to more negative potentials. These effects result from a voltage sensor trapping mechanism, in which toxins trap the voltage sensor in its activated conformation. Determinants of β-scorpion toxin (CssIV) binding and action on sodium channel (Nav1.2) are located in the S1-S2 and S3-S4 extracellular linkers in the voltage-sensing module in domain II. To completely map these regions, we made substitutions for previously unstudied amino acid residues and examined modulation by $CssIV^{E15A}$, a highly active toxin derivative. Of 11 positions studied in IIS1-S2, only one significantly altered the toxin effect from wild-type by reducing binding to the resting state and almost abolishing trapping activity. In IIS3-S4, five positions surrounding a previously identified key binding determinant, G845, define a hotspot of high impact residues. Three of these substitutions reduced toxin binding and voltage-sensor trapping. The other two, V843A and E844N, increased voltage-sensor trapping approximately 4-fold and decreased apparent EC_{50}. The rate of voltage sensor trapping upon depolarization was unchanged for V843A and increased approximately 2.5-fold for E844N. The rate at which the toxin releases the voltage sensor upon repolarization was increased 2.2-fold for the V843A but was unchanged for E844N. Thus $CssIV^{E15A}$ interacts with a short segment of IIS1-S2 and a broader region of DIIS3-S4. The bidirectional effects of mutations on toxin efficacy suggest that native residues make both positive and negative interactions with the toxin. Substitutions that increase toxin effects do so by increasing affinity of resting channels for the toxin and further increasing the relative affinity of the activated voltage-sensor for the toxin. These results provide further support for the voltage sensor-trapping model.

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A Cytotoxic Peptide from a Marine Sponge, Polytheonamide B; II. Properties for Ion Conduction and Voltage Dependent Gating

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A 48-mer peptide from a marine sponge *Theonella swinhoei*, polytheonamide B (pTB), is highly cytotoxic against eukaryotic cells. Alternating D- and L-chirals throughout the molecule suggest formation of β-helix, which was supported by channel activity having high permeability to Cs^+. The 48-mer β-helix is long enough to span the membrane and was shown to form the channel by single peptide. In this study we evaluated the ion conduction and gating properties of pTB channel by use of planar lipid bilayer technique. Single-channel *I-V* curve in CsCl solution exhibited weak outward rectification. Concentration-dependency of single-channel conductance of pTB channel was examined. For Cs^+ permeation, clear saturation in unitary conductance was observed in the concentration range up to 3.0 M, which was contrast to gramicidin A (gA) channel. Gating manner of pTB channel was characteristic. Fast transition between open and closed state was observed, suggesting that the structural changes of single pTB molecule directly link to the gating. In addition to asymmetrical single-channel conductance, distinctive voltage-dependent gating was observed in single-channel and macroscopic current traces of pTB channels. On the basis of the structure of pTB channel, mechanism of ion conduction and voltage-dependent gating will be discussed.

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Physalia physalis Poison Depolarizes Beta Cell Membrane and Increases Insulin Secretion

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Peptide toxins isolated from marine cnidarians are considered as useful tools for studying ionic channels and promising drugs for therapeutics. Hemolytic and cardiotoxic activities have been described in Physalia physalis venom, and two toxins with anticholinergic and antiglutamatergic effects have been isolated in its high molecular weight fraction.
In this work, we explored some crude extract of P. physalis and its low molecular weight fractions on ionic currents and insulin secretion of pancreatic rat beta-cells. P. physalis specimens were collected at the north littoral of La Habana, Cuba. Crude extract were purified by a gel filtration (Superdex 200). Mass spectrometry MALDI-TOF and RP-HPLC (C18 column) were performed on each fraction collected from gel filtration. The biological activity on insulin secretion was tested in a reverse hemolytic plaque assay on primary cultures of pancreatic beta cells from male Wistar rats.
In basal glucose (5.6 mM), the crude extract (25 protein ug/mL) increased by 77% the average immunoplaque area, which is directly proportional to insulin secreted by isolated cells, without disrupting of beta cells or erythrocytes integrity. Low molecular fractions from gel filtration did not exceed of 20 kDa, and most of them eluted before the 20 % of acetonitrile on RP-HPLC. These fractions increased both, the percentage of insulin-secreting cells and the average immunoplaque area at a basal glucose level, suggesting a direct effect on TRP-type channels that are responsible for beta cell depolarization.
Physalia physalis poison contains polar bioactive compounds capable of enhance the secretory behavior of pancreatic beta cells from male Wistar rats by depolarizing the membrane, in non stimulant glucose conditions.
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Insights on Channel-Like Activity of Membrane Bound Alpha-Synuclein

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Alpha-synuclein (syn) is a natively unfolded protein with the ability to acquire secondary structure upon interaction with membranes or with itself. It is linked to Parkinson's disease (PD) by two evidences: the accumulation of amyloid fibrils of the protein and the autosomal dominant forms of the disease (A53T, A30P and E46K mutants). Both the biological role of this protein as the mechanisms involved in the ethiopathogenesis of PD are still unknown. The protein is located at the presynaptic terminal of neurons in all the Central Nervous System, where it exists free in the citosol or bound to synaptic vesicles. The membrane binding causes the formation of an amphipatic alpha-helix in the first part of the protein, which lies at the membrane surface without crossing the bilayer. A recent paper by Zakharov et al. (Biochemistry, 2007) reports that upon the application of a potential a channel like activity of syn can be recorded. Authors suppose that the helices of the first hundred amino acid of syn can pass the membrane bilayer to compose a pore only upon the application of a potential. Both the mechanism and the biological implication of this behaviour are still unknown and potentially of interest for the role that syn channel may play. In this study we extended this approach to syn deletion mutants. Furthermore, the effect of monomer topology in the construction of the purported channels have been explored thought the design of several types of syn dimers. A comparative analysis of the electrophysiological properties of these constructs will be presented and discussed.

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Developing a Functional Screening Assay of Small Molecules That Can Reduce Leakage of Liposomes Induced by Amyloid-Beta Peptides

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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, especially Aβ(1-40) and Aβ(1-42), are the major components of plaques formed in the brain of patients with AD and are considered to be pathologically important. Both peptides aggregate rapidly in aqueous solution to form Aβ oligomers as well as Aβ fibrils over time. Increasing evidence indicates that Aβ peptides, especially in their oligomeric state, play an important role in pathogenesis of AD. One possible pathogenic mechanism of these Aβ oligomers is the formation of pores through neuronal membranes, resulting in cell death. This research examined the effect of Aβ on permeabilization of liposomes by monitoring the change of fluorescence intensity of pH-sensitive dye which was encapsulated in the liposomes due to the leakage of protons. These experiments showed that the effect of Aβ depended on the lipid composition and on the aggregation state of Aβ. The developed liposome leakage assay makes it possible to screen several potential drug candidates for their ability to inhibit or reduce the leakage of liposomes.

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Efficacious in vivo Electrophysiological Screening of Neuromodulatory Compounds: Using Drosophila to Evaluate the Activity of Conotoxins

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Finding compounds that affect neuronal function is central for the development of probes or potential therapeutic agents. We have devised an efficacious