T-Pos87 EFFECT OF METALS ON THE DNA HELIX. Yong Ae Shin, Susan Feroli, and Michele Gay. National Institutes of Health, National Institute on Aging, Gerontology Research Center, Baltimore, Maryland 21224.

Changes in the double stranded DNA helix in response to metal binding have been investigated by monitoring, by two dimensional agarose gel electrophoresis, the movement of covalently closed circular DNA topoisomers in the presence of different metal ions. Since the linking number (LN) is invariant for covalently closed circular DNA, $\Delta Tw = -\Delta Wr$ when no nicking is involved in the reaction. Tw is the Watson-Crick helical turns, and Wr is the number of superhelical turns; the latter can be monitored by gel electrophoresis. Therefore, by following the movement of topoisomers on gels run in the presence of different metal ions, accompanying changes in the helical twist between the adjacent base pairs of the DNA double helix may be deduced. It was found that: 1. Co(III) unwinds the pBR 322 double helix, while Mg(II) increases the winding. 2. Tris ethylenediamine Co(III) unwinds more effectively than hexammine Co(III); $\sim$ 0.3 degrees per base pairs versus $\sim$ 0.2 degrees, respectively, in 50 μM Co(III) at 25°. Either 5 mM Mg(II) or lowering the reaction temperature from 25° to 4° increase the winding angle by $\sim$ 0.3. 3. The presence of Co(III) complex ions reduces the number of negative supercoiling turns required for the conformational transition of the d(GC)16 insert in the plasmid pTR 161*. While the tris ethylenediamine Co(III) induced change is compatible with a complete B-Z transition of the insert, hexammine Co(III) seems to only make the transition incompletely.

T-Pos88 MODELS FOR TWO tRNA's BOUND TO SUCCESSIVE CODONS ON RNA

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A number of changes in tRNA structure have been proposed to occur on binding of the codon. This report covers a possible model complex with two molecules of phenylalanine tRNA bound to successive codons in poly rU. For initial modelling, we have started with the loop structures alone. The binding of this single stranded helical rU with the anticodon was modelled without perturbation to any of the helical constituents. The second tRNA loop was manipulated such that the anticodon forms a helical structure with the remaining mRNA with optimal hydrogen bonds for base pairing. Interlinking of the two tRNAs was avoided, and the van der waals overlapping was minimized through computer graphics. Energy minimization and low temperature molecular dynamics were used to anneal the structure, constraining the basepair hydrogen bonds between tRNAs and mRNA. The remaining tRNA structures were embedded on the loop structures to complete the model. The amino acid acceptor termini of the tRNAs are found to be separated by a large distance. We have followed our earlier adiabatic minimization procedure to bend the tRNAs and brought the amino acid acceptor termini closer so that peptide bond formation would be possible.

T-Pos89 DETECTION OF INOSINE·ADENINE BASE PAIRING IN THE AQUEOUS SOLUTION OF AN OLIGODEOXYRIBONUCLEOTIDE WITH NMR SPECTROSCOPY. L.-S. Kan, N. Kanhouwa, and P. Pramanik, Division of Biophysics, The Johns Hopkins University, Baltimore, Maryland 21205.

The decadeoxynucleotide d-(CCAAIATTGG) forms two self-associated duplexes in aqueous solution, as determined from the proton nmr study. One contains $I_{anti}{\cdot}A_{anti}$ and the other duples contains $I_{anti}{\cdot}A_{syn}$ orientation of base pairing. The relative concentrations of the two duplexes did not change noticeably with the variation of sodium chloride concentration (0 - 0.5 M) and oligonucleotide concentration (0.14 - 3.44 mM is single strand). The duplex with $I_{anti}{\cdot}A_{stn}$ is thermally less stable than the duplex with $I_{anti}{\cdot}A_{anti}$. From the line broadening pattern with the variation of temperature, it is concluded that I·A base pairing is less stable than the G·C as well as A·T base pairing. (supported by N.I.H. grant GM 34252-08)

T-Pos90 THE COVALENT MODIFICATION OF OLIGONUCLEOTIDES BY THE ENANTIOMERS OF 7,8-DIOL,9,10-EPOXY,7,8,9,10-TETRAHYDROBENZO (A)PYRENE. Camille J. Roche and Nicholas E. Geacintov, Department of Chemistry, New York University, New York, NY 10003.

Base sequence specificity is an important factor in determining the reactivity and the covalent modification of DNA by the diol epoxide derivatives of polycyclic aromatic hydrocarbons. One of these derivatives, (+)-7,8-diol,9,10-epoxy,7,8,9,10 tetrahydrobenzo (a)pyrene (BaPDE) is highly tumorigenic in mammalian systems, while its mirror image is not. Though both diol epoxides bind primarily to guanine, the extent of modification and the resulting structure of the covalent adducts is very different. Alternating G:C oligonucleotides, that varied from six to ten base pairs long were used to study the base specific properties of the covalent adducts of the enantiomers of BaPDE. The extent of modification was evaluated by absorption, and the characteristics of the covalent adducts were analyzed by absorption, circular dichroism and fluorescence. In the case of (+)BaPDE the extent of modification of the oligonucleotides was similar to the extent of modification of the long chain alternating G:C polymer. However, in the case of (-)BaPDE, the extent of modification of the oligonucleotides was greater than that of the long chain polymer. When absorption was used to determine the properties of the covalent adducts of each of the enantiomers, the structural characteristics seem to be dependent on chain length. The fluorescence excitation and emission spectra are also blue-shifted from those observed in the long chain alternating G:C polymer. These characteristics are addressed.

T-Pos91 COMPARISON OF OPTICAL MELTING THEORY WITH EXCHANGE BROADENING OF THE NMR SPECTRUM FOR A SELF-COMPLEMENTARY DNA MINICIRCLE. A. S. Benight[1], J. M. Schurr[2], D. E. Wemmer[3], P. Flynn[2] and B. R. Reid[2]. Departments of Chemistry, (1) University of Illinois, Chicago, (2) University of Washington, Seattle and (3) University of California, Berkeley.

We have calculated melting curves for the sixteen base-pair duplex DNA $^{5'}$GTATCCGTACGGATAC$^{3'}$ linked on the ends by TTTT single strand loops. An excellent fit of the previously reported experimental melting curve in 0.2 M NaCl (Wemmer and Benight (1985) Nucl. Acids Res. 13, 8611) was obtained. From our analysis we evaluate the free energy of closing a TTTT end-loop to be 2.1 kcal/mole. The loop free energy of the 40-base single strand open minicircle is 1.3 kcal/mole, thus favoring the melting of two end-loops into the large open minicircle. A comparison of our results with those reported for d(T-A) oligomers (Scheffler, et al. (1970) J. Mol. Biol. 48, 145) reveals that TTTT forms a more stable end-loop, or hairpin, than TATA by ~2 kcal/mole.

Catalytic rate constants for the imino proton-transfer step in the standard exchange model were calculated by extending the theory of diffusion-controlled reactions to account for the electrostatic potential of the DNA. Using these rate constants, prevailing buffer catalyst concentrations, and the equilibrium constants to form the unstacked open state (from optical melting theory) we predicted the imino proton exchange rates under catalysis-limited conditions. Experimentally observed exchange rates of the AT base-pairs significantly deviate from the theoretically predicted values. These results strongly suggest that the solvent-accessible open state of the standard model for imino proton exchange is not identical to the unstacked open state of optical melting theory.

T-Pos92 THE EFFECT OF A:T BASE PAIR ON THE B-Z CONFORMATIONAL TRANSITIONS. Fu-Ming Chen, Department of Chemistry, Tennessee State University, Nashville, Tennessee 37203

Effects of A:T base pairs on the propensity of B to Z conformational transitions have been investigated by the salt titrations on d(CGCGCGCGCG), d(GCGCGCGCGC), and decamers obtained by replacing the terminal or central G and C bases with the A and T bases, respectively. Dodecamers obtained by adding A:T base pairs to both ends of the parent decamers, while maintaining the purine-pyrimidine alternating feature, have also been compared. The results indicate that the presence of A:T base pairs at the center greatly inhibit the B to Z transition of both GC decamers. Only slight inhibition is observed when A:T base pairs are added to the terminals of d(CGCGCGCGCG) while a stronger inhibition is apparent when the terminal G:C base pairs are replaced by the A:T base pairs. The addition and replacement with the A:T base pairs at the terminals of d(GCGCGCGCGC), on the other hand, facilitate the B to Z conversion, with the replacement exhibiting somewhat more pronounced effect. These results may be rationalized in terms of the number of contiguous CG sequence present in an oligomer and the relative inhibitory effects of other dinucleotide sequences. Supported by NIH Grant CA-42682 and in part by MBRS Grant S06RR0892.

T-Pos93 RAMAN SPECTROSCOPIC ANALYSIS OF THE B-DNA DODECAMER d(CGCAAATTTGCG) REVEALS SIGNIFICANT CONFORMATIONAL DIFFERENCES BETWEEN CRYSTAL AND SOLUTION STATES. J.M. Benevides and George J. Thomas, Jr., Division of Cell Biology and Biophysics, School of Basic Life Sciences, University of Missouri-Kansas City, Kansas City, MO 64110

X-ray crystallography (A.H.-J. Wang, unpublished results) shows that the self-complementary dodecamer d(CGCAAATTTGCG) crystallizes as a double helix of the B-form. Knowledge of the crystal structure permits the bands of the observed Raman spectrum to be assigned unambiguously to specific phosphodiester backbone geometries and nucleotide conformations associated with B-DNA. The Raman bands proposed as markers of the crystalline B-DNA structure are compared and contrasted with previously proposed markers of Z-DNA and A-DNA crystals. The results indicate that the three canonical forms of DNA can be readily distinguished by Raman spectroscopy. We have also examined the Raman spectrum of the dodecamer in aqueous solution. Raman bands which are sensitive to backbone geometry remain within the ranges expected of B-DNA for the solution structure. However, unlike Z-DNA and A-DNA, which retain their characteristic Raman fingerprints in aqueous solution, the B-DNA Raman spectrum is not completely conserved between crystal and solution states. Raman markers specific to GC pairs show a much greater dependence upon the state of aggregation than corresponding markers specific to AT pairs. The results suggest that conformations which are unstable in the solution structure may be stabilized in the crystal structure, at least with respect to the terminal domains containing GC pairs. A model for the solution structure is proposed on the basis of the Raman results.

Supported by N.I.H. Grants AI11855 and AI18758

T-Pos94 Stability and Exchange Kinetics of the N-2-Acetylaminofluorene Modified d(CCACGCACC)•(GGTGCGTGG) Duplex. **Guanjin Huang and Thomas R. Krugh**
Department of Chemistry University of Rochester Rochester, NY 14627

N-2-Acetylaminofluorene(AAF) and Aminofluorene (AF) are polycyclic aromatic carcinogens. We have studied the stability and measured the lifetimes of the AAF-d(CCACGCACC)•(GGTGCGTGG) duplex. All nine imino protons have been assigned by sequential T.O.E. experiments at 0 °C. Non-selective and selective saturation recovery measurements were used to determine the lifetime of the imino protons in both the AAF-modified and the unmodified duplexes. The lifetime is significantly shorter at the site of modification and at the 3' neighbouring CG base pair when compared to the equivalent data from the unmodified duplex. An transfer of magnetization experiment was used to determine that chemical exchange, and not magnetic relaxation, is the major contributer to the decrease in the lifetime of these two imino protons. A thermal denaturation experiment showed the AF-d(CCACGCACC)•(GGTGCGTGG) duplex is less stable than the unmodified duplex, but more stable than AAF-modified d(CCACGCACC)•(GGTGCGTGG) adduct duplex.

T-Pos95 IONIC STRENGTH DEPENDENCE OF THE PERSISTENCE LENGTH OF DNA. Marcia Fenley, Gerald S. Manning & Wilma K. Olson, Department of Chemistry, Rutgers University, Piscataway, NJ 08855-0939.

The aim of this study is to compare two different theoretical approaches to an understanding of the observed ionic strength dependence of the persistence length of DNA. One approach makes use of an extended "counterion condensation" polyelectrolyte theory to obtain the electrostatic contribution to the persistence length. The other approach involves chain statistics where long-range electrostatic forces are used to account for the salt dependence of the average chain extension. This study will consider 1:1 as well as 2:1 aqueous salt solutions. Segments of DNA of finite length are treated so that we can analyze the end effects. Experimental measures of the persistence length of DNA are used to analyze and test predictions from the above theoretical approaches. (Supported by USPHS grant GM-22724 and USPHS grant GM-34809).

T-Pos96 SEQUENCE DEPENDENT MODELS OF DNA BENDING IN DNA-PROTEIN COMPLEXES. Minghong Hao and Wilma K. Olson, Department of Chemistry, Rutgers University, Piscataway, NJ 08855-0939

The structures of the very bent DNA's in DNA-protein complexes have been modeled by the homogeneous coordinate transformation of 20 base pair fragments through a series of gradually decreasing radii followed by the energy minimization between successive transformations. The energies of the resulting bent structures are within 0.4 kcal/mole bp of that of the corresponding straight DNA. In contrast to earlier studies of DNA bending (Sussman & Trifonov, PNAS, 75, 103 (1978); Levitt, PNAS, 75, 640 (1978)), the structures show significant differences from the smoothly bent model, the bending concentrating around the points where the minor and major grooves bend in the direction of chain curvature. The minor groove bending is achieved by large base pair rolling, while the major groove bending is effected by base pair sliding, no significant tilting of adjacent base pairs being found. The bending extent as measured by the width of grooves is sequence dependent, the sequences 5'-..AATT..-3' and 5'-..AAAA..-3' showing the largest bending towards the minor groove, and the sequences 5'-..GGCC..-3' and 5'-..GGGG..-3' showing the largest bending towards the major groove. Moreover, the sequences 5'-..GGCC..-3' and 5'-..TTAA..-3' resist bending towards the minor groove and the sequences 5'-..AATT..3' and 5'-..AAAA..-3' resist bending towards the major groove. These sequence-dependent bent structures are closely correlated with the preferred positions of selected base pairs and the variation in groove widths found in nucleosomal DNA (Travers & Klug, Nature, 327, 290 (1978)). (Supported by USPHS grant GM-34809).

T-Pos97 ELECTROSTATIC INTERACTIONS IN DNA B. Jayaram, K. Sharp and B. Honig Department of Biochemistry and Molecular Biophysics, Columbia University, 630 West 168th Street, New York, New York 10032.

A discrete-continuum approach to the treatment of electrostatic interactions in nucleic acids is described. The nucleic acids are represented as a low dielectric medium containing a discrete set of real and partial charges whose coordinates are known from X-ray analyses. The solvent is treated as a high dielectric continuum which contains a simple electrolyte. Electrostatic potentials are obtained by solving the non-linear Poisson-Boltzmann equation with a finite-difference algorithm used previously for proteins. With regard to nucleic acids, the most important new feature of this work is that, in addition to the actual charge distribution, the detailed shape of the dielectric boundary between the nucleic acid and solvent is explicitly taken into account. The effect of this improved description of the macromolecule on a number of electrical properties is explored. These include effective dielectric constants between different phosphates, the extent of counterion condensation and the sequence dependence of the calculated electrostatic potentials. Results are compared with those obtained from simpler models for the shape, charge distribution and dielectric boundary of the macromolecule.
Supported by the NIH (GM-30518).

T-Pos98 ANISOTROPIC MOBILITY IN QUINACRINE/DNA COMPLEXES. Pei Fan, Torleif Härd, Doug Magde and David R. Kearns, Department of Chemistry, University of California, San Diego, La Jolla, California 92093-0314.

The anisotropic motions of quinacrine and 9-amino-6-chloro-2-methoxyacridine (ACMA) in intercalative complexes with DNA and various double-stranded polynucleotides were studied using time-resolved fluorescence polarization anisotropy (FPA). The FPA decay (80 ns) was monitored following excitation of the UV or visible transitions of the dyes. The limiting FPA of the UV transitions is negative due to a large angle between the excitation and emission transition moments, but in the DNA-complexes it changes sign after 10 ns owing to the predominance of torsional motions in DNA. Both FPA decays (UV and visible excitation) can be deconvoluted using a model for elastic deformations in DNA (derived by Barkley and Zimm, and by Allison and Schurr) with the DNA torsional rigidity as the single adjustable parameter. A simultaneous (global) deconvolution of the two decays improves the accuracy in determining the DNA torsional rigidity. The observation that the value of the DNA torsion constant is independent of the choice of intercalating dye (ethidium, quinacrine or ACMA) further justifies using fluorescence measurements to probe DNA motions on the nanosecond time scale. The torsional rigidity of the studied polynucleotides decreases in the order poly(rI)·poly(rC) > poly(dI)·poly(dC) ≅ calf thymus DNA > poly(dA-dT)·poly(dA-dT) ≅ poly(dA)·poly(dT). The magnitude of dye "wobbling" within the intercalative pocket can be judged from differences between the initial FPA of the DNA/dye complexes and the limiting FPA for dyes in viscous solvents, monitored as an "amplitude reduction factor" (ARF). Differences in ARF values between the UV and visible excitation transitions might arise from DNA to dye energy transfer in the UV band and/or anisotropic "in-plane" wobbling motions of the intercalated dyes.

T-Pos99 ORIGIN OF DNA HELICAL STRUCTURE AND ITS SEQUENCE DEPENDENCE. A. Sarai, J. Mazur*, R. Nussinov† and R.L. Jernigan, Lab. Math. Biol., NCI, NIH, Bethesda, MD 20892 USA, *Advanced Scientific Computing Lab. PRI, NCI-FCRF, Frederick, MD 21701 USA, †and Sackler Institute of Molecular Medicine, Sackler Faculty of Medicine, Tel-Aviv University, Ramat Aviv, 69978 Israel.

Double helical DNA plays the essential role of storing genetic information in a stable form, and its stability probably affects the control of gene expression. Although the structures of DNA have been studied in some detail, it is not well understood why DNA is stabilized in a particular helical conformation. Hydrogen bonding between bases leads to double-stranded forms, but not to a specific type of helix. Within the major families, B, A, Z etc., and depending upon the base sequence, DNA exhibits smaller scale local conformational variations, from small base pair orientational changes to DNA bending. Implications for biological function have recently been a focus of intensive research. To date, the most popular model of the sequence dependence of B-form variants was proposed by Calladine, who stated that the steric clash between base pairs may be responsible for the conformational variation. Here, conformational analysis of DNA shows that the origin of the B-form double helix can be attributed simply to the atomic charge pattern in the base pairs. The charge patterns favor the helical stacking of the base pairs. Base pairs alone - without backbones - have a tendency to form helix, indicating that the backbones play a rather passive role in determining the basic helical structure of DNA. In our previous calculation without backbones, the fit to the X-ray structure of B-form DNA gives RMS deviation of less than 0.8 Å. The charge pattern in the base pairs appears to be responsible for much of the sequence dependence of DNA conformation rather than the steric clash proposed by Calladine.

T-Pos100 X-RAY CONFORMATIONAL STUDY OF THE DNA SUPERHELIX, S.W. Chen, S. Rothenberg, D. Schaak, D.B. Fein and G.W. Brady, Wadsworth Laboratories, NY State Department of Health, Albany, NY 12201. Rensselaer Polytechnic Institute, Troy, NY 12181.

Since X-ray diffraction techniques allow the direct observation on dissolved DNA molecules, this fact makes it an ideal method for the study of the conformation of the DNA superhelix (1). We had previously reported that except for a minority of cases (5%), the superhelix was found to exist in the non-interwound for (2). A much more extensive number of experiments done on plasmids of a range of length ranging from 2.6 kb to 8.2 kb has shown that the non-interwound and interwound forms appear to occur with nearly equal probability. The size of the molecule does not influence the occurrence of one form over the other nor does the presence of protein impurities. Repeating the experiments with fresh reagents had no effect. Since the two forms can be isolated from the same growth batch, the differentiation must occur during the extraction procedure. We conclude that the two forms are energetically quite similar, and that some slight bias in solvent conditions will result in one of the two forms being preferred. We conjecture that since electron microscopy always seems to favor the interwound form, during the EM sample preparation the sample passes through a condition where the conditions are just right for the occurrence of that form. (Supported by NSF Grant DMB-8519150.)

(1) Brady, G.W., Fein, D.B., Lambertson, H., Grassian, V., Foos, D. and Benham, C.J. (1983) PNAS USA 80, 744-741.
(2) Brady, G.W. and Foos, D. (1984) Biopolymers 23, 2963-2966.

T-Pos101 EFFECT OF SUPERHELIX DENSITY ON THE STRUCTURE AND DYNAMICS OF SUPERCOILED DNA. EVIDENCE FOR A CHANGE IN SECONDARY STRUCTURE. Lu Song, Bryant S. Fujimoto, and J. Michael Schurr, Department of Chemistry, University of Washington, Seattle, WA 98195.

Supercoiled DNA samples with different median superhelix densities have been prepared by relaxing pUC8 dimer (5434 bp) using topoisomerase I in the presence of various amounts of ethidium bromide. After removing the ethidium by dialysis, the median numbers of superhelical turns of these samples are +4, 0, -8, -13, -20, -25 (native). These samples were investigated using dynamic light scattering (DLS) from K^2 = (0.47 to 20.56) x 10^{10} cm^{-2}, time-resolved fluorescence polarization anisotropy (FPA) of intercalated ethidium dye (1/300 bp), high resolution gel electrophoresis (GEL), and circular dichroism (CD). The apparent diffusion coefficient at large scattering vector, $D_{plat} = D_{app}(K)$ at $K^2 = 20 \times 10^{10}$ cm^{-2} reflects in part the local rigidity and dynamics of the filament. FPA measurements provide information about the magnitude and uniformity of the torsion elastic constant α. Plots of D_{plat}, α, and CD parameters vs. median number of superhelical turns all show striking, anomalous behavior at -13 turns, corresponding to a superhelix density σ = -0.025. This is taken as evidence for a change in secondary structure at that point. The +4 sample shows a greater spacing of its topoisomer bands than the negatively supercoiled samples in GEL studies. Interesting variations of the center-of-mass diffusion coefficient D_0 and the GEL mobilities are also noted.

Evidence is also obtained for a marked effect of the presumed double-spiral tertiary structure on curves of D_{app} vs. K^2.

T-Pos102 EXCITATION TRANSFER AND TORSION DYNAMICS OF ETHIDUM/DNA COMPLEXES. Bryant S. Fujimoto, Pengguang Wu, James B. Clendenning, and J. Michael Schurr, Department of Chemistry, University of Washington, Seattle, WA 98195.

Time-resolved fluorescence polarization anisotropy (FPA) measurements were performed on complexes of ethidium with both linear and supercoiled DNAs over a wide range of binding ratios (bound dye/bp) from r = 0.002 to 0.2. Dynamic light scattering (DLS) measurements were also performed on the ethidium/linear DNA complexes. In order to determine the effect of intercalated ethidium on the torsional rigidity at high binding ratios, it is necessary to deconvolve the effects of fluorescence excitation transfer from one dye to another. The contribution of excitation transfer to the mean squared angular displacement of the transition dipole around the symmetry axis was determined using the Monte Carlo method of Genest and Wahl. Excitation translation along an equilibrium distribution of bent DNAs contributes to the mean squared angular displacement around a transverse axis, and was also taken into account. In analyzing the FPA data, these contributions were superimposed on the respective mean squared angular displacments due to deformational motions in order to determine the optimum torsion constant.

The torsion constants of both linear and supercoiled DNA/ethidium complexes remain uniform and essentially identical up to r = 0.1. However, in both cases the apparent torsion constant drops by about 30 % from r = 0.002 to 0.02, but remains constant from r = 0.02 clear up to r = 0.1. These and other considerations indicate that ethidium (and other dyes, excluding chloroquine) may induce a structural change at low binding ratios r ≤ 0.02, but induce no further change until much higher binding ratos r = 0.2 are reached.

T-Pos103 INTERACTION OF CHLOROQUINE WITH LINEAR AND SUPERCOILED DNA. EFFECT ON THE TORSIONAL DYNAMICS, RIGIDITY, AND TWIST ENERGY PARAMETER. J. Michael Schurr, Pengguang Wu, Lu Song, James B. Clendenning, and Bryant S. Fujimoto, Department of Chemistry, University of Washington, Seattle, WA 98195.

The magnitude and uniformity of the torsion elastic constant (α) of linear pBR322, and high twist (σ = -0.083) and normal twist (σ = -0.048) supercoiled pBR322 DNA were measured as a function of chloroquine concentration up to binding ratos (bound dye/bp) r ≥ 0.28. This information is obtained from the time-resolved fluorescence polarization anisotropy (FPA) of intercalated ethidium dye (1/300 bp). The equilibrium constant K for chloroquine binding to linear DNA, and the twist energy parameters E_T for chloroquine/supercoiled DNA complexes were determined by analyzing the ratio (A_b/A_f) of ethidium fluorescence decay amplitudes corresponding to bound (A_b) and free (A_f) dye. r was determined as a function of added chloroquine per bp (chl/bp) in the same analysis. E_T values for ethidium/supercoiled DNA complexes were determined by competitive dialysis. E_T is the free-energy to introduce the first superhelical turn times the number N of bp, in units of k_BT. The torsional rigidities of all three DNAs are uniform, identical in magnitude, and completely unaffected by chloroquine binding up to r = 0.2! A structural transition occurs as r increases from 0.2 to 0.3. The E_T values determined for either chloroquine or ethidium binding are somewhat lower than those obtained in previous dye-binding studies. The two (or more)-fold discrepancy between E_T values obtained by dye-binding (smaller) and by ligation (larger) methods holds also for chloroquine, and cannot be attributed to any direct effect of chloroquine on the torsional rigidity.

T-Pos104 EFFECT OF 9-AMINOACRIDINE, PROFLAVINE, AND QUINACRINE ON THE TORSIONAL RIGIDITY AND DYNAMICS OF LINEAR AND SUPERCOILED DNA. Pengguang Wu, Albert S. Benight, and J. Michael Schurr, Department of Chemistry, University of Washington, Seattle, WA 98195.

Complexes of linear and supercoiled pBR322 DNA with 9-aminoacridine, proflavine, and quinacrine were studied over a wide range of binding ratios (bound dye/bp) from r = 0 to 0.35 by time-resolved fluorescence polarization anisotropy (FPA) of intercalated ethidium dye (1/300 bp). Such FPA measurements provide information about the uniformity and magnitude of the torsion elastic constant (α). The equilibrium constant for binding each dye to linear pBR322, and the twist energy parameter E_T for each dye/supercoiled DNA complex were determined by analyzing the ratio (A_b/A_f) of ethidium fluorescence decay amplitudes corresponding to bound (A_b) and free (A_f) dye. The binding ratio r was determined as a function of added dye per bp (dye/bp) in the same analysis. E_T is the free-energy to introduce the first superhelical turn times the number N of base-pairs, in units of k_BT.

The torsional rigidities of complexes of 9-aminoacridine and proflavine with linear DNA remain uniform for r ≤ 0.35. For complexes with supercoiled DNA, they remain uniform for r ≤ 0.2. For complexes with linear DNA, the magnitude of the torsion elastic constant decreases by about 30 % as r increases from 0 to about 0.1, and then remains approximately constant up to about r = 0.35. For supercoiled DNA, the behavior of proflavine is similar to that for linear DNA. However, the torsion elastic constant of 9-aminoacridine/supercoiled DNA complexes is independent of r up to about r = 0.2. The torsional rigidities of complexes of quinacrine with both linear and supercoiled DNAs likewise remain uniform up to relatively high binding ratios. For both linear and supercoiled supercoiled DNAs the magnitude of the torsion elastic constant decreases by about 30 % as r is increased from 0 to about 0.1.

T-Pos105 SEQUENCE SPECIFICITY OF DNA CLEAVAGE BY BIS - (1,10) PHENANTHROLINE Cu (I). James M. Veal and Randolph L. Rill. Inst. of Molecular Biophysics and Dept. of Chemistry, The Florida State University, Tallahassee, FL 32306.

The complex bis - (1,10) phenanthroline Cu(1) (CuOP) recognizes DNA in a sequence dependent manner and, in the presence of molecular oxygen and a reducing thiol, causes strand scission in the local region of binding. We examined the CuOP cleavage preferences for linear DNA sequences encompassing > 2000 bases: complementary strand data was obtained on > 500 base pairs. Sequence level resolution revealed that the local base sequence governed specificity and that the sequences TAT, TGT, and PyAGPy (Py = pyrimidine) were preferred relative to all other sequences. Cleavage data on complementary strands supports CuOP binding in the minor groove. In particular, the triplet TAT was consistently and strongly cleaved (> 10x average) with cleavage occurring primarily at the central adenine and to a lesser extent at the 3' thymine. The 5' thymine was not significantly cleaved when flanked by a pyrimidine i.e. PyTAT and cleavage was variable when flanked by a purine to the 5' side. Single base permutations of TAT - CAT, TGT, and TAC - showed reduced affinity for CuOP; CAT and TAC were not significantly recognized. Cleavage at the central adenine of CAT in an undecamer increased to a level comparable to that of TAT when the cytosine was paired with inosine instead of guanine. The triplet TIT in an undecamer was likewise cleaved to a degree comparable with TAT. These observations imply that guanine amino groups in the minor groove inhibit the binding of CuOP to DNA. Moreover, the CuOP cleavage pattern when interpreted in terms of local DNA helix geometry indicates that CuOP favors a locally underwound B type DNA helix such as provided by the 5' T - A 3' step. Supported by grant EV058888 from the DOE.

T-Pos106 THE EFFECTS OF EXCITON COUPLING ON THE LINEAR DICHROISM OF DNA. Stephen P. Edmondson, Shermila B. Singham, Gary C. Salzman. Life Sciences Division, Los Alamos National Laboratory, Mail Stop M880, Los Alamos, NM 87545.

Flow linear dishroism (LD) studies indicate that single-stranded poly(U) is in an ordered helical conformation in solution. The reduced dichroism (RD = LD divided by isotropic absorption) spectrum contains more bands than the number of individual electronic transitions, and thus the RD spectrum of poly(U) cannot be explained within the oriented gas model. Good agreement with experiment, however, can be obtained when the transition dipoles on neighboring bases are allowed to interact as coupled dipoles. The effects of coupling between degenerate and nondegenerate states on the RD spectrum of poly(U) are described, and the implications for the interpretation of LD measurements on other polynucleotides are discussed. The results of calculations on poly(U) indicate that for polynucleotides with simple sequences and well characterized electronic transitions it is possible to determine not only the orientation of the bases from the RD spectrum but also the helical parameters.

T-Pos107 RAMAN SPECTROSCOPIC STUDY OF THERMALLY INDUCED STRUCTURAL CHANGES IN NUCLEIC ACIDS AND THEIR COMPONENTS. R. Savoie, A. Tajmir-Riahi, P. Audet, and M. Langlais, Département de chimie, Université Laval, Québec, Canada, G1K 7P4. (Intr. by J.-P. Caillé)

Nucleic acids and their components often exist in highly organized macrostructures (double, triple, quadruple helices, etc...) which fall apart cooperatively upon heating. A large number of studies of these melting phenomena have been based on the monitoring of specific intensity changes in the spectra (u.v., Raman, c.d., etc...). These changes, however, often reflect an unstacking of the nucleic bases (hypo- or hyperchromic spectral effects) and they do not always provide a complete picture of the various processes involved in the overall disruption of the organized three-dimensional network. In many cases interesting complementary information is gained from frequency shifts of characteristic peaks in the Raman spectra. For example, small frequency changes in the 1340 cm^{-1} band of adenine and of those mostly attributable to guanine at 1490 and 1580 cm^{-1} in the Raman spectrum of a concentrated aqueous solution of DNA indicate that base pairs in A•T-rich and G•C-rich regions break apart quite selectively in this biopolymer, at temperatures which are well below the accepted T_m. Another example is found with aqueous poly(rI) with a normal T_m of 45°C, for which frequency shifts of the two ring modes at 1465 and 1552 cm^{-1} indicate a multistep process, with minor changes in structure at 38°C and a complete disruption of higher structure at 52°C only. Finally, we would like to cite the case of 5'-GMP, for which the 1476 cm^{-1} band shifts by 7 cm^{-1} and the intensity increases by a factor of 3 at ca. 18°C, whereas the 1334 cm^{-1} band is not appreciably affected at this temperature, although it increases in intensity by 40% and shifts to 1320 cm^{-1} at 55°C, pointing to another structural change at that temperature.

T-Pos108 DETERMINATION OF THE SHAPE AND MOLECULAR WEIGHT OF MAMMALIAN CHROMOSOMAL DNA BY VISCOELASTOMETRY, J.Y. Ostashevsky, N.M.S. Reddy, and C.S. Lange, SUNY Health Science Center at Brooklyn, Brooklyn, NY 11203

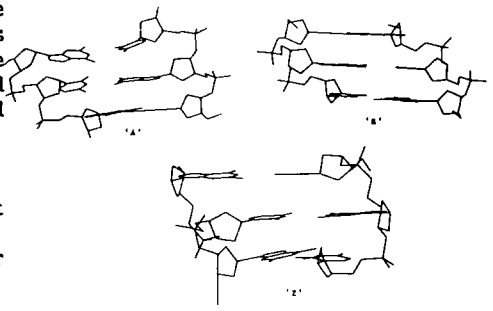
In a recent paper (Ostashevsky & Lange, 1987, Biopolymers, 26: 59), we have shown by viscoelastometry (VE) and the induction of double strand breaks (DSBs) in DNA, that one can distinguish linear from circular DNA molecules. Thus, to determine if mammalian DNA molecules are circular, we studied the X-ray dose-dependence of the VE parameters of DNA (25°C, 1.5 M NaCl) released from G_1 phase cells of Chinese hamster line V-79, by sarcosyl and protease. The retardation time (τ_{11}) increases from 1000-1500 sec (for unirradiated cells) to a maximum of 4000-5000 sec after a dose of 3 Gy, and decreases again with higher dose. This behavior of τ_{11} can be explained by the transition of originally circular DNA molecules to linear after the first DSB, and the creation of smaller fragments by additional DSBs. Using $\tau \sim M_r^{1.5-1.67}$ and τ_{11} for linear DNA of T4c phage (174 kbp) one can estimate M_r of V-79 mammalian DNA in the range 30-60 Mbp ($2\text{-}4\times10^{10}$). The good agreement of our data with those of Shafer et al., (Radiat. Res., 85: 47, 1981) for 9L rat brain tumor DNA suggests a similarity of size of DNA molecules from cells with widely different numbers of chromosomes (22 vs 72). Funded by NSF (DMB-8416242) and NIH/NCI (RO1CA39045) grants.

T-Pos109 Analysis of the site-specific binding of Benzo[a]pyrenediolepoxide to restriction fragments of pBR322 DNA. Karen A. Dittrich and Thomas R.Krugh, Department of Chemistry, University of Rochester, Rochester, NY 14627

The binding sites of the ultimate chemical carcinogen (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene, (BPDE), covalently adducted to DNA of a known sequence are determined by a technique developed by Boles, T.C. and Hogan, M.E., PNAS, 81, 1984, p.5623-5627. BPDE-modified DNA undergoes a photodissociation at the site of the BPDE adduct when the sample is irradiated with 355nm laser light. The BPDE moiety selectively absorbs the photons and there is a subsequent scission in the DNA sugar-phosphate backbone. This photodegradation reaction results in DNA fragments similar to those produced in Maxam-Gilbert sequencing reactions. By analyzing the reaction on a sequencing gel, along with the Maxam-Gilbert sequencing reactions for the DNA, the binding profile of BPDE and the relative reactivity of BPDE with each DNA base can be assessed. We have used this technique to analyze the binding of BPDE to pBR322 DNA. The EcoRI-EcoRV and BamHI-SalI restriction fragments of pBR322 have been analyzed in this way. The binding profile to both supercoiled and linear DNA is determined as well as the quantitative analysis of binding to both the 5' and 3' strands of these fragments.

T-Pos110 PREDICTION OF DNA STRUCTURE FROM SEQUENCE. B. E. Hingerty, Health and Safety Research Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, S. Broyde, Biology Department, New York University, New York, NY 10003, and S. Figueroa and T. Hayden, Mathematics Department, University of Kentucky, Lexington, KY 40506.

A build-up technique has been developed which predicts DNA structure from sequence using minimized semi-empirical potential energy calculations. First, a global search of the conformation space was made entailing 2,000 trials for d(CpG) and d(GpC). In the next stage the minimum energy conformations below 5 kcal./mole were combined to form trimers. Larger single stranded polymers can be generated by further build-up. Duplexes were produced by combining the single strands. By this method canonical A, B and Z form duplex helices have been computed a priori for the double stranded trimer d(CpGpC)·d(GpCpG), as well as novel duplexes.



Research supported jointly by OHER, U.S. DOE, under contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc. (BEH) and PHS Grant 1RO1 CA28038-07, U.S. DOE, under contract DE-AC02-81ER60015, and NSF Grant DMB8416009 (SB).

T-Pos111 THEORETICAL CONSIDERATIONS OF THE HYDRATION OF A, B, AND Z FORMS OF DNA: MONTE CARLO COMPUTER SIMULATION.

David L. Beveridge, P. S. Subramanian and S. Pitchumani, Chemistry Department, Wesleyan University, Middletown, CT 06457.

Environmental effects, including hydration and ion atmosphere, are well known to influence significantly the conformational stability of various forms of DNA, RNA and oligonucleotide prototypes thereof. In this presentation the information available on nucleic acid hydration from thermodynamic, spectroscopic and crystallographic investigations will be considered, and a series of computer simulations aimed at providing additional information about the subject will be presented. Theoretical descriptions of the hydration of nucleic acid bases, sugars and phosphates will be reported, followed by results on oligonucleotide hydration in crystals and in aqueous solution. Hydration Density Analysis will be reported for the A and B forms of $d(CGCGAATTCGCG)_2$ and the B and Z forms of $d(CGCGCG)_2$ in aqueous solutions. The results will be discussed in terms of solvation sites, water bridges and networks. Theoretical considerations on the "spine of hydration" in the B-form dodecamer will be provided.

T-Pos112 MECHANICAL FLUCTUATION-ENHANCED DECAY OF ELECTROSTATIC DOUBLE-LAYER AND HYDRATION FORCES BETWEEN LINEAR DNA POLYMERS, Rudi Podgornik, Donald C. Rau and V. Adrian Parsegian, National Institutes of Health, Bethesda, MD 20205

Recent direct measurements of forces between parallel DNA double helices have made us aware of two unexpected facts: (a) near contact (intersurface separation smaller than 1 nm), these polyelectrolyte molecules repel by an exponentially decaying force whose approx. 3 Angstrom decay constant is virtually independent of ionic strength; (b) at greater distances, ionic strength dependent forces act between the molecules but in a manner that is qualitatively modified by the entropic forces of confinement through soft collisions effected by long-range potentials.

The observed effect of configurational confinement is to double the apparent decay length of the underlying electrostatic or hydration force. This expansive behavior appears to depend only weakly on DNA stiffness as defined by its persistence length or on the species of salt solution into which the DNA is dissolved. At no point do the molecules interact with the forces expected of them from traditional double-layer theory.

The natural interplay between force and motion, first explicitly recognized in phospholipid multilayer systems (PNAS 83:7132 (1986)) is a central feature of assembling macromolecular systems. By simultaneously measuring the strength of forces and the extent of motion, we finally begin to see the enactment of this essential relationship.

T-Pos113 EFFECTS OF CIS-DIAMMINEDICHLOROPLATINUM II(cis-DDP) ON THE DENATURATION OF SYNTHETIC DNA R.V. Rosal and H. Mizukami. Division of Regulatory Biology and Biophysics, Department of Biological Sciences, Wayne State University, Detroit, Michigan 48202

The anti-tumor efficacy of platinum compounds seems to arise from possible Pt-induced denaturation/renaturation effects on DNA. We investigated the effect of cis-DDP on a specific sequence of synthetic double stranded DNA(16-mer with 12 repeating G-C base pairs).

Single stranded oligomers were synthesized, then purified and characterized by reverse phase HPLC and PAGE-urea. Double stranded oligos were formed by mixing complimentary single strands in 0.5mM phosphate buffer at 50°C for 1 hr, and cooling the mixture slowly. Unannealed strands were removed by HAP chromatography. The annealed oligos were incubated with cis-DDP in the dark at 40°C for 24 hrs. Hyperchromicity before and during heat denaturation was monitored spectrophotometrically at an increasing molar ratio (cis-DDP/oligo).

The control exhibited a sigmoidal melting curve, T_m=62°C. Addition of cis-DDP induced an initial hyperchromic effect of 0.04 OD, with a decrease in T_m by 8°C (at 0.1 molar ratio) and 0.17 OD, with a decrease in T_m by 11°C (at 0.5 molar ratio). These effects suggest the intrastrand cross-linking of cis-DDP. The decrease in the slope of the linear portion of the sigmoidal melting curve by 59% and 96% from the control for 0.1 and 0.5 molar ratio cis-DDP/oligo, respectively, is supportive of interstrand cross-linking and may be responsible for the anti-tumor efficacy. Further investigation of cis-DDP's intra and inter cross-linking effects in relation to sequence dependency will be examined using alternating sequences of G-A-C and G-T-G.(supported by NIHRS)

T-Pos114 CONFORMATIONAL ANALYSIS OF AN RNA HAIRPIN LOOP STRUCTURE BY NUCLEAR OVERHAUSER EFFECT SPECTROSCOPY K. D. BISHOP, S. R. LAPLANTE, J. MILLIGAN*, O. UHLENBECK*, P. N. BORER BIOPHYSICS DEPT., BOWNE HALL, SYRACUSE UNIV., SYRACUSE, N.Y. 13244-1200 & *UNIV. OF COLORADO, DEPT. OF CHEMISTRY AND BIOCHEMISTRY, BOULDER, COLORADO 80309-0215.

The coat protein binding site for the R17 virus is a RNA hairpin loop. The replicase gene begins near the 3'-end of the loop and the coat protein acts as a translational repressor of the replicase gene. The presence of the bulged-A residue in the stem greatly enhances the coat protein binding. A variant of this hairpin loop has been synthesized by J. Milligan and O. Uhlenbeck using bacteriophage T7 RNA polymerase starting with a DNA primer. This RNA analog which is 25 residues long, is similar in structure to the viral RNA hairpin loop strongly binds to the coat protein. NOESY spectra of the synthetic variant have been obtained and are now being analyzed. Two-dimensional maximum entropy method (2D-MEM) analysis greatly simplified the spectra by resolving obscured peaks. The proton resonance assignments are now being made from these spectra. Structural features of the loop from the NOESY data will be discussed.

T-Pos115 NONSPECIFIC BINDING OF A PROTEIN INTO MEMBRANE LIPID CAN BE INDISTINGUISHABLE FROM SPECIFIC BINDING TO ONE OR MORE MEMBRANE RECEPTOR PROTEINS. R.D. MacGregor and C.A. Hunt. University of California, San Francisco, CA 94143.

The binding of proteins into pure lipid systems can exhibit binding plots indistinguishable from high affinity, site-specific binding to a receptor protein. This lipid binding mechanism is highly sensitive to the stereochemistry of the protein and to the planar extent of the lipid bilayer.

It is often assumed that linear Scatchard plots (or linear components) that have a well defined number of high affinity sites arise from binding to a receptor molecule(s), which is likely to be a protein. However, we find that subdivision of a large, cell-sized lipid bilayer into smaller areas results in the appearance of high affinity, lipid only, binding sites for some proteins. The Scatchard plots resulting from nonspecific binding of model proteins into the subdivided lipid bilayer can be identical to Scatchard plots arising from a site-specific binding mechanism. We suggest that cytoskeletal and other membrane proteins may produce an effective subdivision of the membrane and thereby control binding of proteins into the membrane lipid. If this is true, proteolysis of any of these proteins should decrease the number of lipid binding sites. Proteolysis would also decrease binding by receptor proteins, thus obscuring the mechanistic interpretation of binding experiments on proteolytically treated membranes.

We demonstrate that the binding of apocytochrome c to outer mitochondrial membranes (Hennig et al. 1983: PNAS, 80, 4963) is adequately explained by binding into subdivided lipid. However, insufficient data is available to determine whether the actual mechanism involves site-specific or subdivided-lipid binding, or both.

T-Pos116 ACID-TRIGGERED ENTRY PATHWAY OF PSEUDOMONAS EXOTOXIN A. Zohreh T. Farahbakhsh and Bernadine J. Wisnieski. Dept. of Microbiology and the Molecular Biology Institute, University of California, Los Angeles, CA 90024

The goal of this study was to examine the effect of pH on the kinetics of Pseudomonas toxin (PTx) binding to vesicles and the degree of reversibility of conformational changes observed in soluble and membrane-bound forms. PTx binding to vesicles and insertion into the bilayer increase with decreasing pH. Acid-pulsed toxin exhibits pH 7.4 binding and insertion levels, suggesting that any hydrophobic regions that become exposed upon toxin acidification become buried again at pH 7.4. In contrast, the change in PTx conformation that occurs upon membrane binding is irreversible. Returning samples to pH 7.4, incubation with excess toxin or dilution with buffer up to 1000-fold has very little effect on bound toxin. Compared to free toxin, bound toxin is 300 to 500-fold more susceptible to trypsin (at both pH 4 and pH 7.4). At pH 4, membrane-associated toxin slowly proceeds to a trypsin-protected state; neutralization halts this process. With fluid targets, the proportion of bound toxin that was photolabeled from within the bilayer peaked rapidly and then decreased with time. With frozen targets, the efficiency of photolabeling peaked but then remained fairly constant. The data indicate that after acid-triggered insertion, PTx can cross a fluid bilayer much more efficiently than it can a frozen one. We conclude that the reversible pH-dependent changes in toxin conformation (Farahbakhsh, Z.T., Baldwin, R.L., and Wisnieski, B.J. (1987) J. Biol. Chem. 262, 2256-2261) have a functional role in promoting membrane binding, insertion and translocation. The kinetics of translocation is regulated by pH and the physical state of the target membrane. Supported by USPHS GM22240 and the UCLA Academic Senate.

T-Pos117 MEMBRANE PERMEABILITY TO MACROMOLECULES RESULTING FROM THE MEMBRANE ATTACK COMPLEX OF COMPLEMENT. Justine A. Malinski and Gary L. Nelsestuen, Biochemistry Dept., University of Minnesota, St. Paul, MN 55108.

A well defined system of purified phospholipids and human complement proteins was used to study membrane permeability mediated by the membrane attack complex (MAC) of complement. Large unilamellar vesicles (LUVs) which contained trapped macromolecules (pancreatic trypsin inhibitor [R_h = 15 Å], thrombin [R_h = 25 Å], or glucose-6-phosphate dehydrogenase [R_h = 48 Å]) provided the means to measure permeability during MAC assembly. Membrane permeability was detected by release of the protein from the vesicle and lesion size was estimated from the hydrodynamic dimensions of the released macromolecule. Transmembrane macromolecular communication was also detected by inhibition of trapped thrombin by an externally added thrombin inhibitor (either hirudin [R_h = 20 Å] or antithrombin-III [R_h = 44 Å]). LUVs of phosphatidylcholine (PC) or phosphatidylserine (PS) were used. Macromolecular permeability due to C5b-9 occurred without fragmentation, fusion, or aggregation of the vesicles. Quantitative membrane binding by C5b-7 as well as essentially quantitative release of thrombin was obtained with PS vesicles. Titrations with complete MACs approximated the theoretical Poisson distribution curve for full release of vesicle contents by one complex per vesicle. The composition of the complexes determined the size of the membrane lesion and the full tubule of 12-18 C9 molecules was not required for macromolecular permeability. For example, membrane penetration by thrombin required only 5 or 6 C9 molecules. Pore size varied throughout the range of C9 additions to C5b-8. Relative to thrombin, 50% release of pancreatic trypsin inhibitor required 33% fewer C9 molecules while glucose-6-phosphate dehydrogenase required 75% more C9 molecules per MAC complex. (Supported by grant HL 15728).

T-Pos118 AGGREGATION OF MELITTIN IN LIPID BILAYERS. Phyllis J. Fisher, Salah Sedarous, and Franklyn G. Prendergast, Department of Biochemistry and Molecular Biology, Mayo Foundation, Rochester, Minnesota 55905.

There are two disparate views regarding the state of aggregation of melittin (MLT) in lipid bilayers. One view holds that tetrameric melittin predominates and the other that lipid bound melittin is monomeric. In an attempt to resolve the controversy we have used fluorescence spectroscopy to examine the interaction of synthetic melittin, dansylated on the α-amino terminus, with lysolipid and diacyl-glycerophosphoryl cholines. Dansyl melittin was only marginally less cytolytic (to human erythrocytes) than normal melittin. FAB-MS measurements proved the purity of the dansyl melittin and indicated a single site of labeling at the α-amino group. The fluorescence properties of the dansyl and indole moieties change markedly with formation of either MLT tetramer (e.g. the intrinsic fluorescence of the tryptophan is about 97% quenched in the tetramer) or with MLT lyso-or diacyl lipid interaction. The changes are the consequence of both differences in environment and in degree of fluorescence energy transfer from the indole side chain to the dansyl moiety. By measuring the fluorescence spectra, anisotropy and extent of energy transfer, we have shown that a tetramer akin to that seen in solution does not form in lipid peptide micelles and that the formation of an MLT oligomer in lipids in which all melittin molecules are similarly oriented, is also improbable. Supported by GM 34847.

T-Pos119 KINETICS OF SPONTANEOUSLY INSERTING HELICAL HAIRPINS INTO LIPID BILAYERS. Mark A. Roseman, Department of Biochemistry, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814-4799.

Current models cannot account for the rapid insertion of helical hairpins into lipid vesicles because the ΔG° of burying the exposed backbone CONH groups of the bend region is thought to be about +18 kcal/mole [Engelman and Steitz, Cell 23, 411 (1981)]. If this were correct, one can readily show (by assuming the off-rate is diffusion controlled) that the on-rate constant, k_{on}, could be no larger than $1\times10^{-4}M^{-1}s^{-1}$. In contrast, k_{on} for the nonpolar tail of cytochrome b_5 is $2\times10^{7}M^{-1}s^{-1}$ [Leto and Holloway, J. Biol. Chem. 254, 5015 (1979)]. Therefore, the rate of insertion is underestimated by a factor of $\sim 10^{11}$.

There appear to be two shortcomings of present models: 1) overestimation of CONH hydrophilicity [Roseman, Biophys. J. 51, 167a (1987)], and 2) failure to take into account the polarity gradient in lipid bilayers [Griffith, J. Membr. Biol. 15, 159 (1974)]. In the present study, it is proposed that the free energy of transferring the backbone CONH from water to organic solvents is only 36% of the values that are currently employed. This change alone makes an enormous difference in the maximal k_{on} because ΔG° and k_{on} are logarithmically related. Hypothetical polarity gradients were constructed, and insertion-progress curves for a helical hairpin with hydrophobic side chains were calculated from water/organic solvent partition data. The results show that the high on-rate constants can now be largely accounted for. Supported by NIH grant AM30432.

T-Pos120 CRITICAL MIXING IN PC-GRAMICIDIN AND PC-AMPHIPHILIC PEPTIDE MODEL MEMBRANES M.R. Morrow[a], J.P. Whitehead[a], and J.H. Davis[b] (Intro. by K.M.W. Keough) Physics Departments, [a]Memorial University of Newfoundland, St. John's, Newfoundland, CANADA, A1B 3X7 and [b]University of Guelph, Guelph, Ontario, CANADA, N1G 2W1

Two phase coexistence in the phase diagrams of gramicidin-DPPC-d62 and gramicidin-DMPC-d54 has been explored using DSC and ^2H NMR. For both mixtures, two phase coexistence appears to be restricted to a few degrees below the pure lipid transition temperature and to gramicidin mole fractions less than 2%. Beyond this concentration, the phase transition seems to be replaced by a continuous phase change. These observations suggest a 'tear-drop' shaped two phase region with a critical mixing point. This suggestion is supported by DSC and phase diagram simulations using a phenomenological model based on a Landau expansion of the bilayer free energy in terms of area per lipid. Simulated DSC scans approximate the dependence of observed scan shape and transition enthalpy on gramicidin content. Previously reported phase diagrams and DSC results for DPPC model membranes containing a bilayer spanning amphiphilic peptide (1) (2) are also approximately reproduced by this model suggesting the presence of a critical mixing point for this system as well. The model also illustrates the distinction between the critical point for the homogeneous mixture and the critical mixing point. (Supported by the Natural Sciences and Engineering Research Council, Canada.)

(1) J.C. Huschilt, R.S. Hodges, and J.H. Davis (1985) Biochemistry 24, 1377-1386.
(2) M.R. Morrow, J.C. Huschilt, and J.H. Davis (1985) Biochemistry 24, 5396-5406.

T-Pos121 CHARACTERIZATION OF DIPHTHERIA TOXIN-MEDIATED MARKER RELEASE FROM ASOLECTIN VESICLES. Gui-sen Jiang and Valerie W. Hu, Dept. of Biochemistry, Uniformed Services University of the Health Sciences, Bethesda, Md. 20814-4799.

The interaction of diphtheria toxin with the lipid bilayer has been studied by following the release of a fluorescent dye and radiolabeled markers from large unilamellar vesicles composed of asolectin. Dye release is strongly dependent on pH, increasing sharply at pH 5.2 and below. The mode of dye release is an all-or-none process. The extent of marker release is dependent on toxin concentration within a certain range as well as on the size of the entrapped molecules. Fluorescence polarization studies with diphenylhexatriene indicate that toxin has no effect on the bulk membrane fluidity at lower concentrations and increases the anisotropy at higher toxin concentrations. This increase in membrane order is opposite to the effect exhibited by the detergent $C_{12}E_9$ on the same membranes. Photolabeling studies with a membrane-restricted photoprobe show that insertion of fragment B into the lipid bilayer parallels the extent of dye release. Fragment A, however, is not labeled by the photoprobe in these membranes.

T-Pos122 THE TARGETING SEQUENCES FROM AN EXPORTED BACTERIAL PROTEIN AND A MITOCHONDRIAL PRECURSOR PROTEIN INTERACT DIFFERENTLY WITH PHOSPHOLIPID BILAYER MEMBRANES. M. Kodama, M. S. Briggs, C. J. McKnight, L. Gierasch and E. Freire. Dept. of Biology, The Johns Hopkins Univ., Baltimore, MD 21218 and Dept. of Chemistry, Univ. of Delaware, Newark, DE 19716

The interactions of the signal peptide of the E. coli λ receptor protein (LamB protein) (MMITLRKLPLAVAVAAGVMSAQAMA) and the targeting sequence of the mitochondrial protein cytochrome c oxidase subunit IV (MLSLRQSIRFFKPATRTLCS) with phospholipid bilayer membranes composed of DPPC and DPPG (DPPC:DPPG = 5:1) have been investigated by high sensitivity differential scanning calorimetry. The LamB peptide induces a monotonic decrease in the transition temperature (T_m) of the phospholipid bilayer as well as a broadening of the heat capacity profile. The cytox IV peptide induces a monotonic increase in T_m up to a peptide:lipid ratio of 1:50 and a decrease at higher concentrations. This behavior indicates that the LamB peptide interacts preferentially with the fluid phase and that the cytox IV peptide interacts preferentially with the gel phase of the lipid bilayer. Increasing the ionic strength induces an increase in T_m and a decrease in ΔH on both systems. The decrease in ΔH is larger for the LamB peptide and the magnitude of the effect increases at higher peptide:lipid ratios. For the cytox IV peptide, the salt effect is insensitive to the peptide:lipid ratio. The concentration and ionic strength dependence of the thermodynamic parameters are consistent with a model in which the LamB peptide penetrates and perturbs the hydrophobic core of the bilayer whereas the cytox IV peptide binds to the negatively charged lipids in the membrane surface without significant perturbation of the hydrophobic core of the membrane. This is also consistent with additional isothermal calorimetric binding studies, spectroscopic measurements and the expected secondary structure of these peptides based upon their amino acid sequences. (Supported by NIH grants GM-37911 (E.F.) and GM-34962 (L.G.).

T-Pos123 CONFORMATION STUDIES OF MODEL ION CHANNELS (ALAMETHICIN, MELITTIN) IN PERFECTLY ALIGNED HYDRATED LECITHIN MULTILAYERS* -- Glenn A. Olah and Huey W. Huang, Physics Dept., Rice University, Houston, TX 77251

Model ion channels are embedded in aligned hydrated lecithin (DLPC, DMPC) multilayers sandwiched between two electrode coated fused silica plates. Sample preparation involves a combination of mechanical stressing, shearing and temperature annealing. Perfectly aligned samples that are up to 50μ thick with a 1.5cm² area have been achieved. These samples are ideal for conformation studies of ion channels in an electric field by CD and small-angle neutron (or X-ray) scattering. Here we present some results of a CD study.

Alamethicin and melittin both have a large α helical content. The orientational effect of these α helices on the far UV CD is consistent with the Moffitt theory. We were concerned about a previous linear dichroism study (Yamaoka et al., (1986) J. Am. Chem. Soc. 108, 4619) which seemed to contradict the theory. In fact, our CD measurements of the static electric field effect on a polypeptide (PBLG) confirmed their data. However, we believe that the apparent disagreement with the Moffitt theory is due to either the flexibility of the polypeptide or an ordered side-chain contribution.

The conformation of alamethicin shows a dependence on the degree of hydration and the temperature. Two distinct CD spectra exist depending on whether the samples are in the low or high end of the liquid crystalline phase. The data also appears to support the barrel model rather than the dipole flip-flop model.

* This research was supported in part by the Office of Naval Research, the National Institute of Health, and Robert A. Welch Foundation.

T-Pos124 ^{19}F NMR STUDIES OF FLUOROTRYPTOPHAN SUBSTITUTION MUTATIONS IN THE MEMBRANE-BOUND D-LACTATE DEHYDROGENASE FROM *E. coli*. Olve Peersen, E. A. Pratt, Gordon S. Rule, and Chien Ho. Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA 15213.

D-Lactate dehydrogenase (D-LDH) is a 571 residue membrane-bound respiratory enzyme from *E. coli* which contains an FAD cofactor and needs a lipid environment for maximal activity. D-LDH oxidizes D-lactate to pyruvate and shuttles an electron to a membrane bound ubiquinone as part of the electron transport chain. We have incorporated 5-fluorotryptophan into the enzyme and studied the five wild-type Trp residues by ^{19}F NMR. Site directed mutagenesis was used to construct a series of mutants containing an additional tryptophan at various positions in the protein, and these fluorotryptophans were then used as internal probes for localized sensitivity to substrate, fatty acids, and solvent changes. NMR spectra show that all of the mutants in the region of residues 279 to 361 are sensitive to a nitroxide-spin label incorporated into the lysophosphatidylcholine lipid phase and there are residues on both sides of this region which are sensitive to substrate addition. The chemical environment of the wild-type Trp469 residue changes as a result of both Phe39 and Leu517 mutations, indicating some residue-residue interactions. Large chemical shifts due to substrate addition are seen in mutants from Phe39 and Tyr243, and Trp469 has previously been shown to be sensitive to FAD reduction. The contribution of these results to our understanding of structure-function relationships in D-LDH will be discussed. [This work is supported by a research grant from the NIH (GM-26874) and O.P. was supported by a NSF grant (BBS-8712919) during the summer of 1987.]

T-Pos125 NMR, BIOCHEMICAL AND MOLECULAR GENETIC STUDIES OF THE MEMBRANE-BOUND D.LACTATE DEHYDROGENASE OF *E. COLI*. Hoai-Thu N. Truong, E. A. Pratt, Gordon S. Rule, Olve Peersen, and Chien Ho. Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA, 15213, U.S.A.

The membrane-bound respiratory enzyme D-lactate dehydrogenase (D-LDH) of *E. coli* is being used as a model system to investigate interactions between protein and lipids in membranes. D-LDH is an FAD-containing enzyme of MW 65 000; it requires lipids or detergent for full activity. The effect of D-LDH on small unilamellar vesicles was studied using ^{1}H- and ^{31}P-NMR to examine phospholipid headgroups, and ^{19}F-NMR to observe the acyl chains of fluorinated phospholipids. The results suggest that the inner monolayer phospholipids are more perturbed by addition of protein than the outer monolayer phospholipids, even though the enzyme is added to the external face of the vesicles. The effect of lipids and substrate on the ^{19}F-NMR spectrum of D-LDH labeled with 5-fluorotryptophan was examined. The tryptophan residues exposed to solvent and to the external medium were determined. There are no significant differences in the ^{19}F-NMR spectrum of labeled D-LDH in lysophosphatidylcholine or vesicles composed of varying phosphatidylcholine to phosphatidylglycerol ratios. However, proteolytic digestion patterns of D-LDH suggest that the conformation of the protein is altered in the differing environments. Site-specific mutagenesis was used to introduce additional tryptophan residues into selected regions. These additional reporter probes show that the region comprising amino acids 300-370 is accessible to the lipid phase. Data on these mutant forms of D-LDH are presented. [This work is supported by a research grant from the NIH (GM-26874) and H.-T. N. T is supported by a postdoctoral fellowship from the American Cancer Society (PF-2877).]

T-Pos126 **RAMAN AND RESONANCE RAMAN STUDY OF THE INTERACTION OF FERRICYTOCHROME c WITH DIMYRISTOYLPHOSPHATIDIC ACID.** James S. Vincent[a] and Ira W. Levin[b], [a]Chemistry Department, University of Maryland Baltimore County, Catonsville, Maryland 21228, and [b]Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892

The vibrational Raman spectra of both pure l-α-dimyristoylphosphatidic acid (DMPA) liposomes and DMPA multilayers reconstituted with ferricytochrome *c* under varying conditions of pH and cation are reported as a function of temperature. Total integrated band intensities and relative peak height intensity ratios, two spectral scattering parameters used to determine bilayer disorder, are sensitive to the presence of ferricytochrome *c* and the cation in the reconstituted liposomes. Protein concentrations were estimated by comparing the 1636 cm^{-1} resonance Raman line of known ferricytochrome *c* solutions to intensity values for the reconstituted multilayer samples. Temperature dependent profiles of the 3100-2800 cm^{-1} C-H stretching, 1150-1000 cm^{-1} C-C stretching, 1440 cm^{-1} CH_2 deformation and the 1295 cm^{-1} CH_2 twisting mode regions characteristic of acyl chain vibrations reflect bilayer perturbations due to the interactions of ferricytochrome c. The DMPA multilamellar gel to liquid crystalline phase transition temperature T_M, defined by either the C-H stretching mode I_{2935}/I_{2880} or the C-C stretching mode I_{1061}/I_{1090} peak height intensity ratios, is decreased by ~12 C for the ferricytochrome *c* reconstituted DMPA liposomes at pH 4.0 and a 1:1 mole ratio of Ca^{++} to lipid. Other spectral features, such as the increase in the 2935 cm^{-1} C-H stretching mode region, which arise in bilayers containing ferricytochrome *c*, are interpreted in terms of protein penetration into the hydrophobic region of the bilayer.

T-Pos127 EXTRACTION OF PHOSPHATIDYLGLYCEROL FROM MIXED BILAYERS OF PHOSPHATIDYLCHOLINE AND PHOSPHATIDYLGLYCEROL BY POLYMYXIN B. Yves Babin and Michel Pézolet, Département de chimie, Université Laval, Québec, Canada, G1K 7P4.

Polymyxin B (PXB) is an amphiphilic antibiotic that attacks the outer and cytoplasmic membranes of Gram-negative bacteria. It is known that anionic molecules are the binding sites of PXB on membranes and that the antibiotic binds five phosphatidylglycerol (PG) molecules and induces the interdigitation of bilayers of this lipid, while it nearly does not affect phosphatidylcholines (PC). In this study, we have use FTIR and Raman spectroscopy to investigate complexes of PXB with dipalmitoylphosphatidylglycerol (DPPG) and DPPG/dimyristoylphosphatidylcholine-d_{54} (DMPC-d_{54}) mixtures. Both IR and Raman results indicate that the binding of PXB to DPPG bilayers lowers the melting temperature from 41°C to 37°C. While PXB does not affect the conformation of the acyl chains of DPPG in the gel phase, it increases the intermolecular vibrational coupling over the whole temperature range studied. This effect may be due to a change in the tilt angle of the acyl chains and to their interdigitation in the gel phase, and to the penetration of the acyl chain of PXB in the hydrophobic core of the bilayer in the liquid crystalline phase. The PXB/DPPG complex precipitates under the charge saturation condition, and at a lipid to PXB incubation molar ratio (R_i) higher than 5, the unbound DPPG remains in the supernatant. In DPPG/DMPC-d_{54}/PXB (5:5:1) mixtures, all the lipid molecules are precipitated. When such a mixture is enriched in PC, the R_i of PG/PXB being kept at 5, the excess of PC is detected in the supernatant. It is, to our knowledge, the first report of a macroscopic phase separation in a phospholipid mixture induced by a polypeptide. This could explain how PXB breaks the membranes of bacteria, leading to a release of the cytoplasmic material.

T-Pos128 A MOLECULAR MODEL FOR THE BINDING OF TWO EXTRINSIC MEMBRANE PROTEINS TO PHOSPHATIDYLGLYCEROL AND PHOSPHATIDYLSERINE-CONTAINING MEMBRANES. B.R. Lentz*, G.A. Cutsforth* and R.N. Whittaker. Department of Biochemistry, University of North Carolina, Chapel Hill, NC 27599-7260.

Model membranes containing phosphatidylserine (PS) effectively support thrombin formation whereas model membranes containing phosphatidylglycerol (PG) do not. We have developed a molecular model for the binding of prothrombin and Factor X to PG-containing and PS-containing small unilamellar vesicles (SUVs). Relative light scattering measurements were used to generate the binding isotherms. The measured dissociation constants were one-tenth to ten micromolar over a range of eight to sixtyfive mole percent negatively-charged phospholipid. Stoichiometries calculated from the ratios of the molecular weight of the protein-vesicle complex to the molecular weight of the vesicle indicated that, at saturation of the vesicle surface, the proteins cover 40-50 phospholipids, of which 20 or 12 were PG or PS, respectively. These results indicate that twice as much PG is required to bind Factor X and prothrombin to a vesicle surface, and once bound the proteins are held tightly. Analysis of the binding isotherms in terms of model of equivalent, independent protein binding sites for negatively-charged phospholipid revealed both tight and loose binding sites on Factor X and prothrombin. Only 3-5 sites were involved in each case. In general, fewer tight sites were available for binding PG as compared to PS, although the total number of light and loose sites was comparable for both lipids. A model of lipid binding is suggested in which a small number (3-5) of negatively-charged lipid molecules are weakly recruited to a number of specific sites on protein resulting in a not tight association. Supported by USPHS (SCOR) Grant HL-26309-07.

T-Pos129 **CALCIUM-DEPENDENT AND CALCIUM-INDEPENDENT INTERACTIONS OF AN EXTRINSIC MEMBRANE PROTEIN WITH PHOSPHATIDYLSERINE/PHOSPHATIDYLCHOLINE SMALL UNILAMELLAR VESICLES.** Susan C. Windes and Barry R. Lentz. Department of Biochemistry and Nutrition, The University of North Carolina at Chapel Hill, Chapel Hill, N.C., 27514. Intr. by Kenneth A. Jacobson.

The phase behavior of mixed dimyristoylphosphatidylserine (DMPS) and dimyristoylphosphatidylcholine (DMPC) small unilamellar vesicles (SUV) in the presence and absence of bound bovine prothrombin was measured by monitoring diphenylhexatriene fluorescence anisotropy. The shape of the membrane temperature-composition phase diagram was essentially unaltered by the binding of prothrombin in the presence of Ca^{2+}, although the two-phase (gel/fluid) region was narrowed and shifted by 0-6°C to higher temperatures. This result does not support the popular idea that extensive domains rich in negatively charged DMPS are induced in response to prothrombin binding. This 0-6°C shift was much smaller than the 2-15°C shift seen previously with dipentadecanoylphosphatidylglycerol($DC_{15}PG$)/DMPC SUV (Lentz *et al.*, Biochemistry, 24, 6697). This result suggests that the lipid packing in DMPS/DMPC vesicles was less altered by the binding of prothrombin than was the packing in $DC_{15}PG$/DMPC vesicles. In the absence of Ca^{2+}, the effect of prothrombin was to make the phase transitions of the small vesicles resemble those of large vesicles. This effect was seen even with pure DMPC vesicles. As previously concluded for $DC_{15}PG$/DMPC vesicles (*ibid*), this supports a Ca^{2+}-independent mechanism for the interaction of prothrombin with DMPS/DMPC membranes in addition to the commonly accepted Ca^{2+}-dependent bridging mechanism. Supported by USPHS(SCOR) Grant HL-26309.

T-Pos130 INTERACTIONS OF ENDONEXIN AND LIPID VESICLES STUDIED BY LIGHT SCATTERING AND NMR. M. Junker, C.E. Creutz, C.M. Grisham, Biophysics Program, Univ. of VA, Charlottesville, VA.

Endonexin (a mammalian calelectrin[1]) is a member of the annexin family of Ca^{2+} dependent membrane binding and vesicle aggregating proteins[2]. Endonexin binds secretory vesicle membranes at low (1-10μm) Ca^{2+} concentrations and aggregates these vesicles at higher (300um) Ca^{2+} concentrations[1,3]. Similar to other annexin proteins, the Ca^{2+} affinity of endonexin is believed to be modulated by membranes, perhaps by lipid-Ca^{2+} interactions. To further understand the interactions of endonexin, membrane lipid, and Ca^{2+}, studies are being performed using purified endonexin and defined synthetic lipid vesicles. In the presence of Ca^{2+}, endonexin binds and aggregates vesicles containing acidic phospholipids but not those consisting solely of phosphatidylcholine. Vesicles composed of 30% phosphatidylserine or phosphatidic acid are half maximally aggregated at pCa^{2+}= 4.5, while those composed of 30% phosphatidylinositol are half maximally aggregated at pCa^{2+}= 3.5.

Proton nuclear relaxation rate studies, substituting Gd^{3+} for Ca^{2+}, have been undertaken to study metal-lipid interactions and the possible influence on endonexin membrane binding. Vesicles containing acidic phospholipids bind Gd^{3+}, as does endonexin in the absence of lipids. In the presence of both membranes and endonexin, Gd^{3+} is bound to a site with a unique environment and affinity, varying with the type of lipid used, suggesting a transformation in the state of the lipid and/or protein and the formation of a ternary complex of metal, lipid, and protein. [1]Südhof et al (1984) Biochem. 23: 1103. [2]Geisow et al (1986) Nature 320: 636. [3]Creutz et al (1987) JBC. 262: 1860.

T-Pos131 CHARACTERIZATION OF THE RELEASE OF DIPHTHERIA TOXIN FROM LIPID BILAYERS. Laura A. Chung and Erwin London, Department of Biochemistry, State University of New York at Stony Brook, Stony Brook, NY 11794-5215.

It has been proposed that diphtheria toxin enters target cells via a receptor-mediated endocytotic pathway. A critical step in the cytotoxic pathway of the toxin is the low pH-dependent insertion into and translocation across an endocytotic membrane. Previously, we have determined in detail the parameters involved in the binding and insertion of toxin into vesicles at low pH. We have now started to study factors involved in the release of toxin from unilamellar vesicles. One question is whether reversal of pH releases toxin bound to vesicles at low pH. Using column chromatography and sucrose gradients, we have found that both nicked and unnicked toxin molecules remain associated with vesicles upon neutralization of pH. Control studies show that this association is not due to the presence of toxin trapped within the vesicle lumen. In addition, it can be shown that after reversal of pH the toxin remains an intergral protein because high ionic strength does not cause release as would be expected for an extrinsic membrane protein. Studies on dithiothreitol-treated, nicked toxin show that there is release of at least some portion of the toxin under reducing conditions. Supported by N.I.H. grant GM 31986.

T-Pos132 INTERACTION OF SUBUNIT A OF DIPHTHERIA TOXIN WITH MODEL MEMBRANES AND BRIJ MICELLES AT LOW pH. Jian-Min Zhao and Erwin London, Department of Biochemistry, State University of New York at Stony Brook, Stony Brook, NY 11794-5215.

Membrane penetration triggered by the low pH in acidic organelles is a critical factor in the entry of diphtheria toxin into the cytoplasm. In order to understand this process the effect of pH on the interaction of subunit A of diphtheria toxin with liposomes (DOPC or DOPG/DOPC mixtures) and with Brij 96 micelles was examined using fluorescence quenching. At low pH subunit A rapidly and efficiently binds and inserts into both liposomes and micelles. The binding at low pH is tight, with half-maximal binding of 24 nM subunit A at about 10 μM lipid for 20%PG/80%PC (w/w) SUV at pH 3.5. Binding to liposomes is completely reversible when pH is returned to neutral, as judged by quenching. The pH at which insertion becomes significant ranges from about 3.5 for Brij 96 to pH 5 for 20%PG/80%PC SUV at 23°C. Vesicle size affects the pH of insertion, resulting in a decrease with increasing size. Increasing temperature also affects the pH of insertion, causing it to increase. We previously described the conformational changes in subunit A that are induced by low pH and high temperature. It appears that the partial denaturation of the subunit under these conditions is closely linked to its interaction with liposomes. In this regard, its behavior is very similar to that of whole toxin. Supported by N.I.H. grant GM 31986.

T-Pos133 **Phosphatidylserine Differentially Modulates Protein Kinase C Substrate and Auto-Phosphorylation.** Alexandra C. Newton and Daniel E. Koshland, Jr., Department of Biochemistry, University of California, Berkeley, California 94720

The Ca^{2+}/phosphatidylserine-dependent protein kinase C phosphorylates a number of membrane-bound substrates, including itself, in response to increased diacylglycerol (DG) levels. We report that protein kinase C substrate and auto-phosphorylation are modulated by the intrabilayer phosphatidylserine (PS) concentration. At relatively low PS concentrations (1-10 mol % PS in phosphatidylcholine liposomes containing 2 mol % DG) autophosphorylation is the dominant mode of activity. At higher PS concentrations (>10 mol %) substrate phosphorylation dominates and auto-phosphorylation is inhibited. The differential modulation of the two modes of activity likely does not reflect different minimum PS requirements for auto- versus substrate phosphorylation. Substrate (histone) and auto- phosphorylation have the same requirement for PS in a mixed micelle system in which one monomer of protein kinase C binds to one Triton X-100:PS:DG micelle. Low PS concentrations may favour intramolecular phosphorylations, while higher PS concentrations may promote intermolecular reactions. In contrast to the modulation exerted by PS, DG activates protein kinase C equally towards substrate and auto- phosphorylation. The PS content of most biological membranes is on the order of 15 mol % of the inner leaflet lipid, suggesting that local fluctuations in the PS content of the plasma membrane may affect protein kinase C activity.

T-Pos134 **COMPLEMENTARY NMR AND FLUORESCENCE STUDIES OF BACKBONE AND SIDECHAIN DYNAMICS OF ^{13}C-LABELED SYNTHETIC MELITTIN AND MELITTIN ANALOGUES.** Arthur J. Weaver*, Marvin D. Kemple**, Franklyn G. Prendergast*, *Dept. of Biochemistry and Molecular Biology, Mayo Foundation, Rochester, MN 55905, **Dept. of Physics, Indiana University-Purdue University at Indianapolis, Indianapolis, IN 46223

Melittin (MLT), a surface-active single-tryptophan peptide continues to be vigorously studied as a model for protein-membrane interactions. However, the spatial and dynamic properties of the MLT-membrane complex remain obscure. $^{13}C\delta_1$-L-tryptophan has been incorporated into synthetic native MLT (Trp^{19}) and into three single-Trp MLT analogues (Trp^{9}, Trp^{11}, Trp^{17}) in order to study the disposition and dynamics of discrete segments of this peptide. $^{13}C\alpha$-glycine has also been incorporated to directly monitor the overall tumbling rate. ^{13}C-NMR relaxation data were analysed by an adaptation of the "model-free approach" proposed by G. Lipari and A. Szabo (1982, *J. Amer. Chem. Soc.* 104, 4546). A generalized order parameter $\mathcal{S}$ and an effective correlation time $\tau(e)$ describe the "internal dynamics" of the $^{13}C\delta_1$ label in the context of an overall peptide correlation time $\tau(m)$. An empiric relation between NMR- and fluorescence-derived parameters (steady-state anisotropy and lifetime data) is proposed which reconciles data obtained by both methods. The monomeric peptides show Trp $\mathcal{S}$ values consistent with restrained sidechain mobility and correlation times of ~300 ps. The "internalized" Trp^{19} residue of MLT tetramer demonstrates a slightly increased order parameter and a decreased correlation time (~100 ps). Aqueous peptide-lysolipid mixtures yield $\tau(m)$ values consistent with micelle formation, while the rate and mobility of the indole sidechain in the micelle environment are dependent on the intrachain position of the Trp residue. Supported by GM34847.

T-Pos135 LABELING OF MYELIN BASIC PROTEIN IN LIPID VESICLES WITH A HYDROPHOBIC PHOTOLABEL. Joan M. Boggs, Kalavelil M. Koshy, and Godha Rangaraj, Research Institute, Hospital for Sick Children, Toronto M5G 1X8, and Dept. of Clinical Biochemistry, University of Toronto, Toronto, Ont.

The hydrophobic photolabel 3-(trifluoromethyl)-3-(m-(^{125}I)iodophenyl)diazirine (TID) was used to label myelin basic protein (MBP) and polylysine in aqueous solution and bound to lipid vesicles. Although MBP is a water soluble protein which binds electrostatically only to acidic lipids, unlike polylysine it has several short hydrophobic regions. Results from a variety of techniques have suggested that some of the hydrophobic residues of MBP penetrate partway into the bilayer. MBP was labeled to a significant extent by TID when in aqueous solution indicating that it has a hydrophobic site. However, it was labeled 2-4 times more when bound to acidic lipids than when bound to phosphatidylethanolamine, or when in the presence of phosphatidylcholine vesicles. It was labeled 5-7 times more than polylysine bound to acidic lipids. These results suggest that when MBP is bound to acidic lipids, it is labeled from the lipid bilayer rather than from the aqueous phase. However, it cannot be ruled out that changes in the protein conformation or degree of aggregation may occur upon binding to lipid, which allow increased binding of TID from the aqueous phase. Within this limitation, the results are consistent with the model that some hydrophobic residues of MBP penetrate partway into the bilayer, while its basic residues are on the surface of the bilayer bound electrostatically to the lipid head groups.

T-Pos136 REVERSIBLE BINDING OF SYNEXIN AND THE CALELECTRINS TO LIPOSOMES. Paul Meers, Demetrios Papahadjopoulos and Keelung Hong, Cancer Research Institute, University of California, San Francisco, CA 94143, U.S.A.

Several cytosolic synexin-like proteins bind to membranes, aggregate vesicles and affect fusion rates of liposomes in a Ca^{2+}-dependent manner. We have studied the Ca^{2+}-dependent binding of synexin and the 32 kilodalton and 67 kilodalton calelectrins from bovine liver using fluorescent membrane probes. When these proteins bind to liposomes containing 5 mol% chain labeled pyrene-phosphatidylcholine, an increase in the monomer-to-excimer fluorescence ratio is observed. The increase in fluorescence of the monomer peak at 377 nm was used to monitor binding of these proteins to liposomal membranes. Under the conditions of our experiments, we found that synexin binds to phosphatidylserine (PS) liposomes at Ca^{2+} concentrations ranging between approximately 20 and 100 μM, where no significant aggregation or fusion of liposomes occurs. Lower Ca^{2+} and protein concentrations were sufficient to observe similar binding of the calelectrins. Binding of synexin to PS and phosphatidate (PA) was observed but no binding to phosphatidylcholine, consistent with the results of binding measured by sedimentation of liposomes. The affinity of these proteins was approximately the same for PS as for PA by these criteria. The rate and extent of the increase in fluorescence is dependent on the protein concentration, and the fluorescence increase is completely and rapidly reversible by EDTA. When unlabeled liposomes are added to liposomes with the pyrene label, bound synexin does not exchange between liposomes on the time scale of these experiments (minutes). There is also no leakage of encapsulated calcein from the same types of liposomes under conditions where synexin and the calelectrins bind. The observed fluorescence increases occur only with synexin and the calelectrins but not with other proteins, such as parvalbumin or bovine serum albumin. The reversible, non-leaky binding of these proteins and the small fluorescence change per protein molecule suggest that if there is bilayer penetration, it may involve only a small part of the protein. Non-exchangeability between liposomes suggests a tight binding to the surface of the bilayer, while preference for negative surface charge suggests ionic interactions play a role in protein binding.

T-Pos137 MECHANISM OF ACTION OF PULMONARY SURFACTANT SPL(pVAL). George P. Kreishman and John E. Baatz, Department of Chemistry; Barry Elledge and Jeffrey E. Whitsett, Department of Pediatrics, University of Cincinnati, Cincinnati, Ohio 45221.

A novel pulmonary surfactant-associated proteolipid of M_r=5000 (SPL(pVal)) has been isolated and shown to confer virtually full biophysical activity to surfactant phospholipids at protein concentrations in the membrane of 0.2% by weight. The amino acid sequence has been determined from SPL(pVal) cDNA. The middle section is extremely hydrophobic and contains only Val, Leu, and Ile residues. Preliminary 2-D NMR results indicate that the protein winds back on itself with the N-terminus in close proximity to the C-terminus. In this configuration, one end of the protein contains only hydrophobic residues and the other end contains the hydrophilic residues. The alterations in the lipid packing in the bilayer induced by SPL(pVal) have been studied by various NMR and Fluoresence Anisotropy techniques. The action of the protein is to impart greater order to the surface of the membrane while increasing the disorder of the interior of the bilayer. A model is proposed for the mechanism of action of SPL(pVal). First, the hydrophobic domain of the protein inserts into the bilayer, followed by rearrangement of the lipids to coat this hydrophobic portion. Now the head groups are crowded together and the surface tension of the bilayer is decreased.

T-Pos138 **STRUCTURAL CHANGES OF POLY-L-LYSINE HOMOPOLYMERS AND RANDOM COPOLYMERS FOLLOWING INTERACTION WITH DPPG VESICLES.** Shelia Loughran*, Ram Mohan^, Anne Walter~, Robert Blumenthal^, David G. Covell^, and Robert W. Williams*. *Dept. of Biochemistry, USUHS, Bethesda, MD, ^LMMB, NIH, Bethesda, MD, and ~Wright State University, Dayton, OH.

Secondary structural features of peptides play a significant role in their interaction with membranes; the formation of amphiphilic conformers appears to be an important step. A peptide's affinity for and penetration into membranes, factors that determine weak adsorption to or fusion and disruption of membranes, is related to the hydrophilic-lipophilic balance of the amphiphilie. Using Raman spectroscopy, we have examined the order parameters for DPPG vesicles and the secondary structural changes of the homopolymer poly-L-lysine (PLL) and random copolymers (PLL with serine, phenylalanine and alanine) in the absence and presence of DPPG vesicles at 14°C.

sample	C-C stretching			Amide I	
Protein:Lipid	1:3	1:1	3:1	solution	DPPG
PLL		.19		coil	helix
PLL-S	.20	.21	.21	coil	helix/beta
PLL-F	.35	.37	.50	beta	beta
PLL-A	.20	.20	.31	helix	helix
pure DPPG		.21			

These results A) suggest that the stability of the acyl chain region of DPPG i) increased in the presence of PLL and PLL-S, ii) decreased in the presence of PLL-F, B) both beta and helix secondary protein structures interact favorably with DPPG, and C) provide a basis for examining interactions of peptides with membrane affinity.

T-Pos139 CONCENTRATION DEPENDENCE OF THE TRANSLATIONAL DIFFUSION OF MONOCLONAL ANTIBODIES SPECIFICALLY BOUND TO PHOSPHOLIPID LANGMUIR-BLODGETT FILMS. Lois L. Wright, Arthur G. Palmer III and Nancy L. Thompson, Department of Chemistry, University of North Carolina, Chapel Hill, NC 27599-3290.

Clustering of complexes of antibodies and antibody receptors in the regions of contact between immunological cells and their target cells may be an initial step in antibody-mediated cellular recognition and response. To begin to address the role of antibody diffusion and clustering in the recognition of targets by immunological cells, the translational mobility of fluorescent-labelled monoclonal antibodies specifically bound to supported phospholipid bilayers, which consist of dimyristoylphosphatidylcholine (68 mol%) and hapten-conjugated phospholipids (32 mol%), has been measured as a function of the surface concentration of bound antibodies, using fluorescence recovery after pattern photobleaching. At low surface concentrations, bound antibodies diffuse with a coefficient approximately equal to that of fluorescent phospholipids in the bilayers ($\sim 4 \times 10^{-9}$ cm^2/sec). At high surface concentrations, one population of antibodies diffuses rapidly ($\sim 4 \times 10^{-9}$ cm^2/sec) and another population diffuses much more slowly ($\sim 2 \times 10^{-10}$ cm^2/sec). The fraction of the fluorescence recovery due to slowly diffusing antibodies increases from 0 to ~0.7 with increasing concentration of bound antibody. These results suggest that bound antibodies may form submicroscopic, mobile clusters at high surface concentrations. According to the theory of Saffman and Delbruck (1975, Proc. Natl. Acad. Sci. 72, 3111), the observed 20-fold decrease in the diffusion coefficient would correspond to clusters of 50-100 antibodies. This work was supported by NIH grant GM-37145 and NSF grant DCB-8552986.

T-Pos140 EFFECT OF MEMBRANE PHOSPHATIDYLCHOLINE ON THE ACTIVITY OF BOVINE LIVER PHOSPHATIDYLCHOLINE TRANSFER PROTEIN. Elizabeth A. Runquist and George M. Helmkamp, Jr., Department of Biochemistry, University of Kansas Medical Center, Kansas City, KS 66103

Protein-mediated transfer of phosphatidylcholine (PC) by bovine liver phosphatidylcholine transfer protein (PC-TP) was examined using a vesicle-vesicle assay system. Donor and acceptor membranes were prepared from *E. coli* phospholipids and limiting amounts of egg yolk PC. PC transfer between vesicles of *E. coli* lipid/egg PC was markedly higher than transfer of PC from vesicles of *E. coli* lipid/egg PC to vesicles of *E. coli* lipid. Kinetic parameters of the interaction between PC-TP and *E. coli* lipid vesicles with or without PC was investigated. The apparent dissociation constants of the complex formed between PC-TP and these vesicles were determined kinetically and from double reciprocal plots of PC-TP intrinsic fluorescence intensity increase versus vesicle concentration. The magnitude of the dissociation constant decreased as the PC content of the vesicles increased from 0 to 5 mol%. In addition, kinetic analysis revealed that the presence of PC in acceptor vesicles increased both the association and dissociation of PC-TP from vesicles. Quenching of the intrinsic protein tryptophan fluorescence was achieved to an equivalent extent by a series of *trans*-parinaroyl phospholipids, including transferable and non-transferable species. The effect of membrane PC molecules on transfer rates was examined using *bis*-phosphatidylcholine, a dimeric PC which is not transferred by PC-TP. Rates of PC transfer to vesicles comprised of *E. coli* lipid/*bis*-PC were virtually identical to rates observed to vesicles containing only *E. coli* lipid. The results suggest that transfer of PC by PC-TP is enhanced only when insertion of protein-bound PC occurs concurrently with the extraction of a molecule of membrane PC, *i.e.*, a concerted, one-step catalytic mechanism for phospholipid exchange. *(This research was supported by NIH grant GM 24035.)*

T-Pos141 ^{13}C AND ^{31}P NMR INVESTIGATIONS OF 1-PALMITOYL LYSOPHOSPHATIDYLCHOLINE BOUND TO PHOSPHOLIPID VESICLES AND BOVINE SERUM ALBUMIN. Shastri P. Bhamidipati and James A. Hamilton, Biophysics Institute, Departments of Medicine and Biochemistry, Housman Medical Research Center, Boston University School of Medicine, Boston, MA 02118

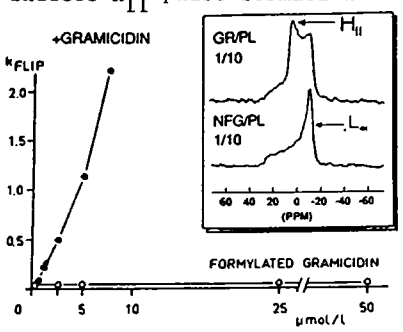
The carbonyl and the polar headgroup phosphate environments of 1-palmitoyl lysophosphatidylcholine (LPPC) have been monitored in different biological environments by ^{13}C and ^{31}P NMR. ^{13}C=O LPPC (90% enriched) was used in ^{13}C NMR studies. The carbonyl chemical shift value for aqueous, micellar (1.0-18.0 mM) LPPC was 175.00 ppm. In vesicles prepared by cosonicating 5 mol% LPPC and 95 mol% egg phosphatidylcholine (PC), two carbonyl peaks (174.73 and 174.61 ppm) of unequal intensity, well separated from the PC carbonyl peaks, were seen. After addition of aqueous LPPC (3 mol% with respect to PC) to preformed egg PC vesicles, a single peak which corresponded to the more intense peak in the cosonicated system (174.73 ppm) was observed. This peak was assigned to LPPC in the outer monolayer of the vesicles. In LPPC/bovine serum albumin (BSA) mixtures with 1-5 moles of LPPC/mole of protein, a single peak with increasing intensity was observed at 175.30 ppm. In ^{31}P NMR spectra, micellar LPPC had a chemical shift of -0.24 ppm whereas LPPC complexed to BSA had a chemical shift of -0.35 ppm. In egg PC vesicles treated with phospholipase A_2 (Crot. Adam.), lyso-PC appeared as a single peak at -0.48 ppm close to the PC phosphate signal (-0.96 ppm); the ^{13}C NMR spectrum showed a single peak corresponding to lyso-PC in the outer monolayer. These chemical shift data were also used to monitor the partitioning of LPPC between egg PC vesicles and BSA. For example, it was found that BSA, when added in 2:1 (LPPC/BSA) molar ratio, extracted LPPC from the outer monolayer of the PC vesicles alone.

T-Pos142 DEUTERIUM NMR STUDIES OF THE EXCHANGE RATES OF INDOLE AND AMIDE HYDROGENS IN TRYPTOPHAN PEPTIDES ORIENTED IN LYOTROPIC LIQUID CRYSTALS. S. Michael Strain and Frederick W. Dahlquist, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403.

The conformation and dynamics of hydrophobic polypeptides can be studied by deuterium NMR when these molecules are oriented as guests in the micelles of lyotropic liquid crystals. The exchange rates of tryptophan indole and amide deuterons of indole, trp-NH_2, gly-trp, and trp-gly oriented in potassium laurate/decanol/KCl/D_2O micelles were measured by ^2H NMR. Quadrupole doublets with splittings of up to 11.3 and 6.4 kHz were observed (trp-NH_2) and were assigned to the indole and primary amide deuterons, respectively, based on their chemical shifts (center frequency of the doublet). Base catalyzed exchange rates were calculated from the excess linewidths of these resonances as a function of pH in the range 9.5-12. The apparent exchange rate for indole in the micellar phase was about two orders of magnitude slower than that reported for tryptophan derivatives in solution (Waelder and Redfield (1977), Biopolymers 16, 623-629). To assess the degree of altered hydroxyl ion activity in the liquid crystal, the ionization of potassium monophosphate added to the aqueous component was studied by measurement of its ^{31}P NMR chemical shift as a function of pH. While differences in hydroxyl ion activity were noted, it was concluded the principal reduction in exchange rate must be due to the limited access of aqueous phase hydroxyl ions to the indole or trp moieties intercalated into the hydrocarbon domain of the lyotropic micelle. Supported by NIH Grant GM-35787.

T-Pos143 GRAMICIDIN INDUCED ENHANCEMENT OF FLIP IS RELATED TO INDUCTION OF H_{II} PHASE FORMATION. H.Tournois, J.Classen, C.W.M.Haest, J.de Gier and B.de Kruijff (intr. by J.A.Killian) Dept of Biochemistry,Univ.of Utrecht,Padualaan 8,3584 CH Utrecht,The Netherlands.

Incorporation of the channel forming antibiotic gramicidin (GR) into the membrane of human erythrocytes highly(upto 30 fold) enhances rates of reorientation (flip) of lysophosphatidylcholine and palmitoylcarnitine as determined by the albumin extraction method.Increase in flip is already detectable at a 1:2000 molar ratio of GR to phospholipid (PL).This is 1000 fold higher than the ratio required for detection of K^+ permeability mediated by the GR channel.^{31}P NMR,Small angle X-ray scattering and Freeze-fracture electronmicroscopy showed that GR induces H_{II} phase formation in both total lipid extract and ghosts prepared from human erythrocytes at molar ratio's >1:80 GR to PL.The parallel between H_{II} phase formation and transbilaver reorientation induced bv GR as welI as the lack of effects in both H_{II} phase formation (figure) and flip enhancement of trp-N formylated gramicidin in which the four tryptophan residues are formylated, strongly suggest that both processes are mechanistically related. The formation of GR aggregates of specific structure,which are thought to be intermediates in H_{II} phase formation,might lead to enhancement of transbilayer movement of phospholipids. (H.Tournois et al. (1987) Biochemistry (october issue,inpress),J.Classen et al. (1987) Biochemistry (october issue, inpress).



T-Pos144 CONFORMATIONAL DYNAMICS OF FLUORESCENTLY LABELED APOLIPOPROTEIN A-II IN SOLUTION AND IN RECOMBINANT LIPID COMPLEXES. William W. Mantulin, Laboratory for Fluorescence Dynamics, Departments of Physics, Biochemistry and Biophysics, University of Illinois at Urbana-Champaign, 61801.

Apolipoprotein A-II (apoA-II) is a major protein component of plasma high density lipoprotein. It is a homodimer (MW 17400) of known sequence (77 residues) linked through a sulfhydryl bridge at cystine 6. Reduced apoA-II was specifically labeled at cystine 6 by 6-acryloyl-2-dimethylaminonaphthalene (ACRYLODAN). Recombinant lipid complexes with dimyristoylphosphatidylcholine (DMPC) and the labelled protein (100:1 lipid to protein molar ratio) were prepared by cholate solubilization. To assess the conformational dynamics of apoA-II, we compared the temperature dependence (15-46C) of the fluorescence properties of ACRYLODAN-apoA-II in solution and in lipid complexes. Association of ACRYLODAN-apoA-II with DMPC blue shifts the emission center of mass by 23nm to 471nm; however, the fluorescence polarization remains high at about 0.3. Increasing temperature 1) decreases the polarization, 2) does not detect the lipid phase transition, and 3) red shifts the emission and broadens the emission half width ($20cm^{-1}$). The fluorescence lifetime data is well-described by two component discrete exponentials (in solution: τ_1=2.4nsec, τ_2=0.65nsec, f_1=0.5; in complex: τ_1=3.5 nsec, τ_2=1.9nsec, f_1=0.8) or continuous discrete distributions (in solution: center=1nsec, width= 1nsec; in complex: center=3.1nsec, width=1.3nsec). Global analysis of the temperature dependent lifetime data in terms of a physical model of two non-interacting states recovers activation energies of 1-2kcal for thermal quenching. The ACRYLODAN-apoA-II fluorescence data are consistent with a fairly immobilized probe, which senses the lipid-protein interfacial region. Supported by NIH grant RR03155.

T-Pos145 CHROMAFFIN GRANULES SWELL IN SITU DURING CATECHOLAMINE SECRETION FROM BOVINE ADRENAL CHROMAFFIN CELLS. R. L. Ornberg, S. Furuya, and G. A. J. Kuijpers. LCBG, NIDDK, NIH, Bethesda, MD 20892.

Changes in the size of chromaffin granules accompanying secretagogue-induced cathecholamine release have been examined to search for possible structural correlates of an osmotic mechanism in exocytosis and membrane fusion. Bovine adrenal chromaffin cells were quick frozen and freeze-substituted following stimulation with nicotine (15 μM, 1-3 min) or high external K^+ (50 mM, 1-3 min). Granule diameters were calculated, assuming a spherical shape, from granule cross-sectional areas and circumferences digitized from 8 - 12 serial electron micrographs. Each granule was measured in the sectioned volume only once, at its largest diameter. The mean granule diameter from area measurements in unstimulated control cells measured 220 $\pm$ 67 nm (s.d., n = 1847). After 1 min of K^+ or nicotine stimulation, the mean diameter had increased to 277 $\pm$ 76 nm (n=986) and 261 $\pm$ 66 nm (n=957), respectively. This increase was due to a shift in the distribution of diameters to larger values, ie. granule swelling, although the selective release of a population of small granules from the control distribution may have also occurred. In control cells, diameters calculated from circumference measurements were consistently larger (1.1-1.2 x) than those from area measurements. In stimulated cells, the two measures produced identical results suggesting that swelling results in an ellipsoid to spherical shape change. Granule swelling was accompanied by the swelling of the storage contents. These results indicate that secretory chromaffin granules increase in size following stimulation, prior to exocytotic membrane interaction, and suggest that osmotic forces may play a role in membrane fusion.

T-Pos146 DYNAMICS OF EXOCYTOTIC PORE FORMATION IN MAST CELLS OF THE BEIGE MOUSE. M. Curran, J. Zimmerberg, DCRT/NIDDK, National Institutes of Health, Bethesda, MD, 20892; and F. S. Cohen, Dept. of Physiology, Rush Medical College, Chicago, IL 60612.

We are continuing to study the aqueous pore which initiates fusion of large secretory granules during exocytosis. We have previously shown that when capacitance is monitored using the tight-seal, whole-cell patch clamp technique and a phase-sensitive detector, the conductance of the exocytotic pore can be calculated from the real and imaginary components of the complex admittance (Zimmerberg, Curran, Cohen, & Brodwick, PNAS 84:1585, 1987). By monitoring the discharge of the granule potential through a fusion pore, the earliest detectable pore was subsequently shown to be 230 83 pS (Breckenridge and Almers, Nature 328:814, 1987). We report here the the effect of granule potential on the complex admittance, the time course for the enlargement of the pore from its initial value, and the effect of hyperosmotic conditions on pore growth. Initially, a large, biphasic transient in admittance was seen in both components. This transient can be explained by the discharge of granule potential at the instant of pore formation. Following the transient, we measure a variable time course - the pore can be as small as 0.11 nS or larger than 10 nS within 3 ms of initial pore formation. In hypertonic solutions (660 mOsm/kg), the time course is retarded and the pore conductance flickered between discrete states more often than in isotonic conditions. The conductances of these states varied between granules. Further, a pore that was continuously and smoothly increasing in conductance could suddenly begin flickering only to resume its smooth increase to a fully enlarged pore. Because these pores show rich and varied kinetics we envision this exocytotic fusion pore to be an extremely dynamic structure.

T-Pos147 MEMBRANE PENETRATION OF VIRAL PROTEINS DURING FUSION OF SENDAI VIRUS WITH LIPOSOMES SHOWN BY HYDROPHOBIC PHOTOAFFINITY LABELING. Steven L. Novick[1], John D. Baldeschwieler[1], and Dick Hoekstra[2], [1]Division of Chemistry, 127-72, California Institute of Technology, Pasadena, CA 91125 and [2]Laboratory of Physiological Chemistry, University of Groningen, Bloemsingel 10, 9712KZ Groningen, The Netherlands.

The hydrophobic photoaffinity label 3-(trifluoromethyl)-3-(m-[^{125}I]iodophenyl)diazirine (TID) was used to probe protein interaction with target membranes during fusion of Sendai virus with TID containing liposomes composed of cardiolipin (CL) and phosphatidylserine (PS). Conclusive evidence is provided for hydrophobic penetration of the fusion (F) protein into the target membrane as an initiating event of fusion at neutral pH. At low pH, fusion of Sendai virus and CL liposomes also involves hydrophobic interaction of the hemagglutinin/neuraminidase (HN) with target membranes, consistent with previous observations.[1] Furthermore, under conditions in which binding occurs without fusion, HN labeling suggests that in addition to electrostatic binding, hydrophobic interactions may also be important in viral attachment. Labeling during fusion with either vesicle type was similar, suggesting a common fusion mechanism, in spite of quantitative differences in the kinetics and extent of fusion.[1] When reconstituted envelopes (RSVE) were fused with liposomes, labeling was quite different, implying an altered protein orientation in the reconstituted membrane. Initial experiments show that this method may be useful for investigation of virus fusion with biological membranes. Identification of labeled peptide fragments is also underway. This work was supported by ARO grant DAAG29-83-K-0128 and a gift from Monsanto. One of us (S.L.N.) is the recipient of an NSF Graduate Fellowship. [1]Klappe, K., Wilschut, J., Nir, S., and Hoekstra, D., Biochem.25 8252 (1986).

T-Pos148 FUSION OF GLYCOPHORIN CONTAINING VESICLES WITH SENDAI VIRUS AND RED CELLS.
Ruby I. MacDonald, Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL 60208.

By an adaptation of the method of Umeda et al., reverse phase vesicles composed of egg phosphatidylcholine, cholesterol, dicetylphosphate and glycophorin and labeled with NBD-phosphatidylethanolamine and rhodamine-phosphatidylethanolamine were demonstrated to fuse at pH 7 with about 20% of the combined lipid in Sendai virus and human red cells on consecutive incubation with virus and red cells. These values do not appear to reflect lipid exchange, since the labeled vesicles which did fuse participated in lipid mixing exceeding that possible if NBD in the inner monolayer remained quenched and NBD in the outer monolayer mixed with the available, unlabeled membrane lipid. The degree of lipid mixing was determined from standard curves relating the amounts of unlabeled vesicles mock-fused with labeled vesicles to NBD F/F_o values of those samples. Negligible fusion occurred between glycophorin containing vesicles and Sendai virus alone. If not incubated at 37°C for 60 min., about 6% of the combined virus and red cell lipid fused with glycophorin containing vesicles. Furthermore, vesicles without glycophorin fused with about 8% of the combined virus and red cell lipid and vesicles without dicetylphosphate fused with a few % of the combined virus and red cell lipid. Lipid mixing was detectable between unlabeled vesicles composed of egg PC and cholesterol and NBD-PE + Rh-PE labeled vesicles with or without dicetylphosphate and/or glycophorin when incubated under conditions otherwise leading to lipid mixing among vesicles, virus and red cells but not to a degree which could account for lipid mixing among the latter membranes.

Supported by NIH AI20421.

T-Pos149 FUSION OF SYNAPTIC VESICLES WITH LIPOSOMES.
Lan-rong Hu and R.C. MacDonald, Dept. of Biochem., Mol. Biol. & Cell Biol., Northwestern University, Evanston, IL 60208

The possible fusion of synaptic vesicles with phosphatidylethanolamine (PE)/phosphatidylserine (PS) bilayers was investigated. A fluorescence energy transfer assay (based on NBD- and rhodamine-labeled lipids in PS/PE liposomes) and an assay for the ATP in synaptic vesicles (based on luciferin and luciferase encapsulated by PS/PE liposomes) were used to assess membrane and contents mixing, respectively. The former assay involved the common configuration in which dequenching of NBD fluorescence occurs as the liposome lipid is diluted into the target membrane--here, the synaptic vesicle. Luciferin+luciferase-containing large and giant, unilamellar vesicles were prepared by one cycle of freezing and thawing of small, unilamellar vesicles suspended in a solution of enzyme containing a high concentration of a salt of an oxy-acid such as a phosphate or a sulfate. Some fluorescence dequenching, indicating mixing of liposome lipid with rat brain synaptic vesicle membranes,occurred in the absence of added calcium ion, but subsequent additions of calcium ion (1 mM or more) elicited additional dequenching. Only the former and not the latter phase was inhibited by trypsin treatment (but not heat-denatured trypsin) of the synaptic vesicles. A trypsin- and heat-sensitive factor isolated from synaptosomal cytoplasm markedly stimulated the fluorescence dequenching that occurs in the absence of added calcium ion. Hydrolysis of ATP in synaptic vesicles from fish electric organ, catalyzed by luciferase trapped in liposomes, occurred on addition of calcium ion to a concentration of at least 1 mM. Evidence from the two types of experiments is consistent with fusion occurring at millimolar calcium and either lipid exchange or a low level of fusion in the absence of added calcium. Supported by NIH NS20831.

T-Pos150 **EFFECTS OF OSMOTIC GRADIENTS AND AMPHIPATHIC COMPOUNDS ON FUSION OF DIPALMITOYLPHOSPHATIDYLCHOLINE LARGE, UNILAMELLAR VESICLES BY LOW CONCENTRATIONS OF POLY(ETHYLENEGLYCOL).**
G.F. McIntyre and B.R. Lentz. Department of Biochemistry and Nutrition, The University of North Carolina at Chapel Hill, Chapel Hill, N.C. 27514. Intr. by Francine R. Smith.

Poly(ethyleneglycol) [PEG]-induced fusion of DPPC vesicles prepared by extrusion (LUVETS) or by reverse evaporation (REVS) was monitored by lipid mixing and contents mixing assays at 48°C. Low concentrations of PEG (3.8 w/w%) induced fusion of REVS exposed to a positive osmotic gradient (50% of one round of fusion). The same concentration of PEG caused vesicle aggregation (increased turbidity) and lipid exchange (70%) with LUVETS but minimal fusion (11% contents mixing). The presence of amphipathic compounds, such as monooleoylglycerol, increased the extent of LUVET fusion to 33%. Surprisingly, a positive osmotic gradient had no effect on the extent of PEG-induced LUVET fusion. For both LUVETS and REVS , the extent of lipid mixing was independent of the extent of contents mixing and of lipid concentration. The results suggest that low concentrations of PEG will cause intimate membrane contact between vesicles but are not sufficient to induce fusion. Amphipathic agents, such as monooleoylglycerol, or changes in bilayer surface tension were needed to obtain fusion of vesicles aggregated by PEG. Supported by NIH GM 32707.

T-Pos151 MECHANISMS OF ANNEXIN ACTION IN CHROMAFFIN GRANULE AGGREGATION AND FUSION. W.J. Zaks and C.E. Creutz (Intr. by W.H. Martin) Dept. of Pharmacology, University of Virginia, Charlottesville, 22908.

The annexins are a group of Ca^{2+}- and phospholipid-binding proteins which may play a role in exocytosis by promoting Ca^{2+}-dependent membrane interactions. Included in this group are synexin, endonexin and the 67kDa calelectrin. The mechanisms of action of these proteins in model systems for exocytosis including chromaffin granule binding, aggregation and fusion, were investigated. The binding of ^{125}I-labelled annexins to chromaffin granule membranes was measured using a centrifugation protocol. The Ca^{2+}-dependence of membrane binding of endonexin was multiphasic and varied with the nature of the lipid substrate, suggesting a lipid effect on the protein's Ca^{2+} affinity. In the presence of cis-unsaturated free fatty acids, membrane binding of the annexins as well as membrane aggregation and fusion, occurred in the micromolar Ca^{2+} range. Ca^{2+} also affected the maximum amount of annexin which could be membrane bound, suggesting a separate membrane site of Ca^{2+} action. Cooperative and synergistic binding interactions between identical and distinct annexin species, respectively, was observed at millimolar Ca^{2+}, suggesting the existance of intramembrane interactions between annexin molecules. Annexin-dependent chromaffin granule aggregation, measured by a turbidometric assay, revealed both synergistic and antagonistic effects of the different proteins with each protein functioning as a partial agonist. Chromaffin granule fusion, monitored by the R-18 fluorescent lipid dequenching assay, was inhibited at high annexin concentrations, and provided evidence for the importance of intermembrane annexin interactions in membrane aggregation and fusion.

T-Pos152 ABSCISIC ACID ENHANCES PHOSPHOLIPID MEMBRANE AGGREGATION AND FUSION by W. Stillwell*, B. Brengle* and S.R. Wassall+, Departments of Biology* and Physics+, Indiana University-Purdue University at Indianapolis, Indianapolis, IN 46223

The plant hormone abscisic acid (ABA) has been shown to affect solute permeability across several types of biological membranes as well as bilayers composed of two different types of phospholipids. Here we report that ABA can also enhance the aggregation and fusion of bicomponent phospholipid bilayers. Bilayers containing phosphatidylethanolamine (PE) and phosphatidylcholine (PC) were unaffected by the hormone when both lipids were in the liquid crystalline state. However when the temperature was held between the phase transition temperatures of the membrane lipids, or, in a few cases, when both lipids were in the gel state, ABA enhanced aggregation (followed by an increase in absorbance at 350 nm) and fusion (followed by fluorescence resonance energy transfer with vesicles containing N-Rh-PE and N-NBP-PE). Fusion was confirmed by following aqueous compartment mixing with a terbium-dipicolinic acid fluorescence assay. Enhancement of membrane fusion is therefore proposed as a possible function for ABA.

T-Pos153 PH DEPENDENT FUSION BY A SYNTHETIC AMPHIPATHIC PEPTIDE. Roberta A. Parente, Shlomo Nir*, and Francis C. Szoka, Jr. Departments of Pharmacy and Pharmaceutical Chemistry, University of California, San Francisco, CA 94143 and *Seagram Center for Soil and Water Sciences, Faculty of Agriculture, Hebrew University of Jerusalem, Rehovot 76100, Israel.

A synthetic, 30-amino acid peptide with the major repeat unit Glu-Ala-Leu-Ala (GALA) is water soluble with an aperiodic conformation at neutral pH and becomes an amphipathic α-helix as the pH is lowered to 5.0. It was designed to mimic the hydrophobicity and membrane interactions of viral fusogenic sequences. Fluorescence energy transfer measurements indicated that GALA induced lipid mixing between phosphatidylcholine small unilamellar vesicles at pH 5.0 but not at neutral pH. Dynamic light scattering and internal volume measurements showed an increase in vesicle diameter concomitant with lipid mixing. GALA induced leakage of small molecules (450 MW) at pH 5.0 was too rapid to permit detection of contents mixing. However, retention of larger molecules (4100 MW) under the same conditions suggests that vesicle fusion occurs. A mass action model simulates the kinetics of increase in fluorescence intensity and yields rate constants of aggregation and fusion. As the lipid to peptide ratio decreases from 100/1 to 50/1 both rate constants of aggregation and fusion increase, indicating that GALA is a genuine inducer of vesicle fusion. GALA analogs with shortened sequences or an altered sequence but the same amino acid composition did not induce lipid mixing or an increase in vesicle size. These results indicate that the fusogenic activity of GALA requires a sequence greater than 16 residues and a defined topology of the hydrophobic residues. The fusogenic activity of GALA is currently being studied using large unilamellar vesicles composed of mixid phospholipids. Supported by NIH-GM29514 (FCS), Damon Runyon-Walter Winchell Cancer Fund Fellowship DRG-907 (RAP) and BSF-8600010 and NIH-GM31506 (SN).

T-Pos154 DIVALENT CATION BINDING ON ACIDIC PHOSPHOLIPID SONICATED VESICLES.

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The molecular mechanism by which divalent cations induce the fusion of unilamellar vesicles made of acidic phospholipids is not understood clearly. The most recent advances in this regard imolies that fusion occurs via defects in molecular packing and dehydration of the polar head group of phospholipids at the region of interbilayer contact.

In the first goal to clarify this hypothesis, the binding constants of divalent cations on phospholipids were determined and related to the fusion threshold concentration and se for various acidic phospholipids in vesicle systems. The method of determination of the binding constants of Mg^{2+}, Ca^{2+} and Mn^{2+} on PA, PS and PG sonicated vesicles differs from other authors in the way that we dialysed the preincubated systems over a buffer that doesn't contain any metallic cation. The values of the binding constants such obtained may be compared to the one's obtained from various technics of quantitative measurements.

These results let's suppose that there exist some specific interactions between the fixed cations and the functional groups of the phospholipids head group since the cations dilution induce by the desalting dialysis doesn't desorb them. The binding data let's suppose that the initial conditions of incubation are determinant in the post-dialysis measurements and in fact to the destabilization of the membranes.

This report was supported by a CRSNG grant and a CRSNG award to F.B. .

T-Pos155 PHOTOINDUCED DESTABILIZATION OF TWO-COMPONENT VESICLES OF PHOSPHATIDYLETHANOL AMINES AND POLYMERIZABLE PHOSPHATIDYLCHOLINES. Ulrich Liman, David A. Frankel, and David F. O'Brien, Department of Chemistry, University of Arizona, Tucson, Arizona 85721

Stable phosphatidylethanolamine (PE) vesicles can be prepared at physiological pH when the PE is mixed with natural phosphatidylcholines (PC). In this report we show that dioleoyl PE (DOPE) or transesterified egg PE (TPE) form stable two-component vesicles when combined with a polymerizable chain terminal sorbyl PC (-OCOCH=CH-CH=$CHCH_3$). Photopolymerization of the PE/sorbylPC vesicles by irradiation of the 260 nm band (ε 30,000) leads to vesicle destabilization. The vesicle stability was evaluated with encapsulated water soluble fluorescent markers (calcein or ANTS/DPX). Thus TPE/sorbylPC (2/1) vesicles were prepared in the presence of 75 mM calcein, 70 mM NaCl, 20 mM Tris pH 7.4. After desalting the calcein leakage was determined at selected temperatures after photopolymerization. The release was less than 10% at 25 ^{o}C, and increased to 40% at 55 ^{o}C and 70% at 80 ^{o}C. Leakage was not significant prior to polymerization or in vesicles that did not contain PE. Release experiments with DOPE/sorbylPC (2/1) showed 60% release at 25 ^{o}C, and 70% at 45 ^{o}C. In each vesicle system leakage was observed after polymerization and after the sample was brought to a temperature above the lamellar to hexagonal phase transition. The photoinduced membrane destabilization of PE containing membranes is likely due to polymerization initiated phase separation of the polymerized PC and the PE, which allows the enriched PE domains to undergo a transition to nonlamellar phase(s).

T-Pos156 EVIDENCE FOR A FUSION INTERMEDIATE IN ELECTROFUSION. Arthur E. Sowers, Cell Biology, American Red Cross/Holland Laboratory, Rockville, MD 20855.

A single electric field pulse was used to fuse human erythrocyte ghosts held in contact by alternating current (AC)-induced dielectrophoresis. Fluorescence microscopy was used to detect individual fusion events with either DiI as a membrane mixing indicator or mw = 10 kD FITC-Dextran (FD) as a contents mixing indicator. Fusion yields (FY) were scored in terms of events in which DiI or FD moved to unlabeled membranes or cytoplasmic compartments, respectively. However, FD-based FYs nearly always much higher than DiI-based FYs. However, where FD was the label and the AC was turned off after the pulse and the cytoplasmic compartments which became labeled after the pulse were continuously observed for several minutes, then some groups of contiguously labeled compartments separated from one another by Brownian motion while others remained irreversibly attached to each other. This revealed that only the membranes which showed both contents mixing and the irreversible attachment were actually fused. Subtracting the unfused membrane fraction from the number of contents mixing events resulted in a FY which matched, within experimental error, the DiI-based FY. DiI-based FY and the corrected FD-based FY agree over a broad range of conditions and move up, and parallel, with ionic strength. Conversely the observation of non-leaky contents mixing events which do not lead to fusion events suggests the presence of a reversible fusion intermediate structure. Supported by ONR contract N00014-87-K-0199.

T-Pos157 EFFECT OF MONOVALENT CATIONS ON POLYVALENT CATION-INDUCED FUSION OF SMALL UNILAMELLAR PHOSPHATIDYLSERINE VESICLES. Dinesh K. Sukumaran and Shinpei Ohki, Department of Biophysical Sciences, School of Medicine, State University of New York at Buffalo, Buffalo, NY 14214.

Internal contents mixing fluorescence assay was used to monitor the fusion of small unilamellar phosphatidylserine (PS) vesicles, induced by metal ions (Ca^{2+}, La^{3+} and Tb^{3+}), at various concentrations of monovalent cations (Li^{+}, Na^{+} and K^{+}). The influence of ionic strength (0.02M - 1.0M) on the threshold concentration of the cations required to induce fusion was measured. The threshold concentrations increased monotonically (1mM at 0.1M NaCl, and 3.1mM at 1.0M NaCl) with the increasing ionic strength of the solution for Ca^{2+}, but remained unchanged for both La^{3+} and Tb^{3+}. Changes in the ionic strength of the encapsulated solution did not alter the threshold concentration for all the ions studied, in the range 0.02M to 0.3M. The results are analysed in terms of competetive binding between the monovalent ions and the "fusogenic" ions (Ca^{2+}, La^{3+} and Tb^{3+}). It is shown that there is a critical value for Ca-bound PS, below which no massive fusion occurs. The calculations of the bound and free fractions of PS are based on the Gouy-Chapman model, taking ionic strength corrections to activity coefficients into account. Our experiments show that monovalent ions do not induce fusion even at high concentrations. Supported by a grant from NIH (GM24840).

T-Pos158 CHANNELS OF GRAMICIDIN A ANALOGUES : TYR[9,11,13,15] AND TYR (BZL)[9,11,13,15] GRAMICIDIN A.
F. HEITZ[1], P. DAUMAS[1], N. VAN MAU[1], and Y. TRUDELLE[2]. 1) Laboratoire de Physicochimie des Systèmes Polyphasés, BP 5051, F-34033 MONTPELLIER Cedex, France ; 2) Centre de Biophysique Moléculaire CNRS, F-45071 ORLEANS Cedex, France.

Comparison of the single channel conductances of Gramicidin A (GA) and Gramicidin M$^-$ (GM$^-$) (Heitz et al. 1982, Biophys. J., 39 : 87) pointed out the importance of the nature of the aromatic side-chains. In order to precise this role, two analogues, Tyr[9,11,13,15] GA (GT) and Tyr (Bzl)[9,11,13,15] GA (GTBzl) have been synthetized. Single channel data reveal that GT behaves similarly to GA (λGA = 85 ps and λGT = 67 ps in 1 M CsCl GMO membranes) while GTBzl ressembles GM$^-$ (the conductance is strongly voltage dependant and much lower λ = 12 ps at 100 mV applied voltage). An analysis of the variation of the conductance with the electrolyte concentration, based on a 3 barriers-2 sites model with oneor two ions in the channel (Finkelstein and Andersen 1981, J. Membr. Biol. 59 : 155) shows that the main difference when going from GA or GT to GTBzl lie in the height of the entry and translocation barriers ($\sim$ 2 kcal). As both, GT and GTBzl have nearly the same aromatic dipole moment these result indicate that other properties such as the hydrophobicity could also govern the conductance of the Gramicidin channel.

T-Pos159 FLASH PHOTOLYSIS OF GRAMICIDIN CHANNELS IN LIPID BILAYERS.
David Busath and Elie Hayon*. Section of Physiology and Biophysics, Brown University, Providence, RI 02912 and *Dept. of Chemistry, Queen's College, Flushing, NY 11367.

Monolein/hexadecane bilayers (50 mg/ml, 0.83 mm diameter) exposed to gramicidin D (5.0 nM in 1.0 M KCl) develop a stable conductance of about 300 μS within about 5 min of bilayer formation. We exposed bilayers thus doped to flashes from a xenon flash lamp lasting ~100 μs. The UV light from a single flash (Busath and Waldbillig, BBA 736:28-38, 1983) caused 15-25% reductions of membrane conductance, the entire drop occuring during the 100 μs period of the flash. This implies that the channel photolysis process occurs on the sub-millisecond time scale or faster, consistent with a photo-oxidation process. Gramicidin M, (obtained from F. Heitz, Montpellier, FR) produced a lower specific conductance: ~10 μS using V_m=100 mV, [Gram M]=25.0 nM. The flash had no discernable effect on this analog of gramicidin which has the photosensitive Trp's replaced with Phe's, consistent with other evidence implicating the Trp in gramicidin as the component responsible for its photosensitivity. (Supported by NIH grant GM33361 to DB)

T-Pos160 CALCULATION OF DEFORMATION ENERGIES AND CONFORMATIONS IN LIPID MEMBRANES CONTAINING GRAMICIDIN CHANNELS.
Helfrich, P., and E. Jakobsson, Department of Chemistry, Department of Physiology and Biophysics, and Program in Bioengineering, University of Illinois, Urbana, IL 61801

Huang (1) calculated surface conformation and deformation free energy in a thin lipid bilayer membrane by a linearized form of smectic liquid crystal theory. We have extended the smectic liquid crystal theory to thicker membranes by solving numerically the full nonlinear equations including compression, surface tension, and splay terms. By the principle that the surface conformation and the lipid surface-protein contact angle adjust themselves to minimize free energy, we have calculated the lipid surface shapes and energies as a function of gramicidin monomer separation. For a wide range of membrane thicknesses and compressibilities, the calculated lipid surface deformation energy to accommodate one formed channel is less than 5kT. This result suggests the hypothesis that thermal fluctuations of opposing membrane surfaces (2,3) containing gramicidin monomers bring the monomers into "docking" position to form the dimeric channel. We will attempt to test the plausibility of this hypothesis by calculating the spectrum of surface fluctuations by the liquid crystal theory, and by direct molecular simulations using the GROMOS package to calculate the energetics of separating the gramicidin monomers. An incidental result of our calculations is that the form of the lipid surface will make possible refined calculation of the access resistance for ions to enter the channel mouth. (1) Huang, H.W. 1986. Biophys. J.,50:1061-1070 (2) Miller, I.R. 1984. Biophys J., 45:643-644 (3) Hladky, S.B., and D.W.R. Gruen 1984. Biophys. J.,45:645-646. This work is supported by the Bioengineering Program at the University of Illinois and PHS grant 1 R01 GM32356.

T-Pos161 GRAMICIDIN A STRUCTURE IN LIPID BILAYERS. P. V. LoGrasso, F.Moll III, L.K. Nicholson, C.G. Fields and T.A. Cross. Department of Chemistry and Institute of Molecular Biophysics Florida State University, Tallahassee, Florida 32306-3006.

Solid State ^{15}N Nuclear Magnetic Resonance experiments have been performed using uniformly ^{15}N and single site ^{15}N labeled gramicidin A. The chemical shift and ^{15}N-^{1}H dipolar interactions have been observed in oriented samples for a variety of sites in the polypeptide backbone which lines the monovalent cation selective channel. The dipolar splittings have been interpreted in light of the backbone dynamics to determine the orientation of the N-H bonds which lie in the plane of the peptide linkages. The orientation of these planes is of special interest because it has been suggested that the planes librate about the C_α- C_α axis allowing the carbonyl oxygens to protrude toward the channel center so that monovalent cations can be more adequately solvated during transport.

While the channel state appears to be the most stable conformation in hydrated lipid bilayers, other "non-channel" states do exist in which gramicidin is in intimate contact with the lipids. The conformation of gramicidin in hydrated lipid bilayers is solvent history dependent. When gramicidin and DMPC are cosolubilized in 97% chloroform and 3% methanol, dried and hydrated, a non-channel structure is generated. This structure can be completely converted to the channel state only after dilution, sonication and incubation at elevated temperature for hours. However, when trifloroethanol is used as the cosolubilizing solvent, the channel state is achieved directly in the lipid bilayers without resorting to sonication and heat treatment. This work has been supported by NSF (DMB-8451876 DMB-8504250), Procter and Gamble and NIH (AI-23007).

T-Pos162 ENERGETICS AND DYNAMICS OF ION TRANSLOCATION IN NORMAL AND MODIFIED GRAMACIDIN CHANNELS. Shankar, S., S.W. Chiu, J.A. McCammon, and E. Jakobsson, Department of Chemistry, University of Houston, Houston, TX 77004, and Department of Physiology and Biophysics and Program in Bioengineering, University of Illinois, Urbana, IL 61801.

Replacement of methyl groups in the no. 1 valines in the gramacidin channel with more polar groups has major effects on ionic permeabilities.(1) Phenomenological studies indicate these effects may be summarized by postulating that these substitutions increase the height of the central barrier for translocation and create more favorable energetics for ion entry into the channel mouth.(2) It would be desirable to test these conclusions by calculations based on the underlying molecular physics. A molecular simulation technique particularly suited for calculating the effects of such modifications is the thermodynamic cycle-perturbation method.(3) We have begun such calculations using the GROMOS molecular simulation package, and will report on results achieved to date. An incidental result of the simulation will be the ionic mobility within the channel, to be determined by applying time-correlation analysis to the molecular dynamics output.

(1) Barrett Russell, E.W., L.B. Weiss, F.I. Navetta, R.E. Koeppe II, and O.S. Andersen. 1986. Biophys. J. 49:673-686.
(2) Jakobsson, E. and S.W. Chiu. 1987. Biophys. J. 52:33-45.
(3) Lybrand, T.P., J.A. McCammon, and G. Wipff. 1986. Proc. Natl. Acad. Sci. USA 83:833-835.
This work is supported by the Bioengineering Program at the University of Illinois, PHS grant 1 RO1 GM32356, and NCSA, and at Houston, by NSF.

T-Pos163 FATTY ACID COMPONENTS OF GRAMICIDIN K. Roger E. Koeppe II, Ruth A. Corder, Department of Chemistry and Biochemistry, University of Arkansas, Fayetteville, AR 72701; Olaf S. Andersen, Elizabeth J. Narcessian, Linda M. Peart, Department of Physiology and Biophysics, Cornell University Medical College, New York, NY 10021; and George R. Waller, Department of Biochemistry, Oklahoma State University, Stillwater, OK 74078.

The gramicidin K family of channel-forming peptides was discovered as a set of more hydrophobic relatives of the gramicidin A family [Biochemistry 24: 2822 (1985)]. Based on NMR spectroscopy and amino acid analysis, we previously suggested that the gramicidin K molecules were lipopeptides consisting of gramicidin A, B or C with a fatty acyl chain esterified to the ethanolamine hydroxyl group. We now report that mild alkaline hydrolysis of a gramicidin K mixture in 0.06 N methanolic NaOH at room temperature yields a mixture of six peptides that occur in the same ratios as valine- and isoleucine-gramicidins A, B and C in commercial isolates from B. brevis. The chromatographic and single-channel properties of the individual gramicidins released by base hydrolysis are identical to those of gramicidin A, B and C, respectively (see accompanying abstract by Peart et al.). We have analyzed the methylene chloride-soluble products that are released by the NaOH treatment by gas chromatography-mass spectrometry and find a mixture of octadecanoic, hexadecanoic, pentadecanoic, and 12-methyl-tetradecanoic acids, and several other minor products. The lipopeptide nature of the gramicidin K species is therefore confirmed. The fatty acyl tail helps to stabilize the dimeric gramicidin channel in a membrane, as the gramicidin K channels have longer mean lifetimes than gramicidin A or C channels.

T-Pos164 MOLECULAR CHARACTERISTICS OF CHANNELS FORMED BY GRAMICIDIN K. L.M. Peart-Williams, E.J. Narcessian, O.S. Andersen, Dept. Physiol. Biophys., Cornell Univ. Med. Coll., New York, NY 10021, and R.E. Koeppe II, Dept. Chem. Biochem., Univ. Arkansas, Fayetteville, AR 72701.

Recently, three new naturally occurring gramicidins were isolated (Koeppe et al., Biochemistry 24:2822-2826 [1985]). These new compounds, the gramicidin K's, have the same amino acid compositions but about 20% higher molecular weights than the normal gramicidins, and are strongly retained on reverse-phase liquid chromatography columns. To further establish the identity of these compounds, we examined their channel-forming ability. They form channels that have similar conductances but about five-fold longer average durations than standard gramicidin channels. Results of hybrid channel experiments, where we monitor the formation of channels between chemically dissimilar peptides, show that the gramicidin K's form channels that are structurally equivalent to channels formed by the normal gramicidins. From a combination of HPLC chromatography, NMR spectroscopy and single-channel studies on gramicidin K that was subjected to mild alkaline hydrolysis (see accompanying Abstract by Koeppe et al.), we conclude that the new gramicidins differ from their normal counterparts by having a fatty acid esterified to the COOH-terminal ethanolamine - at the channel entrance. The ethanolamine -OH may be critical in mediating ion entry into the channel (Etchebest et al., FEBS Letters 173:301 [1984]). If so, our finding that its modification has little effect on the single-channel conductance would suggest that the major limitation to ion entry resides in the aqueous phase adjacent to the channel entrance. Our results are consistent with a diffusion-limited ion entry (Andersen, Biophys. J. 41:147 [1983]).

T-Pos165 NON-IONIC DETERGENTS INCREASE THE FREQUENCY OF GRAMICIDIN MINI CHANNELS. D.B. Sawyer, O.S. Andersen, Dept. Physiol. Biophys., Cornell Univ. Med. Coll., New York, NY 10021, and R.E. Koeppe II, Dept. Chem. Biochem., Univ. Arkansas, Fayetteville, AR 72701.

Channels formed by the linear gramicidins have conductances that mostly fall within a quite narrow range. The gramicidins, however, also form less conductive "mini" channels. The relative frequency of mini channels formed by valine gramicidin A varies among laboratories, with a range of ~5% to ~50%, and the molecular origin(s) of mini channels remain(s) unknown (Busath et al., Biophys. J. 51:79 [1987]). Here we show that the mini frequency of channels formed by valine gramicidin A, B, and C can be increased, from ~5% to ~30%, by the addition of small amounts of the non-ionic detergent Triton X-100. Triton from two separate sources, of different purity, was used at a final nominal concentration of 8 μM (critical micellar concentration, ~3 mM). For both sources, the mini frequency was increased by the same factor. In addition, the conductance of channels in the main peak in the amplitude histograms was decreased by ~20%, and the average channel duration increased four-fold. Similar results were obtained with reduced Triton X-100, where the benzene ring has been replaced by a cyclohexane ring. The increase in mini frequency is not specific for the Tritons, as 200 μM β-octyl-glucoside induced comparable changes (critical micellar concentration, ~70 mM). Incubation of gramicidin A in 300 μM Triton X-100 overnight at room temperature did not affect the mini frequency, indicating that the increase in mini frequency cannot result from covalent modifications of the peptide. The results demonstrate that molecules that adsorb to the membrane/solution interface, and possibly perturb its structure, can have profound effects on channel behavior.

T-Pos166 A THEORETICAL STUDY OF THE ANTIPARALLEL DOUBLE STRANDED HELICAL DIMER OF GRANICIDIN AS A ION CHANNEL. Shen-Shu Sung and Peter C. Jordan, Dept. of Chemistry, Brandeis University, Waltham, MA 02254.

Recent experimental studies by Durkin et al. (Biophys. J. 1987, 51 451a) have suggested that the antiparallel double stranded helical (APDS) dimer of gramicidin can form a transmembrance cation channel. Crystallographic evidence indicates that this channel, when crystallized from alcoholic cesium electroytes, has the same length and pore diameter as that inferred for the head-to-head (HH) dimer (Koeppe et al., Nature 1979, 279, 723; Kimball et al, Ann. N. Y. Acad. Sci. 1984, 435, 551). The APDS appears to have much longer lifetime than, and to be essentially as cation selective as the more familiar electrically active form, HH. We have carried out theoretical studies designed to account for these observations. The calculations are based on the molecular Architecture deduced by Koeppe (personal communications) using his method of conformational analysis. Energy minimization and structural analyses were performed. The energy of an isolated APDS is higher than that of the HH; however the monomer-monomer interaction energy in the APDS is much greater than in the HH due to the formation of many more hydrogen bonds between the monomers. This is consistent with the observation of a much longer channel lifetime. As in the case of the HH, APDS exhibits a high energy barrier as anions approach the channel mouth, accounting for the observation of valence selectivity. The energy profiles of hydrated cations in the APDS show a larger pseudo-periodicity and higher intermediate energy barriers than do corresponding profiles in the HH, an observation consistent with suggestions that the APDS exhibits a lower single channel conductance. Interaction structures for hydrated cations in the APDS are illustrated and contrasted with those for the HH.

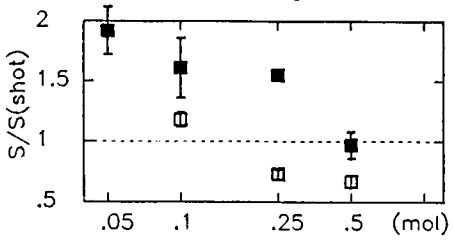
T-Pos167 ION DEPENDENCE OF CURRENT NOISE IN OPEN GRAMICIDIN A CHANNELS.

Stefan H. Heinemann and Frederick J. Sigworth, Dept. of Cellular and Molecular Physiology, Yale School of Medicine, New Haven CT 06510.

Ionic currents flowing through open Gramicidin A channels exhibit fluctuations about their mean value with a 'white' frequency characteristic up to 20 kHz as shown by Sigworth et al. (Biophys.J., December 1987). The measured spectral densities deviate from that expected for independent ion movement according to Schottkys formula, S=2*i*q, where i is the single-channel current and q the unitary charge.

In the figure the averaged spectral densities normalized to the expected shot-noise are shown for symmetrical solutions of KCl (open squares) and CsCl (filled squares) respectively and 200mV membrane potential. The permeation of Cs^+ produces a consistently higher current noise than the flow of K^+. This may give further insight into the mechanism of ion permeation.

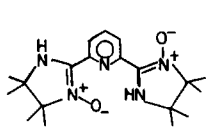
In addition we have to note that these measurements performed in Glycerol- Monooleate membranes with hexadecane as solvent yielded considerably lower excess noise levels than in Phosphatidecholine/decane membranes. A contribution of fluctuations in thick membranes with relaxation times higher than 20 KHz to the current noise can therefore so far not be excluded.



T-Pos168 MODELING OF ION COMPLEXATION AND MOLECULAR RECOGNITION VIA NOVEL TYPES OF MACRORINGS.

Valeria Balogh-Nair and Claude E. Brathwaite, Department of Chemistry, City College of New York, New York 10031.

The design and synthesis of novel types of structures which mimic biological recognition sites such as the ones which are found in ion transport, in receptor and enzyme interactions, in biological redox processes, and in recognition of DNA sequences important for biological control, is important because it permits study of the biological systems at the molecular level. We have synthesized prototypes of new classes of recognition sites, podand 1, and macrorings 2-5. All these crystalline materials were characterized by analysis, reactions and extensive spectroscopic studies. These compounds incorporate hitherto unexplored nitrone and oxaziridine moieties within the macrocyclic framework, to serve as tailored ligands for the development of new families of metal ion receptors. Photochemical properties, oxidizing ability, and complexing behavior of these new macrocycles as well as their relevance to molecular recognition in biological systems will be discussed. (Supported by PSC-CUNY Award 85/87 to V.B-N)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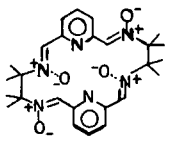
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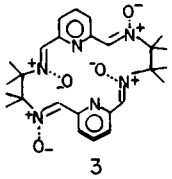
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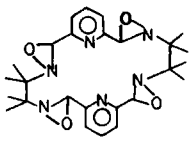
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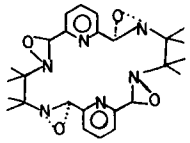
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T-Pos169 FREE DIFFUSION COEFFICIENT OF IONIC CALCIUM AND CHLORIDE IN CYTOPLASM. Brian S. Donahue and Ronald F. Abercrombie, Department of Physiology, Emory University School of Medicine, Atlanta, GA 30322. (Introduced by T. R. Nichols.)

The free diffusion coefficients of ionic Ca and Cl were measured in isolated samples of Myxicola axoplasm by following the migration of radioactive tracers. When sequestration and chelation of ^{45}Ca were minimized using inhibitors, energy deprivation, and saturation of Ca chelation sites, we measured a calcium diffusion coefficient of $5.3 \pm 1.5 \times 10^{-6}$ cm^2 s^{-1}. The diffusion coefficient was not appreciably changed by lowering free calcium from 100 µM to ≈10 µM, suggesting that the saturation of Ca chelation sites was unchanged over this range. Under the same conditions, we measured a chloride diffusion coefficient of $20 \pm 7 \times 10^{-6}$ cm^2 s^{-1}. The values of these parameters in dilute aqueous solution are 7.78×10^{-6} cm^2 s^{-1} for calcium, and 18.9×10^{-6} cm^2 s^{-1} for chloride (Wang, J. Am. Chem. Soc. 75:1769, 1953). When no inhibitors were used, the migration of Ca was more complex and could not be described by a single diffusion coefficient. In ten minutes, ^{45}Ca partitioned into the cylindrical tube of axoplasm from an adjacent drop of solution and spread evenly over a region 400 µm to 500 µm in depth. In contrast, only a small increase in free Ca could be detected with microelectrodes placed at a depth of 125 µm from the axoplasmic surface. A possible explanation for the distribution of ^{45}Ca in the untreated axoplasm could be bulk migration of Ca-sequestering organelles; however, Myxicola axoplasm contains few microtubules for axoplasmic transport. Supported by NIH NS19194.

T-Pos170 INITIAL CHARACTERIZATION OF ANION EXCHANGE IN PROMYELOCYTIC HL60 CELLS. D. Restrepo, D.J. Kozody and P.A. Knauf. Dept. of Biophysics, Univ of Rochester Med. Ctr., Rochester, NY 14642

In previous studies we found that Cl/HCO_3 exchange is at least partially responsible for maintaining intracellular pH above electrochemical equilibrium in HL60 cells and that it is involved in recovery of intracellular pH from alkaline loads. To learn more about the anion exchange system in these cells, we are studying the kinetics of Cl/Cl exchange. When HL60 cells are incubated in HCO_3 free media with 152mM Cl^- for one hour or longer, intracellular Cl increases from 35.7±1.8mM to 49.5±4mM. Under these conditions, tracer (^{36}Cl) efflux occurs at a rate of 68.2±5 mmols/(lt cell water x min). If chloride is removed from the medium, the rate of chloride efflux drops to 4±.2 mmolxlt^{-1}xmin^{-1}. The exchange component of Cl^- efflux follows Michaelian kinetics as a function of extracellular Cl^-. The K_m for Cl_o is 4.70±0.51mM and the maximum rate (J_{max}) 66.9±6 mmolxlt^{-1}xmin^{-1}. Both the K_m and the J_{max} decrease when intracellular chloride is lowered. The exchange system is inhibited by classical inhibitors of the red cell anion exchanger capnophorin (H_2DIDS, ethacrynic acid , furosemide and 3,5-diiodosalicylate). Cl/Cl exchange is sharply dependent on intracellular pH. Increasing intracellular pH from 6.5 to 8.0 increases the exchange rate by over 10 fold. This is consistent with the role of the anion exchanger in recovery form alkaline loads.

T-Pos171 REGULATION OF THE Na^+,K^+-ATPase AND GASTRIC H^+,K^+-ATPase BY THE CORRESPONDING ENDOGENOUS ACTIVATORS: ASPECTS OF SIMILARITIES AND DIFFERENCES. Ranjan Chakrabarti and Tushar K. Ray. Dept. of Surgery, SUNY Health Science Center, Syracuse, New York 13210.

We recently demonstrated the presence of cytosolic protein activators for the Na^+,K^+-ATPase and gastric H^+,K^+-ATPase. The endogenous activator (HAF) for the H^+,K^+-ATPase has recently been demonstrated to have the characteristics of an intracellular regulator (J.B.C. 262:5564-5670, 1987). The activator (NaAF) for the Na^+,K^+-ATPase, on the other hand, has been partially purified from the kidney and brain of different animals and characterized demonstrating its universal occurence (Fed. Proc. 46(3):261, 1987). In view of striking similarities between these cation transporters, studies on the similarites and differences between the HAF and NaAF appear important. Both activators are polymeric proteins, the HAF being a dimer of two 40 KDa subunits and the NaAF over 200 KDa. Both exist in soluble as well as membrane-bound forms. Studies on cross-activation revealed that while the HAF is equally effective in stimulating both the H^+,K^+-ATPase and Na^+,K^+-ATPase, the NaAF is specific for the latter. Unlike HAF effects on H^+,K^+-ATPase, the NaAF stimulates both basal and Na^+,K^+-stimulated portions of the ATPase. Both Va^{+3} and Ca^{+2} showed some interesting effects. Thus in both cases the activator-stimulated part was found to be relatively insensitive to Va^{+3} inhibition, but were obliterated by µM concentration of Ca^{+2}.

T-Pos172 DIRECT DETERMINATION OF THE DISSOCIATION CONSTANT FOR CALCIUM SENSITIVE INDICATORS
M.D. Yeager and G.W. Feigenson, Section of Biochemistry, Molecular and Cell Biology, Clark Hall, Cornell University, Ithaca, NY 14853

Calcium dissociation constants (Kd) for the "new generation" of calcium sensitive indicators (fura2, quin2, indol, Bapta, etc.) have been reported at only a few specified conditions, which often differ considerably from the experimental conditions where they are used. A spectrophotometric method is described which exploits the high extinction coefficients of the indicators and requires titrating the indicator with a calcium standard under the conditions of interest (pH, ionic composition, temperature), taking care that contaminating divalent cations have been removed from the buffer by storage over a Chelex bed. Absorbance changes at a sensitive wavelength are normalized by their isosbestic absorptions, and the resulting values are fit to a model describing a chelator with a 1:1 stoichiometry undergoing dilution by the titrant. Using bromo-Bapta to test the validity of the method, four parameters of the model are fit by non-linear least squares analysis: the concentration of the indicator in the cuvette, the normalized absorbance in the absence and in the presence of excess Ca, and the Kd. The values for all the fitted parameters agree with independently determined values. The method obviates the need for using laboriously prepared and calibrated EGTA-Ca buffers to set the free Ca concentration. The results for bromo-Bapta show a Kd insensitive to pH over the range 7.0-7.5 as expected, but a significant sensitivity to temperature, ionic strength, and the type of buffer used.

T-Pos173 Subcellular localization of G-protein and phospholipase c activity in rabbit skeletal muscle. G. Salviati, R. Betto, V. Tegazzin and A. Della Puppa. (Introduced by Roger Sabbadini, San Diego CNR-C.S. Biologia e la Fisiopatologia Muscolare, Istituto di Patologia Generale, Universita' di Padova, Padova, Italy.

Evidence from several laboratories has implicated a role for a regulatory guanine-nucleotide binding protein (G-protein) in transducing the effect of hormones on phospholipase c mediated breakdown of phosphoinositides. We have studied the sub-cellular distribution of G-protein and phospholipase c activity in rabbit fast-twitch skeletal muscle. A total membrane fraction and a soluble fraction were obtained by centrifuging a 6.500g supernatant from muscle homogenate at 150.000g for 60 min. Purified vesicles preparations from sarcoplasmic reticulum (SR) longitudinal tubules, SR terminal cisternae, and enriched (50%) preparations from transverse tubules (t-tubules) were obtained according to Saito et al. (J. Cell Biol. 99: 875-885, 1984). G-proteins were identified by ADP-ribosylation with 32P-NAD using pertussis toxin and autoradiography after SDS-Page. Phospholipase c activity was measured by following the hydrolysis of 3H-phosphatidylinositol 4,5 bisphosphate.Pertussis toxin labelled a protein band of 39kDa both in soluble and total membrane fractions. Labelling was most intense in the t-tubule enriched fraction. By counting the radioactivity in the band cut out from the gel, the amount of G-protein in the t-tubules enriched fraction was estimated to be about 2.0 fmoles per mg membrane protein. Similar resultes were obtained in pig skeletal muscle. Phospholipase c activity was found to be present both in soluble and total membrane fractions. The membrane activity was not extracted by 0.6M KCl. The activity was stimulated by 0.1uM Ca2+ and 10mM NaF. Most of the membrane bound activity was recovered in the t-tubule enriched membrane fraction. These results indicate that skeletal muscle contain both soluble and membrane-bound G-proteins and phospholipase c activity and that most of the membrane-bound proteins are localized in the t-tubule membrane. Supported by institutional funds from the CNR and by grant from MDA.

T-Pos174 MECHANISM OF CALCIUM/CALMODULIN REGULATION OF CARDIAC ADENYLATE CYCLASE.
Joseph A. Oibo and Robert A. Colvin, Dept. of Pharmacology,Oral Roberts Univ. School of Medicine, Tulsa, OK 74137

Adenylate cyclase activity was assayed in the presence of the G-protein dependent activators GTP, Gpp(NH)p,or NaF.Calcium inhibition curves with each activator showed a high affinity site (IC_{50}= $3.0x10^{-7}$M) and a low affinity site ($IC_{50}>10^{-4}$M).When vesicles were calmodulin depleted,calcium inhibition curves demonstrated only the low affinity site ($>10^{-4}$M),and showed increased adenylate cyclase activities at all concentrations of calcium tested. Addition of exogenous calmodulin (1μm) to calmodulin depleted vesicles reversed the effects of calmodulin depletion on both the high affinity site and the adenylate cyclase activity.Free Mg^{2+} activated adenylate cyclase in the presense of 10^{-4}M Gpp(NH)p.The concentration for half maximal effect was 2mM.Calcium at $3x10^{-6}$M had no effect on Mg affinity but did inhibit maximal activity by 30%,while 10^{-4}M calcium decreased the apparent Mg^{2+} affinity twofold and inhibited maximal activity by 52%.In the presence of 10mM Mg^{2+} Gpp(NH)p activated adenylate cyclase with a half maximal concentration of $5x10^{-7}$M.There was no effect of $3x10^{-6}$M or 10^{-4} calcium on Gpp(NH)p affinity but maximal activity was inhibited 51% and 54%,respectively.[^{3}H]-GTP binding was complete and reached equilibrium after 10 min.Scatchard analysis gave binding constants of B_{max}=85.9 pMole/mg and K_d=183nM.A slow rate of [^{3}H]-GDP release (.009 min^{-1}) was obtained in the absence of quanine nucleotide,addition of 100μM Gpp(NH)p increased the rate to .03 min^{-1}.Calcium had no effect on maximum [^{3}H]-GTP binding or rate of release.The results show that calcium/calmodulin dependent inhibition of adenylate cyclase,although dependent on the presence of guanine nucleotides or NaF,is not a result of changes in the kinetics of Gs activation or deactivation.

T-Pos175 PHYSIOLOGICAL LOCALIZATION OF A RECEPTOR-SENSITIVE Ca POOL IN A SECRETORY CELL.
J.K. Foskett, P. Gunter-Smith, J.E. Melvin, J.R. Turner, Physiology Dept., Armed Forces Radiobiology Research Institute, and National Dental Institute, NIH, Bethesda, MD 20814.

Cholinergic stimulation of fluid secretion by rat parotid salivary acinar cells is associated with elevated intracellular Ca (Ca_i) due to mobilization of intracellular stores and Ca influx across the plasma membrane. To determine whether receptor-induced increases in Ca_i stimulate maxi Ca-activated K-channels (observed in patch-clamp studies) in intact cells under physiologically relevant conditions, we have measured simultaneously Ca_i and membrane voltage in single acinar cells with high temporal resolution. Fura-2 loaded cells were perfused on the stage of a microscope (37°C). Ca_i was determined at 15 Hz by ratioing photomultiplier (PMT)-detected flourescence emmisions from 350 and 380 nm excitations. Carbachol caused rapid (complete <1 sec) elevations of Ca_i and 10-40 mV hyperpolarizations. The earliest changes in Ca_i usually lagged by 66 msec the earliest detectable hyperpolarizations, in both Ca-replete and 0-Ca media. The data indicate that a) Ca_i is not the activator of the K-channels or b) there is a region of elevated Ca_i near the plasma membrane which cannot be optically detected. To test (a), Ca_i was buffered with Bapta and cells were stimulated in 0-Ca media. This completely blocked the Ca_i elevation and changes in membrane potential, supporting Ca as the physiological messenger. Bapta-buffered cells stimulated in Ca-replete media exhibit hyperpolarizations of normal size with slower activation kinetics which precede detectable increases in Ca_i by >500 msec. Thus it is possible for the plasma membrane to sense a region of elevated Ca which is not detected by the PMT, supporting alternative (b). The data indicate that receptor-mobilized intracellular stores of Ca are localized in close proximity to the basolateral membrane in these cells.

T-Pos176 PROPERTIES OF SINGLE CELL-TO-CELL CHANNELS IN PANCREATIC ACINAR CELLS
R. Somogyi & H.-A. Kolb. University of Konstanz, Faculty of Biology, D-7750 Konstanz, F.R.G.

The properties of single gap-junctional channels of electrically coupled cell pairs were studied using the double whole cell patch-clamp technique. Acinar cell pairs were isolated from the mouse pancreas by collagenase treatment. The initial electrical coupling corresponds to the simultaneous opening of 400-600 cell-to-cell channels of 130 pS. After about 15-20 min, the cells uncouple spontaneously and conductance steps of 130 pS occasionally become visible. The spontaneous uncoupling can be suppressed by use of cAMP and ATP containing pipette filling solutions. Addition of 0.4 mM octanol or 0.3 mM benzhydrol to the bath medium induces a rapid electrical uncoupling. The effect of these alcohols can be washed out. Amplitude histograms of the current fluctuations during the time course of uncoupling yield several conductance levels. Besides the 130 pS level, those of 70-80 pS and 30 pS could be identified. Different conductance levels appear simultaneously in the corresponding records of the junctional current. The analysis gives evidence for conducting sublevels of cell-to-cell channels.

T-Pos177 A NOVEL CYTOLYTIC ACTIVITY OF DIPHTHERIA TOXIN. Michael P. Chang, John Bramhall, Scott Graves, Benjamin Bonavida and Bernadine J. Wisnieski. Dept. of Microbiology and Dept. of Microbiology and Immunology, University of California, Los Angeles, CA 90024

The cytotoxic action of diphtheria toxin (DTx) is well known for its potent ability to inhibit protein synthesis. Here, we report evidence for a cytolytic pathway that is independent of protein synthesis inhibition. DTx-mediated cytolysis was measured through marker release assays; high molecular weight markers were detected within 10 hours. In contrast, inhibition of protein synthesis by cycloheximide, by metabolic poisons such as sodium azide/2-deoxyglucose, or by incubation in medium deficient in specific amino acids did not result in target cell lysis over a period of up to 20-30 hours. The lytic property of DTx could be a direct effect of the toxin on the boundary membranes of target cells (pore formation) or the consequence of enzymic activity (transfer of ADP-ribose moieties to target substrates) within the cells. We therefore examined the ability of CRM 197 (a mutant form of DTx that lacks enzymic activity) to mediate marker release. CRM 197 failed to lyse cells despite its capacity to form pores. The fact that target cell lysis triggered by DTx appears to be independent of direct pore formation suggests a novel autolytic mechanism which involves the activation of endogenous enzymes. Supported by NIH GM22240, CA35791, CA43121, CA9120 and USPHS/NRSA CA09056.

T-Pos178 LYOTROPIC ANIONS AND CHARGE MOVEMENT IN FROG SKELETAL MUSCLE. D. Garcia-Diaz and J.A. Sanchez. Department of Pharmacology, CINVESTAV, Mexico City, A.P. 14-740, 07000, Mexico.
Lyotropic anions are well known potentiators of contraction in skeletal muscle (Hodgkin & Horowicz, J. Physiol. (1960) 153:404-412). Our experiments examined the role of these anions on charge movement. Methods: The triple vaseline gap voltage clamp technique for cut fibers was used (Hille & Campbell, J. G. P. (1976) 67:265-293). Membrane currents were filtered at 5 kHz and sampled at 12 bits every 250 μs. Solutions (mM). External: Mg^{2+}=10, TEA=108, TTX=0.1, pH=7.2 and $CH_3SO_3^-$ as anion. Internal: TEA_2EGTA=20, Cs glutamate=89, Na_2ATP=2, MOPS=4, pH=7.1, T=15°C. Holding potential (Eh)-90, -100 mV. Linear components were subtracted out by scaling a -20 mV step from Eh -100 mV or by scaling a +80 mV from Eh=0 mV. Results: Membrane capacitance was constant from -160 to -65 mV and averaged 7.3±0.3(3) μF/cm^2. In control experiments the fitting of a Boltzmann function to the ON-charge (-20 mV control pulse) was: Qmax=23.4±0.3 nC/μF, Em 1/2=-19.7±3.1 mV, km=7.0±0.3 mV(7). In the same experiments, a 80 mV control pulse from Eh=0 mV resulted in a 57% increase in Qmax. SCN^- (12 mM) in the External solution shifted the Q_{ON} towards more negative potentials (-20 mV control pulse): Qmax=21.6±0.4 nC/μF, Em 1/2= -30.7±3.6 mV, km=7.0±0.5 mV(5). SCN^- does not produce simple shifts since pronounced slowing of OFF-kinetics was apparent. SCN^- (12 mM) in the Internal solution also produces similar effects: Qmax=22.0±0.5 mC/μF, Em 1/2=-29.8±3.6 mV, km=6.9±0.5 mV(4). Control pulses of +80mV (Eh=0) gave qualitatively similar results, i.e. shifts in the negative direction were always present and slowing of OFF-kinetics was observed. Supported by CONACyT.

T-Pos179 SILVER-INDUCED CALCIUM RELEASE FROM CARDIAC SARCOPLASMIC RETICULUM VESICLES: INHIBITION OF Ca^{2+}/K^{+}-ATPase. J.A.WISLER and H.R.BESCH,Jr. Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, Indiana 46223.

Recent reports suggest that Ag^{+} interacts with the Ca^{2+} release channel of skeletal sarcoplasmic reticulum (SR) membrane vesicles (MV) rather than directly with the Ca^{2+}/K^{+}-ATPase (CKA) pump. The CKA of skeletal MV, however, is a target for Ag^{+}: low (uM) concentrations of Ag^{+} have been reported to stimulate CKA whereas high concentrations inhibit it. In canine cardiac MV and subfractions, we report nanomolar concentrations of Ag^{+} as the threshold for inhibition of CKA and no stimulation at any concentration. CKA inhibition by Ag^{+} was unchanged in the additional presence of the Ca^{2+} ionophore A23187. Inhibition of basal Mg^{+}-ATPase did not occur. Na^{+}/K^{+}-ATPase (NKA) of a more highly purified sarcolemmal preparation was also inhibited by Ag^{+} with an approximately equivalent K_i to that of CKA in MV. The concentration-effect curve describing calcium efflux from the cardiac MV paralleled that for the inhibition of CKA. Ryanodine failed to alter Ag^{+} induced Ca^{2+} efflux from cardiac MV regardless of the state of Ca^{2+} load of the vesicles or order of addition of ryanodine or Ag^{+}. These data suggest that the effect of Ag^{+} ions on calcium efflux from canine cardiac MV probably lies with its inhibition of the CKA pump rather than the SR calcium efflux channel. [Supported in part by the Showalter Trust].

T-Pos180 PERMEABILITY CHARACTERISTICS OF CARDIAC SARCOPLASMIC RETICULUM WITH DETERGENT AND IONOPHORE. C. Tate, S. Blaylock, K. Youker, R. Bick, and M. Entman. Section of Cardiovascular Sciences, Baylor College of Medicine, Houston, Texas 77030.

At concentrations where solubilization does not occur, detergents alter the permeability of the SR. The rate of net oxalate-supported, ATP-dependent Ca^{2+} uptake is abolished when a low concentration of detergent is included. In order to examine the detergent-activated permeability of cardiac SR, the nonionic detergent, $C_{12}E_8$, was added well below the CMC of 50 µg/ml (10 µg $C_{12}E_8$/ml to 20 µg cardiac SR/ml). When added before starting the reaction, net Ca^{2+} uptake was obliterated; CaATPase activity (V_{max}) was stimulated by 50%. The Km of the low affinity Ca^{2+} site(s) was not affected by $C_{12}E_8$. These effects mimicked A23187, suggesting that the detergent stimulation of CaATPase activity resulted from reduced intravesicular Ca^{2+}. When $C_{12}E_8$ was added after Ca^{2+} uptake reached equilibrium, however, only 50% of the accumulated Ca^{2+} was released, whereas nearly 100% was released with A23187. Yet the stimulation of CaATPase activity remained at 50% when $C_{12}E_8$, A23187, or both were added to the reaction. Permeability of K^{+} was unaltered under identical conditions, suggesting a selective permeability increase to Ca^{2+}. Vanadate (10 µM) inhibited CaATPase activity by 50% in the presence and absence of $C_{12}E_8$ and by 100% only in the presence of A23187, indicating that vanadate sensitivity is rendered by a pool of Ca^{2+} which is released only to A23187 in "tight" vesicles. Thus, there may be two classes of low affinity Ca^{2+} binding sites, only one of which is affected by $C_{12}E_8$ or A23187 while both are modulated by A23187. The one class held in common may regulate net Ca^{2+} flux.

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T-Pos181 HALOTHANE AFFECTS THE CONTRACTILE APPARATUS AND SARCOPLASMIC RETICULUM OF MECHANICALLY SKINNED RAT VENTRICULAR FIBERS. JS Herland, DG Stephenson*, and FJ Julian, Dept. of Anesthesia Research Laboratories, Brigham & Women's Hospital, Boston, MA, 02115 and *Dept. of Zoology, LaTrobe University, Bundoora, Victoria 3085, Australia.

Mechanically skinned cardiac preparations were obtained from the left ventricle of female rats by mild homogenization. Contractility was measured over the range 1-20 µM $[Ca^{2+}]$. Sarcoplasmic reticulum (SR) function was investigated by loading for 10 minutes in 1-2 µM weakly buffered $[Ca^{2+}]$. The area under the force transient produced by exposure to 30 mM caffeine was used to indicate the amount of releasable Ca^{2+}. Maximum force in saturating $[Ca^{2+}]$ was reversibly decreased by 30-50% in 10 mM halothane and by 0-5% in 2 mM halothane. 50% relative force was reversibly shifted to lower $[Ca^{2+}]$ by .12 pCa in 2 mM halothane and by .26 pCa in 10 mM halothane. SR loading in 2 mM halothane markedly and reversibly diminished the amount of Ca^{2+} released by caffeine. Following a standard loading procedure, substitution of 2 mM halothane as a releasing agent caused an immediate force transient 50-60% as large as that produced by caffeine. Addition of up to 100 µM ruthenium red, a known Ca^{2+} efflux channel blocker, to either the loading or releasing solution or both had no effect upon halothane's reduction of Ca^{2+} loading nor upon halothane-induced Ca^{2+} release. The results suggest that: 1) halothane increases contractile apparatus sensitivity to $[Ca^{2+}]$ in a dose-dependent manner; 2) significant SR effects occur at a lower level of halothane than do contractile apparatus effects; 3) halothane itself is a releasing agent of SR Ca^{2+}, though not as effective as caffeine; 4) it is unlikely that halothane's releasing action can be explained by a stimulation of Ca^{2+} release via Ca^{2+} efflux channels. This work supported by NIH Grant HL35032 (FJJ).

T-Pos182 ABNORMAL RYANODINE RECEPTOR PROPERTIES CORRELATE WITH ALTERED CALCIUM RELEASE OF SARCOPLASMIC RETICULUM FROM MALIGNANT HYPERTHERMIA SUSCEPTIBLE PIGS. James R. Mickelson, Kay M. Johnson, Lynn A. Litterer and Charles F. Louis. Department of Veterinary Biology, University of Minnesota, St. Paul, MN 55108.

It has previously been shown that in the skeletal muscle disorder malignant hyperthermia (MH) an abnormality exists in the regulation of SR Ca release. Because of the likely association of the ryanodine receptor with the SR Ca release channel, we have examined various aspects of the binding of [^{3}H]ryanodine (Ry) to heavy SR isolated from MH susceptible (MHS) and normal (N) pigs. The Ca^{2+} dependence of Ry binding was determined at 10, 30, 100, 300, and 1000 nM Ry. At all 5 Ry conc. the Ca^{2+} dependence followed a bell-shaped curve, with the optimal binding of Ry in the range 3 - 10 µM Ca^{2+} for both MHS and N SR. The Ry binding to MHS SR was significantly greater than to N SR at all Ry conc., but the magnitude of the difference diminished as the Ry conc. increased. Hill plots demonstrated that the $Ca_{0.5}$ for activation of Ry binding to both MHS and N SR decreased from 0.6 - 0.8 µM at 10 nM Ry, to 0.2 µM at 1000 nM Ry. The $Ca_{0.5}$ for the inhibition of Ry binding increased from 15 - 30 µM at 10 nM Ry, to 100 - 400 µM at 1000 nM Ry. Interestingly, between 30 and 1000 nM Ry, the $Ca_{0.5}$ required for inhibition of Ry binding to MHS SR was significantly greater than for N SR. Furthermore, Scatchard plots indicated that at 6 µM Ca^{2+}, for MHS and N SR respectively, the K_d of the Ry receptor was 110 vs 590 nM, while the B_{max} was 14.0 vs. 11.4 pmoles/mg. These results demonstrate that the abnormal release of Ca seen in MHS SR may be attributable to a defect in the Ry receptor/Ca release channel complex. Supported by NIH GM-31382.

T-Pos183 ABNORMALITIES IN CALCIUM REGULATION OF SKELETAL MUSCLE TRANSVERSE TUBULE VESICLES ISOLATED FROM MALIGNANT HYPERTHERMIA SUSCEPTIBLE SWINE. J.M. Ervasti, M.T. Claessens, J.R. Mickelson and C.F. Louis, Dept. of Veterinary Biology, University of Minnesota, St. Paul, MN 55108

The primary defect in malignant hyperthermia (MH) is considered to be an abnormality in the regulation of skeletal muscle sarcoplasmic Ca. We have recently reported alterations in Ca transport by MH-susceptible (MHS) sarcolemma vesicles (Mickelson et al., 1987; Biochim. Biophys. Acta 897, 364) suggesting transverse tubule (TT) Ca regulation may also be abnormal. MHS and normal (N) TT did not differ in the content of cholesterol (1.08 µmole/mg) or phospholipid (2.12 µmole Pi/mg), maximal ouabain binding (100 pmol/mg at 37°C), maximal (Na^++K^+)-ATPase activity (31 µmole Pi/mg/hr at 37°C), or Ca-ATPase activity (0.067 µmole Pi/mg/min at 25°C). ATP-dependent Ca transport by N and MHS vesicles had a similar dependence on Ca^{2+} concentration ($K_{½}$ for Ca^{2+} of 0.21-0.25 µM), as well as a similar Vmax (21-24 nmole Ca/mg/min at 37°C). Calmodulin and cAMP-dependent protein kinase stimulated Ca uptake to the same extent in N and MHS TT. However, maximal accumulation by MHS TT was significantly less than by N TT (90±10 vs. 130±9 nmole Ca/mg after 15 minutes). Ca transport in N and MHS TT were similarly inhibited by halothane concentrations greater than 2 mM. Of the other agents examined (dantrolene, nitrendipine, Bay K8644) only Bay K8644 (1 µM) had any effect on active Ca transport; a small (10%) but significant inhibition was observed only in MH TT. These differences were not due to differences in the passive permeability of N and MHS vesicles. Interestingly, maximal ^{3}H-nitrendipine binding in MHS TT was only 63% of that observed in N TT (B_{max}=26±5 vs 41±4 pmole/mg) while binding affinity was not significantly different (K_D=3 nM). Our results suggest that a defect in TT Ca regulation may contribute to the abnormal sarcoplasmic Ca homeostasis in MH skeletal muscle. Supported by NIH grant GM 31382.

T-Pos184 **EFFECT OF [Mg^{2+}] ON THE Ca^{2+} UPTAKE KINETICS AND STRUCTURE OF THE SARCOPLASMIC RETICULUM MEMBRANE.** **F.J. Asturias, J.K. Blasie, Dept. of Chemistry, U. of Pennsylvania, Phila. PA 19104**

Direct measurements of Ca^{2+}ATPase phosphorylation have shown that the lifetime of the first phosphorylated intermediate in the Ca^{2+} transport cycle, E_1~P increases with decreasing [Mg^{2+}] (Y.Dupont, Eur.J.Biochem. **109**, 231 (1980)). Earlier studies of Ca^{2+} uptake kinetics at high [Mg^{2+}] (8mM) identified the "fast" phase of the uptake with Ca^{2+} occlusion and E_1~P formation, and the ionophore sensitive "slow" phase with Ca^{2+} translocation across the membrane. The present work at low [Mg^{2+}] (<20µM) shows a slowing in the kinetics of both phases and particularly an increase in the duration of the plateau of the fast phase before the onset of the slow phase, indicating an increase in the lifetime or "transient trapping" of E_1~P. Calcium uptake kinetics at low [Mg^{2+}] and moderately low temperature (~0°C) are similar to those observed at much lower temperatures (~-10°C) at high [Mg^{2+}]. Previous X-ray diffraction work (D. Pascolini, et.al. Biophys. J. 51, 347a (1987)) indicated that changes in the Ca^{2+}ATPase profile structure were responsible for E_1~P transient trapping, at temperatures below the upper characteristic temperature t_h for lipid lateral phase separation in the membrane. The structural changes that we observed at low [Mg^{2+}] are analogous, but they are much more pronouced and are referenced to a higher t_h, i.e., ~8°C instead of ~2°C. Both kinetic and structural data suggest that the behavior of the system can be best described in terms of a "reduced temperature", T/t_h. Lipid phase behavior and conformational changes in the protein seem to be closely related, and our studies suggest that the effect of [Mg^{2+}] on E_1~P lifetime is structural, and not due to simple chemical effects in the E·metal·ATP complex. Supported by NIH HL-18708.

T-Pos185 TRYPTIC PROTEOLYSIS STIMULATES THE CALCIUM CHANNELS OF SARCOPLASMIC RETICULUM
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We have investigated the effects of limited tryptic proteolysis on the Ca2+ permeability of the isolated SR vesicles. For proteolysis carried out for 10 minutes at room temperature at a trypsin: SR protein ratio of .005 (TMSR), with 1 mM free Mg2+ we observe 1). Passive Ca2+ efflux from TMSR vesicles is 20x faster than from native SR in the absence and presence of cAMP and doxorubicin. 2). Ruthenium red (Ru red) inhibits Ca2+ efflux from TMSR. 3). The Ca2+ uptake capacity of TMSR is less than native SR but is restored in the presence of Ru red. 4). The binding affinities and Hill coefficients of Ca2+ efflux activation by cAMP and doxorubicin are the same in TMSR as in native SR. 5). Proteolysis stimulates efflux from both light SR and heavy SR, though heavy SR is inhibited by Ru red while light SR is not. 6) Hg2+-activated Ca2+ efflux is only weakly enhanced by proteolysis.

Though the rates are greatly stimulated by proteolysis, 7). with .1 mM Mg2+, the Ca2+ dependence of cAMP-activated Ca2+ efflux from TMSR is similar to that of native SR, with peak rates at 1 to 10 uM Ca2+ and 8). the Mg2+ dependence of Ca2+ efflux from TMSR is similar to that of native SR. 9). Under conditions promoting maximal channel open time (i.e. no Mg2+, 5mM ATP, 5 uM Ca2+), the rate constant of Ca2+ efflux from TMSR is not significantly different from that of native SR.

The above data suggest that tryptic proteolysis strongly affects the gating of the SR's Ca2+ channels. Supported by AHA, Oregon and Western PA affiliates of AHA. J.A. is an Established Investigator of AHA, G.S. is supported by RCDANS00909 and J.T. is a Postdoctoral Fellow of the Oregon affiliate of AHA.

T-Pos186 ISOLATION AND RECONSTITUTION OF THE RYANODINE RECEPTOR FROM CARDIAC SARCOPLASMIC RETICULUM. KRISTIN ANDERSON, F. ANTHONY LAI, QI-YI LIU, ERIC ROUSSEAU, HAROLD ERICKSON*, AND GERHARD MEISSNER. Department of Biochemistry, University of North Carolina, Chapel Hill, NC 27599 and *Department of Anatomy, Duke University Medical Center, Durham, NC 27710.

Ryanodine is a plant alkaloid which binds specifically and with high affinity to the Ca^{2+} release channel of skeletal and cardiac muscle sarcoplasmic reticulum (SR). Using [^{3}H]ryanodine as a channel marker, a~30S complex has been isolated by solubilization of canine cardiac SR membranes in 1 M NaCl and CHAPS or digitonin, followed by centrifugation through a linear sucrose gradient. Ryanodine binding was found to be stabilized by phospholipid and micromolar Ca^{2+}. The ~30S peak of ryanodine binding activity contained a single polypeptide of apparent relative molecular mass (M_r) 350,000, as indicated by SDS PAGE. A 100-fold enrichment of specific [^{3}H]ryanodine binding over the initial SR membranes was achieved. The maximum specific activity observed was 600 pmole ryanodine bound per mg of protein. This value corresponds to a stoichiometry of one binding site per four M_r 350,000 subunits. Negative stain electron microscopy of the ryanodine peak fractions revealed tetrafoil structures with ~35 nm diameter. The purified receptor fused into planar lipid bilayers exhibited a unit Na^+ conductance of 70 pS and a Ca^{2+} conductance of 50 pS. Subconductance states were typically observed. This suggests the ~30S ryanodine receptor complex from cardiac SR is able to form a cation selective channel.

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T-Pos187 RAPID COOLING CONTRACTURES IN ISOLATED RABBIT VENTRICULAR MYOCYTES, by Larry V. Hryshko and Donald M. Bers, Division of Biomedical Sciences, University of California, Riverside, CA 92521. Rapid cooling contractures (RCC) in cardiac tissue can be used as a relative index of the calcium available for contraction from the sarcoplasmic reticulum (SR) (Bridge, J. Gen. Physiol, 88, 437-473, 1986). We have examined the effect of rapid cooling in rabbit ventricular myocytes. Superfusate temperature was changed from 30°C to 5°C in less than 1 second. If RCCs were invoked immediately following a cessation of stimulation (0.5Hz), shortening occurred within 2 seconds and typically reached plateau in less than 5 seconds. RCCs produced shortenings from 10-30% of resting myocyte length, whereas electrically evoked twitches resulted in less than 10% shortening. In contrast, RCCs in rabbit ventricular tissue develop tension over 20-30 seconds and only reach 30-50% of twitch force. Upon rewarming, both preparations exhibit an additional transient shortening (rewarming spike) prior to relaxation. When RCCs are invoked following increasing periods of rest (2-300 secs), a progressive decrease in magnitude is observed (decay $t_{\frac{1}{2}}$ = 90 secs). The rest decay of twitch shortening in myocytes ($t_{\frac{1}{2}}$ = 75 secs) is similar to that reported for intact tissue (Bers et al. Can. J. Physiol. Pharmacol, 65, 610-618, 1987) suggesting that diastolic calcium fluxes are unchanged. This suggests that the rest decay of force production is due to loss of SR Ca during diastole. These data reveal that RCCs occur very much faster in myocytes than in multicellular preparations. Therefore, suggested mechanisms for the onset of RCCs must account for the rapidity with which this phenomenon occurs.

T-Pos188 IDENTIFICATION OF THE HIGH AFFINITY CALCIUM BINDING PROTEIN IN MUSCLE AND NON-MUSCLE TISSUES. L. Fliegel, M. Opas*, M. Michalak. (Intr. by L.B. Smillie) Dept. of Pediatrics, Univ. of Alberta, Edmonton, Alberta; *Dept. of Anatomy, Univ. of Toronto, Toronto, Canada

The high affinity calcium binding protein (HACBP) is a 55-kDa calcium binding protein initially identified and purified from skeletal muscle sarcoplasmic reticulum membranes. We investigated the presence of proteins homologous to the HACBP in cardiac and smooth muscle and in non-muscle cells. Using a goat anti-rabbit HACBP polyclonal antibody, we identified immunoreactive HACBP-like proteins of 55-kDa in bovine heart and in rabbit uterine muscle. These proteins were partially purified from muscle homogenates and stained blue with the cationic carbocyanine dye Stains-all, confirming their Ca binding properties. The HACBP was not immunoreactive with polyclonal antibodies against skeletal muscle or cardiac muscle calsequestrin and polyclonal antibodies against the skeletal muscle HACBP did not cross react with calsequestrin. These results indicate that despite their common properties and localization, HACBP and calsequestrin are distinct proteins. Uterine muscle microsomes and homogenates in addition to the HACBP also contain a distinct calsequestrin-like protein which was immunoreactive with polyclonal antibodies against cardiac calsequestrin, but did not react with antibodies against skeletal muscle calsequestrin. The HACBP was also identified in non-muscle cells. Using immunoblotting and immunofluorescence microscopy, the HACBP was found to be associated with endoplasmic reticulum membranes in human fibroblasts and retinal pigmented epithelial cells.

In conclusion, our results indicated that HACBP may be a membrane associated Ca binding protein common for both muscle and non-muscle cells.

T-Pos189 NOVEL Cl CHANNELS FROM SKELETAL MUSCLE SARCOPLASMIC RETICULUM RECORDED IN NATIVE MEMBRANE. G.D. Hals and P.T. Palade, Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550.

Skeletal muscle sarcoplasmic reticulum (SR) is known to be permeable to anions, but the single channel properties of the anion channel(s) remain largely unexplored. Using the "sarcoball" preparation presented elsewhere at this meeting (Stein & Palade), we are able to record SR Cl channels in native internal membrane of skinned frog semitendinosus muscle.

The Cl channels have a high conductance, with a slope conductance of 505 ± 25 pS (n=35) for the predominant state in symmetrical 200 mM TrisCl, and are observed in nearly every patch. The slope conductance vs. Cl^- ion concentration saturates at 620 pS, with a K_m=60 mM. The steady-state open probability vs. holding potential relationship produces a slightly asymmetric bell-shaped curve, with P_o values reaching a maximum near 1.0 at 0 mV, and falling off to 0.05 by -30 mV or by +40 to +50 mV. Preliminary data, obtained by reversal potential shifts, gives the following selectivity sequence: Cl^-, HCO_3^- (1.0) > SO_4^{2-} (0.40) > K^+ (0.05). The channels are still fairly selective for Cl^- over K^+ ions, despite their high conductance. Both SO_4^{2-} and phosphate ions produce large conductances (250 to 300 pS), but events are much more brief than with Cl^- as the charge carrier. In addition, gluconate currents are unresolvable. These properties are very unlike those described for mitochondrial VDAC or T-tubule Cl channels. Further, since SR Ca channels are found in great abundance in sarcoball patches, and we have observed similar Cl channels in isolated SR (Hals & Palade, Neurosci. Abs., 13:96, 1987), we conclude these sarcoball Cl channels originate from SR. Supported by PHS 1 PO1 HL37044.

T-Pos190 DIRECT MEASUREMENT OF Mg.ATP AFFINITY FOR SARCOPLASMIC RETICULUM Ca-ATPase, IN THE PRESENCE OR IN THE ABSENCE OF CALCIUM.

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In the presence of calcium, Mg.ATP reacts rapidly with the Sarcoplasmic Reticulum Ca-ATPase to form a covalent phosphoenzyme. Using both rapid filtration technique (Biologic system) and multimixing quench flow, we have directly measured association and dissociation rate constants for Mg.ATP (respectively 3.5 10+6 M-1s-1 and 9 s-1, at pH 7.0, 5oC and 5 mM MgCl2, in the absence of potassium). These measurements lead to the true dissociation constant of Mg.ATP for the E1 conformation (Kd = 2.6 10-6 M).

In the absence of calcium, using similar kinetic approach, we have measured association and dissociation rate constants for Mg.ATP (respectively 7.5 10+6 M-1s-1 and 25 s-1). The dissociation constant deduced from kinetic measurements (Kd = 3.3 10-6 M) is in good agreement with that obtained from equilibrium measurements with radiaoactive ATP.

Comparison of the rates of binding of Mg.ATP and calcium on a Ca-deprived enzyme (E2) showed that Mg.ATP binds much faster than Ca. As it is generally accepted that the slow calcium binding represents the E2 to E1 transconformation, Mg.ATP binds to the E2 species. Therefore, in the absence of calcium, Mg.ATP binds to the E2 conformation without inducing the E2 to E1 transconformation.

These results obtained in the presence and in the absence of calcium demonstrate that the E1 and E2 conformations have similar affinities for Mg.ATP.

T-Pos191 THE EFFECT OF LIGAND CONDITIONS ON THE THERMODYNAMIC STABILITY OF THE Ca, Mg-ATPase OF CARDIAC AND SKELETAL SARCOPLASMIC RETICULUM. Barry S. Lutzke, Uta M. Rykert, and Frederic Mandel, The Upjohn Company, Kalamazoo, MI 49001.

It is generally accepted that sarcoplasmic reticulum(SR) removes and sequesters calcium from the contractile apparatus of both cardiac and skeletal muscle at a rate and amount consistent with muscle relaxation. The translocation of calcium from the outside of the SR into the SR lumen is accomplished by a Ca,Mg-ATPase present in the SR membrane. It is known that different ligand conditions cause that ATPase to assume different conformational states corresponding to the various ligands present. In the present study, we have examined the effects of varying ligand conditions on the stability of the Ca,Mg-ATPase using heat denaturation as a probe of thermodynamic stability. Rabbit skeletal SR (RSSR) canine cardiac SR (DCSR), and purified canine cardiac Ca,Mg-ATPase(P-DCSR) were kept at 37°C in the presence of various ligands and the loss of activity as a function of time examined. The ligand conditions studied were Ca (2μM, 1mM and 10mM), Mg(0.2mM and 3mM), ATP(10uM and 1mM), ADP(0.5mM) and absence of ligands. We found RSSR to be more stable than DCSR under all conditions studied. However, DCSR is more sensitive to changing ligand conditions than RSSR i.e. certain ligands are capable of stabilizing DCSR to a greater extent than RSSR. P-DCSR was more stable than either DCSR or RSSR. Other results indicate that calcium stabilizes DCSR in a dose dependent manner over a large concentration range (500nM to 10mM calcium). With calcium, the activity decays biphasically with an initial fast activity loss followed by a long slow decay. Other ligands such as ATP, ADP, and Mg also protect the Ca, Mg-ATPase but to a lesser degree than calcium and their decay curves seem to be monophasic.

T-Pos192 TRYPSIN DIGESTION OF JUNCTIONAL SARCOPLASMIC RETICULUM VESICLES

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Junctional terminal cisternae (JTC) vesicles contain morphologically intact processes (feet structures) that connect the terminal cisternae of sarcoplasmic reticulum and the transverse tubules of the sarcolemma in situ. The main component of the processes is a 350,000 Da protein (350k) that binds ryanodine (RY) and is sensitive to Ca^{2+} channel modulators. The JTC are subjected to stepwise trypsin digestion. It is found that even under extensive digestion, the ultrastructure of the junctional processes is not changed. However, the 350k is selectively cleaved with mild digestion as evidenced by SDS-PAGE. Trypsinization does not alter the blocking effect of Mg^{2+} on rapid Ca^{2+} release from actively loaded JTC, nor the activation and potentiation by Ca^{2+} and ATP. When RY is first preincubated with JTC, high affinity RY binding sites are not digested away, and the binding is approximately stoichiometric with the 350k. However, RY binding is reduced when JTC are digested first and then subjected to prolonged RY incubation at 37^0C. The results suggest that trypsin produces proteolytic fragments of the 350k that remain stabilized by multiple noncovalent interactions and are only dissociated by strong detergents. RY binds to a protein domain where it does not interfere with trypsin binding, and can regulate the Ca^{2+} channel through allosteric mechanisms. [Supported by NIH and MDA grants (to G.I.) and AHA fellowship-Calif. Affil. (to A.C.)]

T-Pos193 THERMAL DENATURATION OF THE CALCIUM ATPASE OF SARCOPLASMIC RETICULUM. James R. Lepock, A. Michael Rodahl, Ching Zhang and *Kwan-Hon Cheng, Department of Physics, University of Waterloo, Waterloo, Ontario, N2L 3G1, CANADA and *Department of Physics, State University of New York, Buffalo, NY, 14214.

The thermal inactivation of the Ca ATPase of rabbit SR was determined and compared to the thermal denaturation of the enzyme. Inactivation of both Ca-uptake and ATPase activity was measured as a function of time at temperatures from 32 to 51°C in the absence of Ca^{2+} (in 1mM EGTA) and in the presence of Ca^{2+} (in 1mM $CaCl_2$). Differential scanning calorimetry (DSC) and fluorescence spectroscopy was used to determine fractional denaturation as a function of temperature. In EGTA, the enzyme denatures as a single unit with a transition temperature (T_m) of 49°, but in Ca^{2+} a new domain appears with T_m = 57°C. Fluorescence measurements of intrinsic trp fluorescence show that this domain includes the transmembrane helices in addition to the Ca^{2+} binding sites. FITC labelling was used to show that the domain with T_m = 49°C includes the ATP binding site. The denaturation of the ATP binding domain correlates with the loss of ATPase activity when SR is heated in medium with and without Ca^{2+} and to the loss of the ability to transport Ca^{2+} when heated in medium with Ca^{2+}. Each of these activities is inactivated with a $T_{1/2}$ for inactivation near 49°C. The inactivation of Ca-uptake in EGTA ($T_{1/2}$ = 37°C) does not correlate with any large-scale conformational changes in the Ca ATPase.

T-Pos194 INTERACTION OF CR•ATP WITH THE SARCOPLASMIC RETICULUM ATPASE. Zhida Chen, Lee Fielding, and Carol Coan, Dept. of Physiology, Univ. of the Pacific, 2155 Webster St., San Francisco, CA 94115.

It has been established for some time that Cr•ATP forms an extremely stable complex with the SR ATPase. This occurs very slowly, as the Cr (or the Cr-P moiety) exchanges ligands with the enzyme under conditions where a phosphorylated intermediate is normally formed. However, we find that a similar complex can be formed with Cr•AMP-PCP (Ca present), and with Cr•ATP in the presence of EGTA. In the latter case the reaction must be driven with higher temperatures (37°C) and longer incubation periods.

Both sets of experiments, one in which the enzyme is not actually phosphorylated, and an another in which the enzyme would not be expected to be phosphorylated, but apparently is, point to a potential role for conformation in regulating the enzyme mechanism. Cr•AMP-PCP should produce a good structural analogue of the Mg•ADP•E-P•Ca_2 complex, but one in which the γ P can not actually transfer to the enzyme. Alternatively, the formation of Cr•ADP•E-P (no Ca) is likely due to a shift in equilibria to a complex which is formed when a very small fraction of the Cr•ATP binding sites come into contact with the phosphorylation domain. However, in the case of Cr, the complex once formed is essentially irreversible. Normally this complex would require Ca^{2+} for stablization. Experiments are now underway to isure that E-P is actually formed, as opposed the γP being attached only through a Cr linkage.

T-Pos195 **MECHANISM OF THE PROTON PUMPING ASSOCIATED WITH PLANT ROOT TONOPLAST H^+-ATPase**
Shu-I Tu, Edwin Nungesser, and David Brauer. U. S. Department of Agriculture, ARS, Eastern Regional Research Center, Philadelphia, PA 19118

The effects of changing temperature on ATP hydrolysis and proton pumping associated with the H^+-ATPase of tonoplast membrane vesicles isolated from maize root microsomal fraction were determined. In the range of 5 to 45 °C, the maximum initial rate of ATP hydrolysis obeyed the simple Arrhenius model and the activation energy was estimted as 14 Kcal/mol. On the other hand, the initial proton pumping rate showed a bell-shaped temperature dependence with a maximum observed at ~25 °C. The Michaelis Menten constant (K_m) of the ATP hydrolysis remained nearly unchanged in the tested temperature range. In order to quantitatively describe the effects on proton pumping, a steady-state kinetic model which allows the measurement on the linkage between ATP hydrolysis and proton movement and the H^+ leakage of the membrane in the presence and absence of ATP, was employed. The results indicated that the observed temperature effects on proton pumping were mainly due to enhanced membrane H^+ leakage and decreased function-link with ATP hydrolysis at elevated temperature. This information and other ion effects were used to support an indirect link mechanism for H^+ pumping in the tonoplast system.

T-Pos196 EVIDENCE FOR A FUNCTIONAL OLIGOMER IN THE MECHANISM OF Na^+-H^+ EXCHANGE.
Kinya Otsu, James Kinsella, Bertram Sacktor, and Jeffrey Froehlich. National Institute on Aging, National Institutes of Health, Baltimore, MD 21224.

Previous transient state kinetic studies from our laboratory have demonstrated that $^{22}Na^+$ uptake by the Na^+-H^+ exchanger in renal brush border membranes exhibits a monoexponential burst phase at 0°C which corresponds to the first turnover of the system. Between 1 and 10 mM Na^+ the burst amplitude showed a sigmoidal dependence on $[Na^+]$ ($K_{0.5}$=4.25 mM) whereas steady state Na^+ uptake obeyed Michaelis-Menten kinetics. Over this range the turnover number, evaluated from the steady state uptake velocity and burst amplitude, fell to about half its original value. Experiments conducted over a ten-fold lower range of Na^+ concentrations (0.1-1 mM) revealed the presence of a second class of Na^+ binding sites ($K_{0.5}$=0.67 mM) with positive cooperativity. At saturating Na^+, treatment with gramicidin completely abolished $^{22}Na^+$ uptake implying that these Na^+ sites are exclusively involved in Na^+ transport as opposed to having a regulatory function. Experiments carried out at alkaline internal pH (7.7), which permits only a single turnover of the exchanger, gave a simple dependence of the burst amplitude on $[Na^+]$, similar to the effect of internal $[H^+]$ on burst amplitude. These results suggest that the Na^+-H^+ exchanger is an oligomer with two distinct classes of Na^+ transport sites, each having at least two sites which interact cooperatively. The minimum transport model consistent with these results is a tetramer consisting of 4 interacting polypeptide chains. Activation of the exchanger at acidic pH occurs through binding of H^+ to an internal site which then triggers oligomerization of the polypeptide chains into a functional transport unit.

T-Pos197 FUNCTIONAL EXPRESSION OF THE HUMAN ERYTHROCYTE GLUCOSE TRANSPORTER IN *ESCHERICHIA COLI*.
Hemanta K. Sarkar*, Bernard Thorens†, Harvey F. Lodish† and H. Ronald Kaback*. *Roche Institute of Molecular Biology, Nutley, NJ 07110 and †Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02142.

Recently, Mueckler *et al.* cloned the gene encoding the human erythrocyte glucose transporter from HepG2 hepatoma cells into lambda gt11 DNA [Mueckler *et al.* (1985) *Science 229,* 941]. By subcloning the gene into the T7 promoter/T7 polymerase expression system [Tabor, S. & Richardson, C.C. (1985) *Proc. Natl. Acad. Sci. USA 82,* 1074] and transforming a strain that is *ptsG*$^-$, *ptsM*$^-$ and *gal*$^-$ and totally defective in glucose transport [Bouma *et al.* (1987) *Proc. Natl. Acad. Sci. USA 84,* 930], we have expressed the erythrocyte glucose transporter in *Escherichia coli*. Cells bearing plasmids encoding T7 promoter/T7 polymerase with the gene encoding the transporter, unlike cells bearing the plasmids without the gene, take up D-glucose and 2-deoxy-D-glucose. Moreover, 2-deoxy-D-glucose uptake is inhibited by unlabeled D-glucose, cytochalasin B or mercuric chloride, but not by L-glucose. Finally, the glucose transport protein is inserted into the membrane of *E. coli*, as evidenced by immunoblotting experiments with two site-directed polyclonal antibodies, one directed against the carboxyl terminus of the glucose transport protein and the other directed against a synthetic peptide containing amino acid residues 225-238 of the glucose transporter. With both antibodies, the protein migrates with an M_r of ~34,000 on a 12% $NaDodSO_4$/polyacrylamide gel, a position similar to that of the unglycosylated glucose transport protein synthesized *in vitro*.

T-Pos198 *LAC* PERMEASE OF *ESCHERICHIA COLI* CONTAINING A SINGLE HISTIDINE RESIDUE IS FULLY FUNCTIONAL. Irene Püttner and H. Ronald Kaback*. *Roche Institute of Molecular Biology, Nutley, NJ 07110.

Recent application of site-directed mutagenesis to *lac* permease of *Escherichia coli* suggests that Arg-302, His-322 and Glu-325, neighboring residues in putative helices IX and X, play an important role in lactose/H^+ symport, possibly as components of a catalytic triad similar to that postulated for the serine proteases. By using restriction fragments of *lac Y* genes harboring specific site-directed mutations, a fusion gene has been constructed that encodes a permease in which His-35 and His-39 are replaced with Arg, and His-205 with Gln. Thus, the resultant molecule contains a single His residue at position 322 and exhibits all of the properties of the wild-type permease. In addition, an analogous single His permease was engineered with Ala at position 325 in place of Glu. The single His permease with Ala-325 is defective in active transport, but catalyzes exchange and counterflow normally. This permease, like the single His permease with Glu-325, also shows normal behavior with respect to N-ethylmaleimide inactivation, substrate protection and binding. In addition to providing strong support for previous experiments [Padan *et al.* (1985) *Proc. Natl. Acad. Sci. USA 82,* 6765; Püttner *et al.* (1986) *Biochemistry 25,* 4483; Carrasco *et al.* (1986) *Biochemistry 25,* 4486.], the engineered permease molecules will be useful for determining the apparent pK of His-322 under various conditions.

T-Pos199 EVIDENCE FOR "REVERSE MODE" Na/Mg EXCHANGE IN DIALYZED GIANT SQUID AXONS. H. Gonzalez-Serratos, *H. Rasgado-Flores, R. A. Sjodin, and J. G. Montes. Departments of Biophysics and *Physiology. University of Maryland School of Medicine, Baltimore, MD - 21201.

The electrochemical Na gradient provides energy for the uphill extrusion of Ca (Na/Ca exchange) in numerous cells. A simultaneous increase in intracellular Na (Na_i) and decrease in extracellular Na (Na_o) promotes reverse mode Na/Ca exchange in excitable cells manifested by a Na_o-dependent Ca efflux and intracellular Ca-dependent Na influx (Rasgado-Flores and Blaustein. Amer. J. Physiol. 252:C499-504. 1987). An analogous mechanism (Na/Mg exchange) has been proposed to account for the extrusion of Mg from excitable cells, although evidence for the operation of the putative Na/Mg exchanger is incomplete and based primarily on observations consistent with its forward mode of operation. More definitive evidence of such an exchanger requires a description of its operation in the reverse mode. To determine if reverse mode Na/Mg exchange could be observed in squid giant axons, we internally dialyzed these cells with solutions containing various Na_i (and ^{22}Na) while maintaining them in Na-free (Na replaced with Tris) solutions containing 0.1 mM ouabain, and measured ^{22}Na efflux as a function of external Mg (Mg_o). In 100 mM Na_i, reductions of extracellular Mg (replaced by Ba) produced reversible decreases in Na efflux. The observed relationship fit Michaelis-Menten kinetics indicating that there is one binding site for external Mg. Half-maximal activation was at Mg_o = 10.8 mM and the maximal Mg_o-dependent Na efflux was 11 pmoles/cm^2 sec. In 25 mM Mg_o, Na_i activated the Mg_o-dependent Na efflux with a sigmoid relationship displaying half maximal Na_i = 56 mM and maximal Mg_o-dependent Na efflux = 8.7 pmoles/cm^2 sec, fitting the Hill equation with a coefficient of 3. These results are consistent with the operation, under our experimental conditions, of a Na/Mg exchange process in the reverse mode, mediated by an exchanger possessing three binding sites for Na and one for Mg.

T-Pos200 **ACTIVATION BY $[Ca]_i$, AND BLOCK BY 3',4'-DICHLOROBENZAMIL, OF OUTWARD Na/Ca EXCHANGE CURRENT IN GUINEA-PIG VENTRICULAR MYOCYTES.** Mami Noda, R. Neal Shepherd, and David C. Gadsby. *Laboratory of Cardiac Physiology, The Rockefeller University, New York, N.Y. 10021.*

Whole-cell currents were recorded in response to 100-ms pulses from -40 mV to potentials between -140 and +60 mV in guinea-pig ventricular cells voltage-clamped and internally-dialyzed via wide-tipped pipettes, at 36°C. Internal and external solutions were designed to minimize ion channel currents and Na/K pump current but sustain Na/Ca exchange current. $[Na]_o$ and $[Na]_i$ were set at 145 mM and 20 mM, respectively; all internal solutions included 10 mM MgATP and 40 mM HEPES (*p*H 7.4), as well as 50 mM EGTA to buffer $[Ca]_i$ which was varied from ~0 to 500 nM. Outward Na/Ca exchange current was elicited by briefly raising $[Ca]_o$ from nominally zero to 1 mM and its size was determined by subtracting steady current levels obtained at ~0 mM $[Ca]_o$ from those obtained at 1 mM $[Ca]_o$: it increased almost exponentially with voltage (exponent ~0.4VF/RT) from an immeasurably small value at large negative potentials. Although the shape of this outward Na/Ca exchange current-voltage relationship was unaltered, its amplitude showed a hyperbolic dependence on $[Ca]_i$ and, at +40 mV, this "catalytic" effect of $[Ca]_i$ was characterized by an apparent K_m of 47±16 nM and a Hill coefficient of 1.0±0.02 (±SEM, n=4-7).

At a fixed $[Ca]_i$ of 50 nM, outward Na/Ca exchange current activated by 1 mM $[Ca]_o$ was reduced by 3-48 μM 3',4'-dichlorobenzamil (DCB) in an approximately voltage-independent manner. The vehicle, 0.01-0.16% DMSO, was shown in control experiments to have no effect. Hill plot analysis of the block at +40 mV yielded a half-maximal effect at 13±5 μM DCB and a Hill coefficient of 1.5±0.5 (±SEM, n=1-4), similar to its potency for inhibition of [Na]-dependent Ca uptake by guinea-pig cardiac sarcolemmal vesicles (IC_{50}=17 μM; Siegl et al., *PNAS* **81**; 3238-42, 1984). Over a similar concentration range, DCB also inhibited steady-state, $[Ca]_i$-dependent, inward Na/Ca exchange current. Supported by NIH grant HL 14899; DCB was a gift of Dr. G. Kaczorowski (Merck, Sharp & Dohme, NJ).

T-Pos201 THE AMINO ACID SEQUENCE OF A PEPTIDE FROM THE ATP-BINDING SITE OF Na,K-ATPASE LABELED BY 8-N3-ATP. TRAN, C.M.*, SCHEINER-BOBIS, G.#, SCHONER, W.# AND FARLEY, R.A.* *USC SCHOOL OF MEDICINE, DEPARTMENT OF PHYSIOLOGY AND BIOPHYSICS, LOS ANGELES, CA 90033, USA; AND #FACHBEREICH VETERINARMEDIZIN UND TIERZUCHT, INSTITUT FUR BIOCHEMIE UND ENDOKRINOLOGIE, JUSTUS-LIEBIG-UNIVERSITAT GIEBEN, FEDERAL REPUBLIC OF GERMANY.

[α-^{32}P]8-N_3-ATP, [^{3}H]8-N_3-ATP, and non-radioactive 8-N_3-ATP have been used as photoaffinity labels for the ATP binding site of the dog kidney Na,K-ATPase. The radioactive 8-N_3-ATP was specifically incorporated into the α-subunit of the enzyme and not the β-subunit. The labeling of the α-subunit was prevented in the presence of 2 mM ATP. The site of labeling is on the T58 fragment derived from the carboxy-terminus of the α-subunit by limited trypsin digestion in the presence of KCl. A Ki of 4.1 uM was obtained for 8-N_3-ATP inhibition of the equilibrium binding of [^{3}H]-ATP. This Ki corresponds well to a reported Kd of 3.1 uM for the binding of 8-N_3-ATP to Na,K-ATPase . HPLC separation of non-radioactive 8-N_3-ATP-labeled, trypsin-digested Na,K-ATPase, using dual wavelength detection (254 nm and 280 nm) showed one peptide peak that had an elevated absorbance ratio of A254/A280 relative to unlabeled Na,K-ATPase. This peptide eluted at the same position as the [^{32}P]8-N_3-ATP labeled tryptic peptide. The peptide in this peak was purified and sequenced, and was shown to have the amino acid sequence:

I-V-E-I-P-F-N-S-T-N-K-Y-Q-L-S-I-H-K-N-P-N-T-S-E-P-R.

This sequence corresponds to amino acids 470 - 495 of the dog kidney Na,K-ATPase α-subunit, and is highly conserved among other Na,K-ATPases. Manual sequencing of a purified peptide from pig kidney Na,K-ATPase labeled with [^{3}H]8-N_3-ATP also showed the amino-terminal sequence I-V-E-.

T-Pos202 CALCIUM REGULATION BY LENS PLASMA MEMBRANE VESICLES. Aurora Galvan and Charles F. Louis. Department of Veterinary Biology, University of Minnesota, St. Paul, MN 55108.

The role of the plasma membrane in the regulation of lens fiber cell cytosolic Ca^{2+} concentration has been examined using a vesicular preparation derived from calf lenses. Ca accumulation by these vesicles was ATP-dependent, and was releasable by A23187, indicating that Ca was transported into a vesicular space. The rather low amount of Ca accumulated (approx. 1 nmol Ca accumulated/hr) likely reflects both the small proportion of inside-out vesicles in this preparation (18% based on the latency of Na^+, K^+-ATPase activity), and the significant quantity of extrinsic proteins (mainly crystallins) that remain associated with the lens membrane vesicles (approx. 90% of the vesicle protein). The Ca^{2+} sensitivity of this Ca accumulation indicates that this pump could maintain lens cytosolic Ca^{2+} in the submicromolar range. Ca accumulation was stimulated by K (maximally at 50 mM K), and cAMP-dep. protein kinase; it was inhibited both by vanadate (IC_{50} = 5 uM), and the calmodulin inhibitor R24571 (IC_{50} = 5 uM), indicating that this pump was plasma-membrane derived and likely calmodulin-dependent. Inhibition of Ca uptake by selenite and p-chloromercuribenzoate demonstrates the presence of an essential -SH group(s) in this enzyme. Ca release from Ca-filled lens vesicles was enhanced by Na, demonstrating that these vesicles also contain a Na:Ca exchange carrier. p-Chloromercuribenzoate also promoted Ca release from Ca-filled vesicles suggesting that this release, like Ca uptake, is in part mediated by a cysteine-containing protein. The sensitivity of both Ca uptake and release to -SH reagents has implications in lens cataract formation, where oxidation of lens proteins has been proposed to account for the elevated cytosolic Ca^{2+} in this condition. Supported by NIH grant EY-05684.

T-Pos203 THE INTERACTION OF EXTRACELLULAR Na AT HIGH AFFINITY SITES ON THE Na/K-PUMP. Bliss Forbush III, Linda J. Kenney, and Jack H. Kaplan. Depts. of Physiology, Yale University School of Medicine, New Haven, CT 06510, and Univ. of Pa., Phila., PA 19104.

We have examined the interactions of Na with the ^{86}Rb-occluded state of Na,K-ATPase (J. Biol. Chem. *262*, 11104-11127, 1987), and with the red cell Na/K pump in the ^{86}Rb-Rb exchange mode. In each case, Na acts with an affinity in the range of 1-3 mM, consistent with action at a high affinity Na_{ext} site previously reported in transport studies. 1) The rate of ^{86}Rb release from the occluded state in $NaMgP_i$ is twice the rate observed in $cholineMgP_i$; this can be explained by Na causing immediate release of the ^{86}Rb ion from the "s" (slow) site when the first ion leaves the "f" (fast) site. 2) When the two sites are labeled individually with ^{86}Rb, it is seen that Na has no effect on release of ^{86}Rb from the "f" site, but that it causes release from the "s" site without a lag. 3) After incubation with amine-MgP_i and removal of amine, the "s" site is occupied and the "f" site is not; in this situation Na causes an extremely rapid release of ^{86}Rb from the "s" site. 4) Na competetively prevents the action of K or Rb in blocking release of ^{86}Rb from the "s" site. 5) The ordered release model predicts that during ^{86}Rb-Rb exchange, only one site is effectively used in transport, the "s" site always remaining occupied by Rb from the intracellular medium; since Na can act to cause the release of the ion from the "s" site, Na (at low concentrations) should *stimulate* the rate of ^{86}Rb-Rb exchange. With resealed red cell ghosts containing 10 mM Rb and no nucleotides, in the presence of 250 μM Rb_{ext}, Na_{ext} stimulated ^{86}Rb uptake at concentrations below 5 mM and inhibited at higher concentrations, consistent with the prediction. 6) Using tight right-side-out membrane vesicles from dog kidney and monitoring ^{86}Rb release from the occluded form, we have found that: a) both ^{86}Rb ions are released to the extracellular medium with MgP_i, b) Rb_{ext} blocks the release of one ion, c) Na_{ext} causes the rapid release of the ^{86}Rb ion from the "s" site, and d) the release is to the extracellular medium. These actions of Na are all consistent with the conclusion that extracellular Na does not affect the Na/K pump when both K transport sites are occupied, but that it interacts at high affinity sites to cause the rapid release of Rb or K when only one of the two transport sites is occupied by ^{86}Rb. (Supported by NIH GM-31782, and HL-30315).

T-Pos204 EVIDENCE FOR POTASSIUM-ACTIVATED MAGNESIUM EXTRUSION IN BARNACLE MUSCLE FIBERS. J. G. Montes, R. A. Sjodin, H. Gonzalez-Serratos, and *H. Rasgado-Flores. Department of Biophysics and *Department of Physiology, University of Maryland School of Medicine, Baltimore, MD - 21201.

Bundles of giant muscle fibers from the barnacle Balanus nubilus were isolated and placed in solutions with various Na (replaced with Tris), K, and Mg concentrations. Single fibers were removed from the bundles at regular intervals to measure changes in muscle contents of the above cations as a function of time. Analyses for K and Na content were performed by flame photometry, while Mg analyses were performed by atomic absorption spectroscopy. Muscles placed in Na-free media containing ØK and $[Mg^{2+}]_o$ = 400 mM showed greater Mg net uptake rates and final levels than when in $[Mg^{2+}]$ = 400 but $[K^+]_o$ = 10 mM. Gain in Mg content did not correlate with decreases in fiber Na content when fibers were placed in Ø Na and 10 mM K, indicating that Na-Mg exchange did not significantly regulate internal Mg levels under these conditions. In general, however, Mg gains correlated with loss of K from the fibers. When the ionized internal Mg level was studied by dual-wavelength differential absorption spectroscopy using Eriochrome Blue as an indicator, ionized Mg levels also showed a greater rate of increase and final values in the absence of external K ions. When K ions were readmitted to the medium, ionized Mg levels dropped with time, while Mg levels remained unchanged if solutions remained K free. Fibers placed in 100 mM Mg containing either high K (388 mM K) or zero K gained Mg in the K-free medium but lost Mg ions in the high K medium; this net Mg extrusion was against an electrochemical gradient. Our results show that, under our experimental conditions, a K-dependent extrusion of Mg ions can be demonstrated in barnacle muscle.

T-Pos205 EXTERNAL Mg STIMULATED Na EFFLUX IN SKELETAL MUSCLE. B.G. Kennedy and S.D. Knight. Indiana Univ. Sch. of Med. Northwest Center for Medical Education, Gary IN 46408.

Though free Mg_i is regulated below electrochemical equilibrium in various cells, the transport mechanisms controlling Mg concentration have not been well characterized. We have measured Na efflux in skeletal muscle to determine if a Na:Mg exchange mechanism may exist. Frog sartorius muscles were exposed to Ringer solution containing 2 uCi/ml 22-Na at 4 °C for 24 hours to label the intracellular compartment with 22-Na and to elevate total Na_i content (Na increased from 10 to 18 mM). Unidirectional efflux was determined into a Na-free (isosmotic Li substitution), 10^{-4}M ouabain containing Ringer solution. The rate constant for Na efflux under these conditions was 0.0017 (0.00007) min^{-1} (mean with SEM in parentheses). Isosmotic substitution of Mg for external Li resulted in a large (on the average 70%), rapid increase in Na efflux to 0.0029 (0.00013) min^{-1}. This Mg_o stimulated Na efflux remained constant for over 3 hours in the continued presence of Mg and was rapidly reversed upon return to a Mg-free solution. The Mg effect was completely blocked by 1 mM amiloride and approximately 75% inhibited by 400 uM quinidine. Application of the metabolic inhibitor 0.38 mM dinitrofluorobenzene, which rapidly decreases ATP concentration, reduced Mg stimulated Na efflux to the level observed in the absence of Mg_o. These results are consistent with the existence of a Na:Mg exchange mechanism which can extrude Na, driven by an inwardly directed Mg gradient. However, any ongoing Na:H exchange, if modulated by either Li or Mg or both, could affect the magnitude of the postulated Na:Mg exchange. Supported by a grant-in-aid from the American Heart Association, Indiana affiliate, Inc.

T-Pos206 DETERGENT EXTRACTION AND RECONSTITUTION OF Na-Ca EXCHANGE FROM PORCINE CARDIAC SARCOLEMMAL MEMBRANES. R. S. Slaughter, M.L. Garcia, and G.J. Kaczorowski, Merck Institute for Therapeutic Research, Rahway, NJ 07065.

The Na-Ca exchange system has been extracted from porcine cardiac sarcolemmal membranes utilizing the nonionic detergent, Triton X-100, in the obligatory presence of more than 40 mM NaCl. Stability in solution does not depend on the presence of phospholipids and the original activity can be recovered after a 4h incubation at 0°C, although half of the activity is lost at 18 h. Recovery of Na-Ca exchange activity upon reconstitution into proteoliposomes depends on 500 mM NaCl, the presence of asolectin phospholipids, the dilution factor of the detergent solution, and the presence of BioBeads SM-2 (50 mg/mg detergent). In the Na-Ca exchange assay, polyethylenimine (0.3% w/v) pretreatment of the glass fiber filters used to collect the reacted proteoliposomes results in the trapping of more vesicles than is found using untreated GF/C filters and thus significantly enhances the measured Na-Ca exchange activity. This optimized Triton X-100 extraction and reconstitution procedure resulted in a 50% increase of recovered Na-Ca exchange activity in the proteoliposomes compared to the unextracted sarcolemmal vesicles, with a stimulation corrected 10-fold purification of transport activity. When these proteoliposomes are subjected to a second Triton X-100 extraction and reconstitution procedure, the recovery of Na-Ca exchange activity is equal to the activity found in the original proteoliposomes. The hydrogenated form of Triton X-100 has low absorbance in the 250-280 nm region used for monitoring protein, and behaves similarly to Triton X-100 with respect to Na-Ca exchange, although slightly more is needed and optimization parameters are more critical. These procedures are being employed in the purification of the porcine cardiac Na-Ca exchanger.

T-Pos207 LAC PERMEASE OF ESCHERICHIA COLI: ARGININE-302 AS A COMPONENT OF THE POSTULATED PROTON RELAY. D.R. Menick, L. Patel, and H.R. Kaback. Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

The lac permease of Escherichia coli was modified by site-directed mutagenesis such that Arg-302 in putative helix IX was replaced with His, Gln or Leu. Permease with His, Gln or Leu at position 302 manifests properties that are similar to permease with Arg in place of His-322 [Püttner, I.B., Sarkar, H.K., Poonian, M.S. & Kaback, H.R. (1986) Biochemistry 25, 4483]. Thus, permease with each of these replacements is markedly defective in active lactose transport, efflux, exchange and counterflow, but catalyzes downhill influx of lactose at high substrate concentrations without H^+ translocation. By molecular modeling, Arg-302 may be positioned in helix IX so that it faces the postulated His-322/Glu-325 ion pair in helix X. In this manner, the guanidino group in Arg-302 may interact with the imidazole of His-322 and thereby play a role in the H^+-relay suggested to be involved in lactose/H^+ symport [Carrasco, N., Antes, L.M., Poonian, M.S. & Kaback, H.R. (1986) Biochemistry 25, 221]. In addition, molecular modeling suggested that the hydroxyl group in Ser-306 may be sufficiently close to hydrogen bond to one of the guanidino nitrogens. For this reason, Ser-306 was replaced with Ala. Permease with this mutation catalyzes lactose/H^+ symport in a manner indistinguishable from that of wild-type permease.

T-Pos208 EVIDENCE FOR HIGH MOLECULAR WEIGHT CARDIAC NA-CA EXCHANGE.

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Three groups have reported conflicting results in attempts to identify protein responsible for catalyzing cardiac Na-Ca exchange. Thus far, proteins with molecular weights of 33 (140 multimer), 82, 125 kDa have been reported. Using target sizing analyses, HPLC protein fractionation, and detection of Ca^{2+} binding protein, we have evidence that native Na-Ca exchange in cardiac sarcolemmal vesicles may be much larger than individual proteins thus far identified. Radiation inactivation studies indicated a native molecular weight of 220 kDa ± 20 kDa (n=6). Further evidence in support of this was obtained by fractionation of SL vesicle proteins by HPLC sizing columns. Na-Ca exchange activity was reconstituted from column fractions containing high molecular weight protein. SDS-PAGE analyses of HPLC fractioned SL protein indicated that exchange activity best correlated with a protein of apparent Mr of 220 kDa. The 220 kDa protein was determined to be a calcium binding SL protein by autoradiography of electrophoretically blotted proteins (J. Biochem. 95:511-519, 1984). Taken together, these data suggest the native molecular weight of cardiac Na-Ca exchange is 220 kDA. (Supported by NSF DCB-8602234, AHA-Missouri Affiliate and Edward Mallinckrodt Foundation).

T-Pos209 SEARCH FOR RESONANCE RESPONSE IN Ca^{++} TRANSPORT IN CELLS IN VITRO. W. C. Parkinson, Dept. of Physics and C. T. Hanks, School of Dentistry, University of Michigan, Ann Arbor, Michigan 48109. Reports [1,2] that efflux and/or influx of Ca^{++} ions from brain tissue display a resonance type response to an applied electromagnetic field has led to a cyclotron-resonance model for the interaction.[3] We have searched for resonances in the Ca^{++} transport for Balb c/3T3, L929, V79, and rat osteosarcoma cells by measuring changes in the cytosolic Ca^{++} concentration using fluorescence spectroscopy on cells loaded with the fluorescent chelating agent Fura-2.[4] The movement of Ca^{++} into and out of the cytoplasm under the action of ionomycin and EGTA are readily observed as changes in the fluorescent intensity, but no change was observed when DC and AC magnetic fields were applied under the reported resonant conditions (DC fields comparable to the geomagnetic field). The frequency and field amplitudes were also varied over a large range without an observable effect.

(1) S. M. Bawin and W. R. Adey; Proc. Nat. Acad. Sci. USA 73, 1999 (1976).
(2) C. F. Blackman; Bioelectromagnetics 6, 327 (1985).
(3) A. R. Liboff; "Geomagnetic Cyclotron Resonance in Living Cells," J. Biol. Phys. 13, 99 (1985).
(4) Grynkiewicz, Poenie, and Tsien; J. Biol. Chem. 260, 3440 (1985).

T-Pos210 CONCERNING THE INFLUENCE OF 4-β-PHORBOL-12,13-DIBUTYRATE (PD) ON THE OUABAIN-INSENSITIVE SODIUM EFFLUX IN BARNACLE MUSCLE FIBERS. E. Edward Bittar & Jude Nwoga*, Department of Physiology, University of Wisconsin, Madison, WI 53706.

There is ample evidence that the ouabain-insensitive Na efflux in barnacle fibers suspended in 10 mM-Mg^{2+}-ASW is stimulated by external or internal application of PD and that the response is dose-dependent, the minimal effective concentration being $\sim 10^{-8}$ (ext) or $\sim 10^{-6}$M (int. before dilution). In either case, the response reaches a peak in about 20 min and persists. The size of this response depends on the ext. Ca^{2+} concentration and is reduced by replacing Na_e with Li. It is also reduced by preinjecting (but not post-injecting) 250 mM-EGTA. Prior external application of 10^{-4}M-verapamil almost completely abolishes the response to external or internal application of PD. However, Cd^{2+} or Co^{2+} are less effective. Prior or post-injection of a 17 kd protein which is thought to act as a specific inhibitor of PK c fails to even reduce the response to PD. PKI is also ineffective. However, injection of Mg^{2+} or raising the external Mg^{2+} concentration reduces the response. Importantly, fibers injected with PD (e.g. 10^{-3}M) undergo a sustained contracture 30-50 mins later, while fibers treated with 10^{-5}M-PD externally undergo a sustained contracture about 5 mins later. Collectively, these results support the view that activation of PK c by PD results in the opening of Ca^{2+} channels, which, in turn, leads to stimulation of the Na efflux as the result of a fall in myoplasmic pCa. This view, though incomplete, is strengthened by the fact that the enzyme is present in barnacle fibers and that luminescence from aequorin increases when PD is applied externally or internally. In the former case, the increase in light output is preceded by a latent period of 5-10 mins and occurs slowly or rapidly as multiple, bursts of increased light output. Further work along these lines is in progress.

T-Pos211 FUNCTIONAL CHARACTERISTICS OF A Na^+-Ca^{2+} EXCHANGE SYSTEM IN SARCOLEMMAL MEMBRANE VESICLES OF VASCULAR SMOOTH MUSCLE*. Mohammed A. Matlib, Department of Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, 231 Bethesda Avenue, Cincinnati, Ohio, 45267-0575

The functional characteristics of a Na^+-Ca^{2+} exchange system in the cell membrane of vascular smooth muscle were explored in vitro in isolated sarcolemmal membrane vesicles of dog mesenteric artery. Na^+-loaded vesicles rapidly accumulated Ca^{2+} when an outwardly directed Na^+ concentration gradient was created by suspending them in a Na^+-free medium. This Ca^{2+} uptake process was reversible depending on the direction and the magnitude of the Na^+ concentration gradient across the membrane of the vesicles. Low temperature, monensin and external Na^+ drastically decreased Ca^{2+} uptake in Na^+-loaded vesicles. Monovalent cations K^+, Rb^+, Li^+ and Cs^+ could not substitute for Na^+ in the exchange process. The rate of Ca^{2+} uptake in Na^+-loaded vesicles was dependent on intravesicular Na^+ concentration. Divalent cations Ba^{2+}, Cd^{2+}, Mg^{2+}, Mn^{2+}, and Sr^{2+} inhibited Ca^{2+} uptake in Na^+-loaded vesicles. The order of potency of these divalent cations was $Cd^{2+} > Sr^{2+} > Ba^{2+} \gg Mn^{2+} > Mg^{2+}$. The trivalent cation La^{3+} also inhibited Ca^{2+} uptake (IC_{50} = 0.175 μM). The Km for free Ca^{2+} in vesicles loaded with 150 mM NaCl was 2.64 ± 0.5 μM and the maximum velocity was 14.8 ± 1.9 nmoles/min/mg protein. Valinomycin in the presence of K^+ increased the magnitude of Ca^{2+} uptake by 16% in Na^+-loaded vesicles, indicating that the process may be electrogenic. These data clearly indicate the existence and operation of a specific carrier-mediated Na^+-Ca^{2+} exchange system in sarcolemmal membrane vesicles isolated from a small blood vessel. Supported by a grant from NIH (RO1-HL 34664).

T-Pos212 Enrichment of Na/Ca Exchange From Cardiac Sarcolemma (SL). Robert H. Smith, Eldwin Van Alstyne, and George E. Lindenmayer, Depts. of Pharmacology and Medicine, Med. Univ. S.C., Charleston, SC 29425

Enrichment of Na/Ca exchange from canine SL was achieved by alkaline extraction (Philipson et al., BBA 899: 59, 1987), cholate solubilization and reconstitution. SL was loaded in 150 mM NaCl, 10 mM MOPS/Tris, pH 7.4, and diluted with 10 mM CAPS/NaOH (final pH 12.2). After centrifugation, pellets were resuspended in 500 mM NaCl, 20 mM MOPS/Tris, pH 7.4, and solubilized in 1.75% cholate, 670 mM NaCl, 20 mM MOPS/Tris, pH 7.4, with 25 mg/ml soybean phospholipids. After centrifugation, proteoliposomes (PL) were formed by dilution, washed and assayed for Na-dependent Ca uptake. One sec assays were (nmol/mg) 4.1 (SL) and 1213 (PL), representing 296-fold increase over SL with 109% and 0.37% recovery of total activity and protein, respectively. PL, obtained from procedure with protease inhibitors, were TCA-precipitated and delipidated. The following analyses were carried out in presence of DTE. Exposure to SDS (95°; 5 min) yielded a pellet upon centrifugation and, upon SDS-PAGE of supernatant, a prominent 89 kDa band, less prominent bands and significant protein at interface between stacking and running gels. Extract of pellet with SDS + Triton X-100 yielded minimal protein at interface, four major bands of 155, 116, 95 and 89 kDa and variable amounts of a 33 kDa band. Antibodies against an 82 kDa protein obtained by protease treatment (Hale et al., PNAS 81:6569, 1984), reacted with 116, 95 and 89 kDa bands. Thus, 116 kDa protein might be precursor for the latter two. The 155 and, possibly, the 116 kDa band may reflect constituents of the Na/Ca exchanger along with the 33 kDa protein (Longoni & Carafoli, BBRC 145:1059, 1987) in cardiac SL.

T-Pos213 **COMPARISON OF ENDOGENOUS AND EXOGENOUS SOURCES OF ATP IN SUPPORTING Ca^{2+}-UPTAKE IN ISOLATED SMOOTH MUSCLE PLASMA MEMBRANE VESICLES (PMV).** **C. Hardin, L. Raeymaekers, F. Wuytack, R. Casteels and R.J. Paul. Departments of Physiology, Universities of Cincinnati (45267-0576) and Leuven (Belgium).**

We have shown that glycolytic substrate (F-1,6diP) and cofactors (ADP, Pi, NADH) can support Ca^{2+}-uptake in PMV. Based on serial dilutions of the membrane concentration, we demonstrated that glycolytically fueled Ca^{2+}-uptake was independent of bath [ATP], suggesting that glycolysis preferentially fuels Ca^{2+}-uptake (Biophys. J. 51:181a). We further tested this hypothesis, using a hexokinase-based "trap" to consume free ATP in solution, and compared the Ca^{2+}-uptake fueled by membrane associated enzyme cascades, glycolysis I (F,1,6dP), glycolysis II (PEP), creatine kinase (PCr), as well as ATP supplied to the reaction mixture at a similar rate via a perfusion pump. An ATP-trap utilizing hexokinase (HK) inhibited both ATP and glycolytically supported Ca^{2+}-uptake. However, an ATP-trap using agarose-bound HK completely eliminated ATP driven Ca^{2+} -uptake while only partially reducing that supported by glycolysis I. This PMV fraction contains PK (1400) and CPK (50) activity (nmol/(min·mg)). By varying [ADP], the rates of ATP production (J_{ATP}) from PEP and PCr were adjusted to match those of glycolysis I (4 nmol/(min·mg)). This J_{ATP} could also be matched mechanically with a perfusion pump. All 4 systems supported Ca^{2+}-uptake, with initial experiments indicating that glycolysis I was somewhat more effective than glycolysis II or PCr-CPK, and all endogenous systems better than the exogenously supplied ATP. Moreover, the agarose-bound ATP-trap had little effect on the Ca^{2+}-uptake fueled by endogenous sources, while that supported by exogenous ATP was abolished. Our results are consistent with the hypotheses that membrane-bound enzymes provide ATP to the Ca^{2+}-pump independent of "free solution" and that glycolysis preferentially supports Ca^{2+}-pump activity. Supported in part by NIH HL 23240, NSF-Belgian American Program, and SW Ohio AHA (RJP) and HL 07517 (CH).

T-Pos214 CHARACTERIZATION OF AN INCREASED K^+ PERMEABILITY ASSOCIATED WITH THE STIMULATION OF THE RECEPTOR FOR IMMUNOGLOBULIN E (IgE) ON RAT BASOPHILIC LEUKEMIA (RBL) CELLS. Gary F. Labrecque, David Holowka, and Barbara Baird, Dept. of Chemistry, Cornell University, Ithaca, NY 14853

An increase in plasma membrane K^+ permeability has been observed to follow the aggregation of IgE-receptor complexes by multivalent antigen in RBL cells, a tumor analogue of rat mucosal mast cells. We have studied this increased permeability by measuring the timecourse of $^{86}Rb^+$ efflux and have found it to be correlated with the recovery of plasma membrane resting potential following antigen-stimulated depolarization and the stimulated influx of Ca^{+2}. At 37°C, the observed efflux of $^{86}Rb^+$ begins about 1 min after the addition of antigen and lasts for 8-9 min. Both the stimulated $^{86}Rb^+$ efflux and the recovery of the membrane potential are inhibited by quinidine in a dose-dependent manner which parallels the inhibition of the degranulation response. Neither the efflux nor the membrane potential recovery are affected by the absence of K^+ from the external buffer or by 4-aminopyridine at doses up to 5mM, indicating that the putative channel responsible for the increased Rb^+/K^+ permeability is not the previously characterized K^+-selective inward rectifier. Depolarizing the plasma membrane with 150mM K^+ or 1μM CCCP does not cause an observable efflux of $^{86}Rb^+$, indicating that this putative channel is not likely to be a standard voltage-gated type. Inhibition of Ca^{+2} influx by 0.1mM La^{+3}, 1μM CCCP, or 150mM K^+ also inhibits the antigen-stimulated $^{86}Rb^+$ efflux, indicating that influx of external Ca^{+2} may be neccessary for the opening of this putative K^+ channel. (Supported by NIH grants AI18306 and AI22449 and the Cornell Biotechnology Program.)

T-Pos215 THE EFFECTS OF DRUG CHARGE AND MEMBRANE STRUCTURE ON THE PARTITIONING AND LOCATION OF 1,4-DIHYDROPYRIDINES IN MODEL AND NATIVE LIPID BILAYERS. R. Preston Mason, D.W. Chester, G.E. Gonye and L.G. Herbette. Dept. of Medicine, Biochemistry, Radiology, and the Biomolecular Structure Analysis Center. Univ. of Connecticut Health Center, Farmington, CT 06032.

Nonspecific interaction of drugs with lipid bilayers appear to play an important role in subsequent recognition and binding to specific receptor sites in the membrane. We have used small angle X-ray diffraction to examine the location of Bay K 8644, an uncharged calcium channel agonist, Bay P 8857, an uncharged calcium channel antagonist, and Amlodipine, a charged antagonist in cardiac sarcolemmal lipid bilayers and dioleoyl phosphatidylcholine in the liquid crystalline state. Electron density profiles show these DHPs to be near the hydrocarbon core/water interface with the charged amlodipine displaced closer to the headgroup than the uncharged Bay K 8644 and Bay P 8857. This location may in turn define a region of localized drug concentration in equilibrium with a high affinity receptor site. The position of the amlodipine closer to the headgroup region suggests a specific charge interaction which may account for its reported longer **in vivo** halflives and longer association (i.e. "washout times") with native membranes **in vitro** compared with the uncharged DHPs. We have also examined the effect of thermal phase transition on DHP location and partitioning in dipalmitoyl phosphatidylcholine bilayers. Whereas DHPs are at the hydrocarbon core/water interface in the liquid crystalline phase, they appear to be reversibly excluded to the interbilayer water space when the membrane reaches gel phase. Further, partition coefficients for the DHP were over 2 orders of magnitude greater for membranes above their phase transition than below. Supported by HL33026 and RJR Nabisco, Inc. L.H. is an Established Investigator of the AHA.

T-Pos216 PURIFICATION OF NEURAL 5-HYDROXYTRYPTAMINE RECEPTOR PROTEINS BY AFFINITY CHROMATOGRAPHY. Timothy K. Gallaher, Cristina Weaver and Howard H. Wang, Department of Biology, University of California, Santa Cruz, CA 95064.

Pharmacological profiles of neural 5-hydroxytryptamine (Serotonin, 5-HT) binding sites by a number of researchers indicate a non-homogeneous receptor population in which an increase of cyclic-AMP or inositol triphosphate occurs upon 5-HT binding. D-lysergic acid diethylamide (LSD) is a specific ligand for all pharmacologically defined types of 5-HT receptors. In order to purify and identify components of these serotonin receptor/effector systems, an affinity column consisting of an agarose matrix with a covalently bound LSD molecule is used to purify serotonin receptors from bovine brain. Reconstitution of CHAPS-solubilized membrane fragments (after affinity chromatography using serotonin as the specific eluent) by dialysis in the presence of soybean lecithin yields active receptors. By Scatchard analysis, these receptors were shown to have an apparent K_d of 17 nM for [^{3}H]5-HT and a K_i of 27 nM for 5-methoxytryptamine. These values correspond well with sites observed in crude membranes. The specific activity indicates a greater than one thousand fold purification has been achieved. SDS-PAGE of concentrated affinity purified fractions resulted in a number of polypeptides as visualized by silver staining. Four high molecular weight bands (range from 56 to 96 kD) may represent the individual ligand binding proteins. A number of lower molecular weight peptides (between 32 to 48 kD) may correspond to effector proteins with the multiple receptor/effector complexes.

T-Pos217 CROSS-LINKING OF MOUSE MACROPHAGE SURFACE IgG2B/IgGI Fc RECEPTORS INDUCES MEMBRANE POTENTIAL CHANGES: FLUORESCENCE SPECTROSCOPY AND ELECTROPHYSIOLOGICAL STUDIES. Michael P. Blanton,[1] Brett A. Premack,[2] Stuart H. Thompson,[2] and Howard H. Wang,[1] Department of Biology,[1] University of California, Santa Cruz, CA 95064 and Hopkins Marine Station[2] of Stanford University, Pacific Grove, CA 93950

Previous work has demonstrated that the binding of IgG2B/IgGI immune complexes to immunoglobulin specific Fc receptors at the mouse macrophage surface resulted in functional ion channel formation [Young et al., Nature 306: 186]. Inducibility of membrane potential changes by multivalent but not by monovalent ligands suggests that receptor clustering is necessary for channel formation and/or activation. In order to more directly confirm this hypothesis in intact cells, we set out to correlate membrane potential changes with fluorescence energy transfer between the immunoglobulins. IgG2B immunoglobulin was labeled with either T-490 or FITC a fluorescent energy transfer couple. Mouse macrophages of the J774 cell line were incubated with labeled immunoglobulin and the excess immunoglobulin removed by washing. The cell suspension was excited at a wavelength within the donor (FITC) excitation spectrum and the emission spectra of the T-490 acceptor was monitored. Addition of an immunoglobulin cross-linking agent (Protein A) resulted in a time-dependent increase in fluorescence energy transfer. Transfer had reached 60% of its maximum by three minutes. The fluorescent voltage sensitive dye, oxonol, was allowed to equilibrate across the membranes of mouse macrophages. Addition of IgG2B immunoglobulin and subsequently protein A produced a membrane potential change characterized by a maximum depolarization by 3 minutes and a prolonged hyperpolarization followed over 25 minutes. We also have begun studies using whole cell and single channel patch clamp techniques. The J774 cell line has been shown to express two voltage-dependent K^+-conductances, one of which is inwardly rectifying with a single channel conductance of 29 pS and is open 50% of the time at the resting potential (Gallin and Sheehy, J. Physiol., 1985; McKinney and Gallin, Biophys. J. Abstracts, 1986). Bath application of IgG2B/protein A produced a large transient membrane conductance increase which appears to be independent of gating modifications to the preexisting K^+-conductance. The unitary event underlying this response is currently being studied to determine the role of protein cross-linking in the activation of ligand-dependent ion-channels.

T-Pos218 DISULFIDE BONDING PATTERNS WITHIN NATIVE ACETYLCHOLINE RECEPTOR MEMBRANES. Andrew L. Palma[1] and Howard H. Wang,[2] Departments of Chemistry[1] and Biology,[2] University of California, Santa Cruz, CA 95064.

Enriched nicotinic acetylcholine receptor (nAcChR) membranes from the electroplax of Torpedo californica were labeled with monobromobimane (mBBr), a fluorescent thiol alkylating agent. Analysis by gel electrophoresis showed that, in these membranes, mBBr incorporated primarily into the alpha and gamma and to a lesser extent, the beta and delta subunits of both the resting and desensitized states of the nAcChR. mBBr also labeled 43K protein and polypeptides of approximately 32, 83 and 35 kDa (all molecular masses referred to are those of the nonreduced form). A drastic increase in fluorescence appearing in the vicinity of the nAcChR beta subunit was observed when 2-mercaptoethanol (BME) was added during electrophoresis. This was most likely due to an increase in protein migrating to the same area, rather than BME producing additional thiol groups which were then labeled with mBBr. This result suggests crosslinking of β-subunits to other polypeptides in the non-reduced preparation. The membrane proteins were also subjected to a modification of the method developed by A. Gurusinghe et al. (Electrophoresis 7: 96-98, 1986) to detect disulfide-bonded polypeptides. Interchain disulfide bonds are present in polypeptides of approximately 230 and 128 kDa, and possibly one of 83 kDa. Intrachain disulfides were detected in the nAcChR alpha subunit and polypeptides of approx. 39 and 40 kDa. A peptide map of S. aureus V8 protease generated fragments of the nAcChR beta subunit were analyzed for the presence of disulfide bonds as above. Both the resting and desensitized states displayed intrachain disulfide bonds in fragments of approx. 40 and 21.3 kDa. We are attempting to identify which cysteine groups undergo disulfide bonding by comparing the amino acid sequence of these fragments to that of the nAcChR beta subunit.

T-Pos219 DESCRIPTION OF A NUCLEOSIDE TRANSPORT SYSTEM IN LYSOSOMES OF HUMAN FIBROBLASTS. Ronald L. Pisoni and Jess G. Thoene, Dept. of Pediatrics, Univ. of Michigan, Ann Arbor, MI 49109.

Lysosomes contain enzymatic activities capable of degrading nucleic acids to their constituent nucleosides, but the manner by which these degradation products are released from the lysosome is virtually unknown. To investigate this process, human fibroblast lysosomes, purified on Percoll density gradients, were incubated with 0.018 mM ^{3}H-adenosine at pH 7.0 and the amount of adenosine taken up by the lysosomes was measured. Adenosine uptake into fibroblast lysosomes attained a steady state by 12 min at 37°C, was unaffected by the presence of 2 mM MgATP, and was insensitive to pH over the range from 5.0 to 8.0. An Arrhenius plot of lysosomal adenosine uptake was linear, displaying an activation energy of 12.9 kcal/mol and a Q_{10} of 2.0. Adenosine uptake by fibroblast lysosomes occurs by a saturable process with a K_m of 8 mM at pH 7.0 and 37°C. Uptake of 8 uM ^{3}H-adenosine is inhibited 70-80% by adenosine (27 mM), 2'-deoxyadenosine (27 mM), inosine (27 mM), uridine (55 mM), or thymidine (55 mM), is inhibited 30 % by 2.7 mM adenine, but is unaffected by 69 mM D-ribose. In addition, the plasma membrane nucleoside transport inhibitors, dipyridamole (25 uM) and nitrobenzylthioinosine (25 uM), inhibit lysosomal adenosine uptake 50-60%. The half-time of ^{3}H-adenosine efflux from adenosine-loaded lysosomes was 7.0 min at 37°C and was slowed to 11.5 min when lysosomes were pre-incubated with 50 uM nitrobenzylthioinosine. Trans effects were not observed to be associated with lysosomal adenosine transport. In conclusion, the saturability of lysosomal adenosine uptake, and its specific inhibition by other nucleosides and by the known nucleoside transport inhibitors, nitrobenzylthioinosine and dipyridamole, indicate the existence of a carrier-mediated transport system for nucleosides within fibroblast lysosomal membranes.

T-Pos220 SEROTONIN INDUCES FERTILIZATION RESPONSES IN XENOPUS EGGS INJECTED WITH RAT BRAIN mRNA. Douglas Kline*, Luciana Simoncini†, Gail Mandel††, Robert Maue††, Raymond T. Kado**, and Laurinda A. Jaffe*. *Dept. of Physiology, Univ. of Connecticut Health Center, Farmington, CT 06032, †Dept. of Zoology, Univ. of Washington, Seattle, WA 98195, ††Dept. of Molecular Medicine, Tufts Univ. School of Medicine, Boston, MA 02111, **Lab. de Neurobiologie Cellulaire, C.N.R.S., Gif-sur-Yvette 91190 FRANCE.

To investigate the function of G-proteins at fertilization, we introduced into the Xenopus egg membrane serotonin receptors, which are known to act by way of G-proteins (Nomura et al., 1987, Mol. Brain Res. 2, 113). The serotonin receptors were introduced by injection of rat brain mRNA into oocytes; the oocytes were then matured to the egg stage by exposure to progesterone. In response to 0.1 μM serotonin, such eggs produced an activation potential, resulting from an increase in conduct ance comparable to that occurring at fertilization. Capacitance measurements, made by applying a 400 Hz AC signal, indicated that the serotonin-treated eggs underwent cortical vesicle exocytosis, with the same time course and extent as that observed at fertilization. A characteristic cortical contraction and endocytosis also occurred. These responses to serotonin were not observed in control, non-injected eggs. We propose that the exogenously introduced serotonin receptors interact with an endogenous G-protein in the frog egg membrane that is normally activated by sperm. The ability of serotonin to substitute for sperm supports the hypothesis that receptor-mediated activation of a G-protein initiates the response of the egg to fertilization. Supported by NIH.

T-Pos221 ADENINE NUCLEOTIDE-INDUCED CHANGES IN CONCENTRATION OF CYTOSOLIC-FREE CALCIUM IDENTIFY P_{2Y}-PURINOCEPTORS IN CARDIAC VENTRICULAR RAT MYOCYTES. O.G. Björnsson and J.R. Williamson. Dept. of Biochemistry & Biophysics, Univ. of Pennsylvania, Philadelphia, PA 19104, USA.

Adenosine 5'-triphosphate (ATP) has direct inotropic effects on the myocardium (Brit. J. Pharmacol. 73:879,1981). We therefore exposed isolated Fura2-loaded cardiac ventricular rat myocytes to various adenine nucleotides and measured concentration of cytosolic free calcium $(Ca^{2+})_i$, a likely determinant of muscle contractility. Extracellular ATP ($5x10^{-7}$ M - $1x10^{-4}$ M) increased $(Ca^{2+})_i$ in a dose-dependent manner (max. increase 124% ± 14, SEM, n = 8, $P<0.01$). The increase in $(Ca^{2+})_i$ was usually biphasic with an initial fast phase (<1 sec.) of low amplitude, followed by a slow phase (20 - 25 sec.) of higher amplitude. Thereafter $(Ca^{2+})_i$ gradually declined towards basal levels. A second application of ATP had little effect, and ATP abolished the effect of a subsequent electrical stimulation. Isoproterenol and the dihydropyridine calcium agonist BAY K 8644 enhanced the effect of ATP while the calcium antagonist nifedipine or verapamil greatly attenuated it, and binding of extracellular free calcium, $(Ca^{2+})_o$, by EGTA completely abolished the effect of ATP. ADP or AMP had little effect, and adenosine had no effect on $(Ca^{2+})_i$. The following rank of potency was established when the effect of ATP analogues on $(Ca^{2+})_i$ was studied: 2-methylthioATP $\geq$ ATP $\gg$ ATP-γS $>$ β,γ-methyleneATP = α,β-methyleneATP $>$ adenosine (n = 6), indicating that the purinoceptor for ATP was of the P_{2Y}-type. - The data suggest that there are purinoceptors (P_{2Y}) in cardiac ventricular myocytes which upon activation lead to an increase in $(Ca^{2+})_i$, and which is dependent on $(Ca^{2+})_o$ and voltage-sensitive Ca^{2+}-channels. (Supported by grants from the NIH (HL-14461) and the Icelandic Science Foundation).

T-Pos222 MEMBRANE BINDING OF ADRIAMYCIN USING TERBIUM LUMINESCENCE. Robert G. Canada, William Saway and Errol Thompson. Department of Physiology and Biophysics, Howard University College of Medicine, Washington, D.C. 20059.

Terbium (Tb^{3+}) luminescence has been used to investigate the interaction of adriamycin with a specific Ca^{2+} binding protein, in the plasma membrane of tumorigenic cells (Canada, R.G., Biophys. J., 51, 521a, 1987; and, Anal. Chim. Acta, in press, 1987). The binding of adriamycin to GH3/B6 cells was found to quench the luminescence intensity and lifetime of the Tb^{3+}-GH3/B6 complex. According to Stern-Volmer quenching analysis, the apparent association constant for adriamycin binding to the membrane was approximately 3.3×10^5 M^{-1}; and, the bimolecular quenching constant was about 7.3×10^7 $M^{-1}s^{-1}$. The quenching of Tb^{3+} luminescence by bound adriamycin was via a dipole-dipole resonant energy transfer mechanism. However, the quenching of Tb^{3+} luminescence by free adriamycin was dominated by an energy exchange interaction arising from the overlap of their electron clouds. Further, adriamycin was established to quench the intrinsic tryptophan fluorescence of the GH3/B6 cell membrane. The data suggests that, the Ca^{2+} binding site at the outer surface of the membrane is collisionally accessible to freely diffusing adriamycin; and, that the toxin receptor site is located near the bound metal ion.

T-Pos223 **TRANSFECTED MURINE B-82 CELLS EXPRESSING HUMAN β_2-ADRENERGIC RECEPTORS EXHIBIT ISOPROTERENOL STIMULATED CHANGES IN MEMBRANE EXCITABILITY AND $[Ca^{2+}]_i$.** P. A. Sheehy[1], M. B. Goodman[1], D. A. Robinson[2], F-Z. Chung[2], J. Venter[2], J. L. Barker[1] and C. M. Fraser[2]. (Intr. by M. Mayer) [1]LNP and [2]LMCN, NINCDS, NIH, Bethesda, MD 20892.

Murine B82 cells were transfected with the human β_2-adrenergic receptor gene using the $CaPO_4$ precipitation technique. Stable cell lines expressing the β receptor (JBC **262**:14843, '87) were used to study mechanisms of stimulus/response coupling. Whole-cell patch-clamp recordings show that isoproterenol (ISO, 100 nM) causes an increase in membrane conductance and an apparent hyperpolarization (K-Asp pipette, 0.1/1.1 Ca/EGTA, pH 7.2). These effects, elicited by brief applications of ISO (2-5 sec), are irreversible whether or not 2.5 MgATP & 0.5 mM NaGTP are included in the recording pipette. Using the cell-attached recording conformation, ISO elicits a transient burst of ion channel activity after a delay of 5-10 sec. Ion channel activity is refractory to immediate reapplication of ISO but responsiveness returns after 2-3 minutes. Single cell quantitative fluorescence microscopy using the Ca^{2+}-sensitive dye Fura-2 shows that ISO produces a dose-dependent, propranolol-antagonized (1 μM) transient rise in $[Ca^{2+}]_i$ not seen in control B82 cells. Preliminary dose-response curves indicate half-maximal Ca response near 100 nM. The Ca response is temperature dependent, and is not seen at T<22°C. We propose that ISO stimulation of transfected B82 cells expressing the β receptor is coupled to a subsequent rise in $[Ca^{2+}]_i$ which in turn activates ion channels. This system may serve as a model system for study of physiologic examples of activation/modulation of transmembrane ion channels by intracellular and extracellular ligands.

T-Pos224 PHOTOAFFINITY LABELLING OF THE HIGH AND LOW AFFINITY d-TUBOCURARE BINDING SITES OF THE NICOTINIC ACETYLCHOLINE RECEPTOR (AChR) BY [^{3}H] d-TUBOCURARE (d-Tc). S.E. Pedersen and J.B. Cohen, Dept. of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO.

Photoincorporation of [^{3}H] d-Tc into polypeptides of nicotinic postsynaptic membranes isolated from Torpedo nobiliana electric organ has been studied by SDS-PAGE and fluorography. Irradiation at 254 nm resulted in specific incorporation of label into α, γ, and δ subunits of the AChR, i.e. labelling that was inhibited by carbamylcholine (an agonist), α-bungarotoxin, or pancuronium (competitive antagonists), but not by the noncompetitive antagonist proadifen. This demonstrates that the labelling reflects reaction of d-Tc at acetylcholine binding sites and not at the noncompetitive antagonist binding site. As judged by isolation of individual subunits, about 0.5% of the α and γ subunits were specifically labelled. As judged by gel-slice counting of SDS-PAGE, about 0.2% of the δ subunit was specifically labelled. Labeling of the α and γ subunits was seen at low (<100nM) d-Tc concentrations. In contrast, significant labelling of the δ subunit required the presence of proadifen and high (>1 μM) d-Tc concentrations. The results suggest that the γ and δ subunits are part of the high and low affinity d-tubocurare binding sites respectively. This provides a structural basis for the observed differences between the two acetylcholine binding sites of the AChR without invoking differences in the two alpha subunits. The results also suggest an arrangement of the subunits in the lipid bilayer consistent with placement of either the γ or δ subunit, but not the β subunit, between the two α subunits.

T-Pos225 LAMININ MEDIATED LIPOSOME-METASTATIC CELL ASSOCIATION. S. M. Sullivan and J. D. Baldeschwieler, Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125.

An in vitro model system was developed to investigate liposome targeted drug delivery specific for metastatic cells. Metastatic cells express a cell surface receptor for laminin, and this receptor participates in the process of extravasation resulting in a metastatic lesion. Laminin is a multifunction subbasement membrane glycoprotein which has separate binding domains for a cell surface receptor and for sulfatide. By incorporating sulfatide into the liposomal membrane, the liposomes would bind to cells having occupied laminin receptors. Human breast carcinoma cell lines MCF-7 and T47-D were used as target cells in the in vitro model system. Sulfatide liposome-cell association was shown to be dependent upon sulfatide surface density, and degree of laminin receptor occupancy. Antibody raised against the laminin sulfatide binding domain reduced liposome-cell association to that of non-specific binding. Liposome-cell association was sensitive to the following factors: 1) SUVs gave a higher degree of cell binding than LUVs or MLVs; 2) incorporation of CDH or GM1 decrease cell binding with increasing molar ratios; 3) liposomal PE routinely gave a higher degree of cell binding than PC; 4) liposome cell binding was sensitive to PE acyl chain composition; and 5) liposome cell binding was completely inhibited in the presence of serum. Serum albumin, laminin, fibronectin or complement were found not to be involved in this inhibition. This system demonstrates a unique strategy for liposome targeting which may be applicable for other receptor-ligand systems. This research was supported by ARO Grant DAAL-03-87-K-0044 and a gift from Monsanto.

T-Pos226 A PORE FORMING FACTOR FROM ENTAMOEBA HISTOLYTICA DEPOLARIZES CELLS ONLY IN ITS AGGREGATED FORM. Edna Kalef, Ian Rosenberg, Carlos Gitler, Mei-de Wei, Valerie Montana, and Leslie M. Loew, Department of Membrane Research, Weizmann Institute, Rehovot, Israel and Department of Physiology, University of Connecticut Health Center, Farmington, CT 06032

It has been suggested that contact killing by Entamoeba Histolytica is mediated by a protein which inserts into and permeabilizes target membranes - amoebapore. Amoebapore has been isolated and partially purified. It permeabilizes lipid vesicles and produces channels in planar bilayers. It appears to be packaged in the amoeba as an aggregate within a particulate fraction which precipitates at 150Kg. A freeze thaw step in the isolation procedure solubilizes the pore forming activity as an apparent dimer with a subunit molecular weight of 14 KD. While both forms are active in permeabilizing artificial membranes, we have found that only the aggregated form is effective in depolarizing cells. Presumably, only the larger lesions formed by the aggregate are able to overwhelm the cell. We have used digital video fluorecence microscopy and a voltage sensitive dye to follow this process on individual cells. Because the method requires only enough medium to form a thin layer between slide and coverslip (ca. 25 µl), the cells can be exposed to highly concentrated amoebapore suspensions. Thus, the method is intrinsically very sensitive. The kinetics show significant variability from cell to cell. Interestingly, antibody grown against the soluble form of amoebapore is able to protect cells from the aggregated form (Supported by USPHS Grant AI22106).

T-Pos227 RESULTS OF AUTOMATED TRACKING OF LDL RECEPTORS ON CELL SURFACES. Richik N. Ghosh and Watt W. Webb, Applied and Engineering Physics, Cornell University, Ithaca, NY 14853.

Cell surface receptors for low density lipoprotein (LDL) are labeled with the fluorescent ligand diI-LDL (Barak and Webb, J. Cell Biol. 90, 595, 1981) and time lapse fluorescence images are collected using digital video fluorescence microscopy. We have developed software that automatically identifies and tracks receptors on the living cell's surface for many minutes without user intervention. This permits a large number of receptor trajectories to be mapped out rapidly and enables us to see patterns of movement over the entire cell with ease. We see fast concerted motion (velocities $>1\times10^{-6}$ cm/sec) of LDL receptors towards the cell's interior on a retracting lamellipodia, while some receptors in patches near the main cell body show concerted velocities an order of magnitude smaller with different directionalities. Correlated motion over large regions of the cell surface possibly indicate some bulk motion of a "matrix-sheet" to which the receptors are anchored. The implied drag forces on these LDL receptors undergoing non-random behaviour are in the order of 10^{-9} dynes, several orders of magnitude larger than viscous lipid drag. LDL receptors lacking a cytoplasmic tail (Brown and Goldstein, Science 232, 34, 1986) still undergo slow diffusion implying that constraints on receptor motion arise within or above the lipid bilayer. Preliminary experiments with treatments disrupting coated pit internalization (Larkin et al., Cell 33, 273, 1983) show suppression of concerted LDL receptor motion on internalization-defective cell surfaces.

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T-Pos228 ALTERATION IN K^+ FLUXES MAY HERALD ANTIPROLIFERATIVE ACTION OF ALPHA INTERFERON.

A Aszalos, E. Balazs and P.M. Grimley. (Introduced by Ira Levin)
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We have established earlier that Daudi cells sensitive to the antiproliferative action of alpha interferon (IFa) or recombinant alpha interferon A (rIFaA) respond with membrane potential shift to IFa or rIFaA treatment (10-1000 U/ml) (Grimley and Aszalos, BBRC 146,300, 1987). This observed membrane potential shift was detected with membrane potential shift sensing fouorescence dyes, $DiOC_6(3)$ and $BiBa_4(3)$ using a FACS system. Now we report that the observed membrane potential shift can be blocked by K^+ channel blockers. verapamil (5×10^{-8}M), nifedipin (1×10^{-4}M) and 4-amino pyridin (2×10^{-3}M) and is insensitive to amiloride (2×10^{-4}M). Furthermore the effect can be observed in Na^+ and Cl free media. We have concluded therefor that this membrane potential shift is due to altered K^+ flux changes. Since no membrane potential shift can be observed in such Daudi mutant cells which are not sensitive to the antiproliferative action of IFa, we theorize that altered K^+ fluxes may constitute IF signal for antiproliferative action.

T-Pos229 DYNAMICS OF CYTOSOLIC Ca^{2+} IN TUMOR MAST CELLS INDUCED BY ANTIGEN STIMULATION WITH MOBILE AND LOCALIZED ANTIGENS. T. A. Ryan, P. Millard, C. Fewtrell and W. W. Webb, Department of Physics (T.A.R.), Department of Pharmacology (P.M., C.F.) and School of Applied Physics (W.W.), Cornell University, Ithaca, New York 14853.

We have extended fura-2 imaging measurements of free cytoplasmic calcium ($[Ca^{2+}]_i$) in rat basophilic leukemia (RBL) cells during antigen stimulation to a temporal resolution of about 2 sec. In addition to the previously reported result that antigen addition leads to a sudden and sustained increase in $[Ca^{2+}]_i$ in most cells after a lag time which varies from cell to cell, the new measurements reveal that considerable oscillations in $[Ca^{2+}]_i$ with about 10-20 sec periods can occur during the response. This phenomenon has been observed over a wide range of soluble antigen concentrations (0.05-10ng/ml DNP_{14}-BGG) and oscillations appear in the absence of extracellular Ca^{2+} as well as in the presence of extracellular La^{3+}. We have also investigated the response of these cells during the application of a localized stimulus using an antigen coated micropipette (about 1-2μm tip). These studies revealed oscillations in $[Ca^{2+}]_i$ similar to those induced with soluble antigen but frequently without a sustained $[Ca^{2+}]_i$ rise. The spatial distribution of $[Ca^{2+}]_i$ responses to local stimulation are not correlated with stimulation location but are frequently inhomogeneous.

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T-Pos230 CALCIUM- AND PROTEIN KINASE C-INDEPENDENT ACTIVATION OF ELECTROPERMEABILIZED NEUTROPHILS BY CHEMOTACTIC FACTOR. Sergio Grinstein and Wendy Furuya. Division of Cell Biology, The Hospital for Sick Children, Toronto, Canada.

Electrically permeabilized human neutrophils were used to study the mechanism of activation of the respiratory burst by the chemotactic agent formyl-methionyl-leucyl-phenylalanine (fMLP). Permeabilization was assessed by flow cytometry of propidium-iodide, trapping of ^{86}Rb or [^{3}H]deoxyglucose-6-phosphate and by the requirement of exogenous NADPH for the respiratory burst, which was measured as oxygen consumption. A respiratory burst could be elicited by fMLP, phorbol ester or diacylglycerol (1,2-dioctanoylglycerol) in permeabilized cells suspended in EGTA-buffered medium with 100 nM free Ca^{2+}, but only if exogenous NADPH was provided. The fMLP response persisted even in cells depleted of intracellular Ca^{2+} stores by pre-treatment with the Ca^{2+} ionophore ionomycin. Therefore, a change in cytosolic free Ca^{2+} is not required for receptor-mediated stimulation of the respiratory burst. The responses induced by phorbol ester and diacylglycerol were largely inhibited by H7, a protein kinase C antagonist. In contrast, the stimulation of oxygen consumption by fMLP was unaffected by H7. The diacylglycerol- and fMLP-induced responses also differed in their sensitivity to changes in intracellular Ca^{2+}, Mg^{2+} and ATP concentration. The fMLP response did not require exogenous guanine nucleotides, but enhanced responses were obtained adding 100 μM GTP. These results suggest that a third signalling pathway, distinct from changes in cytoplasmic [Ca^{2+}] and activation of protein kinase C, is involved in the response of neutrophils to chemoattractants.

T-Pos231 MECHANISMS OF OSMOTIC ADAPTABILITY IN E. COLI. D.S. Cayley, B.A. Lewis, S. Padmanabhan, A.M. Krog and M.T. Record, Jr., Depts. of Chemistry and Biochemistry, University of Wisconsin, Madison, Wisconsin, 53706

To better understand the internal millieu of a living cell and define cellular mechanisms for coping with osmotic stress, we have characterized the chemical environment within E. coli as a function of external osmolarity. By ^{13}C and ^{14}N NMR and analytical measurements, K, glutamate and trehalose are the dominant solutes shown to accumulate in minimal media of increasing osmolarity. The intracellular osmotic activity of these solutes is relatively low since, after adding NaCl to the media, the rise in total cytoplasmic osmolyte concentration significantly exceeds the rise in media [NaCl]. These intracellular solutes may therefore interact with cell macromolecules. Uptake of exogenous betaine stimulates growth of osmotically stressed cultures, leading to a reduction of the steady state content of K while abolishing glutamate and trehalose synthesis. Overall, betaine uptake significantly reduces total solute content while increasing cytoplasmic water content relative to unsupplemented cultures, such that a net increase in media [NaCl] exceeds the corresponding rise in total cytoplasmic osmolyte concentration. Cytoplasmic betaine presumably has a high intracellular osmotic activity and, supported by the nearly 100% ^{14}N NMR visibility of cellular betaine, does not interact with cell macromolecules. Our results are discussed in light of a model suggesting the osmoprotective mechanism of betaine is in part to increase turgor pressure.

T-Pos232 UPTAKE AND EFFLUX OF LIPOSOMAL CONTENTS BY MACROPHAGES. David L. Daleke, Keelung Hong, and Demetrios Papahadjopoulos, Cancer Research Institute, University of California, San Francisco, CA 94143.

The uptake and efflux of liposomal lumen and lipid markers by murine macrophages (J774) is measured using a water soluble fluorophore, pyranine, and radiolabeled dipalmitoylphosphatidylcholine. The excitation spectrum of pyranine (1-hydroxypyrene-3,6,8-trisulfonate, HPTS) displays two strongly pH-dependent maxima (403 nm, 450 nm) and a pH-insensitive isosbestic point (413 nm). HPTS encapsulated into liposomes has been shown recently to accurately measure the endocytosis of liposomes into a low pH compartment in cells (Hong et al., *J. Cell Biol.* 103 (1986) 56a, Daleke et al., *Biophys J.* 51 (1987) 518a). Cell-induced leakage of liposomal contents is measured by two methods: co-encapsulation of HPTS and a non-fluorescent quencher, DPX, into the lumen of liposomes and co-labelling liposomes with HPTS and ^{3}H-DPPC. Liposome leakage results in dilution of contents and an increase in the fluorescence signal of HPTS/DPX containing liposomes, whereas preferential loss of HPTS results in a decrease in the fluorophore:radiolabel ratio with HPTS/^{3}H-DPPC liposomes. When J774 cells are treated with HPTS/DPX liposomes at 37°C, fluorescence dequenching coincided with the uptake of liposomes into an acidic compartment. Similar results were obtained with HPTS/^{3}H-DPPC liposomes at 37°C; the HPTS:^{3}H-DPPC ratio decreased coincident with acidification of HPTS. Subsequent to endocytosis, the HPTS/^{3}H-DPPC ratio gradually decreased indicating loss of HPTS from the cells. A slower decrease in the HPTS:^{3}H-DPPC ratio occurred when cells were incubated with liposomes under endocytosis-inhibiting conditions (0°C). In addition, specific uptake of liposomes by the Fc receptor pathway resulted in less leakage of contents. Thus, in addition to cell surface induced leakage, endocytosis induces leakage of liposomal contents followed by a slow efflux of endocytosed contents from cells.

T-Pos233 SINGLE-CHANNEL STUDIES OF THE ACTIONS OF DITHIOTHREITOL ON UNLIGANDED AND LIGAND ACTIVATED ACETYLCHOLINE RECEPTORS. L. Rojas, *A. Steinacker and C. Zuazaga. Institute of Neurobiology, U. of Puerto Rico Med. Sci. Campus, San Juan, PR 00901 and *Dept. of Otolaryngology, Washington U. Sch. of Med., St. Louis, MO 63110.

We have characterized the unliganded acetylcholine receptor ($AChR_U$) in Xenopus myocytes and the modification of its properties by the disulfide reducing agent dithiothreitol (DTT). These properties were compared to those of the ACh-activated receptor ($AChR_L$) previous to and following reduction by DTT to asses the effect of the agent on ligand binding and/or channel gating. Two forms of the $AChR_U$ were found; they differ in conductance by a factor of 1.5 and in channel open time(τ)by a factor of 2-3. The high conductance (59 pS) form of $AChR_U$, however, has a τ of 0.32 ms (MP, -100 mV; 20-22 °C) whereas the high conductance (64 pS) form of $AChR_L$ has a τ of 0.82 ms. For $AChR_U$, τ is independent of voltage. Following exposure to DTT (2-7 mM, inside pipette), the conductance of both $AChR_U$ and $AChR_L$ decreases to 35 pS, without changes in the reversal potential. τ of $AChR_L$ is reduced 20% (MP, -100 mV) and becomes shorter with hyperpolarization; for $AChR_U$, τ is increased 3-fold and remains independent of voltage. For $AChR_L$, histograms of closed intervals between two high-conductance events were fitted with two exponentials. Following reduction with DTT, the fast component increased 3-fold while the long component decreased 10-fold; a decrease in the long component of the closed time distribution of $AChR_U$ was also observed. Since reduction of a disulfide bond alters many properties of the receptors, both liganded and unliganded, the above data is difficult to reconcile with existing kinetic schemes to link structural domains with specific kinetic rate constants. (Supported by NIH NS07464 and NSF BNS 8218429).

T-Pos234 FUNCTIONAL SODIUM CHANNELS AND NICOTINIC CHOLINERGIC RECEPTORS ARE REGULATED INDEPENDENTLY IN PC12 CELLS. C.K. Ifune, C. Kopta and J.H. Steinbach, Depts. of Anesthesiology and Anatomy/Neurobiology, Washington Univ. School of Medicine, St. Louis, MO 63110.

PC12 cells grown in DME supplemented with 10% fetal calf serum and 5% horse serum, were exposed to 0, 50, or 100 ng/ml β-NGF. Voltage activated Na^+ and ACh activated currents were measured using the whole-cell voltage-clamp technique. Peak currents were normalized by capacitance. A sodium chloride solution was present extracellularly and a cesium chloride solution buffered with 1 or 5 mM EGTA was the intracellular solution. 100μM ACh was applied by a pipette placed near the cell. The fluid exchange around the cell was complete within 500 ms. Treatment with NGF increased the size of the Na current densities compared to untreated cells (controls: 0.07 $pA/\mu m^2$, 0.00-0.23 $pA/\mu m^2$, 7; NGF-treated: .39 $pA/\mu m^2$, 0.25-0.65 $pA/\mu m^2$, 19; median, 95% interval, number of cells). NGF also increased the ACh-activated current densities (controls: .025 $pA/\mu m^2$, 0.00-0.06 $pA/\mu m^2$, 7; NGF-treated: 0.25 $pA/\mu m^2$, 0.15-0.39 $pA/\mu m^2$, 19). There was no significant difference between the cells treated with 50 ng/ml NGF and 100 ng/ml NGF. Though NGF increases both the Na current and ACh-activated current, the size of these currents in a cell were not correlated (r=0.26). Dexamethasone, a glucocorticoid, had been shown to affect the differentiation of PC12 cells. Cells grown in the presence of NGF were then treated with $1x10^{-6}$ M dexamethasone for 5-7 days in the continued presence of NGF. Cells treated with dexamethasone had significantly smaller ACh-activated current densities compared to cells treated with NGF alone (Dex: 0.00, 0.00-0.07 $pA/\mu m^2$, 7, $p<0.01$). On the other hand, Na current densities were not affected (0.39 $pA/\mu m^2$, 0.03-0.63 $pA/\mu m^2$, 7). This suggests that the expression of Na channels and ACh receptors can be regulated independently. (NIH NS 22356, CKI supported by a GPOP fellowship.)

T-Pos235 **SINGLE-CHANNEL GATING AND PERMEABILITY PROPERTIES OF THE NMDA RECEPTOR.** J.A. Dani, C.E. Jahr and C.F. Stevens, Dept of Physiol and Molec Biophys, Baylor College of Medicine, Houston, TX 77030; VIABR, Oregon Health Sci Univ, Portland, OR 97201; Sect of Molec Neurobio, Yale Univ Med Sch, New Haven, CT 06510.

Single NMDA receptor channels from cultured rat hippocampal neurons were studied. Solutions of monovalent permeants and BAPTA at pH7.4 always bathe the inner surface of the membrane. When NaCl and 2mM $CaCl_2$ at pH7.4 bathe the outside, the I-V relation is linear and the conductance is 50pS at 21° and 27pS at 10°. When the external pH is lowered to 6.7, there is a V-dependent decrease in I at negative potentials. When the external solution contains BAPTA to remove all divalent ions, there is a V-dependent increase in I at negative potentials. With pure 114mM $CaCl_2$ in the external solution, the conductance varies from 11pS at negative potentials where Ca carries the current to 50pS at positive potentials where the internal monovalent cation carries the current, 21°. These results indicate that Ca can carry substantial current, and at low concentrations Ca and H ions can enter the channel to influence the permeability of monovalent cations. In nominally Mg free solutions, the mean open time of the channel is about 3ms at 21° and 10ms at 10°, HP=-75mV. When 10μM Mg is present externally, the mean open times are shorter at negative potentials: about 1.7ms at 21° and 4ms at 10°, HP=-75mV. The open time distribution is a multiple exponential, but in 10μM Mg it becomes dominated by a single component. At 10°, however, a fast component seen in Mg free solutions is still seen in 10μM Mg. It is likely that the longer open times are broken up by Mg briefly blocking the open channel. The fast component is less affected by Mg because the channel closes before Mg blocks it. The Mg block shows a strong V dependence, and at very positive potentials the open time seems unaffected by Mg. Supported by NIH grants NS21229, NS21419 and NS12961.

T-Pos236 **INHIBITION BY NALOXONE OF GLYCINE-INDUCED POTENTIATION OF NMDA-ACTIVATED ION CHANNELS.** **P.D. Bregestovski, I.N. Sharonova and L.G. Khaspekov (Intr. by D.J. Adams). Institute of Experimental Cardiology and Institute of Brain, Moscow, USSR**

Two features unique for NMDA-activated ion channels were recently discovered: voltage-dependent inhibition by Mg^{2+} (Nowak et al., Nature **307**: 462-465, 1984) and potentiation of NMDA response by low concentrations of glycine (Johnson & Ascher, Nature **325**: 529-531, 1987). The glycine-effect was not inhibited by strychnine which suggested different pharmacological properties of the glycine receptor from those described previously. The aim of the present study was to characterize the action of glycine and its antagonists on hippocampal neurones. Single channel currents were recorded from outside-out patches in hippocampal neurons isolated from 18-19 days mouse embryoes and cultured for 10-20 days. 10 uM N-methyl-D/L-aspartic acid (NMA) activated single channel currents which have a conductance of ~50 pS and were inhibited by Mg^{2+} in a voltage-dependent manner. The mean channel open time was 12 ± 4.2 ms (mean ± sd, n=9). The glycine-effect was observed in more than 50% of the cases (18 of 30 patches). 1 uM glycine increased the frequency of channel activation and induced the appearance in some patches "long-lived" channels with an open time of about 1 sec. In the presence of glycine, the open probability increased an average 4.7 times. We found that naloxone (1-10 uM) inhibits the glycine-effect and the kinetics of NMDA-activated channels returned to control. Pretreatment of patches by naloxone prevented the glycine-effect. Met- and leu-enkephalins (up to 1 uM) did not affect glycine potentiation. This suggests that the naloxone effect is unlikely to be mediated through either a μ or δ-receptor. The possible site of naloxone interaction may be an ϵ-opiate receptor which has a comparatively low affinity for naloxone.

T-Pos237 POTENTIATION OF GLUTAMATE CURRENTS BY VIP IN CULTURED CORTICAL NEURONS. D. W. Y. Sah. Dept. of Neurobiology, Harvard Medical School, Boston, MA.

A number of peptidergic transmitters are present in the cerebral cortex, where excitatory and inhibitory effects have been described. However, the physiological effects of peptides on cortical neurons are not well-known. I have investigated the effects of vasoactive intestinal polypeptide (VIP) on whole-cell currents in cultured cortical neurons from neonatal rats. Although VIP (1 uM) had no substantial effect when applied alone (<5 pA change at -60 mV, n=15), it usually potentiated the effect of the conventional transmitter, glutamate (1-10 uM), when applied together with glutamate (average increase=152%, n=33). Since glutamate activates both NMDA and non-NMDA receptors in these cells, selective agonists were used to determine which receptor subtype VIP interacted with. Responses to kainate (10-20 uM) and quisqualate (1-5 uM) at -60 mV were not affected by VIP (<20% change, n=5 and 6, respectively), even when VIP potentiation of glutamate currents was found to be present. In contrast, currents evoked by N-methyl-D-aspartate (NMDA, 30 uM) were usually potentiated by VIP (average increase=298%, n=20). Thus, the effect of VIP is similar to that of glycine in that it is specific for the NMDA response. VIP has been reported to increase the firing rate of cortical neurons in vivo. The results here indicate that this excitation may be mediated via an enhancement of ongoing glutamate transmission.

T-Pos238 TWO DISTINCT COMPONENTS OF SYNAPTOSOMAL 3H-GABA RELEASE RESOLVED BY A RAPID SUPERFUSION SYSTEM. T.J.Turner and S.M.Goldin (Intr. by W.A.Toscano), Dept. of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA .

^{3}H-GABA release from rat brain synaptosomes preincubated with 0.5uM ^{3}H-GABA was studied with 50 ms time resolution, using a novel superfusion method. There are two kinetic components of ^{3}H-GABA release stimulated by continuous KCl-induced depolarization. The slow component gradually develops for 200 ms after stimulation, to a steady-state rate 3x the basal rate of 0.15% of the total synaptosomal ^{3}H-GABA per sec. It is independent of $[Ca]_e$, and is partially sensitive to the GABA uptake system antagonist nipecotic acid (IC50=0.3uM). The rapid component reaches a maximal rate of 10x basal within 100 ms and is transient, inactivating exponentially (τ =80 ms). The rapid component requires physiological $[Ca]_e$, with half-maximal release seen at 1mM $[Ca]_e$. The rapid component was further studied by measuring Ca-dependent release evoked by a train of six depolarizing pulses, 350 ms long, delivered @ 0.06 Hz. The rapid component decreased from 0.11% of the total synaptosomal ^{3}H-GABA during the first pulse to a steady level of 0.05% per pulse after 3 pulses. After 3 pulses, >95% of the rapid component was blocked by 0.3uM w-conotoxin; likewise, 50% of the rapid component was blocked by 1uM nimodipine. Neither agent blocked the rapid component during the first pulse. We conclude that: a) the slow component is mediated by electrogenic transport processes including the GABA uptake system; b) multiple, functionally distinct types of Ca channels mediate the rapid component of GABA secretion; and c) dihydropyridine antagonists show "use-dependent" blockade of synaptosomal neurosecretion, which may account for the absence of dihydropyridine blockade of neurotransmitter release reported in some previous studies.

T-Pos239 **PERMEABILITY PROPERTIES OF THE ACETYLCHOLINE RECEPTOR CHANNEL TO CALCIUM.**

E. Radford Decker, and John A. Dani, Dept of Physiol and Molec Bioph, Baylor Col of Medicine, Houston, TX 77030.

Ca ions are vital in regulating the functional state of cells. Since AChR channels are densely packed at nicotinic synapses, a small permeability of the AChR channels to Ca could be important in development, maintenance and regulation of the synapse. The purpose of this work is to quantitatively determine the permeability of the channel to Ca and Ca's interaction with other permeants. Patch clamp techniques were used to study AChRs from the cell line BC3H1. When 1mM Ca is added externally to a pure solution of NaCl or CsCl, there is a voltage-dependent decrease of the inward current. As more Ca is added to the external solution, the inward currents decrease further. At -100mV, with 45mM Cs on both sides of the membrane, the inward currents as a function of added external Ca is 0 Ca,5.6pA; 1mM Ca,2.9pA; 10mM Ca,1.7pA; 25mM Ca,1.5pA; 77mM Ca,1.3pA. With pure 110mM Ca as the external permeant, the current at -100mV is about 0.9pA. These results show that a small amount of added Ca influences the permeation of a monovalent cation dramatically, which indicates that Ca has a high affinity for the channel. At higher concentrations, it is likely that Ca occupies a site within the channel, thereby displacing the monovalent permeant and carrying most of the current itself. The ionic permeation data is described well by a transport model that considers the physical size, shape and charge of the AChR channel. The physical characteristics of the large entrance vestibules of the channel are coupled to a two barrier, one site Eyring rate theory model that describes the strong interactions between the permeant and the protein in the narrow region of the pore. Supported by NIH grant NS21229.

T-Pos240 EFFECTS OF TRIMETHYLOXONIUM MODIFICATION ON ACETYLCHOLINE RECEPTORS IN PATCH-CLAMPED BC3H1 CELLS. G.L. Barchfeld & P.A. Pappone, Department of Animal Physiology, University of California, Davis, CA 95616.

We used the gigaohm-seal patch clamp method to test the effects of trimethyloxonium (TMO) modification on the properties of acetylcholine receptors (AChR's) in BC3H1 cells. TMO reacts specifically and covalently to convert negatively charged carboxyl groups to neutral methyl esters. A previous study of frog NMJ has shown that TMO-modification results in a reduction of the single channel AChR conductance, γ, derived from noise analysis [Adams, (1983) J. Physiol. 343:29]. For the experiments presented here, whole-cell recordings from cultured BC3H1 cells were made with Cs as the major cation inside and outside the cell. Cell-attached recordings were made with 5 μM ACh and Cs as the major cation in the pipette and high K solution in the bath. Comparison of the effects of bath application of 0.1 to 10 μM ACh to cells patched in the whole-cell configuration before and after TMO-modification showed that TMO decreased both the initial transient response and the steady-state conductance in the presence of ACh by 43.3$\pm$9.5%. These effects are apparently not due to a change in the permeability properties of the AChR, since neither γ nor the reversal potential were changed following modification. The slope of single channel current-voltage relations at negative potentials gave values of γ equal to 48$\pm$1 pS (n=4) in controls and 46$\pm$2 pS (n=5) following TMO. The currents reversed at +4 mV (assuming a cell resting potential of 0 mV) in both controls and modified cells. TMO treatments did modify the kinetics of AChR channels, resulting in an increase in "bursting" behavior and a decrease in channel open time. These kinetic effects, rather than a decrease in channel conductance, seem to be responsible for the reduced response to ACh in BC3H1 cells following TMO modification. Supported by the American Heart Association, California Chapter, and NIH grants NS07300 and AR34766.

T-Pos241 RAPID DESENSITIZATION OF AGGREGATED GLUTAMATE RECEPTORS. L.Trussell & G. Fischbach. Dept. Anatomy & Neurobiology, Wash. U. Sch. of Med., St. Louis, MO 63110

In contrast to the submillisecond risetime of excitatory synaptic currents in the vertebrate CNS, conventional responses to exogenously-applied glutamate require tens to hundreds of milliseconds to reach peak amplitude. Such a slow onset would be expected to mask more rapid behavior of ion channels. We have looked for rapid desensitization of glutamate responses in cultured chick spinal neurons by focal ionophoresis. The ionophoretic pipettes were positioned within 2 μm of the cell membrane, often touching the cell. After carefully adjusting the ionophoretic backing current, 0.5-1.0 msec pulses could produce a rapidly rising, transient inward current. The rise time of these responses varied from 0.8 to 3.6 msec. These rapid responses were only elicited at discrete regions on the cells, suggesting that glutamate receptors are clustered. They were insensitive to 2-amino-5-phosphonovalerate (APV) and showed linear I-V relations. When the pulse duration was increased to 50 msec or longer, the evoked inward current declined by half within 50 msec and was followed by a more slowly decaying current that exhibited APV-sensitive and -insensitive components. When glutamate was applied by 1 msec paired pulses, the second pulse was reduced in amplitude when delivered within 500 msec of the first and was often completely blocked at intervals of 10-50 msec. The 1/2 time of decay of the first response varied from 5-15 msec; the double pulse experiments indicate that this time represents the upper limit for the time course of desensitization. This decline in sensitivity was not voltage dependent (range -80 to +50 mV) and occurred when the ionophoretic pipette was located near or far from the whole-cell voltage clamp electrode. Thus, this decay probably represents a genuine rapid desensitization of non-NMDA glutamate receptors.

T-Pos242 CURARE INDUCES A SUBCONDUCTANCE STATE IN SPONTANEOUS OPENINGS OF THE ACETYLCHOLINE RECEPTOR CHANNEL. G. Joseph Strecker & Meyer B. Jackson, Departments of Physiology and Biology, University of California, Los Angeles, 90024.

Acetylcholine receptor (AChR) channel currents were recorded in cell-attached patch recordings in cultured mouse myotubes bathed in physiological saline. Curare induces two conductances of 39 pS and 15 pS. In the absence of ligand, spontaneous openings show only the main conductance of 39 pS while the 15 pS subconductance state is never seen. Treatment of the AChR with dithiothreitol (DTT) and N-ethyl maleimide (NEM) renders myotubes much less responsive to cholinergic agonists by modifying the ACh binding site. However the frequency of spontaneous openings is not reduced and their conductance is unaltered (Jackson, PNAS. 81: 3901, 1984). Perfusion of the patch electrode with curare produced an increase in the frequency of opening of the AChR channel. Curare-free recording followed by perfusion of 20 μM curare into the patch electrode, which is held at +50 mV, reveals that in unmodified patches spontaneous channel openings account for 9 ± 3.6% ($\bar{X}$ ± s.e.) of observed openings in the presence of curare. In DTT+NEM treated cells however, 38.0 ± 7.8% of the openings are spontaneous. In spite of this difference, the ratio of the no. of 15 pS events to the total no. of events is not significantly different from control cells (.019 ± .004 controls vs. .019 ± .005 treated). This suggests that curare induces the subconductance state in spontaneously opening AChR channels which have blocked binding sites. The voltage and concentration dependence of subconductance states have been studied and are consistent with curare inducing subconductance states by binding to a different site within the channel opening and partially obstructing ion flow.

T-Pos243 DEVELOPMENT OF ACETYLCHOLINE RECEPTORS ON CHICK CILIARY GANGLION NEURONS. Joseph F. Margiotta, Dept. of Pharmacol. UCSD, La Jolla, CA 92093. (Intr. by V.E. Dionne)

Nicotinic acetylcholine receptors (AChRs) on neurons and muscle differ in subunit structure and regulation by second messengers. To examine the development of neuronal AChRs, ciliary ganglion neurons were acutely isolated from chick embryos using collagenase at stages 34-45 (8-19 days) of embryogenesis. AChR function was examined with patch-clamp techniques, while receptor number was assessed using a monoclonal antibody probe (mAb 35) that recognizes AChRs on the neurons.

Whole-cell currents induced by perfusion with 100 uM ACh at -70 mV increased between stage 34 (-60 ± 15 pA, n=10) and stage 40 (-2000 ± 200 pA; n=11) remaining elevated up to stage 45 (-1900 ± 300 pA, n=6). The number of surface AChRs appeared to increase in parallel with sensitivity since the number of specific 125I-mAb 35 sites per neuron was about 20,000 at stage 37 and 70,000 at stage 40 (n=3). To determine if the increase in ACh response is also accompanied by a change in AChR properties, single channel recordings were obtained from outside-out patches perfused with 5 uM ACh. A major class of AChR having a conductance of 35-40 pS comprised over 80% of all events observed in patches from both young (stages 34,35; n=3) and old (stages 39,40; n=6) neurons. Open duration histograms for the 35-40 pS AChR event in patches from young and old neurons were well described by two components having fast and slow time constants of 50-150 usec and 0.6-1.2 msec, respectively. In patches from young neurons, the fast component was dominant so that nearly all of the events were brief (<200 usec). In contrast, the slow component was dominant in patches from old neurons, revealing a 7-fold increase in the proportion of long duration events between stages 34-40. These results reveal changes in the number and function of AChRs on ciliary ganglion neurons that coincide with the maturation of synapses on the neurons during development. Supported by NS 24417.

T-Pos244 CHARACTERIZATION OF SINGLE CHANNEL NICOTINIC ACETYLCHOLINE RECEPTOR CURRENTS RECORDED FROM TE671 HUMAN MEDULLOBLASTOMA CELLS. Robert E. Oswald, Roger L. Papke, & Ronald J. Lukas†. Department of Pharmacology, Cornell University, Ithaca, New York, USA and †Division of Neurobiology, Barrow Neurological Institute, Phoenix, Arizona, USA.

Acetylcholine (ACh)-gated single channel events were studied on the TE671 human medulloblastoma clonal cell line by the use of the cell-attached patch clamp technique. Channel activity was detected (96% probability) in the presence of 0.1 to 1 μM ACh but not (0% probability) in the absence of agonist or in the presence of 1 μM αbungarotoxin (αBgt). The effect of αBgt was reversible in that channels could be observed 30 min following the removal of unbound αBgt from the medium. This is consistent with binding experiments indicating that the dissociation of αBgt from the AChR of TE671 cells is much more rapid than its dissociation from AChRs of skeletal muscle origin. The most prominent channel type had a conductance of 50 pS. The mean channel lifetime and burst duration were measured in the range of 0.1 to 2 μM ACh. At all concentrations, the channel lifetime and burst durations were distributed as a sum of two exponentials, with time constants for burst duration of 100 μsec and 15 msec. The percentage of long duration bursts increased with increasing concentration of ACh. These findings are similar to those observed with muscle AChRs and are qualitatively consistent with the notion that the short openings represent singly liganded channels and the long openings represent doubly liganded channels. Closed time distributions exhibited two exponentials at 100 nM ACh and three exponentials at higher ACh concentrations. The additional component in the closed time distribution was consistent with clusters of bursts of channel openings. These results suggest that the AChR from the TE671 neuronal cell line share many of the characteristics of skeletal muscle AChRs, with the exception of binding αBgt in a reversible fashion. One attractive possibility is that the AChRs expressed by TE671 are one of a family of neuronal nicotinic AChRs, a subset of which are sensitive to αBgt.

T-Pos245 **DIVALENT CATION MODULATION OF NICOTINIC RECEPTOR CHANNELS.** H.A. Hartmann and R.E. Sheridan, Department of Pharmacology, Georgetown University Medical Center, Washington, DC 20007.

A previous study in frog muscle has shown that increasing concentrations of extracellular divalent cations increased the decay time constant and decreased the maximum amplitude of the miniature endplate current at all voltages studied (Cohen and Van der Kloot, Nature 271: 77-79, 1978). The divalent cations shifted the voltage dependence of receptor open time and these results suggested a screening of membrane surface charge by the divalent cations. These data are also consistent with the idea that divalent cations are less permeable to channels than Na^+ or K^+ and their prolonged occupancy in channels would delay channel closing and reduce ion flux through the open channels. We have reexamined this phenomenon at the single channel level to differentiate surface charge effects from a possible channel block by the divalent cations. Patch clamp recordings were performed in inside-out patches of the mouse clonal cell line, BC_3H_1. Single nicotinic receptors were activated by acetylcholine or carbachol. The reduction of extracellular divalent cation concentrations reduced the mean open time and increased the conductance of the open channel. Conversely, an increase in the concentration of divalent cations at the intracellular surface of the membrane patch also reduced the mean open time of the open channel. Unlike the effects on open channel lifetime, the effects of divalent cations did not depend on the side of membrane of application. Open channel conductance in these inside-out membrane patches was reduced whenever the divalent cation concentration was increased either intracellularly or extracellularly. The effects of mean open time of the nicotinic receptor channel are consistent with the prediction of the surface charge model of this phenomenon. (Supported by NIH Grant No. 1 R01 NS22958)

T-Pos246 SINGLE CHANNEL ANALYSIS OF THE RESPONSE TO ACH AND GLUTAMATE OF CULTURED INSECT NEURONES. M.Amar, H.G.Horseman, D.J. Beadle and Y.Pichon.C.N.R.S. Lab. Neurobiol. cell. Molec., Gif sur Yvette (France); School Biol. Molec. Sci., Oxford Polytechnic, Oxford (England).

Insect neurones are sensitive to externally applied Ach and glutamate. The single channel events associated with this sensitivity were analysed on cultured cockroach neurones. The cell attached configuration of the patch-clamp technique was used to study the effects of 10^{-6} to 10^{-5}M concentrations of the two agonists. In the presence of Ach, short inward currents were observed with a mean open time of 0.3 ± 0.17 ms (N=321) and 0.29 ± 0.12 ms (N=410) and mean intensities of 1.67 ± 0.37 pA and 3.0 ± 1.2 pA at a holding potential 20 mV more negative than resting potential (H20). The reversal potential of these unitary currents extrapolated from the current-voltage relationship approximated -65 mV relative to the resting level (D65), not significantly different from the estimated absolute 0 mV potential of these cells. The open time distribution of the Ach gated channels could be fitted with two exponentials. The time contant of the fast component was too short to be measured with accuracy. The slow component (0.15 ms at H20) was found to decrease linearly with membrane depolarization between H80 and D20 (slope of 0.06 ms/100 mV, R= 0.745) . Addition of glutamate to the patch pipette also induced fast inward currents with a mean amplitude of 2.52 pA at H20 and mean durations of 0.27 ms (3 cells) or 0.95 ms (1 cell). The reversal potential of these events lied around -60 mV relative to the resting level. The open time distribution could be fitted with a single exponential the time constant of which showed little if any voltage sensitivity (mean values: 0.230 ± 0.14 ms for 3 cells, 0.322 ± 0.2 ms for 1 cell).