Piscidins, which are antimicrobial peptides (AMPs) produced in the mast cells of hybrid striped bass, are found in immune-related organs as well as non-immune tissues, including neuronal cell bodies. While they are unstructured in water, they form amphipathic, cationic and alpha-helical structure in the presence of lipid bilayers at physiological pH. The biologically most active piscidin, piscidin 1 (P1), has broad spectrum antimicrobial activity against various bacteria, fungi, and viruses. Recently, it was found to be selective against HT1080 and HeLa cells as compared to healthy cells. This research investigates the factors that imparts P1 this selectivity against cancer cells. We have been particularly interested in special chemical properties of cancer cells, such as their low extracellular pH, presence of phosphatidylserine (PS) in the outer membrane leaflet, and elevated cholesterol level. We hypothesize that the positive charges on the histidine residues of P1 at the low extracellular pH of cancer cells allow P1 to align parallel to the surface and disrupt the membrane via the carpet-like mechanism of action.
Lipid membranes have been made using 2:2:1 phosphatidylcholine/phoshatidylserine/cholesterol to mimic tumor cells. Circular dichroism experiments were performed using large unilamellar vesicles to investigate the effect of pH, cholesterol, and lipid charge on the peptide's helical content and therefore affinity for lipid bilayers. Using 2H and 15N solid-state NMR, we have investigated the fluidity of the lipid bilayers and bilayer orientation of P1, respectively. These results are contrasted with those obtained on healthy mammalian cell mimics. The principles learned from these studies could help design peptides with enhanced activity against cancer cells.

1227-Pos Board B119
Effect of Membrane Cholesterol on the Structure of Alzheimer's Amyloid β Peptide in Lipid Bilayers
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A hallmark feature of Alzheimer's disease is formation of extracellular plaques of the amyloid β (Aβ) peptide. There is evidence that the Aβ peptide inserts into the membranes of surrounding neuronal cells, causing membrane permeabilization or rupture. The level of cholesterol in membranes has been shown to affect the extent and toxicity of Aβ plaques, as well as the mode of membrane insertion of Aβ and its structure. Here we analyze the effect of cholesterol on the secondary structure of the 40-residue Aβ peptide in artificial lipid bilayers. Multilayers composed of 0.3 mol fraction of 1-palmitoyl-2-oleoyl-phosphatidylglycerol, (0.7 - X_{chol}) mol fraction of 1-palmitoyl-2-oleoyl-phosphatidylcholine, and X_{chol} mol fraction of cholesterol were deposited on a germanium plate, with X_{chol} = 0, 0.05, 0.1, 0.2 and 0.4, containing Aβ at a 1:15 peptide/lipid molar ratio. Fourier transform infrared spectra were collected on lipid-peptide samples under three conditions, i) dry multilayers, ii) humidified with D_2O vapors, and iii) in the presence of a bulk D_2O-based buffer. In all cases, the amide I bands of the peptide indicated comparable fractions of α-helical and β-sheet structures. Increase of X_{chol} from 0 to 0.2 resulted in a significant decrease in the β-sheet structure and a slight increase in α-helix content. Further increase in X_{chol} to 0.4 reversed the β-sheet content and increased the α-helical structure; the latter effect was much stronger in hydrated samples. Considering that cholesterol at $X_{chol} > 0.2$ causes a fluid-disordered to fluid-ordered transition in membranes, the observed structural effects may reflect either conformational changes in membrane-bound Aβ peptide or a squeezing-out effect. These possibilities, as well as the effect of cholesterol on membrane permeabilization will elucidate the role of cholesterol in Aβ structure and function.

1228-Pos Board B120
Antihypertensive Antagonists of L-Type Calcium Channel Exert Enhancement of Alzheimer's Aβ Peptides Effect on Cells
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The Alzheimer's Aβ peptides are shown to increase the cytosolic calcium concentrations of cells in culture. Such an alteration may cause a variety of secondary effects, leading to cell degeneration and reduced cell culture growth. Cytosolic calcium concentration can be increased by the formation of cation-selective ion channels by the Aβ peptides. This property is well documented and has been extensively studied both in artificial membrane systems as well as in living cells. Here we show that L-type calcium channel antagonists, commonly used in the treatment of hypertension and ischemic heart disease, intensify the capacity of Aβ peptides to increase cytosolic calcium concentrations and consequently enhance the cytotoxicity of the Aβ peptides. Calcium imaging experiments were conducted with the fluorescent indicator Fura-2 to measure at different periods of times the levels of cytosolic calcium during exposure to Aβ peptides and to the L-type calcium channel antagonists. Two different 1,4-dihydropyridines (nifedipine, and nitrendipine), and Diltiazem, a structurally unrelated Ca^{2+}-channel antagonist belonging to the phenylalkylamine class were used and found to exert significant excitatory effects on the toxicity of Aβ. The reduced toxicity observed in solutions of combinations of Aβ40 and Aβ42 is also reversed by L-type calcium channel antagonists. The toxicity of Aβ peptides was weighed by quantifying the growth of cultures of PC12 cells using the colorimetric assay XTT and by measuring the release of LDH to the media. The enhancement of the Aβ peptides toxicity by L-type calcium channel antagonists can be constrained by specific Aβ ion channel blockers suggesting the mediation of the ion channel property of Aβ peptides. The relevance of our finding derives from the widespread clinical use of L-type calcium channel antagonists in the treatment of hypertension and angina pectoris.

1229-Pos Board B121
Chronic Cytosolic Calcium Changes Induced by Alzheimer's Peptides Aβ Allows for Sorting of Aβ-Resistant Cell Subpopulations
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The Alzheimer's Aβ peptides interaction with the plasma membrane has been shown to result in a chronic increase in the cytosolic calcium concentrations of cells in culture. Such alteration may cause a variety of secondary effects which may lead to cell degeneration and reduced cell culture growth. We have proposed that the alteration in the cytosolic calcium concentrations induced by Aβ is initiated by early formation of Aβ ion channels in the plasma membrane. To study the Aβ-induced cytosolic calcium change dynamics we monitored the level of cytosolic calcium from hundreds of individual cells in culture exposed to Aβ for prolonged period of time. Because of the continuous cytotoxic effect of Aβ on cells, the number of viable cells in the cultures varied with time of exposure. Histograms based on the individual intracellular calcium levels showed that while most cells in the culture remain unaffected, a defined subpopulation of cells show increasingly higher than normal cytosolic calcium levels. After days of continuous exposure to Aβ cells more sensitive to Aβ died, and consequently the averaged cytosolic calcium for remaining cells in the whole culture approached control values. Aβ ion channel blockers prevented the induced cytosolic calcium changes and preserved cell viability, confirming the participation of Aβ ion channels. Confocal microscopic analysis using fluorescent annexin V on cells loaded with fura 2 showed that those cells that remained after prolonged exposure to Aβ did not display the proposed extracellular Aβ receptor phosphatidyl serine. Further addition of Aβ to this sorted cell subpopulation did not induce cytosolic calcium changes and cells continued to grow and divide. Application of this procedure allows for sorting out Aβ resistant subpopulation of cells.

1230-Pos Board B122
Membrane Destabilization by Alzheimer's Amyloid β Peptide
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Alzheimer's Amyloid-β peptide (Aβ) is a 39-42 amino acid fragment of the amyloid precursor protein. Accumulation of the neurotoxic Aβ in the brains of Alzheimer's patients forms detrimental plaques. Another mechanism of neuronal damage is membrane destabilization by insertion of the peptide into brain cell membranes and disruption of ionic homeostasis. The molecular mechanism of membrane permeabilization by Aβ remains largely uncharacterized. Experiments with live cells and lipid membranes have identified ion channels formed by Aβ. The channel formed by an 11 amino acid residue fragment, Aβ25-35, has been modeled by Molecular Dynamics simulations as an 8-stranded β-barrel, but this structure has not been supported by direct experimental data. The objective of this work was to study the mechanism of membrane permeabilization by Aβ using biophysical techniques. Unilamellar lipid vesicles were prepared in a buffer containing 30 mM $CaCl_2$, followed by removal of calcium from the external medium via a desalting column and addition of 0.1 mM Quin-2, a fluorescent calcium indicator. Before peptide addition, the vesicle sample showed little fluorescence because of spatial sequestration of calcium and Quin-2. Addition of submicromolar concentration of the Aβ25-35 peptide resulted in gradual increase in Quin-2 fluorescence, indicating formation of Ca^{2+}-permeable pores in vesicle membranes. Addition of higher concentrations of the peptide resulted in instantaneous increase in fluorescence, comparable to the effect of detergents like Triton X-100, indicating rupture of vesicle membranes. Thus, the data suggest membrane pore formation at low peptide concentrations and membrane disruption at higher concentrations. These data are consistent with a mechanism of detergent-like action of the Aβ peptide;