristics of the problem.

3079-Pos Potent Kv3.1 block by gambierol

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Gambierol, a polycyclic ether toxin produced by the marine dinoflagellate *Gambierdiscus toxicus*, is a ciguatera toxin that accumulates throughout the food chain causing food poisoning by consumption of contaminated fish. Gambierol has been shown to affect a potassium current (I_K) in mouse taste cells, but the specific molecular target has not been determined yet. In this study we investigated different members of the voltage-gated potassium channel families Kv2, Kv3 and Kv4 as a potential target, after expression of these channels in mammalian Ltk- cells. We found that even 1μM gambierol had no effect on members of Kv2 and Kv4 family. However, Kv3.1 was blocked by the toxin in the nanomolar range, with a K_D of 1.2 ± 0.2 nM. The block by gambierol was not use dependent. The structure of gambierol suggests that it is a highly lipophilic molecule that could easily pass the plasma membrane to reach an intracellular accessible binding site. Faster onset of block in inside-out configuration compared to whole cell, favored the idea that the toxin acts from the intracellular side. To characterize the toxin's binding site we constructed channel chimeras between Kv3.1 and Kv2.1, a sensitive and a non-sensitive channel respectively. Switching the S6 segment was sufficient to convert the non-sensitive channel into a sensitive chimera and vice versa, indicating that S6 forms at least part of the high affinity binding site. In conclusion, this study identifies Kv3.1 as a potent target for gambierol block.

3080-Pos Individual Protein-protein And Protein-DNA Channel Current Blockades Are Studied In The Presence Of Chaotropic Agents And High Salt Concentration

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A nanopore detector based on the α-Hemolysin channel was used to study complex formation between individual molecules. A previously studied DNA hairpin, possessing both high affinity to the channel and easily recognizable blockade signature, is used in the design of several event transduction molecules. Here we describe hairpin molecules that are modified with covalently attached groups capable of targeting a variety of molecules of interest. In one series of experiments biotinylated DNA hairpins were studied, with binding partners anti-biotin mAb and streptavidin. Other approaches along these lines include use of monoclonal antibodies conjugated with a DNA hairpin, and specially designed Y-aptamers. The influence of salt concentration and chaotropic agents concentration on binding was investigated. Independently, the conditions of complex formations were tested by electrophoretic mobility shift analysis based on capillary electrophoresis and by isoelectric focusing (IEF). The formation of the complex is followed by it unique

current blockade signature that gives a direct evidence of a binding event. Nanopore observation of molecular species may prove to be much more sensitive than mobility shift analysis, but the sensitivity and sequential nature of the channel current signal limit the range of applicability of the method. In practice, nanopore detection may offer an augmentation to mobility shift analysis, by taking a 'band' of concentrated analyte of interest in isoelectric focusing experiment, and placing it in a nanopore detector for more detailed analysis. In this way subtle isoforms may be discerned that only appear as a single band in electophoretic separation. In addition to the possibility of monitoring the free probe concentration, it may also prove possible with sophisticated nanopore cheminformatics methods to determine the concentration of complexes not directly interacting with nanopore.

Voltage-gated Na Channels

3081-Pos State-dependent Interaction of Paired Substituted Cysteine Residues in the Voltage Sensor of NaChBac Enhances Activation and Inactivation

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Activation of voltage-gated ion channels is initiated by depolarization-dependent outward movement of the S4 voltage sensor driven by its conserved gating charges. The 'sliding helix' model of voltage sensing, as implemented at the structural level with the Rosetta algorithm (Yarov-Yarovoy, 2006), proposes that S4 arginine gating charges make sequential electrostatic interactions with negatively-charged residues of S2 and/or S3 segments, which catalyze their transmembrane movement during activation. To provide evidence for such voltage-dependent interactions, we replaced the 3rd arginine (R3) of S4 and the first negatively charged residue (D60) of S2 in the homotetrameric bacterial Na^+ channel NaChBac with cysteines. Whole-cell Na^+ currents were recorded from tsA-201 cells transfected with NaChBac WT, single mutants (D60C or R3C) and double-mutant channels (R3C:D60). Repeated depolarizations caused pulse-wise reduction of Na^+ current that did not recover at the holding potential (−120 mV) for R3C:D60C but not for WT or single residue mutants, suggesting disulfide bond formation between the S2 and S4 cysteines. We hypothesize that Na^+ current reduction results from enhanced inactivation subsequent to locking of one or more voltage sensors in the activated conformation. Consistent with such activation-locking, deactivation of the R3C:D60C was slowed upon repolarization and recovery from inactivation was shifted to much more negative membrane potentials. The voltage-dependence of the activation-locking interaction ($V_{1/2}$ = −10 ± 8mV) is more positive than channel activation ($V_{1/2}$ = −30 ± 6mV), suggesting that substantial voltage sensor movement is required for activation-locking of these residues. These data indicate that S4 moves relative to S2 upon channel activation, placing R3C sufficiently close to D60C (~2Å) to allow rapid disulfide bond formation, and thus are consistent with predictions of the Rosetta sliding helix model of voltage sensing.

3082-Pos Selectivity of the Bacterial Sodium Channel, NaChBac, Studied by Experiment and Simulation

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The prokaryotic sodium channel, NaChBac, from *Bacillus halodurans*, is a homotetrameric Na_V channel, with each monomer having 6 putative transmembrane helices. The selectivity filter in the ion-conducting pore of NaChBac is thought to be lined by a ring of 4 glutamate residues (EEEE). We expressed NaChBac channels in HEK293 cells and studied their selectivity to monovalent organic and alkali cations. Reversal potential shifts were determined from whole-cell currents, following substitution of a test cation for extracellular sodium, and relative permeabilities (P_X/P_{Na}) were calculated using the Goldman-Hodgkin-Katz equation. Among the alkali cations, only Na and Li (P_{Li}/P_{Na} = 0.73 ± 0.07, n=3) were measurably permeant. Of 13 organic cations, at pH 7.3, only hydrazinium (HZ; P_{HZ}/P_{Na} = 0.37 ± 0.02, n=3) was permeant, and notably, in contrast to eukaryotic Na_V channels, ammonium was not. In preliminary experiments, two manipulations made NaChBac less selective. At pH=6 for WT NaChBac, both K and hydroxylammonium (HA) were permeant; For NaChBac E191D mutant at pH=7.3, both K and ammonium were permeant. Lacking a high-resolution structure or verified model, we have built a homology model of the NachBac pore domain, based on a reported model of an L-type Ca channel, and several K-channel crystal structures. After molecular dynamics simulations to check stability in a lipid bilayer bathed in explicit solvent, we performed free energy calculations to test the selectivity of the initial model. The resulting selectivity sequence (Na>Ca >K) encourages us to develop the model further to explore the complexities of NaChBac selectivity shown by our data. Overall, our results suggest that the simpler, tetrameric NaChBac channel is even more selective for Na than are eukaryotic Na_V channels, and that NaChBac achieves this high selectivity by a unique molecular strategy.

3083-Pos Structural Model of the Voltage-Dependent Gating of NaChBac

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Voltage-gated sodium channels control the generation and propagation of action potentials in excitable cells. Despite recent progress in determining the high-resolution structures of the voltage-gated potassium channels, the high-resolution structure of the voltage-gated sodium channels remains unknown. We used the Rosetta-Membrane method (Yarov-Yarovoy et al. (2006) Proteins *62*, 1010–1025) and recently developed models of the Kv1.2 channel in the open and closed states (Yarov-Yarovoy et al. (2006) PNAS *103*, 7292–7297, Pathak et al. (2007) Neuron *56*, in press) to construct