tyrosine fluorescence, from quenching experiments with spin labelled phospholipids using A1NT Trp.

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Interaction Of Human Islet Amyloid Poly Peptide With Phospholipid Membrane Vesicles

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Amylin, also known as Human Islet Amyloid Polypeptide (hIAPP), is a 37-residue peptide, suspected to play a major role in the malfunction of insulin secretion in diabetes mellitus type II. Co-secreted with insulin in the beta cells, hIAPP, in higher rates destroys the barrier function of the beta-cells, leading to a failure in insulin production. Because of its amyloidogenity, aggregates of fibrils can be observed in the islands of Langerhans due to its overexpression. We studied the physico chemical properties of hIAPP by observing changes in its structure depending on time and the surrounding media using MALDI-TOF-MS, ATR FT-IR- and fluorescence spectroscopy. In water, hIAPP fibrils grow slowly, after a 37°C incubation for 24 hours some alpha-helices are twisted, and after two weeks, no random coil is detected anymore. We determined membrane binding of dansyl-labeled hIAPP to phosphatidylserine (PS)/ phosphatidylcholine (PC) membranes. Additionally, using confocal laser scanning microscopy the binding of TAMRA labelled hIAPP to giant unilamellar vesicles could be observed. At physiological pH, hIAPP is positively charged and thus negative charges at the phospholipid membrane surface accelerate the process of peptide folding. Being random coil as initial state, a mixture of antiparallel beta-sheets and alpha-helices emerges in time. In the presence of negatively charged PS/PC membranes, hIAPP aggregates can be seen within a few minutes after titration. To understand the process of penetration into cells, we performed leakage measurements of carboxyfluoresceine (CF) filled phospholipid large unilamellar vesicles by means of fluorescence spectroscopy. Titration of hIAPP to CF filled PS/PC liposomes showed different results concerning equilibrium time and maximal extent of leakage depending on the age and preparation of the peptide. In particular the composition of the vesicles seems to determine their stability in the presence of hIAPP.

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Single Particle Analysis of Liposome Leakage Induced by Islet Amyloid Polypeptide

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Islet Amyloid Polypeptide (IAPP) is a 37-residue peptide hormone co-secreated with insulin from pancreatic β-cells. In patients suffering from type-2 diabetes mellitus (T2D), IAPP forms amyloid fibers in the pancreas, which are associated with cell death and the progression of the disease. A possible mechanism of cytotoxicity in T2D is the permeablizing of membranes by oligomeric IAPP, followed by leakage of ions or other molecules. We are examining the IAPP-induced leakage of individual liposomes through the use of single particle methods. Individual fluorescently labeled liposomes are measured post-IAPP exposure through the use of single particle burst analysis to determine the distribution of leakage states. By determining the role of individual residues, solution conditions, and lipid composition in modulating leakage insights are made into the mechanism of oligomer-mediated membrane leakage.

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Amyloid Oligomers Alter The Conductance Of The Gramicidin Channel

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Amyloid oligomers alter the conductance of the gramicidin channel.

Our previous data suggest that Aβ does not itself contribute a new intrinsic conductance such as ion channel to the membrane, but it does seem to alter its physical properties, specifically increasing the apparent dielectric constant of hydrocarbon region. This effect could in turn affect the properties of membrane ion channels.

In order to test this notion we compared the effects of amyloid oligomers on the single channel conductance of gramicidin in 2 M NaCl and CsCl. Amyloid oligomers increase the single channel conductance in NaCl from 13 to 16 pS, but the situation in CsCl is more complicated. In CsCl, the single channel conductance histogram shows two peaks, one with a conductance essentially the same as control (42 pS) and one with a conductance significantly less than control (28 pS). In terms of a simple three barrier two site model such as that used by Barnett et al., 1986 this suggests that amyloid oligomers lower the energies of both Cs and Na ions in the gramicidin channel, but at different critical locations relative to the barrier profile. For Na^+, amyloid oligomers lower the principal central barrier and thus increase the translocation rate of Na^+ at a given voltage. For Cs^+, amyloid oligomers act as if they lower the energy of the Cs ion in the channel, but in such a way as to increase the depth of one or both of the two wells in the barrier profile.

This work was supported by Alzheimer's Association grant IIRG-06-26167 and a grant from the Hillblom Foundation.

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The Insulin-sensitizers Troglitazone And Rosiglitazone Alter Lipid Bilayer Properties

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Thiazolidinediones are widely used to treat hyperglycemia in patients suffering from type 2 diabetes. Three thiazolinediones - troglitazone (Resulin), rosiglitazone (Avandia), and pioglitazone (Actos) - have been marketed; troglitazone was subsequently withdrawn due to hepatotoxicity. The thiazolidinediones are selective peroxizome-proliferator receptor gamma (PPARγ) agonists and they increase insulin sensitivity. They also have been found to have anti-oxidant, anti-inflammatory, anti-atherosclerotic and cardiovascular effects, but PPARγ activation alone does not account for all their actions. All three derivatives, with troglitazone being the most potent, modulate L-type calcium and delayed-rectifier potassium Kv1.3 channels by a seemingly PPARγ-independent mechanism. This could result from the adsorption of amphiphilic molecules to the membrane, which can alter bilayer properties such as thickness, intrinsic curvature and elastic moduli, and thus membrane protein function. We therefore set out to determine whether the amphiphilic troglitazone and rosiglitazone alter lipid bilayer properties. Using gramicidin channels as probes, where we monitor the changes in channel lifetime and rate of appearance, we tested and compared the effects of troglitazone and rosiglitazone on channels of different lengths in DOPC bilayers. Troglitazone or rosiglitazone did not alter gramicidin channel conductances, suggesting that direct interactions are not involved. In contrast, the lifetimes of both channels increased with similar relative changes for both the shorter and the longer channels. Consistent with their effects on calcium and potassium channels troglitazone is more potent than rosiglitazone. Our results show that both troglitazone and rosiglitazone affect bulk membrane properties at the concentrations where they modulate other ion channels.

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The Antimicrobial Peptide Gramicidin S Permeabilizes Phospholipid Bilayer Membranes Without Forming Discrete Ion Channels

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We examined the permeabilization of lipid bilayers by the β-sheet, cyclic antimicrobial decapeptide gramicidin S (GS) in phospholipid bilayers formed either by mixtures of zwitterionic diphytanoylphosphatidylcholine and anionic diphytanoylphosphatidylglycerol or by single zwitterionic unsaturated phosphatidylcholines having various hydrocarbon chain lengths, with and without cholesterol. In the zwitterionic bilayers formed by the phosphatidylcholines, without or with cholesterol, the peptide concentrations and membrane potentials required to initiate membrane permeabilization vary little as function of bilayer thickness and cholesterol content. In all the systems tested, the GS-induced transient ion conductance events exhibit a broad range of conductances, which are little affected by the bilayer composition or thickness. In the zwitterionic phosphatidylcholine bilayers, the effect of GS does not depend on the polarity of the transmembrane potential; however, in bilayers formed from mixtures of phosphatidylcholines and anionic phospholipids, the polarity of the transmembrane potential becomes important, with the GS-induced conductance events being much more frequent when the GS-containing solution is positive relative to the GS-free solution. Overall, these results suggest that GS does not form discrete, well-defined, channel-like structures in phospholipid bilayers, but rather induces a wide variety of transient, differently sized defects which serve to compromise the bilayer barrier properties for small electrolytes.

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The Superstructure of an Antimicrobial Peptide, Alamethicin, in Lipid Membranes

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In this work we investigate the effect of membrane hydration and hydrophobic mismatch on the Alm channel superstructure in an oriented multilayer sample by x-ray scattering. Wide angle x-ray scattering (WAXS) near 1.4 $Å^{-1}$ indicates that the lipid chain region is not much perturbed by the incorporation of up to 10 mole percent Alm. Low angle x-ray scattering (LAXS) indicates that when the sample is very dry, which promotes interactions between neighboring