M-Pos207 ACRIDINE ORANGE BINDING TO RENAL BRUSH BORDER MEMBRANE VESICLES. Eric G. Holmberg and James A. Dix, Department of Chemistry, State University of New York, Binghamton, NY 13901

The fluorescent compound, acridine orange, has been used to examine the dissipation of proton gradients in a variety of membrane systems. We have studied the binding of acridine orange to brush border membrane vesicles (BBMV) isolated from the proximal tubule of rabbit kidney in the absence of a proton gradient. Equilibrium binding experiments revealed an large nonsaturable component of binding. Stopped-flow experiments at low acridine orange concentration (<2.5 uM) revealed a biexponential increase in acridine orange fluorescence, characterized by concentration-independent time constants of 0.14 sec and 1.74 sec. Stopped-flow experiments at higher acridine orange concentrations revealed a fluorescence decrease with time, with concentration-independent time constants of 0.039 and 0.78 sec. We have analyzed our equilibrium and stopped-flow binding data in terms of a lipid solubility model. Analysis of the equilibrium binding data in terms of this model gives a BBMV lipid:buffer partition coefficient of 400. In terms of this model, the stopped-flow data indicates that acridine orange undergoes a fluorescence enhancement when bound to membrane lipid, but that the fluorescence is self-quenched at high acridine orange concentration in membrane lipid. The biphasic binding time course suggests differing rates for partitioning of the monomeric and multimeric forms of acridine orange between buffer and membrane. Our data suggest that the discrepancy between calculated intravesicular volume, obtained from calibration curves of fluorescence vs pH gradient, and actual intravesicular volume is due to a large reservoir of nonfluorescent acridine orange in the BBMV membrane. Supported by NIH HL29488.

M-Pos208 THE ROLE OF CHOLESTEROL IN THE ACTIVITY OF RECONSTITUTED Ca-ATPase VESICLES CONTAINING UNSATURATED PHOSPHATIDYLETHANOLAMINE. Kwan-Hon Cheng*,James R. Lepock+,Sek Wen Hui*and Philip L. Yeagle# (*Biophysics Dept.,Roswell Park Memorial Instit.,Buffalo,+Physics Dept.,U.of Waterloo,Ontario,#Biochemistry Dept.,SUNY)

The effect of cholesterol on the activity of sarcoplasmic reticulum Ca-ATPase was examined,using reconstituted systems containing varying proportions of unsaturated soybean PE,egg PC,and cholesterol. The cholesterol-to-phospholipid molar ratio (from 0.1 to 0.3) of the reconstituted proteoliposomes was controlled by the addition of cholesterol to the exogenous PE/PC lipid mixtures used in reconstitution. A positive PE dependence of the Ca-uptake and coupling efficiency (Ca/ATP) was found in those proteoliposomes with low cholesterol content. The coupling efficiency of proteoliposomes increased with the cholesterol content at each PC/(PC+PE) ratio of proteoliposomes and became more pronounced at high PE. Fluorescence measurements of the incorporated lipophilic probe, DPH,revealed a decrease in the motion and an increase in the order of the phospholipid fatty acyl chains in proteoliposomes with high membrane cholesterol content. A complementary observation was made using ESR spin label 2N14. Freeze-fracture electron microscopy studies on proteoliposomes containing 20% PC/(PC+PE) molar ratio and cholesterol/phospholipid molar ratio of 0.1 or 0.3 revealed predominantly smooth vesicular structures. It is postulated that the cholesterol-induced enhancement of the Ca-transport function of the Ca-ATPase is related to the bilayer-destabilization effect of the cholesterol molecule as revealed by P-31 NMR,which may reflect changes in surface hydration.

M-Pos209 PHOSPHOLIPID TRANSFER BETWEEN LIPOSOMES & CELL MEMBRANES: STUDIES OF AMINOPHOSPHOLIPID ASYMMETRY IN ERYTHROCYTES & PLATELETS. W.H. Huestis, D.L. Daleke, J.E. Ferrell, Jr., & K.J. Lee, Department of Chemistry, Stanford University, Stanford, CA 94305.

Phospholipid (PL) transfer from sonicated vesicles to erythrocytes was studied as a function of PL headgroup and acyl chain composition. The kinetics of transfer were affected profoundly by the lipid acyl chain composition. Homologous saturated phosphatidylcholines (PCs) transfered at rates that decreased exponentially with increasing acyl chain length. Short acyl chain PCs also transfered rapidly between cells. The kinetics of these processes are consistent with lipid monomer transfer through the aqueous phase.

Uptake of exogenous PC converted discoid cells into spiculate echinocytes, consistent with lipid incorporation into the expansion of the membrane outer monolayer. Short acyl chain aminophospholipids (phosphatidylserine (PS) and phosphatidylethanolamine (PE)) also induced transient cell spiculation, followed by rapid reversion to discoid and then indented shapes (stomatocytes). Longer acyl chain PSs induced stomatocytosis without initial spiculation. In all cases, PS- and PE-induced stomatocytosis depended on intracellular Mg^{+2} and ATP. Resting platelets exposed to PS vesicles also became transiently spiculate and then reverted to indented shapes. These observations provide no direct demonstration of the transbilayer distribution of incorporated foreign lipids, but they are consistent with insertion of the lipids into the membrane outer monolayer (at rates dictated by their acyl chain compositions) followed by translocation and sequestration of the aminophospholipids in the inner monolayer. This is evidence for a headgroup-specific lipid translocation mechanism that would be expected to generate phospholipid asymmmetry in erythrocyte and platelet membranes. Supported by USPHS Grant HL 23787.

M-Pos210 ENERGETICS OF THE HEXAGONAL PHASE FORMED BY PHOSPHOLIPIDS IN WATER.
S. M. Gruner, Princeton University, Princeton, NJ 08544; V. A. Parsegian, N.I.H., Bethesda, MD 20205; R. P. Rand, Brock University, St. Catharines, Ont. L2S 3A1.

It has become apparent during the past several years that small perturbations of water solvent even several layers away from a polar surface are what dominate interactions when these surfaces approach contact. Since removal of boundary water can perturb surface structure, we expect aqueous cavities of the inverted hexagonal phase to contain such perturbed water and cavity structure to depend on solvent activity. By the combined application of osmotic stress and x-ray diffraction, we have been attempting to measure the interactions governing the formation of H_{II} phases of dioleylphosphatidylethanolamine (DOPE) and of DOPE/dioleylphosphatidylcholine (DOPC) mixtures in the presence of added dodecane or tetradecane. The alkane allows the formation of large intrinsic radii of curvature of the mixed lipid monolayer lining the cavity. Cylinder diameter and monolayer curvature, intercylinder distance and molecular area are determined as we osmotically remove water from the system and measure the energy. Near full hydration, the work required to remove water is accurately described by $W = K((1/r) - (1/r_0))^2$ where r_0 is a well defined intrinsic curvature and K a bending modulus or rigidity. Beyond a 40% reduction in radius, less rather than more work is required compared to that predicted by this quadratic form. This suggests a softer barrier to lipid packing at small radii of curvature than might otherwise be expected.

M-Pos211 **NEUTRON DIFFRACTION STUDIES OF LIPID MULTILAYERS WITH CARBOHYDRATE-HEADGROUPS.**
Robert V. McDaniel. Department of Physiology and Biophysics, SUNY at Stony Brook, Stony Brook, NY 11794 and Department of Biology, Brookhaven National Laboratory, Upton, NY.

When hydrated with H_2O or D_2O, digalactosyldiglyceride (DG) and phosphatidylinositol (PI) form oriented lamellar arrays on quartz slides. At the high flux beam reactor (Brookhaven), I obtained 4 and 7 orders of discrete neutron diffraction from DG and PI, respectively. The structure factors were phased by D_2O/H_2O exchange, and used to calculate neutron density profiles at 0.75-1.0 nm resolution. The density profiles, continuous transforms, and water profiles were used to calculate the water-spacing between bilayers. When the relative humidity increases from 15% to 86%, the water-spacing in DG multilayers increases as predicted by Parsegian and Rand's theory of hydration repulsion. However, the water-spacing in PI multilayers is insensitive to the relative humidity. These results are explained with a structural model of the carbohydrate-headgroups, where the inositol groups of PI (in distilled water) are extended and the digalactosyl groups of DG are "laying down" on the surface. These results are consistent with the findings of others that the headgroups of neutral glycolipids have low hydration (Weislander et al: diglucosyldiglyceride. Quinn, Sen, Hui et al: DG) and are hydrogen-bonded to the surface (Pascher et al; Ruocco, Shipley et al: cerebroside), while charged glycolipids have headgroups that extend from the bilayer (McDaniel and McIntosh: ganglioside). An extended orientation of inositol in PI is consistent with the inability of free inositol to hydrogen-bond with the surface of lipid membranes (Crowe et al). Supported by NIH grant GM24971 to S. McLaughlin and by the US Department of Energy. The author thanks B. Schoenborn, A. Saxena, J. Schafer, and G. King for the use of the facilities at BNL.

M-Pos212 THE ROLE OF WATER AND CHARGED LIPIDS IN THE LIPOSOMAL ENCAPSULATION OF CHARGED MOLECULES. Martha C. Farmer and Sue A. Johnson, Naval Research Laboratory, Code 6190 Bio/Molecular Engineering Branch, Washington DC 20375-5000.

In studies of the encapsulation of hemoglobin (Hb) by extrusion of an aqueous Hb/phospholipid/cholesterol dispersion, the concentration of Hb [Hb] inside the liposomes was always substantially less than the [Hb] in the precursor solution. Above 3-4 mM Hb (tetramer) the ratio of encapsulate [Hb] to precursor [Hb] decreases as the precursor [Hb] is increased. Theoretically, water for hydration of lipid and protein must be limiting at some point. If water is limiting, an increase in the lipid to Hb ratio should have a similar effect on the ratio of encapsulate [Hb] to precursor [Hb] at high Hb concentrations, but not in the lower concentration range. This hypothesis was substantiated; an increase in the lipid to Hb ratio did result in a decreased ratio of encapsulate [Hb] to precursor [Hb].

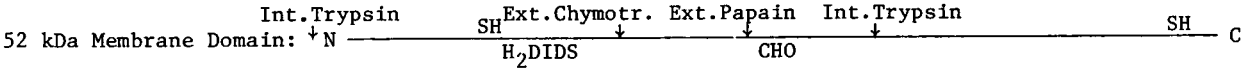
A second major factor influencing encapsulation efficiency is the presence of charge on the lipid membrane. To diminish the potential for serum Ca^{++} or protein induced fusion of liposomes encapsulating hemoglobin, the amount of negatively charged lipid had been held to a minimum, 5 to 10 % of the total lipid. Evidence exists (H. Hauser, 1984, BBA 772:37) that in aqueous dispersions of multi-lamellar liposomes, the distance between the lamellae (aqueous space) was proportional to the percentage of charged lipid, and encapsulation of water-soluble materials was increased by increasing the inter-lamellar distance. Our data indicate that the nature of the polar lipid is also a factor. In initial experiments dimyristoyl phosphatidylglycerol (DMPG) encapsulates Hb and carboxyfluorescein more effectively than does phosphatidic acid (PA) or dicetyl phosphate (DCP), though PA is superior to DMPG or DCP for encapsulating glucose. The results have obvious ramifications for drug delivery systems involving DNA, enzymes, or other macromolecules as well as small hydrophyllic drug molecules.

M-Pos213 STABILITY OF LIPOSOME-ENCAPSULATED OXYHEMOGLOBIN IN PLASMA. J. Kurantsin-Mills, L.J. Fujimoto, L. Leondaridis, R. Muesing. Depts. of Medicine and Physiology, The George Washington University Medical Center, Washington, D. C. 20037

Liposome-encapsulated oxyhemoglobin (hemosome) is a potential erythrocyte surrogate because it may be non-toxic and non-immunogenic. To be useful, hemosomes must be stable in plasma for O_2 delivery to tissues. We have prepared bath-sonicated multilamellar hemosomes from mixtures of phosphatidylcholine (PC) or sphingomyelin (SM) or phosphatidylethanolamine (PE) and cholesterol (Chol) with phosphatidic acid (PA) by entrapping purified oxyhemoglobin (OxyHb) in PBS, pH 6.4. Chloroform:methanol solutions of PC:Chol:PA or SM:Chol:PA or PE:Chol:PA, in molar ratios of 1:0.75:0.1, were vacuum-dried under N_2. OxyHb solutions were added to the dried lipids, the hemosomes briefly sonicated (5 min) and purified by washing and centrifugation. Relative diameters of similar fluorescein-loaded liposomes were determined by laser activated flow cytometer, based on forward light scattering principle. The efficiency of OxyHb entrapment, conversion to deoxyHb or met-Hb, as well as the stability of the hemosomal bilayers in plasma or lipoprotein-depleted plasma (LPDP) were determined spectrophotometrically. Hemosome size distribution was exponential, with greater than 75% averaging ~0.25 μm. The efficiency of encapsulation OxyHb was 15-60% for all phospholipids and showed a linear correlation with fluorescence. The mole % of cholesterol was essential for stability of the hemosomes. OxyHb released into the medium was converted to deoxyHb. Incubation of hemosomes (24 hr, 37° C) released 2.5%, 10%, and 13% of Hb into the buffer, plasma and LPDP, respectively. Met-Hb was less than 2% in all media. Appropriate lipid combinations should provide a model hemosome to mimic the erythrocyte.

M-Pos214 MONOCLONAL ANTIBODIES TO THE MEMBRANE DOMAIN OF HUMAN ERYTHROCYTE BAND 3 PROTEIN. M.L. Jennings, R. Monaghan, and M.P. Anderson, Department of Physiology and Biophysics, The University of Iowa, Iowa City, IA 52242

Six monoclonal antibodies (MAb) have been prepared against the anion-transporting domain (52 kDa) of human erythrocyte band 3 protein. One of the MAb (IVF12) binds to the C-terminal 28 kDa papain fragment. All the other MAb bind to sites within 6 kDa of the N-terminus of the membrane domain. Because these antibodies bind to specific segments of the band 3 primary sequence, they can be of use in studies of the protein structure. Cleavage of the isolated membrane domain by S-cyanylation produces an N-terminal 11 kDa fragment that binds MAb. The same 11 kDa fragment is produced even when 98% of the copies of band 3 have been internally cross-linked with H_2DIDS. Therefore, the site of H_2DIDS reaction is not on this N-terminal S-cyanylation fragment. The MAb also demonstrate that there must be one or more SH groups within 5 kDa of the C-terminus of band 3. The S-cyanylation shows that MAb IVF12 binds to a site within about 5 kDa of the C-terminus. We have used IVF12 to demonstrate that intracellular trypsin, at low ionic strength, cleaves band 3 at a site 22 kDa from the C-terminus. This is the first intracellular cleavage site that has been localized in the membrane domain of band 3. The studies also indicate that the site of glycosylation is 22-28 kDa from the C-terminus. The findings presented here are summarized in the diagram below. (Support: NIH GM26861)



M-Pos215 ACTIVATION VOLUMES FOR ANION EXCHANGE IN HUMAN RED CELLS: BICARBONATE AND SULFATE. Victor A. Canfield and Robert I. Macey, Department of Physiology-Anatomy, University of California, Berkeley, 94720.

We have previously shown that sulfate equilibrium exchange in human red cells has an extremely large activation volume ($\Delta V^{\ddagger}$) of approx. +150 cm^3/mol (Biochim. Biophys. Acta 778:379-384, 1984). We now obtain similar values for $\Delta V^{\ddagger}$ with $SO_4^{=}$ concentrations in the range 30 to 205 mM, indicating that sulfate binding does not contribute significantly to the observed $\Delta V^{\ddagger}$. Since $\Delta V^{\ddagger}$ is independent of pressure (range 0.1 to 83 MPa), we argue that reversible denaturation and subunit dissociation are not responsible for the large $\Delta V^{\ddagger}$.

We have recently extended these results using a second method in which pH equilibration via the Jacobs-Stewart cycle is used as a measure of anion transport. The results confirm our previous estimate for sulfate with estimates of $\Delta V^{\ddagger}$ near +140 cm^3/mol. We have also obtained measurements for bicarbonate transport (in chloride media) which are in the range +70 to +85 cm^3/mol.

We propose that volume changes associated with changes in the conformation of the transporter during its cycle make a major contribution to $\Delta V^{\ddagger}$ for both HCO_3^- and $SO_4^{=}$ transport. The difference between activation volumes for $SO_4^{=}$ and bicarbonate probably results from binding of the H^+ that is cotransported with $SO_4^{=}$. Since this difference exceeds the ΔV for protonation of small molecules (<30 cm^3/mol), it is likely that protonation is also accompanied by changes in conformation.

M-Pos216 TWO-STAGE BINDING OF BAND 3, THE ANION EXCHANGE PROTEIN OF HUMAN ERYTHROCYTES, TO A MATRIX-BOUND INHIBITOR. Sanjay W. Pimplikar and R.A.F. Reithmeier, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7.

The Band 3 polypeptide of the human erythrocyte membrane catalyzes the exchange of anions across the plasma membrane. We have prepared an affinity column by attaching an anion transport inhibitor, 4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonate (SITS; Ki=10 μM) to Affigel-102 matrix. Band 3 binds specifically to the affinity matrix via its inhibitor binding site. When Band 3 was bound to the affinity matrix at 4°C for 15 to 120 min., up to 80% of the bound protein could be eluted by 1 mM 4-benzamido-4'-aminostilbene-2,2'-disulfonate (BADS Ki=1 μM). The remainder of Band 3 could be eluted under denaturing conditions, using 1% lithium dodecyl sulfate (LDS). The amount of Band 3 eluted by BADS was reduced when the experiment was carried out at 37°C with a concommitent increase of Band 3 in the LDS elute. Band 3 bound to the gel at 4°C could no longer be eluted by BADS if the matrix-bound protein was incubated at 37°C for 5 min. This tightly-bound Band 3 could be eluted with 1% LDS. Blocking the inhibitor binding site of one monomer of the Band 3 dimer by 1-ethyl-3-(4-azonia-4,4-dimethylpentyl)carbodiimide did not prevent Band 3 from becoming tightly bound as a result of exposure to 37°C. This suggests that the tight binding is not due to both monomers of the Band 3 dimer binding to the gel as a result of exposure to 37°C. These results support the view that stilbene disulfonate binding to Band 3 is followed by a temperature-dependent conformational change that locks the inhibitor ligand in place. (Supported by the Medical Research Council of Canada, Alberta Heritage Foundation for Medical Research and the Alberta Heart Foundation)

M-Pos217 EFFECTS OF PHLORETIN AND DNDS ON THE CHLORIDE CONDUCTANCE (TUNNELING) IN ERYTHROCYTES. O. Fröhlich, D. T. Bain and L. H. Weimer. Department of Physiology, Emory University School of Medicine, Atlanta GA 30322.

That the chloride conductance of the human red cell membrane is mediated by the same protein that mediates chloride exchange (the Band-3 protein) has been shown qualitatively in a number of studies using different inhibitors. Here we examined how closely related the two pathways are kinetically, in order to test whether anion exchange and net transport by tunneling occur through the same physical pathway in the band-3 protein. For this we tested the inhibitory effects of the competitive inhibitor dinitrostilbene disulfonate (DNDS) and the mixed inhibitor phloretin. We have previously shown that these two inhibitors mutually exclude each other in their binding to the anion transporter. As in chloride exchange, DNDS acts as a competitive inhibitor of chloride net efflux, exhibiting a decreasing inhibitory potency with increasing extracellular chloride concentrations. On the other hand, phloretin, at nominal concentrations up to 20 μM, has no effect on chloride net efflux into media containing 0 or 150 mM chloride, but it reduces the inhibitory potency of DNDS. These phloretin effects provide strong support of our notion of net flux by tunneling in which chloride moves through the same region in band-3 as during exchange, but without the need of a carrier-type conformational change of the protein. Since phloretin acts by preventing this conformational change and not by preventing chloride from binding, one would expect it not to inhibit chloride tunneling. By competing with DNDS for binding to band-3 without itself affecting tunneling, it protects the anion conductance against inhibition by DNDS. (Supported by USPHS GM31269)

M-Pos218 ACTIVE ESTERS OF N-HYDROXYSULFOSUCCINIMIDE AND L-LACTATE TRANSPORT IN RABBIT RED BLOOD CELLS by JERALD A. DONOVAN, Dept. of Physiology and Biophysics, University of Iowa, Iowa City, IA

Esters of N-hydroxysulfosuccinimide strongly inhibit L-lactate transport in rabbit erythrocytes, probably by acylating amino groups on the transport protein. Lactate transport studies using bis-(sulfosuccinimidyl) suberate (BS^3), bis(sulfosuccinimidyl) adipate (BS^2A), dithiobis(sulfosuccinimidylproprionate) (DTSSP), and a variety of monocarboxylate esters suggest that an exofacial amino group on the lactate transport protein is essential for lactate transport. Also, reductive methylation studies show that even when positive charge on modified amino groups is preserved, the transport is strongly inhibited. At pH < 6, Band 3-mediated inorganic anion transport is enhanced in BS^3-treated cells, while at pH > 6, it is inhibited. BS^3-induced inhibition of L-lactate transport does not have this pH dependence. BS^3 reduces the labeling of a 40-50 kDalton membrane polypeptide (Band R) by tritiated 4,4'-diisothiocyanato-2,2'-dihydrostilbenedisulfonate ($(^3H)H_2DIDS$ and by tritiated bis(sulfosuccinimidyl) adipate ($(^3H)BS^2A$). Tritiated sulfosuccinimidyl acetate ($S^2(^3H)$acetate) also labels Band R over a range of concentrations where lactate transport is inhibited in a dose-dependent manner by S^2acetate. BS^3 is a known impermeant protein cross-linker. S^2acetate permeates rabbit red cell membranes by an H_2DIDS-inhibitable mechanism. These labeling experiments support an association between Band R and specific monocarboxylate transport. (Supported by N.I.H. grants GM 26861 and GM 07337.)

M-Pos219 Lio/Hi AND Li/Na EXCHANGE IN HUMAN RED CELLS. EFFECT OF H+ GRADIENTS. Mitzy Canessa, A. Spalvins and Nelson Escobales. Endocrine Hypertension Dept., Brigham and Women's Hosp, Boston, MA 02115 and Dept of Physiology, Univ. of Puerto Rico, San Juan, PR.

The present experiments were designed to test the hypothesis that the Na/H exchanger that we previously described in human red cells can perform Li/H and Li/Na exchange. Li influx (mmol/l cell xhr=FU) into DIDS-treated, acid-loaded cells (pHi=6.5) was measured incubating in (mM):75 LiCl, 75 KCl, 0.1 ouabain, 0.01 bumetanide, 40 sucrose and 10 Tris-Mes (pHo=6) or 10 Tris-Mops (pHo=8), w/o 0.1 phloretin (PL) and/or 1 amiloride (AM). Li influx at pHo=6 was 0.79 ± 0.17 (M+SD, n=4) and was insensitive to PL or AM. When an outward H+ gradient was imposed (pHo=8), Li influx increased to 3.2± 1.3 FU. The pHo-stimulated Li influx (2.3 FU) was partially inhibited by AM but not by PL. Li influx into alkaline loaded cells(pHi=7.8) was also stimulated by a rise in pHo from 6 to 8 (1 FU) and inhibited by PL but not AM, suggesting that is driven by a Nai/Lio exchange. H+ efflux from acid loaded cells into an unbuffered PL, medium containing K was 9.2 0.5 and 11.5 + 1 FU into Li medium, indicating that an inward Li gradient can drive H movement (2.3± 1 FU, n=5) which could be inhibited by 60 uM amiloride analog. At, Nao=0, Li efflux from 25 mM Li loaded cells was stimulated by a raise in pHi=pHo= from 6 to 8 (1.2 FU), the imposition of an outward H+ gradient, and inhibited by an inward H gradient and amiloride, suggesting the presence of a Li-H cotransport pathway. Li/Na exchange was inhibited by pHi=pHo=6 and an inward H+ gradient while pHi=pHo=8 an outward H+ gradient, stimulated. Therefore, Li can be transported by the Na/H and Li/Na exchanger, both sensititive to competitive and thermodynamic effects of H+.

M-Pos220 INVOLVEMENT OF A CYTOPLASMIC PROTEIN IN CALCIUM-DEPENDENT POTASSIUM TRANSPORT IN HUMAN RED BLOOD CELLS. G.A. Plishker, P.H. White and L.S. Seinsoth. Baylor College of Medicine, Houston Texas 77030

In the human red blood cell, Ca-dependent K transport appears to be regulated by a 23,000 dalton (23K-Da) cytoplasmic protein that associates with the membrane in the presence of calcium. The amount associated with the membrane increases with cellular calcium. This effect can be demonstrated by lysing and washing the cells with calcium-containing buffers, increasing the intracellular calcium, or utilizing cells which naturally contain higher calcium, as in sickle cells. Most of the 23K-Da protein is removed when ghosts are prepared from normal cells using hypotonic buffers. However, with cells that have increased intracellular calcium, more of the 23K-Da protein is retained with the membranes after washing with hypotonic buffers. The carboxymethylation of the protein correlates directly with the inhibition of the Ca-dependent K transport. This is not a secondary effect involving a reduction in free intracellular calcium as inhibition is also found in calcium-buffered ghosts. Antibodies raised against this 23K-Da protein inhibit Ca-dependent K transport in resealed ghosts. The dose dependency of this inhibition resembles a typical antibody-antigen interaction. The loss of potassium was inhibited at all dilutions of antisera. The inhibition increases as the serum concentration increases from a 1:10,000 to a 1:1,000 dilution. The inhibition decreases as the serum dilution decreases from 1:1,000 to 1:100. This is typical of reactions that are dependent upon the ratio of antigen to antibody rather than the level of antibody. The incorporation of pre-immune serum had no effect on Ca-dependent K transport. Supported by NIH grants NS-11535 and GM-30594.

M-Pos221 NITROBENZYLTHIOINOSINE-SENSITIVE AND NITROBENZYLTHIOINOSINE-INSENSITIVE NUCLEOSIDE TRANSPORT BY RAT ERYTHROCYTES. S.M. JARVIS (Intro. by M. Poznansky), Department of Physiology, University of Alberta, Edmonton, Canada T6G 2H7.

The sensitivity of nucleoside transport by rat erythrocytes to inhibition by nitrobenzylthioinosine (NBMPR) and p-chloromecuriphenyl sulphonate (PCMBS), was investigated. The dose response curve for inhibition of uridine transport was biphasic - 35% of the transport activity was inhibited with an IC_{50} value of 0.25 nM, and the remaining activity was insensitive to concentrations as high as 1 μM. These two components are defined as NBMPR-sensitive and NBMPR-insensitive, respectively. Uridine influx by both components was saturable and inhibited by other nucleosides. The affinity of the NBMPR-sensitive component was 3-fold higher than that of the NBMPR-insensitive mechanism (apparent Km for uridine 50 ± 18 and 163 ± 28 μM, respectively). The two transport systems also differed in their sensitivity to inhibition by PCMBS. NBMPR-insensitive uridine transport was inhibited by PCMBS with an IC_{50} of 25 μM, while 1 mM PCMBS had little effect on NBMPR-sensitive transport by intact cells. PCMBS inhibition was reversed by the addition of β-mercaptoethanol, and uridine was able to protect the transporter against PCMBS inhibition. NBMPR inhibition of transport was associated with the presence of high-affinity [^{3}H]NBMPR binding sites (apparent Kd 46 ± 25 pM). Exposure of rat erythrocyte membranes to UV light in the presence of [^{3}H]NBMPR resulted in covalent radiolabelling of a membrane polypeptide (apparent Mr 62,000). These results demonstrate the existence of two facilitated-diffusion systems in rat erythrocytes which differ in their sensitivity to inhibition by both NBMPR and PCMBS. (SMJ is an AHFMR Scholar. Supported by MRC Canada).

M-Pos222 HUMAN PLATELET OSMOTIC WATER AND NON-ELECTROLYTE TRANSPORT. Mary M. Meyer and A.S. Verkman, Cardiovascular Research Institute, Univ. of Cal., San Francisco, CA 94143

Stopped-flow light scattering was used to characterize the osmotic water (P_f) and non-electrolyte (P_s) permeability properties of human platelets. Mean platelet volume (V) determined by impedence sizing was 7 μm^3; V was linear with Osm^{-1} of solution NaCl (0.5-1.5 Osm^{-1}) with an extrapolated non-osmotic volume of 3 μm^3. Mixture of platelets with hyper/hypo osmotic buffers in a stopped-flow apparatus resulted in a monophasic time course of increasing/decreasing scattered light intensity. At 37°C, P_f = (7±1) x 10^{-3} cm/s based on a 44,700 cm^{-1} surface to volume ratio. P_f did not depend on osmotic gradient size or flow direction. The activation energy (E_a) for P_f was 4.4 kcal/mole (<36°C) and 25 kcal/mole (>36°C). Mixture of platelets with a hyperosmotic solution containing a permeant non-electrolyte resulted in water efflux followed by solute influx. At 37°C, P_s (cm/s x 10^{-6}) was 2.1 (urea), 3.5 (glycerol), 3.8 (thiourea), 17 (ethylene glycol), 18 (acetamide), 23 (formamide) and 24 (butryamide). σ_{urea}, determined from the solution urea concentration required to give zero initial volume flow, was 0.95±0.04. Platelet P_f, E_a, P_{urea} and σ_{urea} are similar to values known for pure lipid bilayers. In addition P_f and P_{urea} were not inhibited by pCMBS, $HgCl_2$, thiourea or phloretin. Therefore, in contrast to the red cell, platelet osmotic water and urea transport occurs primarily by non-facilitated diffusion through membrane phospholipid. A computer model of platelet circulation through the renal medulla, based on measured P_f, P_{urea} and σ_{urea} indicated that platelets undergo a 40% decrease in volume in the inner medulla and a 20% overshoot in volume as they return to the external isosmotic environment.

M-Pos223 THE DEGRADATION OF SICKLE ERYTHROCYTES INDUCED BY OXYGENATION-DEOXYGENATION CYCLES. K. Horiuchi and T. Asakura. The Children's Hospital of Philadelphia, Department of Pediatrics and Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA19104.

The precise mechanism for damage to the red cell membrane caused by repetitive sickling and desickling has not been well elucidated. Using a device by which red cells can be exposed to cycles of oxygenation and deoxygenation, we investigated the effect of the calsium ion on the density of red cells from patients with HbSS (SS cells) after repeated exposure to such cycles. We found that SS cells from the top layers moved to the bottom layers when these cells were subjected to oxy-deoxy cycles in the presence of 2 mM Ca^{2+} for 4 hr. The density of these cells was slightly less than that of those in the irreversibly sickle cell (ISC)-rich fraction. After 16 hr. of exposure of oxy-deoxy cycles, however, most SS cells returned to the top layers, while a portion (~10%) increased in density, having the same density as those cells in the ISC-rich fraction. It was also found that in the presence of Ca^{2+}, most cells from the bottom layers were hemolyzed after 16 hr. exposure to oxy-deoxy cycles. Under microscope we observed aggregates of deformed cells and ghosts. Such aggregates formed in vivo could initiate vasoocclusive crises. In experiments done in the absence of Ca^{2+}, cell density did not change significantly. The density of red cells incubated under either an oxy or deoxy environment did not change significantly, regardless of the presence or absence of Ca^{2+}. (Supported by NIH grants HL-20750 and HL-18226)

M-Pos224 DIFFUSIVE AND OSMOTIC WATER PERMEABILITY OF MODIFIED RED CELL MEMBRANES. J. Brahm, T. Sisneros, and W.R. Galey, Department of Biophysics, University of Copenhagen, Copenhagen, Denmark and Department of Physiology, University of New Mexico, School of Medicine, Albuquerque, NM

Both the osmotic water permeability (P_f) and diffusive water permeability (P_d) of human red cells are greater than those of lipid bilayers. This coupled with other facts such as the inhibitability of a portion of the water permeability by PCMBS (Naache & Sha'afi, 1974) has led many investigators to believe that there are two pathways by which water penetrates the cell membrane. One of these is through the lipid phase of the membrane and the second through "aqueous pores", the aqueous pathway being composed of integral membrane proteins. Some have even proposed that the membrane protein Band 3, forms a 9 Å aqueous pathway which not only mediates the exchange of anions but also provides a path for cations and water (Solomon et al 1983). We have studied the effects of several enzymes which degrade Band 3, inhibit anion exchange, and/or inhibit water permeability in order to ascertain the similarities and differences in susceptability of the two water permeabilities and anion transport to these agents. Although treatment of red cells with papain and chymotrypsin fragments Band 3 and inhibits anion transport by >80% (Jennings 1980) P_f and P_d are not inhibited. Further, although treatment of red cells with 0.5 mM DIDS inhibits anion transport by ~98% it does not change P_f or P_d. There was no effect of the sulfhydryl reacting agent DTNB on either P_f or P_d but there was a significant and parallel chronological inhibition of both with PCMBS. Both of these agents have been reported to be inhibitors of the water permeabilities. From these studies we conclude that diffusive and osmotic water permeabilities share at least one common pathway but their link to the Band 3 protein is not clearly demonstrated.

M-Pos225 EFFECT OF LANTHANUM ON THE RHEOLOGIC BEHAVIOR OF HUMAN RED BLOOD CELLS R.M. Bauersachs, L. Castro and H.J. Meiselman (Intr. by R.A. Farley), Department of Physiology and Biophysics, USC School of Medicine, Los Angeles, CA 90033.

Although lanthanum (La) is known to have several effects on the human red blood cell (RBC) (e.g., inhibition of Ca-efflux and Ca-Mg-ATPase below 0.25 mM, with cell aggregation, hemolysis and fusion occurring at higher concentrations), its influence on the rheologic behavior of RBC has not yet been explored. The present study was thus designed to evaluate the effects of La (0-0.2 mM) on RBC suspension rheology (Contraves viscometer), RBC deformability (cone-plate Rheoscope) and membrane mechanical properties (micropipette techniques). Fresh human RBC were washed twice in an isotonic, Ca-free HEPES buffer, incubated for 10 min with various La concentrations, then subjected to the rheological tests. Compared to La-free controls, salient results included: 1) no change in cell shape, volume or hemoglobin concentration; 2) higher (40-80%) low shear suspension viscosity, with only minimal effects at higher shear rates; 3) decreased cellular deformability (30-70%) at low to medium stresses (7.5-25 dynes/cm^2), with smaller changes at the highest stress (125 dynes/cm^2). These effects were immediately reversible by resuspension in La-free medium or by the addition of EGTA. The micropipette experiments suggest that the La-induced changes are mainly due to an elevated membrane viscosity rather than to an alteration of the elastic shear modulus. In overview, these results indicate that extracellular membrane binding of La has profound effects on the rheology of RBC, perhaps via a glycophorin-ligand mechanism similar to that suggested for wheat germ agglutinin or for antiglycophorin A. Supported by NIH Research Grants HL15722 and HL15162 and by AHAGLAA Award 537IG.

M-Pos226 COLUMN CHROMATOGRAPHIC SEPARATION OF ERYTHROCYTES USING AQUEOUS POLYMERIC TWO-PHASE SYSTEMS. D.R. Skuse and D.E. Brooks. Departments of Pathology and Chemistry, University of British Columbia, Vancouver, B.C. V6T 1W5.

The use of aqueous polymeric two-phase systems for the separation of cells is well established, the most widely used system being formed by mixing dextran and poly(ethylene glycol) (PEG) solutions. In situations where the partition coefficients of the cells are similar an efficient separation is not obtained using a single-step procedure. In these cases a countercurrent distribution technique is used to achieve the required resolution. However its operation is limited by time and apparatus constraints. For macromolecular separations (e.g. DNA restriction enzyme fragments) a column chromatographic technique has been utilized, in which the more dense dextran-rich phase is immobilized onto agarose beads derivitised with poly(acrylamide) chains and the less dense PEG-rich phase is used as the eluent [Müller *et al*. Eur. J. Biochem., 128, 231 (1982)].

We have adapted Müller's approach to cell isolations and included cell affinity ligands consisting of PEG-fatty acid conjugates in the eluent. These produce separations based on the differential interaction between the fatty acid and cell membrane. A model study in which human and dog erythrocytes are separated by eluting with different concentrations of PEG-linoleate ester has been carried out. Supported by MRC of Canada.

M-Pos227 ELECTROPHORETIC STUDIES OF AGE-FRACTIONATED HUMAN RBC: EFFECTS OF DEXTRAN, PROTEIN AND CALCIUM BINDING S.O. Sowemimo-Coker and H.J. Meiselman, Dept. of Physiology and Biophysics, USC School of Medicine, Los Angeles, CA 90033

Previous reports from our laboratory have indicated differences in the aggregation behavior of density(i.e.,age) separated human red blood cells(RBC): the bottom 10%(BOT) RBC aggregate more than either the top 10%(TOP) or the unfractionated(UNF) population when suspended in dextran/buffer solutions or in autologous plasma. Since RBC aggregation is related to the extent of surface polymer binding and since RBC electrophoretic mobility provides an index of this binding, studies of the mobility of density-separated RBC in different media have been carried out. Fresh human RBC were density separated (12000g), resuspended in plasma, serum or dextran(T-70, 0-6gm%) solutions and measured at 25°C with a cylindrical electrophoresis apparatus. Salient results included: 1) no significant differences ($P>0.20$) between any of the RBC populations in buffered saline (mean=1.09±0.01 um/sec/v/cm); 2) higher mobilities for BOT RBC over the entire range of dextran concentrations (e.g., at 3gm%, BOT=2.14±0.06, UNF=2.07±0.05, TOP=2.03±0.05). Higher mobilities were also recorded for the BOT fraction in both plasma and serum. Further, electrophoretic analyses of calcium ion binding showed identical surface charge distributions for all RBC populations. These data thus indicate that old RBC exhibit higher binding tendencies for both dextran and proteins and are therefore consistent with the abovementioned aggregation results; they may also be relevant to the recognition and elimination of old RBC by macrophages. Supported by NIH Research Grants HL15722 and HL15162 and by AHAGLAA Award 537IG.

M-Pos228 SULFHYDRL REDUCING AGENTS AND SHAPE REGULATION IN HUMAN ERYTHROCYTES. H.-T. N. Truong, J. E. Ferrell, Jr. and W.H. Huestis. Dept. of Chemistry Stanford University, Stanford CA 94305

Metabolic crenation of red cells is reversible; on addition of nutrients echinocytes recover the normal discoid shape. When the shape recovery takes place in the presence of reducing agents such as dithiothreitol (DTT), morphological change continues until the cells are stomatocytic. The degree of stomatocytosis varies depending on the cell morphology when the nutrients and reducing agent are added. DTT has minimal effect on the shape of normal discocytes, but in its presence mildly echinocytic cells become slightly cupped, and advanced stage echinocytes become severely stomatocytic. DTT must be present continuously for development and retention of stomatocytosis; echinocytes preincubated with or metabolically depleted in DTT do not become stomatocytic when supplemented in the absence of DTT, and DTT-induced stomatocytes revert to discocytes when the reducing agent is removed. DTT has no effect on ATP synthesis or equilibrium cell glutathione levels, and the induced stomatocytosis is not inhibited by excluding oxygen from cells during depletion. Spectrin phosphorylation and phosphate turnover are not affected by DTT. The echinocyte-to-discocyte transformation coincides with phosphorylation of membrane inner monolayer lipids (diacylglycerol to phosphatidic acid and phosphatidylinositol to phosphatidylinositol-4,5-biphosphate). Overphosphorylation of these phospholipids is not responsible for the exaggerated shape recovery seen with reducing agents; phosphorylation of inner monolayer lipids proceeds identically in the presence and absence of DTT. (Supported by a grant from the National Institutes of Health (HL23787)).

M-Pos229 QUASI-ELASTIC LIGHT SCATTERING STUDIES OF MEMBRANE MOTION IN SINGLE RED BLOOD CELLS
Roy B. Tishler and Francis D. Carlson, Dept. of Biophysics, The Johns Hopkins University, Baltimore, MD 21218

The cell membrane has been identified as the primary source of the quasi-elastic light scattering (QELS) signal from the human red blood cell (RBC). Measurements of the intensity autocorrelation function of the scattered light were made using a QELS microscope spectrometer (Blank et al., in prep) with a He-Ne laser as the light source. The scattering volume had a cross sectional diameter of 8 um, allowing collection of scattered light from a single cell. RBC studies were made over a range of scattering angles (30^{o}-78^{o}) and sample times (10 us - 1 ms). The average decay rate, angular dependence and general shape of the autocorrelation function measured from these single cells differed from that found for hemoglobin samples at high concentrations (Jones et al., Biopolymers, 17, 1581 (1978); Hall et al., J. Phys. Chem., 84, 756 (1980)). Measurements on RBCs and RBC ghosts using 40 us and 1 ms sample times at a scattering angle of 42^{o} gave similar results. This similarity and the discrepancy between data collected from single cells and hemoglobin solutions implicate the membrane as the source of the intensity fluctuations.
Supported by USPHS grant 5R01 AM12083 (FDC) and NIH 5T32 GM07309 (RBT).

M-Pos230 DIVALENT CATIONS IN LYSING BUFFER MODULATE GHOST MORPHOLOGY
Vicki S. Woolworth and Wray H. Huestis; Department of Chemistry, Stanford University, Stanford, CA 94305

Erythrocyte ghosts undergo a shape change from echinocytic to smooth discocytic to stomatocytic shapes when resealed in the presence of MgATP. This shape change was studied as a function of the divalent cation composition of the lysing buffer. Human red cells were lysed hypotonically in 13mM HEPES buffer at pH 7.4 and 0°C with and without 2mM Mg^{+2}. 2mM MgATP was added and the suspension was made isotonic by the addition of KCl, after which the ghosts were incubated at 37°C and the morphology examined by phase contrast microscopy. When Mg^{+2} was included in the lysing buffer, the ghosts proceeded from echinocytic to discocytic forms. In the absence of Mg^{+2}, the ghosts continued from discocytic to stomatocytic forms. The effect on the shape was half maximal at 0.2mM Mg^{+2}. Similar shape changes were observed when the cation was changed to Mn^{+2} or Ba^{+2} and to a lesser extent with Sr^{+2} or Ca^{+2}. This effect is independent of protein or lipid phosphorylation and oxidation. We have shown that the discocytic to stomatocytic transformation can be controlled by Mg^{+2} and other divalent cations in the lysing buffer, indicating that physical events at the moment of hemolysis influence later stages in the shape change.
(Supported by NIH grant HL 23787)

M-Pos231 SEDIMENTATION POTENTIALS: THE EFFECT OF CELL SHAPE AND CONCENTRATION.
Lawrence C. Cerny, Elaine L. Cerny, Charles L. Cerny and David Huther. Utica College of Syracuse University and The Masonic Medical Research Laboratory, Utica, N.Y. 13502.

It is a well-established fact that the shape of an erythrocyte can be altered by controlling the osmolality of the suspending medium. Therefore it is possible to represent the shape of the red blood cell as a biconcave discoid, an oblate spheroid or a sphere. These shape changes play an important role in several biophysical measurements. Those studied here include sedimentation potentials, electrophoretic light scattering (ELS) and moving boundary electrophoretic mobility. The zeta potentials were calculated and compared from the three techniques. The results of these studies were used to explain the effects of cell shape concentration and charge on the erythrocyte sedimentation rate. Both normal and hardened red blood cells were used.

M-Pos232 ERYTHROCYTE MORPHOLOGY CHANGES AND CYTOSKELETON STRUCTURE
Kong-Joo Lee and Wray H. Huestis. Dept. of Chemistry, Stanford Univ., Stanford, CA 94305

The relationship between cell shape and cytoskeleton structure has been examined in two types of crosslinking experiments. Cells fixed with 0.14% glutaraldehyde were solubilized with Triton X-100 and the residual shells were examined by phase contrast and dark field microscopy. In contrast to the formless ovoids obtained from unfixed discocytes or echinocytes, shells from fixed cells were found to retain structural features of the parent cell. In separate experiments, cells were treated with the sulfhydryl oxidant diamide and then exposed to echinogenic or stomatogenic amphipaths. Oxidized discocytes bound amphipaths normally, but changed shape in an abnormal fashion. Oxidized discocytes were likewise resistant to crenation induced by calcium loading. Triton shells obtained from diamide-treated discocytes appeared discocytic, both before and after crenation by high concentrations of amphipaths. Echinocytes oxidized after crenation and the treated with stomatogenic amphipaths adopted anomalous shapes with membranes both invaginated and evaginated. Triton shells isolated from these species appeared echinocytic. These findings suggest that a) the cytoskeletal protein reticulum is capable of deforming to adopt the shape of the overlying bilayer, provided that its curvature is not severe; and b) shape changes normally seen upon amphipath binding or calcium loading require cytoskeletal deformation, a flexibility which depends on the integrity of sulfhydryl functions. (Supported by a grant from the National Institutes of Health (HL23787))

M-Pos233 SPIN LABEL EPR STUDIES OF SICKLE HEMOGLOBIN-MEMBRANE INTERACTION. L. W.-M. Fung and Gay L. Lilley, Department of Chemistry, Loyola University of Chicago, Chicago, IL 60626.

Spin label EPR techniques were used to monitor the interaction of normal and sickle hemoglobins in increasing concentrations (0- 13.5 mg/ml) with normal human erythrocyte membranes at physiological pH, electrolyte concentration and temperature. The spectra demonstrate a significant hemoglobin concentration dependence with a distinct difference between the normal hemoglobin-membrane and sickle hemoglobin-membrane interactions. The normal hemoglobin-membrane interaction is saturable, and an equilibrium constant was obtained from the simple two state equilibrium model used for earlier work [Fung et al., 1983 (Biochem. 22, 864-869)]. The sickle hemoglobin-membrane interaction does not appear to follow a binding pattern similar to that of normal hemoglobin-membrane interaction. In addition, normal and sickle hemoglobin-membrane interactions exhibited electrolyte concentration dependence and temperature dependence; however the electrolyte and temperature effects differed distinctly for each type of interaction. These results may have implications for the formation of irreversible sickled cells.

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M-Pos234 THERMAL PROPERTIES OF THE ERYTHROCYTE MEMBRANE AS DETECTED BY A SPIN LABELED STEARIC ACID: INVOLVEMENT OF SKELETAL PROTEINS. by M.Minetti, M.Ceccarini, A.M.M.Di Stasi, T.Forte, T.C.Petrucci. Lab. Biol. Cell., Ist. Sup. Sanità, V. Regina Elena 299, 00161 Roma, Italy. Intr. by V.T. Marchesi.

The involvement of skeletal proteins in thermal transitions of erythrocyte membrane detected by a spin label (16-doxyl stearic acid) at 8°C, 20°C and 40°C, has been investigated. Ca-treatment selective extractions, rebinding of purified proteins and antibodies (Abs) specific for spectrin, band 3 and protein 4.1 have been used. Calcium loading caused the lowering of 40°C transition and the disappearance of the 8°C transition. Electrophoretic pattern of Ca-treated membranes showed the presence of high Mw complexes and the disappearance of band 4.1. The 8°C transition was eliminated only by Anti- 4.1 Abs while Anti-spectrin Abs eliminated the 40°C transition. The exhaustive extraction of spectrin-actin removed the 40°C transition. High salt extraction of spectrin-actin depleted vesicles, a procedure which extracts proteins 2.1 and 4.1, was able to eliminate the 8°C transition. Rebinding of purified protein 4.1 restored the 8°C transition. These results suggest the involvement of protein 4.1 in the 8°C transition and of spectrin in the 40°C transition, both these proteins seem to contribute significantly to membrane physico-chemical properties. Purified spectrin showed above 40°C a characteristic thermal unfolding that could be responsible for the change in the freedom of motion of the spin label in the lipid bilayer.

M-Pos235 POSSIBLE MECHANISMS OF ACTION OF VITAMIN E ON ERYTHROCYTE MEMBRANES. Kenneth J. Friedman, Department of Physiology, New Jersey Medical School, U.M.D.N.J., Newark, NJ 07103.

Vitamin E (alpha tocopherol) is a lipid soluble vitamin whose mechanism of action is thought to be the prevention of membrane lipid oxidation. Because individuals with hemolytic anemias have lowered vitamin E plasma concentrations, and published data suggest that restoration of plasma E levels in these patients may be of therapeutic value, the abilities of vitamin E to influence erythrocyte (RBC) lipid composition and membrane fluidity were explored. RBC phospholipid and fatty acid composition of rats maintained on E-deficient and E-sufficient diets were sampled by cardiac puncture at 8, 11, and 16 weeks post-weaning. E-sufficient animals showed a time-dependent increase in PE and a time-dependent decrease in PC. A statistically significant difference in PE and PC concentrations was seen between E-deficient and E-sufficient rats at 16 wks. The alkyl chains of phospholipids derived from E-deficient animals contained more saturated (16:0 and 18:0) fatty acid and lower amounts of 22:x than E-sufficient rats. The only significant difference in alkyl chain composition between the 2 groups was for 22:x. Calorimetric scans of mixtures of DPPC and vitamin E in excess water were used to determine if vitamin E could influence membrane fluidity. Vitamin E broadened DPPC's phase transition and decreased its heat of transition in a linear fashion. A plot of DPPC's heat of transition vs. mole % vitamin E suggests 60 mole percent vitamin E would abolish the phase transition. The RBC data suggest vitamin E may influence phospholipid base and alkyl chain composition. The calorimetric data suggest vitamin E may behave like cholesterol, inserting itself between alkyl chains and introducing disorder into alkyl chain bilayer arrays.

M-Pos236 ^{1}H-NMR STUDIES OF THE INTERNAL MOTIONS AND STRUCTURAL DOMAINS OF SPECTRIN. H.-Z Lu,[1] R. Hjelm,[2] M.E. Johnson,[2] C. Narasimhan[1] and L. W.-M. Fung[1], [1]Department of Chemistry, Loyola University of Chicago, Chicago, IL 60626 and [2]Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL 60680.

Purified human erythrocyte membrane spectrin in its dimeric and tetrameric forms, and in the intact spectrin-actin membrane skeleton have been studied by proton nuclear magnetic resonance as a function of temperature, ionic strength and different denaturation treatments in phosphate buffer at pH 7.4. Standard one pulse, one pulse with presaturation, and spin echo pulse sequences are used to characterize the behavior of the proton resonances. Both gel electrophoresis and circular dichroism were used to analyze spectrin samples used for NMR studies. Our results suggest that the spectrin molecule is composed of at least two types of structural domains. The first type of domain is a flexible, random coil-like segment of the polypeptide chain that exhibits fast internal motions and contributes to the narrow resonances observed in proton NMR spectra of the native spectrin. The second type of domain has more structural features, has moderate internal motions and contributes to the broad envelope under the narrow resonances in the native spectrin spectra. Some parts of this domain may easily convert to flexible conformations by varying solution conditions. Circular dichroism measurements suggest the existence of a third domain that is resistant to all denaturation treatments.

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M-Pos237 REDUCTION OF THE APPARENT AREA COMPRESSIBILITY MODULUS OF RED BLOOD CELL MEMBRANE BY APPLIED ELECTRIC FIELDS. Christopher Katnik and Richard E. Waugh Department of Radiation Biology and Biophysics, University of Rochester Medical Center, Rochester, NY 14642.

Red blood cells (RBC's) and phospholipid bilayers are known to have a large resistance to changes in surface area. This resistance is characterized by the area compressibility modulus of the membrane (K), which relates the isotropic force resultant (T) to the fractional change in membrane area ($\Delta A/A_0$): $T = K\Delta A/A_0$. The modulus (K) is measured by aspirating hypotonically swollen cells into a micropipette. The membrane force resultants are calculated from the pressure applied to the cell surface, and the fractional change in membrane area is deduced from the change in the length of the cell projection in the pipette. We have observed that the application of a voltage across the cell (from the suspending buffer outside the aspirated cell (ground) to the solution inside the micropipette) reduces the apparent value of K. Voltages of ± 200 mv produce changes in the apparent K of ± 40 percent. The apparent value for K also depends on the electrode material or the presence of Cu^{++} or Zn^{++} in solution. Additionally, we have observed that changes in the applied voltage at constant pressure produce reversible changes in the length of the cell projection in the pipette. Applied voltages on the order of -1.0 volt produce changes in projection length equivalent to a 4.0 percent increase in area (assuming constant volume) or an 8.0 percent decrease in volume (assuming constant area). Proper interpretation of these observations will provide important information about the effects of electric fields on biological membranes. It should also provide insight into the physico-chemical events preceding electric-field-induced fusion and point to possible mechanisms for the toxic effects of heavy metal ions on biological membranes.

M-Pos238 MONTE CARLO STUDIES OF MODEL LIPID-POLYPEPTIDE INTERACTIONS IN BILAYERS
H. Larry Scott, Physics Department, Oklahoma State University

The interactions between membrane lipid chains and hydrophobic regions in membrane proteins is of considerable interest in membrane research. As a first attempt to theoretically examine the detailed nature of these interactions a series of Monte Carlo studies have been carried out which calculate average order parameter profiles for aliphatic chains in a monolayer with an embedded model polypeptide. The chains are ten CH_2 subunits long with one end constrained to lie in a plane. The chains are free to translate laterally in the plane and undergo conformational changes via the rotational isomer model. In the center of the simulation cell is the model polypeptide. Chain subunits interact with each other and with the polypeptide via Van der Waals forces, and standard Monte Carlo sampling procedures are used for the lipid chains (the polypeptide remains rigid in these calculations). Order parameter profiles are determined for lipid chains at varying distances from the polypeptide, and periodic boundary conditions in the monolayer plane are imposed. In all cases 36 lipid chains form the simulation cell, which is initialized with hexagonal symmetry and all-trans chains. In order to examine a wide range of possibilities several different models for the polypeptide were considered: i) a rigid smooth cylinder; ii) a cylinder with side chains protruding at alpha-helical positions, for several side chain - lipid interaction strengths, and iii) a model identical to ii) but with a space into which chains are able to fold. Plots of the resulting order parameter profile-vs.-distance to polypeptide data will be presented and discussed for all calculations.

M-Pos239 EFFECT OF MEMBRANE THICKNESS ON GRAMICIDIN CHANNEL LIFETIME*--Huey W. Huang, Physics Department, Rice University, Houston, TX 77251

When a gramicidin (dimeric) channel is formed in a membrane of thickness greater than the length of the channel, the membrane deformation reduces the stability of the channel. The effect of membrane thickness on gramicidin channel lifetime was studied by the Cambridge group (Elliott et al., Biochem. Biophys. Acta 735, 95 (1983)). However, the assumption that the free energy of membrane deformation is mainly due to the work done against surface tension could not account for the effect. Here we consider the elastic free energy involved in membrane deformation. The elastic free energy of bilayer membrane is deduced from that of smetic A liquid crystals. We then derive a differential equation which, with boundary conditions, determines the elastic deformation of a bilayer membrane. For example, the elastic energy of deformation due to a gramicidin channel embedded in the membrane can be calculated. The effect of membrane thickness on channel lifetime, due to the elastic deformation, is predicted. We compare our theoretical results with that of the Cambridge group and their experimental data. The elastic term is about equal to, or larger than, the tension term; but both are smaller than the measured values. However, the experiments were performed with black lipid membranes containing hydrocarbon solvents, to which a theory of bilayer membrane may not apply. It is concluded that in the consideration of protein-membrane interactions the elastic energy is as important as, if not more so than, the surface tension energy. Experiment with solventless bilayer membrane is necessary to resolve the theories.

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M-Pos240 FOURIER TRANSFORM INFRARED STUDIES OF THE INTERACTION OF CA-ATPase WITH BINARY MIXTURES OF PHOSPHOLIPIDS. Richard Mendelsohn and Mark Jaworsky,Department of Chemistry, Rutgers University,73 Warren Street,Newark,New Jersey,USA,07102.

FT-IR spectroscopy has been used to monitor phospholipid order and melting characteristics in reconstituted membrane systems.CaATPase from rabbit sarcoplasmic reticulum has been isolated, purified, and reconstituted into phospholipid vesicles composed of binary lipid mixtures. Use of acyl chain perdeuterated lipids as one of the lipid components permits studies of the thermotropic response of each component in either the binary lipid mixtures (control experiments) or in ternary complexes (two lipids plus protein).

High precision (0.03 cm^{-1}) measurements of the temperature dependence of the acyl chain CH_2 or CD_2 stretching frequencies are used to construct lipid melting curves.Comparison of the curves with those of protein-free lipid mixtures permits the determination of the ability of protein to select a particular lipid component.Several binary mixtures have been utilized,and three types of response have been noted:

I. No preferential selection of a particular component (POPE/DPPC-d_{62})
II.Composition-independent selection of a particular component (DOPC/DPPC-d_{62})
III.Lipid mixtures for which a partitioning preference depends upon the composition of the lipid system (DEPC/DMPC-d_{54} and SOPC/DPPC-d_{62})

The partitioning preferences correlate with the miscibility properties of the lipid pair.

M-Pos241 TIME RESOLVED AND STEADY STATE FLUORESCENCE STUDIES OF LYSINE-TRYPTOPHAN-LYSINE WITH DIMYRISTOYLPHOSPHATIDYLSERINE VESICLES. A.G. Szabo and S. Yamashita (Intr. by J.R. Colvin), Biological Sciences, National Research Council, Ottawa, Canada K1A OR6.

An understanding of the molecular details of changes in protein conformations and dynamics is essential to interactions of proteins and lipids. Studies of small peptides with model membranes provides a basis for the interpretation of spectroscopic parameters obtained from more complex systems. The tripeptide lysine-tryptophan-lysine (KWK) can serve as an example of a basic peptide with an intrinsic fluorescent chromophore, tryptophan. When KWK binds to acidic lipid vesicles such as DMPS the fluorescence of the tryptophan is enhanced but the spectral maximum remains at 350 nm when the vesicles are in the gel phase. In peptide-lipid complexes in the liquid crystalline phase (> 39°C, pH 5.3) a shift of the fluorescence maximum to 335 nm is observed together with a significant intensity enhancement. Temperature studies show that intensity and spectral changes of KWK-DMPS complexes follow the same thermal transition pattern as the anisotropy measurement with diphenylhexatriene. Time resolved fluorescence data show that there is a conformational heterogeneity in KWK. The data are interpreted in terms of a model in which KWK first binds electrostatically to the negatively charged lipid. In the gel phase the tryptophan residue remains on the surface of the bilayer. In the liquid crystalline phase the tryptophan nucleus penetrates into the bilayer.

M-Pos242 TERBIUM PROBE OF CALCIUM BINDING SITES ON THE PROTHROMBIN-MEMBRANE COMPLEX. Leslie E. Sommerville, Robert M. Resnick, David D. Thomas and Gary L. Nelsestuen, Departments of Biochemistry, University of Minnesota, St. Paul, MN 55108 and Minneapolis, MN 55455.

Terbium was used as a probe of Ca^{2+} binding sites on the prothrombin-phospholipid complex. Stoichiometric titrations of prothrombin binding to phospholipid vesicles with either Tb^{3+} or Ca^{2+} showed that a minimum of 8 metal ions were needed for binding prothrombin to vesicles (3 Mn^{2+} + 5 Ca^{2+} for prothrombin or 8 Tb^{3+} for F-1). When Ca^{2+} alone was used, a total of about 11 metal ions were needed for complete binding. These stoichiometries indicated 3 classes of metal ions; one class was needed to induce the conformational change, a second was required for protein-membrane contact, and a third class that bound at other sites on the protein that were not involved in membrane binding. CoEDTA quenching of Tb^{3+} bound to the prothrombin-phospholipid complexes indicated that all metal ions were at least partially exposed to the quencher. Some populations of Tb^{3+} showed lower quenching constants when all of the prothrombin was bound. Tb^{3+} emission lifetimes revealed that some Tb^{3+} in the protein-membrane complex were in a different environment from Tb^{3+} bound to the protein alone. All of the Tb^{3+} bound to the complex were acceptors of fluorescence energy transfer from a donor in the membrane. The results indicated that the metal ions in the prothrombin-membrane complex are close to the membrane surface, relatively open to the solvent, and effect the characteristics of the protein-membrane binding equilibrium. (Supported by NIH grant numbers HL15728 (G.L.N.) and GM27906 (D.D.T.).

M-Pos243 THE EFFECT OF ETHANOL AND OTHER MEMBRANE PERTURBING AGENTS ON MEMBRANE ASSOCIATED PHOSPHOLIPASE ACTIVITY MONITORED BY A NOVEL FLUORESCENCE ASSAY USING NBD-PHOSPHOLIPID SUBSTRATES. C.L. Pryor, C.D. Stubbs, J. Hoek, N. Harada and E. Rubin
Department of Pathology, Hahnemann University, Philadelphia PA 19102

The activity of membrane phospholipases can be modulated by membrane perturbing agents such as alcohols, detergents, non-esterified fatty acids, drugs, etc. We have been studying the relationship between such effects and those on the motional properties of the membrane lipids assessed by fluorescence polarization techniques. Phospholipase activity was measured by determination of the release of the fluorescent fatty acyl moiety from 1-acyl, 2-(6-((7-nitro-2,1,3-benzoxadiazol-4-yl)amino)caproyl-phosphatidylcholine (NBD-PC). The uptake of NBD-PC into liver microsomes was monitored by measuring the fluorescence energy transfer (FET) from fluorescamine (pre-labeled) to the NBD fluorophor. A high FET indicated close proximity, i.e., incorporation of NBD-PC into the membrane, which occurred extremely rapidly. Subsequently, the FET decreased more slowly as the NBD-fatty acyl moiety was cleaved by phospholipase action and expelled from the membrane as NBD-hexanoic acid, presumably due to the polar nature of the NBD moiety. These results led to the development of a novel fluorescence assay for membrane phospholipase activity. Using this assay, we were able to demonstrate elevation of phospholipase activity by the membrane fluidizing agent ethanol at concentrations as low as 50mM.

M-Pos244 STUDIES ON THE MECHANISM OF NON-SPECIFIC LIPID TRANSFER PROTEIN. J. Wylie Nichols, Department of Physiology, Emory University School of Medicine, Atlanta, Georgia 30322.

Resonance energy transfer between (7-nitro-2,1,3-benzoxadiazol-4-yl) NBD-labeled phospholipids and N-(lissamine rhodamine B sulfonyl)-dioleoyl phosphatidylethanolamine (N-Rh-PE) was used to measure the rate of intervesicular transfer of the former in the presence of rat liver non-specific lipid transfer protein (nsLTP) (Nichols and Pagano, 1983, J. Biol. Chem. 258:5368-5371). The observation that the rate of NBD-lipid transferred per nsLTP increases with decreasing acyl chain length argues strongly against a "shuttle carrier" type of mechanism. However, the data are consistent with a model based on the premise that nsLTP binds to the vesicle membranes, increases the vesicle-monomer dissociation rate constant, and thereby increases the rate of phospholipid transfer by diffusion through the aqueous phase. The dependence of the initial transfer rates on donor and acceptor vesicle concentration are consistent with a kinetic model based on this mechanism. Additional studies on the acyl chain length dependence of nsLTP activity indicate that the protein has a differential effect on the phospholipid head group versus the acyl chains. NsLTP increases the free energy of activation for dissociation of the head group region and lowers the free energy of activation per number of carbons in the acyl chains. As a result, nsLTP is relatively more effective in increasing the transfer rates of longer-chain phospholipids. (Supported by USPHS grant GM 32342.)

M-Pos245 **SELECTIVE DETECTION OF THE MOTIONALLY RESTRICTED LIPID POPULATION IN SARCOPLASMIC RETICULUM USING SATURATION TRANSFER EPR.** Thomas C. Squier,*Carl Polnaszek, and *David D. Thomas. Dept. of Biol. Chem., Univ. of Maryland School of Medicine, Baltimore, MD; *Department of Biochemistry, Univ. of Minnesota Medical School, Minneapolis, MN.

The dynamics of lipids in biological membranes can be spectrally resolved into two motional populations using conventional EPR; the motionally restricted spectral component has an effective correlation time, τ_r, greater then 10^{-7} sec. ST-EPR extends the EPR technique into the biologically important microsecond time domain, and is therefore sensitive to the dynamics of the motionally restricted lipid component ($\tau_r \geq 10^{-7}$ sec) over this longer time window. In practice, the large population of more mobile probes (reporting the dynamics of the bulk lipid) can interfere with the analysis of the ST-EPR spectrum. In this study we have used the in-phase spectral intensity to suppress the contribution of the weakly immobilized probes ($10^{-9} \leq \tau_r \leq 10^{-6}$ sec), allowing the selective detection of the motionally restricted lipid population. The amount of spectral saturation decreases as the nitroxide's position is moved toward the center of the bilayer, indicative of increased mobility. This increased mobility has been simulated and may most reasonably be interpreted in terms of a constant rate ($\tau_r \approx 10^{-7}$ sec), but with increasing amplitude. This positional profile has been compared with that obtained using conventional EPR in combination with spectral subtractions. The use of these two EPR methods, in combination with more accurate spectral simulations, provides a better understanding of the physical properties of membranes at the lipid-protein interface.

M-Pos246 DIRECT DETERMINATION OF ENERGETIC PARAMETERS FOR PROTEIN ASSOCIATION AND INSERTION INTO MEMBRANES. G. Ramsay, R. Prabhu, B. Goins and E. Freire. Department of Biochemistry, University of Tennessee, Knoxville, TN 37996

A newly designed, computer controlled isothermal titration calorimetry system with a sensitivity better than 0.15μwatts has been used to measure the enthalpy changes for the association and insertion of myelin basic protein to phosphatidylserine vesicles, and cholera toxin to phosphatidylcholine vesicles containing ganglioside GM_1. Above the phospholipid phase transition temperature, the association of myelin basic protein is highly exothermic and characterized by an enthalpy of -160 kcal/mole of protein. Below the phospholipid phase transition temperature, the enthalpy of association is -98 kcal/mole of protein at low protein/lipid molar ratios. The additional negative enthalpy observed in the fluid phase can be attributed to the protein ordering effect on the surrounding lipid molecules. This contribution amounts to -2.3 kcal per mole of perturbed lipid assuming that each protein molecules perturbs 27 lipid molecules. In the gel phase myelin basic protein induces the formation of large vesicle aggregates at protein lipid ratios higher than 1/150 giving rise to the appearance of an endothermic component in the titration peaks. Cholera toxin binds with almost absolute specificity to ganglioside GM_1 molecules on the membrane surface. The enthalpy change for this association is -44 $\pm$ 4 kcal/mole of protein and is independent of the composition and physical state of the phospholipid bilayer matrix, indicating that the enthalpic contributions arise primarily from the ganglioside-protein interaction. (Supported by NIH grant GM-30819 and NS-20636.)

M-Pos247 THERMODYNAMIC CHARACTERIZATION OF MEMBRANE PROTEIN UNFOLDING USING THERMAL GEL ANALYSIS. C. Rigell and E. Freire. Department of Biochemistry, University of Tennessee, Knoxville, TN 37996

The thermal denaturation of an integral membrane protein is often accompanied by changes in its lipid solubility. These changes in solubility allow separation of the native and denatured protein fractions by detergent solubilization followed by centrifugation, under conditions in which only one of the protein fractions become solubilized. Using this principle, it has been possible for us to study the thermally induced denaturation of cytochrome c oxidase reconstituted into DMPC vesicles. The identification and quantification of the protein subunits undergoing thermal denaturation have been achieved by computer assisted gel electrophoresis analysis. This method allows calculation of transition temperatures, apparent equilibrum constants and Van't Hoff enthalpy changes for individual protein subunits, thus complementing the results obtained by deconvolution analysis of the excess heat capacity function obtained by high sensitivity differential scanning calorimetry. (Supported by NIH grant GM-30819.)

M-Pos248 THE MECHANISM OF ACTIVATION OF PANCREATIC PHOSPHOLIPASE A_2. Guillermo Romero, Kim Thompson and Rodney Biltonen. Departments of Biochemistry and Pharmacology, University of Virginia School of Medicine, Charlottesville, VA 22908.

Previous work of our laboratory and others has shown that the hydrolysis of pure DPPC large unilamellar liposomes by pancreatic phospholipase A_2 occurs only in the vicinity of the gel-liquid crystalline transition of the lipid. The time course of the reaction is characterized by the presence of a slow initial phase (latency period) followed by an apparent burst of activity. During the latency phase the activity of the enzyme increased in a monotonic fashion. Detailed studies of the characteristics of the latency phase showed that its duration was inversely related to the concentration of enzyme and directly dependent on the substrate concentration in a complex manner. Several models to quantitatively describe these data were considered. The most conservative model quantitatively consistent with the kinetic data is one in which the monomeric enzyme initially binds the lipid matrix in an inactive form which subsequently becomes activated via a process that requires the formation of enzyme dimers on the surface of the lipid substrate. The solution of the rate equations of the model indicates that the time course of the reaction during the latency phase is a second order function in time and the inverse of the latency period is identical to an apparent second order rate constant. Detailed analysis of the data obtained at 38°C provided estimates of the binding constant of the enzyme monomer to the lipid substrate and of the second order activation rate constant. The details of the model, including appropriate assumptions, the quality of the fit to the data and its implications will be presented and discussed.

M-Pos249 THE TEMPERATURE DEPENDENCE OF THE ACTIVATION REACTION OF PANCREATIC PHOSPHOLIPASE A_2. Guillermo Romero and Rodney Biltonen. Departments of Biochemistry and Pharmacology, University of Virginia School of Medicine, Charlottesville, VA 22908.

In the preceding paper we demonstrated that the activation of pancreatic phospholipase A_2 in the presence of DPPC large unilamellar vesicles is governed by two parameters, the binding constant of the protein to the lipid substrate and the second order rate constant of activation. We have determined the temperature dependence of these parameters within the gel-liquid crystalline phase transition region. It appears that binding of the inactive protein monomer is best when the lipid is in the gel phase since the apparent lipid binding constant is a monotonically decreasing function of temperature. The apparent second order activation rate constant depends on temperature in a complex manner and reaches a maximum near T_m. This result strongly suggests that a necessary condition for phospholipase A_2 activation is the existence of dynamic structural fluctuations within the lipid matrix. Similar behavior was observed with dimiristoylphosphatidylcholine large unilamellar liposomes. The implications of these results with respect to the modulation and regulation of phospholipase A_2 activity will be discussed.

M-Pos250 THE ASSEMBLY OF THE MEMBRANE ATTACK COMPLEX OF COMPLEMENT (C5b-9) ON PHOSPHOLIPID VESICLES MONITORED BY LIGHT SCATTERING INTENSITY. Ruth E. Silversmith and Gary L. Nelsestuen, Department of Biochemistry, University of Minnesota, St. Paul, MN 55108.

The C5b-9 complex was assembled by sequential addition of purified proteins C5b-6, C7, C8, and C9 to solutions of vesicles while 90° light scattering was monitored and used to quantitate protein binding to vesicles. Measurements in the absence of membranes indicated that C5b-7, the first membrane binding complex, aggregated to form large particles which could bind C8 and C9. This process represented a potential competitive side reaction to vesicle binding which could influence light scattering measurements. However, at C5b-7/vesicle ratios of ≤ 1, there was quantitative binding of C5b-7 to the vesicles and the assembly was studied under these conditions. Assembly did not induce vesicle fragmentation or fusion. The C8/C5b-7 ratio in the phospholipid-C5b-8 complex was 0.97 +/- 0.12 and the maximum ratio of C9/C5b-8 in the final complex was 16.2 +/- 2.0. One C9 molecule associated rapidly with each phospholipid-C5b-8, followed by slower incorporation of the remaining C9 molecules. The initial velocity of the slow phase of C9 addition was easily saturated with C9 and gave an activation energy of 37.0 kcal/mole. This value was identical to the value measured for the analogous process in the absence of membranes and was very similar to the C5b-8-independent polymerization of C9 (40.8 kcal/mole), which was also measured by light scattering intensity. This approach allowed quantitative and continuous analysis of the dynamics of C5b-9 assembly. (Supported by NIH grant HL15728).

M-Pos251 MECHANISM OF APO C-I, C-II, AND C-III BINDING TO SINGLE BILAYER VESICLES. B.J. McKeone, H.J. Pownall, and J.B. Massey, Baylor College of Medicine, Houston, TX 77030

The C-apolipoproteins, apo C-I, apo C-II and apo C-III are low molecular weight proteins of known sequence derived from the high density lipoprotein (HDL), very low density lipoprotein (VLDL) and chylomicron subfractions of the plasma lipoproteins. The apo C are in dynamic equilibrium between the various lipoprotein classes and in part regulate their catabolism. In the metabolic cascade apo C proteins are transferred from VLDL to HDL upon triglyceride hydrolysis and subsequently transfer from HDL to newly secreted VLDL. We have examined the mechanism of binding of the C proteins. Radiolabeled apo C-I binds to VLDL and HDL and transfers reversibly between VLDL and HDL. It also transfers reversibly between 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC) single bilayer vesicles (SBV) and both VLDL and HDL. Upon binding to the vesicles, the fluoresence emission spectra of the C proteins underwent a 1.1 to 1.7 fold increase in relative quantum efficiency and a 7 to 10nm blue shift. Resonance energy transfer from apo C tryptophan residues to 5 mol% N-dansyl-phosphatidylethanolamine incorporated into POPC SBVs was used to measure binding. Protein binding to the dansyl PE containing SBVs resulted in a 1.1 to 1.3 fold increase in relative quantum efficiency at 510nm, the emission maximum of the dansyl moiety. Kinetic studies of binding were measured by stopped-flow with fluoresence detection. Apoprotein binding was first order for both lipid and protein and second order overall. SBV binding half times for 2.5uM apo C-I, C-II and C-III-2 were 17msec, 10msec and 11msec, respectively,at 25C° for a 1:500 protein to lipid ratio. Apo C-I,C-II,and C-III, thus appear to bind rapidly to POPC SBVs, with a concommitant increase in their relative quantum efficiency and a blue shift in their emission spectrum upon binding.

M-Pos252 INTERACTION OF PORIN WITH LIPOSOMES. Sophie S. Fan. (Intr. by L. Lis) Applied Research & Development Dept.,Technicon Instruments Corporation, Tarrytown, New York 10591

It has been reported (T. Nakae et al., FEBS Letter, 106, 85 (1979)) that vesicles formed from phospholipids in the presence of porin (isolated from Escherichia Coli B) allowed rapid diffusion of small solutes (MW<600) through their walls similar to what observed with the outer membrane of E. Coli. In this work, increasing amounts of porin were added to liposomes containing B-galactosidase and the enzymatic color development from a substrate (ONPG) was measured. No color was observed in 10 minutes at 37°C unless the porin: lipid ratio exceeded 1.5 X 10^{-2} (or 1.7 ug protein/nanomole lipid.) Above this ratio the observed rate of color generation increases rapidly with porin concentration. The apparent rate of diffusion of substrate across the membrane is constant with time suggesting that the pore formation by porin is complete within minutes, i.e. before the substrate is added. Photon correlation studies of the liposome suspension containing porin reveal that there is a slight increase in liposome sizes following the substrate permeation. In contrast, mixing of Triton X-100 with the liposomes leads to a marked decrease in particle size. Since porin showed a very different membrane action than Triton X-100, the results suggest that the substrate transport observed in this study is caused by channel formation. In this case, the channels are induced by insertion of porin molecules added externally to the preformed liposomes.

M-Pos253 CORRELATION BETWEEN TORPEDO CALIFORNICA ACETYLCHOLINE RECEPTOR FUNCTION AND STRUCTURAL PROPERTIES OF MEMBRANES, Tung Ming Fong, Department of Biochemistry & Biophysics, University of California, Davis, CA 95616

Protein-lipid interactions were studied using Torpedo californica nicotinic acetylcholine receptor (AChR) as a model system by reconstituting purified AChR into membranes containing various synthetic lipids and native lipids. AChR function was determined by measuring two activities at 4°C: (1) low to high agonist affinity state transition of AChR in the presence of an agonist (carbamylcholine) in either membrane fragments or sealed vesicles, and (2) ion-gating activity of AChR-containing vesicles in response to carbamylcholine. The effects of acyl chain configuration, rigid neutral molecules (such as cholesterol) and negatively charged phospholipids were examined in these reconstituted membranes. The dynamic structures of these membranes were probed by incorporating spin-labeled fatty acid into AChR-containing vesicles and measuring the order parameters. It was found that both aspects of AChR function were highly dependent on the lipid environment even though carbamylcholine binding itself was not affected. An appropriate membrane fluidity was necessarily required to allow the interconversion between the low and high affinity states of AChR. An optimal fluidity hypothesis is proposed to account for the conformational transition properties of membrane proteins. In addition, the conformational change was only a necessary, but not sufficient, condition for the AChR-mediated ion flux activity. Among membranes in which AChR manifested the conformational transition, only those containing both cholesterol and negatively charged phospholipids retained the ion-gating activity.

M-Pos254 TRANSFER OF BAND 3, THE ERYTHROCYTE ANION TRANSPORTER, TO HUMAN PERIPHERAL LYMPHOCYTES USING A PHOSPHOLIPID VECTOR: INTER-MEMBRANE PROTEIN TRANSFER. A.C. Newton & W.H. Huestis Department of Chemistry, Stanford University, Stanford, CA 94305

Band 3, the erythrocyte anion transporter has been shown to transfer between human erythrocytes and sonicated vesicles. Functional band 3 becomes associated with dimyrstoylphosphatidylcholine vesicles incubated with human red blood cells. Proteolytic studies reveal that the transporter is inserted into the vesicle bilayer in native orientation. Band 3 is degraded on the cytoplasmic side of the red cell membrane by trypsin, and on the external side by chymotrypsin. Intact band 3-vesicle complexes are susceptible to digestion by chymotrypsin but not trypsin applied to the external side of the vesicles. In contrast, trypsin encapsulated in the vesicle lumen proteolyzes the transporter, indicating that band 3 traverses the bilayer with the cytoplasmic segment making contact with the vesicle lumen.

Incubation of band 3-containing vesicles with human peripheral blood lymphocytes bestows increased anion uptake capability on the cells. This uptake is DIDS-suppressible, suggesting that it is band 3-mediated. The efficiency of transfer is low: on the order of one or two band 3 molecules are transferred to approximately half the cells. Supported by USPHS Grant HL 23787.

M-Pos255 EFFECTS OF TPA ON SUBCELLULAR LOCALIZATION OF PROTEIN KINASE C AND LIPID BILAYER STRUCTURE. B.A. Lewis, W.D. Dawson, and J.S. Cook, Chemistry and Biology Divisions and Univ. Tenn.-Oak Ridge Graduate School of Biomedical Sciences, ORNL, Oak Ridge TN 37831

The activity of protein kinase C (PKC) requires phosphatidylserine and Ca^{++} and is stimulated by diacylglycerols (DGs), which are transiently produced in vivo by the enzymatic hydrolysis of phosphatidylinositol. Phorbol ester tumor promoters, including 12-O-tetradecanoyl phorbol-13-acetate (TPA) also activate PKC. It has been postulated that PKC is the TPA "receptor" and that activation by TPA mimics activation by DGs. In cultured kidney epithelial cells, PKC is membrane-associated during rapid growth but shifts to the soluble fraction when the cells reach confluence and become quiescent. Exposure of quiescent cells to 10^{-7} M TPA results in a reassociation of PKC activity with cell membranes; TPA has little effect on PKC activity in either compartment in rapidly growing cells. To explore the degree to which TPA exerts its effects via direct action on membranes, two types of experiments have been done. (1) PKC-poor cell membrane fractions from confluent cells are treated with TPA, washed, and added to PKC-rich soluble fractions, with the result that soluble PKC activity decreases with a concomitant increase in PKC activity of the membrane fraction. Thus the increase in membrane-PKC affinity can be accomplished in a fractionated system by exposure of the membrane fraction to TPA without direct exposure of PKC to TPA. (2) We compare the effects of TPA and DG on the structure of model membranes (lipid vesicles), using ^{31}P NMR as a probe for perturbation of bilayer structure. (Supported by ORNL Exploratory Studies Program [B.A.L.], NCI Training Grant CA09336 [W.D.D.], and OHER, USDOE under contract DE-AC05-840R21400 with Martin Marietta Energy Systems, Inc.)

M-Pos256 DETERGENT SOLUBILIZATION OF CHOLESTERYL OLEATE AND PREPARATION OF SPHEROIDAL MODEL HIGH DENSITY LIPOPROTEIN COMPLEXES. C. F. Schmidt and C. G. Brouillette, Department of Pathology, University of Alabama at Birmingham, Birmingham 35294.

The properties of discoidal complexes of phosphatidylcholine with apolipoprotein A-I from High Density Lipoprotein (HDL) have been widely studied by a variety of physical techniques. Relatively little work has been done to characterize spheroidal phospholipid-apo A-I complexes with a cholesteryl ester core, which are more relevant to circulating HDL than the discoidal complexes. In order to develop a simple, reproducable method for the preparation of spheroidal complexes we have investigated the detergent solubilization of cholesteryl oleate (CO). Sodium deoxycholate (DOC) is the only detergent among those investigated that was found to effectively solubilize CO, presumably because it can form larger rod-like micelles. The amount of cholesteryl oleate solubilized depends in a reproducable manner on the deoxycholate concentration, the pH, the ionic strength, and the time spent above the CO phase transition (44°C). The addition of phosphatidylcholine (PC) decreases somewhat the amount of CO solubilized. Further, the CO-DOC suspensions are stable at room temperature for at least 24 hr. after preparation. Data on the size and composition of PC-CO-apo A-I complexes, after removal of the detergent, will be presented for various starting ratios, as well as preliminary results of physical studies.

M-Pos257 MEASUREMENTS OF INTRACELLULAR pH(pHi) IN PARIETAL AND CHIEF CELLS OF RABBIT GASTRIC GLANDS USING DUAL WAVELENGTH MICROSPECTROFLUORIMETRY AND DIGITAL IMAGE PROCESSING, A.M. Paradiso, R.Y.Tsien, & T.E.Machen. Dept. of Physiology-Anatomy, Univ.California, Berkeley 94720.

pHi was measured in parietal cells and chief cells of rabbit gastric glands which were isolated, oaded with the pH-sensitive,fluorescent dye BCECF and mounted in a perfusion chamber on the stage of a microscope attached to a fluorimeter. Fluorescence (em: 520-550nm) was measured during alternate excitation at 439 and 490nm; the fluorescence intensity ratio, R(490/439), was calibrated in terms of pHi using the nigericin-high K method. R(490/439) was measured either (i) using a photomultiplier which focused onto a single parietal or chief cell or (ii) by accumulating separate images from a SIT camera at 439 and 490nm; the logarithm of R(490/439) was calculated at every pixel and displayed in pseudocolors. In this latter case, it was possible to measure pHi in all cells of the gland simultaneously; successive pictures showed changes in pHi in the chief and parietal cells with time. When glands were superfused with NaCl Ringer's, chief cells (pHi 7.17) were slightly more alkaline than parietal cells (pHi 7.06). When the solution was changed to Na gluconate Ringer's, pHi rapidly increased to 7.43 in parietal cells and more slowly to 7.26 in chief cells. When NaCl Ringer's was changed to N-methyl-glucamine Cl, pHi decreased rapidly in chief cells to 6.52 and more slowly in parietal cells to 6.75. Although both parietal and chief cells contain Na/H and Cl/OH (HCO_3) exchangers, it appears that the anion exchanger is more active in parietal cells while the cation exchanger is more active in chief cells. Support: NIH AM19520 and GM31004.

M-Pos258 LOCATION OF THE PATHWAYS USED BY OSMOTIC FLUXES ACROSS THE FROG GASTRIC MUCOSA IN VITRO. L. Villegas (intr. by J. Requena) Instituto Internacional de Estudios Avanzados and Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela.

Effect of viscosity increments in the solutions on the osmotic fluxes were studied in frog gastric mucosa in vitro. Dextran 10% w/v, with an average M.W.= 20,000 was used to increase the viscosity of the solutions. Osmotic flux was induced by adding 300mM sucrose to the secretory solution in contact with the mucosal surface. The nutrient solution in contact with the serosal surface was kept isosmotic. The net serosal-to-mucosal flux measured in these conditions was made up by two fractions: (a) The spontaneous net water flux associated to the ionic transport and secretion and (b) the osmotic flux induced by the concentration difference applied. In the present experiments addition of dextran to the nutrient solution produced no significant effect in the net volume flux ($\Delta\Phi = 0.9 \pm 0.7\ \mu l/cm^2 h$, $P > 0.2$). Use of dextran in the secretory solution produced a significant increment in the net volume flux from 28.0 ± 0.8 to $32.3 \pm 0.8\ \mu l/cm^2 h$, ($P < 0.001$). It was previously shown that the spontaneous net volume flux is reduced by dextran in the nutrient solution and remained unaltered by the use of dextran in the secretory solution (AJP 226, 1338, 1974). Considering that both fractions of the net volume flux in the presence of a concentration differences are induced osmotically, a possible explanation for the observed effect of dextran is the location of the pathways used by the flux associated to the ionic transport and secretion originated in the oxyntic cells at the bottom of the glands and the flux induced by the concentration increment applied to the mucosal surface covered by epithelial cells. Changes in the intercellular spaces associated with the induced fluxes could be observed in the presence of dextran.

M-Pos259 USE OF BARIUM AND HYPOTONIC TECHNIQUE IN DETERMINING SITES OF RESISTANCE CHANGES WITH INHIBITION OF ACID SECRETION IN FROG STOMACH. M. Schwartz, G. Carrasquer, and W. S. Rehm, Departments of Medicine and Physics, University of Louisville, Louisville, KY 40292.

The PD, resistance (R) and H^+ rate were determined with Cl bathing media in the in vitro fundus of Rana pipiens. The standard secretory contained (mM): 156 Na, 4 K and 160 Cl. SCN to either secretory (S) or nutrient (N) increases R by about 100 ohm cm^2. Changing the S fluid to a hypotonic one (mM): 25 Na, 4 K, and 29 Cl resulted in a small increase in R of 25 to 50 ohm cm^2 during secretion. Replacing 25 mM Cl with 25 mM SCN in the above solution reduced the H^+ rate to zero, increased the PD about 20 mV and increased R to over 1,000 ohm cm^2 --then additiion of mannitol (400 mM) to S markedly decreased the resistance. The above and other evidence indicate that inhibition with a hypotonic S collapses area of the luminae resulting in a marked iincrease in resistance via the lumen-tubular cell pathway which is reversed by adding mannitol to S. Hence the resistance of the surface cells and the transintercellular (TIC) pathways are very high. Ba added to N with standard S results for high secretory rates in a marked increase in resistance (e.g. from about 100 to 800 ohm cm^2). With Ba present SCN_2 cimetidine or omeprazole instead of increasing R markedly decreased R by about 300 to 400 ohm cm^2. With a hypotonic S and the fundus inhibited by either cimetidine or omeprazole, SCN did not decrease the resistance of the surface cells and/or the TIC pathways with or without Ba. It is concluded that the resistance via the surface and/or TIC pathways is very much larger than that via the lumen-tubular cell pathway and that in the presence of Ba the resistance of the nutrient membrane of the tubular cells decreases with inhibition of acid secretion.

M-Pos260 ION FLUXES ACROSS GASTRIC MUCOSA IN TERMS OF A TWO-PUMP MODEL. J. Bettencourt, W.W. Reenstra and J.G. Forte. Dept. of Physiology-Anatomy, Univ. of California, Berkeley, CA 94720.

Studies of oxyntic cell basolateral membranes (JBC 264:6641) have demonstrated the presence of Cl^-/HCO_3^- and Na^+/H^+ exchangers, a Na^+/K^+ pump and a K^+ conductance. This, coupled with an apical membrane possessing a H^+/K^+ pump and K^+ and Cl^- conductances, will allow active Cl^- secretion to be driven by either pump, and K^+ secretion or absorption to be driven by the Na^+/K^+ and H^+/K^+ pumps, respectively. In order to test this model, rates of transepithelial Cl^- and K^+ fluxes (in $\mu eq/cm^2/h$) across the frog gastric mucosa were measured. At open circuit, omeprazole inhibited the H^+/K^+ pump, reducing J^H by 5.31±0.65 and J^{Cl}_{net} by 5.43±1.23. At short circuit (SC), where the Na^+/K^+ pump can also drive Cl^- secretion, omeprazole reduced J^H by 5.24±0.39 and J^{Cl}_{net} by 3.30±0.98, to give a J^{Cl}_{net} (5.26±0.54) and a short circuit current (SCC) of 5.73±0.27. During inhibition of J^H with ouabain and omeprazole, J^{Cl}_{net} and SCC were 1.62±0.52 and 1.31±0.17, respectively. We have previously shown (Fed. Proc. 44:1366) that, at SC, the omeprazole inhibited mucosa secretes K^+; J^K_{net} = 0.09± 0.04. This flux was also inhibited by ouabain. The above model predicts that, if omeprazole only inhibited the H^+/K^+ pump, then during inhibition J^{Cl}_{net} would be substantially larger than SCC, with the excess J^{Cl}_{net} balanced by J^K_{net}. Our results are consistent with the model if: (i) omeprazole also inhibits apical K^+ conductance; or (ii) there is an additional barrier to ion flux between the apical membrane and bulk secretory fluid. As the fraction of transmucosal current carried by K^+ does not change with omeprazole, inhibition of K^+ conductance is unlikely. Possible distinctions between the diffusional barrier, due to the gastric lumen, and a membrane surface barrier (e.g., glycoprotein surface layer) will be analyzed. (Supported by USPHS Grant AM10141)

M-Pos261 NETWORK THERMODYNAMIC MODELING OF ISOTONIC SOLUTE-COUPLED FLOW IN 'LEAKY' EPITHELIA. Mark L. Fidelman and Donald C. Mikulecky, Dept. of Physiology and Biophysics, Medical College of Virginia, Richmond, VA 23298.

A Network Thermodynamic model was developed to provide possible insights into the nature of isotonic solute-coupled flow in a "leaky" epithelium. The model contains only one diffusible non-electrolyte solute (s) and there is a fixed amount of an impermeable non-electrolyte (i) inside the cell. The coupled flows of solute and volume at each membrane in this four membrane model are described by the practical phenomenological equations as developed by Kedem and Katchalsky (1957). The cell is assumed to be capable of volume regulation under the steady state experimental conditions simulated. A solute-pump, located in the basolateral membrane, uses feedback regulation to adjust C_s in the cell in order to maintain cell volume at or near control levels in all simulations. Transepithelial volume flow is primarily through the cellular pathway.

Model behavior is consistant with experimental observations with respect to tonicity and magnitude of volume flow over a wide range of lumenal bath osmolarities (produced by varying either lumenal C_s or C_i). Examination of the parameter space suggests the following important features for isotonic coupling: 1) the apical membrane reflection coefficient must be less than that of the basolateral membrane; 2) the basement membrane reflection coefficient must be small; and 3) the apical membrane solute permeability and reflection coefficient are the two most critical parameters and need to vary in an inverse manner with respect to one another in order to maintain isotonicity. Supported by the Whitaker Foundation.

M-Pos262 WATER AND ION CONDUCTANCES IN HUMAN PLACENTAL MICROVILLOUS MEMBRANE VESICLES. Nicholas P. Illsley and A.S. Verkman. Cardiovascular Research Institute, University California, San Francisco, CA 94143.

Microvillous vesicles (MVV) were isolated from fresh human placentae by shearing in isotonic solution, Mg precipitation and differential centrifugation. Enrichment of the MVV marker alkaline phosphatase was 17-fold over crude tissue homogenate. Ion conductances were calculated from effects of test ions on the membrane potential determined by a reference ion (bi-ionic diffusion potential). Membrane potentials were measured using the potential-sensitive probe diS-C_3-(5) (3 μM). Fluorescence intensity (Ex 620 nm, Em 670 nm) was linear with membrane potential as generated by K/valinomycin; %F/mV = 0.35 (0 to +90 mV) and 0.60 (-90 to 0 mV). Relative ion conductances at 37°C were 0.65 ± 0.13 (Na/K), 0.13 ± 0.03 (Cl/K), 0.20 ± 0.09 (Cl/Na) and 0.03 ± 0.01 (gluconate/K) (mean ± SEM, n=5). Several anions quenched diS-C_3-(5) fluorescence in aqueous solution including Br, I and PO_4. Lifetime studies showed that this was due to a collisional quenching mechanism with a decrease in diS-C_3-(5) lifetime from 0.81 ± 0.02 ns (K gluconate, 100 mM) to 0.47 ± 0.08 (K phos, 100 mM) or 0.28 ± 0.05 (KBr, 25 mM). Water and urea permeabilities were measured by stopped-flow light scattering methods. Scattered light intensity (I; 500 nm) was inversely related to MVV volume with a 40% increase in I for a 50% reduction in MVV volume. MVV permeability to osmotic water transport (P_f, 37°C) was (4 ± 1) x 10^{-3} cm/s (S.D.). Urea permeability was (2.9 ± 0.4) x 10^{-6} cm/s with a MVV surface to volume ratio of 3.2 x 10^5 cm^{-1}. The temperature dependence of P_f was biphasic with activation energies of 2.8 ± 0.6 kcal/mole (<20°C) and 8.6 ± 0.4 kcal/mole (>20°C).

M-Pos263 K^+ TRANSPORT BY INSECT MIDGUT: K^+ ENTRY ACROSS THE BASAL MEMBRANE. D. F. Moffett and A. R. Koch. Dept. of Zoology, Washington State University, Pullman, WA 99164-4220.

Previous work showed that Ba^{++} alters the relation between basal side $[K^+]$ and transepithelial K^+ transport in Manduca sexta (tobacco hornworm) midgut. In these studies isolated, short-circuited midgut was penetrated with double-barrelled K^+ and voltage sensing microelectrodes. Addition of Ba^{++} resulted in substantial hyperpolarization of basal membrane with little change in intracellular K^+. Iontophoresis of Lucifer yellow showed that both major cell types responded similarly. Inhibition of the apical electrogenic K^+ pump with anoxia reversed the hyperpolarization. The electrochemical gradient remained favorable for K^+ entry with external K^+ as low as 20 mEq/l. At lower external $[K^+]$, cytoplasmic K^+ remains above the calculated equilibrium value. These results suggest that both cell types participate in the entry step of K^+, that Ba^{++} effects on net K^+ transport are primarily due to effects on the apical potential, and that passive entry into the intracellular transport pool may be augmented by a low-affinity pump.

Supported by NSF PCM 8315739.

M-Pos264 INTRACELLULAR MICROELECTRODE ANALYSIS OF CHLORIDE TRANSPORT IN NORMAL AND CYSTIC FIBROSIS HUMAN REABSORPTIVE SWEAT DUCTS. M.M. Reddy and P.M. Quinton. Div. of Biomedical Sciences, University of California, Riverside, CA. 92521.

Chloride impermeability is a characteristic feature of the reabsorptive sweat duct (RSD) in the genetic disease CF. In an attempt to localize the defective route of chloride (Cl^-) transport in this tissue we have studied the intracellular potentials of microperfused RSD using microelectrodes. Basal membrane potentials (Vb) and transepithelial potentials (TEP) were measured with reference to bath ground. Apical membrane potentials were obtained by subtracting TEP from Vb. In control ducts, luminal Cl^- substitution by gluconate hyperpolarized Vb by 25 ± 4 mV (-36 ± 4 mV to -62 ± 7 mV). Removing bath Cl^- depolarized Vb by 22 ± 7 mV (Vb = -15 ± 3 mV). Removal of Cl^- from bath and lumen simultaneously had no significant effect on Vb. In 150 mM Cl^- (lumen and bath) luminal amiloride eliminated the TEP, but hyperpolarized both Va (-24 ± 2 mV to -61 ± 7.9 mV) and Vb (- 33 ± 1.8 mV to -62 ± 7 mV). Removing Cl^- from the lumen only, or from the bath and lumen simultaneously, limited the amiloride induced hyperpolarization of the apical membrane without any significant effect on Vb. In CF tissue in Cl media, Va was depolarized significantly (+ 36 ± 3 mV) compared to normal ducts (-24 ± 2 mV) and the amiloride induced hyperpolarization occurred only at the apical membrane (one subject). If there is no significant change in the concentration of intracellular ions immediately after changing external $[Cl^-]$ or after adding amiloride, current loop analysis of these results indicate a paracellular pathway for Cl^- reabsorption in the normal sweat duct and implicate this site as the source of the chloride impermeability defect found in cystic fibrosis. (n > 3 in all cases).

M-Pos265 ENDOGENOUS ENZYMES HYDROLYZE EPITHELIAL Na^+ CHANNELS. Simon A. Lewis, William P. Alles and Chris Clausen.* Department of Physiology Yale Medical School, New Haven, CT 06437 and SUNY Stony Brook, NY 11794.*

Cytoplasmic vesicles are inserted into and withdrawn from the apical membrane of the mammalian urinary bladder during bladder filling and emptying. The density of amiloride sensitive Na^+ channels in the vesicles is eight times greater than in the apical membrane suggesting that some constituent of the urine causes loss of Na^+ channels. We tested the effect of two known urinary proteases (urokinase and kallikrein) on the density of apical membrane Na^+ channels. Both of these urine proteases caused a progressive loss of Na^+ channels, and the appearance of two types of amiloride insensitive leak channels, one stable in the apical membrane and the other unstable and seems to partition between the apical membrane and the bathing solution. The loss of Na^+ channels is described by a single inverse exponential where the rate constant is concentration dependent. Amiloride (but not Na^+), protects the Na^+ channel from hydrolysis. In the presence of amiloride, the stable leak channel is hydrolyzed into the unstable leak channel. The loss of stable leak channels is described by a single inverse exponential with a rate constant equal to that for the loss of Na^+ channels. The kinetics of channel loss indicates a sequential model of channel hydrolysis. These results suggest a novel regulatory role by endogenous proteases of epithelial ion transport.

Supported by NIH grant AM-33243

M-Pos266 CHLORIDE CHANNEL REGULATION BY cAMP OR Ca^{++} IN CULTURED HUMAN TRACHEAL CELLS. R.L. Shoemaker, G. Rechkemmer and R.A. Frizzell, Physiology and Biophysics, UAB, Birmingham, AL 35294

Activation of apical Cl conductance pathways by β-adrenergic agonists underlies stimulation of airway NaCl secretion and its accompanying water flow. The ability of isoproterenol to induce a Cl diffusion potential across cystic fibrosis (CF) airways is defective (Boucher et al., Clin. Res. 33:467A, 1985). We studied this problem in human tracheal epithelial cells in primary culture from normal and CF patients. Cl channel activity was assessed using patch clamp techniques. During cell-attached recordings, addition of 5-25μM epinephrine or 0.1mM 8-bromo cAMP activated a ∿25pS Cl selective channel in normal cells. A high degree of Cl selectivity relative to Na was detected by replacing 150mM NaCl in the bath by 300mM NaCl. Activation of these channels by epinephrine or cAMP did not occur in cells derived from subjects with CF. When patches were excised into bath solutions containing 1mM Ca, channels having a conductance similar to those activated by cAMP in normal tracheal cells appeared. This activity could be terminated when Ca was lowered by adding EGTA in excess. Membrane patches excised into solutions containing 20nM free Ca were inactive but Cl channel activity could be evoked subsequently by raising Ca to 180nM - 10μM. During cell-attached recording, Cl channel activity could be elicited by exposure of the cells to 2-4μM A23187 in bathing media containing 0.25mM free Ca but not in 20nM free Ca. After excision, channels remained active in 180nM - 10μM Ca. These results suggest a step in the β-adrenergic activation of apical Cl channels by cAMP is impaired in CF airway epithelia but that Ca-dependent regulation of apical Cl conductance remains operative. (Supported by grants from the CF Foundation and the NIH/NIADDK AM34935).

M-Pos267 **IMPEDANCE PROPERTIES OF THE RABBIT DESCENDING COLON: EVALUATION OF DISTRIBUTED RESISTANCE EFFECTS.** N.K. Wills and C. Clausen, Yale University, New Haven CT 06510 and S.U.N.Y. Stony Brook, N.Y. 11794

Distribution of membrane resistances along narrow, fluid-filled spaces such as the lateral intercellular space (LIS) or crypts can complicate D.C. microelectrode measurements of apical and basolateral membrane properties in epithelia. Using impedance analysis, we evaluated the extent of such distributed resistance effects in the rabbit colon. Transepithelial impedance was measured in seven tissues before and after addition of 10^{-4}M amiloride to the mucosal bathing solution. The results were computer-fitted by three equivalent circuit models: a "lumped" model with no distributed resistors and two morphologically-based distributed models. One model distributed basolateral membrane resistance along the LIS, and the other distributed an apical membrane resistance along the crypt lumen. Improved fits of the data were obtained with the distributed models, however estimates of membrane parameters did not significantly differ among the three models. Average estimates for apical and basolateral membrane conductances were 3.6 ± 0.1 mS/cm^2 and 16± 0.6 mS/cm^2, and were significantly decreased after amiloride by 55% and 17%, respectively. Apical and basolateral membrane capacitances (C_a and C_{bl}) were 26 ± 2 μF/cm^2 and 14±2 μF/cm^2. After amiloride, C_a was significantly decreased by 16%; C_{bl} was unchanged. We conclude that distributed resistance effects are detectable in the rabbit colon but have negligible effects on estimates of membrane parameters for these conditions. The results provide further support for an amiloride-insensitive conductance in the apical membrane of this epithelium, in agreement with previous reports from this laboratory (Supported by N.I.H. grants AM 29962 and AM28074).

M-Pos268 THE CURRENT-VOLTAGE RELATIONS OF Na-COUPLED GALACTOSE TRANSPORT ACROSS THE APICAL MEMBRANE OF NECTURUS SMALL INTESTINE. Jean-Yves Lapointe, R.L. Hudson and S.G. Schultz. Dept. of Physiology & Cell Biology, University of Texas Medical School, Houston, Texas.

The current-voltage (I-V) relations of Na-coupled galactose transport across the apical membrane of Necturus small intestine were determined from the relations between the electrical potential difference across the apical membrane, ψ^{mc}, and that across the epithelium, ψ^{ms}, when the latter was varied over the range ±200 mV under (i) steady-state conditions in the presence of galactose; and, (ii) when Na-coupled galactose entry was blocked with phloridzin. The current due to this rheogenic entry process, I^{m}_{NaS}, showed a marked dependence on ψ^{mc} over the range -50 mV to the "reversal potential" but tended to saturate at large hyperpolarizing or depolarizing values of ψ^{mc} and in a few instances a "negative conductance" was observed. The reversal potentials in the presence of 15 mM, 5 mM and 1 mM galactose in the mucosal solution averaged 28 mV, 11 mV and 6 mV, respectively. The intracellular galactose concentrations, estimated isotopically, in the presence of 15 mM and 5 mM galactose averaged 48 mM and 41 mM, respectively. These values, together with the previously measured intracellular Na activity (14 mM) are consistent with a Na-galactose coupling stoichiometry of unity. The shapes of the I-V relations are consistent with the predictions of a simple kinetic model of a negatively charged carrier (or gated pore) in which the translocation steps are rate-limiting and the rate of translocation of the free carrier is significantly greater than that of the ternary complex.
(Supported by NIH-NIADDK AM 26690. Dr. Lapointe was supported by a Fellowship from the Canadian Medical Research Council.)

M-Pos269 HETEROGENEITY OF CELLULAR AND INTRACELLULAR CALCIUM COMPARTMENTATION AS MEASURED BY X-RAY MICROANALYSIS IN PROXIMAL RENAL TUBULES. A. LeFurgey, P. Ingram*, L.J. Mandel. Dept. of Physiology, D.U.M.C., Durham, N.C.; *Research Triangle Institute, Research Triangle Park, N.C.

Total cell calcium measured in perchloric acid extracts from isolated suspensions of kidney proximal tubules or whole tissue provides values which can range from 4.5 to 40nmol/mg protein (5.6 to 50 mmol/kg dry wt). Furthermore, studies using agents which release mitochondrial calcium have led to the conclusion that about 50% of the total calcium is located within the mitochondria. The present study was designed to measure the intracellular localization of calcium in cryofixed, cryosectioned suspensions of kidney proximal tubule using quantitative electron probe x-ray microanalysis. Two populations of cells were identified: 1) "Viable" cells which represented the majority of cells probed were defined by their relatively normal K/Na concentration ratio of about 4:1. The detectability of the low Ca (in the presence of high K) in these cells was improved by reducing the K content of the tubules by incubation with 10^{-4}M ouabain for 5 min at 37°C; the measured calcium content was 4.00±0.45mmol/kg dry wt in the cytoplasm and 1.34±0.37mmol/kg dry wt in the mitochondria; 2) "Non- viable" cells were defined by the presence of dense inclusions in their mitochondria and K/Na concentration ratio of about 1. The average Ca content of the mitochondria in such cells was obtained by taking small rasters which included the dense inclusions and the mitochondrial matrix space. These measurements gave a value of 678±105 (SEM) mmol/kg dry wt (n=10) for the mitochondrial Ca content. Assuming 30% of the cell volume to be mitochondrial, the presence of these inclusions in 4-6% of the cells would account for the observed total Ca content of the suspension.

M-Pos270 KINETIC TRANSPORT MODEL FOR CELLULAR REGULATION OF pH AND SOLUTE CONCENTRATION IN THE RENAL PROXIMAL TUBULE. A.S. Verkman and R.J. Alpern. UCSF, San Francisco, CA 94143.

The mammalian proximal tubule is a specialized epithelium of one cell type which is responsible for reabsorption of the majority of Na, Cl, HCO_3, glucose, amino acids and H_2O filtered by the glomerulus. Fluxes of NaCl and $NaHCO_3$ are precisely regulated. Transcellular H_2O and solute movement across the luminal membrane, cell cytoplasm and basolateral membrane are driven by a basolateral 3Na/2K ATPase. An open circuit kinetic model was developed to calculate the time course of cell volume, pH and [solute] in response to induced perturbations in luminal or peritubular fluid composition. Fluxes of H_2O and solutes were calculated from electrokinetic equations containing terms for known carrier saturabilities and allosteric dependences. Apical and basolateral membrane potentials were determined iteratively from the requirement of cell electroneutrality and equal opposing transcellular and paracellular currents. Fitted potentials were used to generate ion fluxes required to integrate the electrokinetic equations. Known transporters include apical Na/H, K, Na/glucose and Na/amino acid, and basolateral $Na/3HCO_3$, K, glucose and amino acid. Apical Na/H is regulated allosterically by cell pH. There is likely, but not proven, to be apical NaCl and basolateral KCl transport and an apical H ATPase. Both membranes have high osmotic water permeability. There are significant paracellular conductances for Na, K, Cl and HCO_3. The model is stable and is convergent to membrane potentials accurate to 0.01% in 1-4 iterations. The model makes specific predictions which are confirmed by microperfusion data and requires that there exist (1) apical H ATPase, (2) basolateral $Na/3HCO_3$ transport saturable with HCO_3, (3) basolateral K transport regulated allosterically by cell pH and (4) neutral transcellular Cl transport.

M-Pos271 SINGLE CHANNEL INVESTIGATION OF THE BASOLATERAL MEMBRANE IONIC PERMEABILITY IN THE RABBIT PROXIMAL CONVOLUTED TUBULE. L. Parent, J. Cardinal and R. Sauvé. Département de physiologie, Groupe de recherche en transport membranaire, Université de Montréal, Montréal, Québec.

The patch clamp method was used to investigate the presence of ionic channels in the basolateral membrane of the rabbit proximal convoluted tubule. The PCT were microdissected from rabbit kidney; incubated 20 min. in a collagenase solution (Worthington: 456 units/ml) and transferred in a bath containing: a) normal Earle Hepes (5 mM KCl); b) modified Earle Hepes (127 mM KCl). A first series of cell attached experiments was undertaken in which both the patch electrode and the bathing medium contained the normal Earle solution. Under these experimental conditions, inward current jumps of small amplitude were observed that could be associated to a voltage insensitive 8 pS channel. In a second series of experiments, the 127 mM K-Earle solution was used as the bathing medium. Single channel I-V curves were measured with pipette solutions containing NaCl and KCl in ratios chosen to keep the ionic strength constant (150 mM). The single channel conductance associated to these inward currents ranged from 30 to 35 pS for a 75 mM KCl-75 mM NaCl pipette solution . When the pipette solution was changed from 127 mM KCl to 75 mM KCl, the extrapolated value of the reverse potential shifted from 0 to -13 mV, indicating that the channel, observed under these conditions, is mainly permeable to K ions. Chloride permeability was tested by experiments performed with a pipette solution of 25 mM Na_3-citrate and 75 mM KCl and was not found to be involved in these conditions. The channel did not seem to be Ca-activated since an internal Ca concentration of 2 mM (in inside-out configuration) failed to increase single channel activity.

M-Pos272 LOCATION AND FREQUENCY OF INTERCELLULAR JUNCTIONS IN FROG SKIN. SHARIF SHAHIN AND JAMES T. BLANKEMEYER (Intr. by H.J. Harmon), Department of Physiological Sciences, Oklahoma State Univ., Stillwater, OK 74078.

The morphology and distribution of intercellular junctions were investigated in frog skin epithelium using electron microscopic techniques. Tight junctions were found only at the apicolateral surfaces of the upper two layers of the epithelium (Stratum corneum and Strateum granulosum). Desmosomes were observed in all epithelial layers. Desmosome frequency increased from the basal layer to the upper layers of the epithelium reaching the highest frequency at the Stratum granulosum, but declined in Stratum corneum. Gap junctions were observed in the Stratum germinativum, spinosum, rarely in the Stratum granulosum, but never between the cells of Stratum corneum. Lamellar bodies were encountered in the intercellular space of Stratum spinosum and Stratum granulosum. The structure and function of lamellar bodies have not been fully established in this epithelium. These findings suggest that: (1) the presence of the permeability barrier was restricted to the Stratum corneum and Stratum granulosum. (2) Cells of Stratum granulosum are not coupled to cells of S. granulosum or to cells of other strata by means of classical gap junctions.

M-Pos273 SODIUM CONCENTRATION DEPENDENCE OF APICAL MEMBRANE SINGLE CHANNEL Na^+ CURRENT AND DENSITY OF NONDEPOLARIZED FROG SKIN (THREE STATE MODEL). Lynn M. Baxendale and Sandy I. Helman. University of Illinois, Urbana, IL 61801

Blocker-induced (CDPC, 6-Chloro-3,5-diamino-pyrazine-2-carboxamide) noise analysis was used to investigate the apical solution Na^+ concentration (Na_a^+) dependence of single channel Na^+ current (i_{Na}) and density of open channels (N^o). Data were analyzed according to a 3-state model of closed $\underset{\alpha}{\overset{\beta}{\rightleftharpoons}}$ open $\underset{k_{10}}{\overset{k_{01}}{\rightleftharpoons}}$ blocked states. k_{01} and k_{10} were measured from the relationship between $2\pi f_c$ and [CDPC] while $\beta/\beta+\alpha$ were estimated from the increases in N^o associated with increases of [CDPC]. For epithelia bathed symmetrically in $Cl\text{-}HCO_3$ or Cl/Hepes Ringer solution and short-circuited, i_{Na} and N^o averaged 0.414 ± 0.012 pA and 53.2 ± 2.2 (n=82) millions/cm^2 at Na_a^+ of 100 mM. Decreasing Na_a^+ in steps (75, 50, 25, 10, 3, 1 mM) caused i_{Na} to decrease in a nonlinear, saturable-like manner to 0.116 ± 0.008 pA (n=18) at 10 mM and 0.021 ± 0.001 pA (n=13) at 1 mM. N^o was increased correspondingly to 262 ± 15 % (n=17) and 392 ± 37 % (n=11) of control. Total channel density (open + closed) was increased to 149 ± 16 % (n=17) and 155 ± 12 % (n=11) of the control value of 299 ± 21 (n=73) millions/cm^2. The fraction of channels in the open state ($\beta/\beta+\alpha$) was increased from 0.192 (100 Na_a^+) to 0.321 and 0.515 at 10 and 1 mM Na_a^+ respectively. The increase of open channels is due predominantly to changes of the rate coefficients α and β and not due to Na^+ self-inhibition of open Na^+ channels. USPHS AM 30824. LMB is a fellow of the AHA (Ill. Affiliate).

M-Pos274 SINGLE CHANNEL CURRENTS IN BASOLATERAL MEMBRANE OF ISOLATED FROG SKIN EPITHELIUM. Robert E. Yantorno and Mortimer M. Civan, Dept. of Electrical Engineering, Temple University, Philadelphia, PA 19122, and the Depts. of Physiology and Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

Patch-clamping sheets of epithelial cells such as frog skin would permit simultaneous monitoring of channel activity and net Na^+ transport by measuring short circuit current. Such cells have been patched in culture, but technical difficulties have until now precluded patching whole tissue.

In an initial series of experiments, isolated epithelia from frog skins were bonded to cover slips. Satisfactory seals (>20 GΩ, and usually 50-100 GΩ) were formed and channel activity noted with 40 patches in the cell-attached mode and with 14 excised patches (either inside-out or outside-out). Several different channels were noted, with conductances of approximately 9-120 pS. More recently, giga-seals have been formed with superfused epithelia. In 4 experiments, the basolateral membranes were depolarized with high serosal K^+ concentrations; a single channel was noted, having a linear I-V relationship and conductance of 10.2 ± 0.9 pS (mean ± SE). In 2 experiments in which the patching electrode was filled with 140 mM KCl, the apparent reversal potential was -9 to -19 mV (relative to serosal bath). Under similar conditions, the basolateral membrane potential has previously been measured to be -10 ± 3 mV in this laboratory.

The current study demonstrates the feasibility of patch-clamping superfused frog skin epithelium. Several channels have already been noted, one of which is likely to be a K^+ channel.

M-Pos275 MULTI-SITE OCCUPANCY PROPERTIES OF SQUID AXON DELAYED RECTIFIER K CHANNELS AS DETERMINED IN SYMMETRICAL PERMEANT CATION SOLUTIONS. Kay W. May* and Gerry S. Oxford. (Intr. by A. Finn). Department of Physiology, University of North Carolina, Chapel Hill, NC 27514

Experiments were performed on internally perfused, voltage-clamped squid axons to assess the permeability properties of delayed rectifier K channels under conditions of varying concentration and type of permeant monovalent cation. Permeability and conductance estimates were taken from instantaneous current-voltage (I-V) data in which the activating prepulse was either to a large positive value (to saturate the conductance-voltage curve) or to the apparent reversal potential (to eliminate accumulation/depletion phenomena). Values obtained for the two cases showed no qualitative differences. Conductance ratios (g_X/g_K) obtained from I-V slopes and permeability ratios (P_X/P_K) obtained from changes in reversal potentials were functions of ion concentration in the range 100mM to 2M. The nature of the concentration dependence of these ratios differed for various permeant cations. Anomalously low slope conductances were observed for various mole fractions (mixed with K^+) of NH_4^+, Rb^+, or Tl^+ as compared with external solutions of each cation alone. The conductance minima occurred at different mole fractions for different cations. Statistically different values of bionic permeability ratios (P_X/P_K) were obtained from reversal potential measurements during cation substitutuion for external vs. internal K^+. Internal substitution yielded higher apparent Rb^+ and NH_4^+ permeabilities than external substitution. I-V relations in symmetrical K^+ solutions were linear over a wide voltage and concentration range. In contrast, I-V shapes in NH_4^+ solutions were superlinear below 300mM, but became linear at higher concentrations. These data are inconsistent with models restricting occupancy to a single ion.

M-Pos276 LOCAL ANESTHETICS BLOCK POTASSIUM CHANNELS AT THE INTERNAL QUATERNARY AMMONIUM ION RECEPTOR SITE. Mei-Ven Lo and Joan J. Kendig, Department of Anesthesia, Stanford University School of Medicine, Stanford, CA 94305

Previous studies of local anesthetics employing primarily agents of moderate lipid solubility reported no unusual characteristics of potassium channel block. We present evidence that the highly lipid soluble agent bupivacaine acts in part at the internal quaternary ammonium ion receptor site. Nodes of Ranvier of Xenopus sciatic nerve were arranged for vaseline gap voltage clamp, with TTX (10^{-8}M) to block sodium channels. Control records were made of the delayed outward current (I_K) response to a series of 45 msec depolarizing pulses. Lidocaine (5 mM) or bupivacaine (1 mM) was applied to the external surface of the node. Concentrations were based on the relative potency of these agents at the sodium channel, adjusted for the reported potency of lidocaine at the potassium channel. Rate of onset of block with both agents was very slow. Lidocaine block was linear and time-independent. Bupivacaine block by contrast became progressively more time- and voltage-dependent as block increased. At the more depolarized test potentials, I_K activated with apparently normal kinetics, but declined during a 45 msec depolarization with a time constant of 8.75 msec at 22°C. There was also a tonic component of block; at room temperature complete block required 60 min. Time- and voltage-dependence characterizes block by internally applied quaternary ammonium ions, particularly the larger more lipophilic long-chain compounds. The similarity of bupivacaine block suggests that the larger more hydrophobic local anesthetics act at least in part at the internal quaternary ammonium receptor site; potassium channel block may also involve other sites.

M-Pos277 THE RESPONSE OF SQUID GIANT AXON POTASSIUM CHANNELS TO OSMOTIC STRESS. F. Bezanilla, Dept. Physiology, UCLA, Los Angeles, CA 90024, V.A. Parsegian, J. Zimmerberg, N.I.H., Bethesda, MD 20205

To measure the change in the internal volume of squid axon K channels, we have been subjecting perfused preparations to positive and negative osmotic stress (OS). (Sucrose or sorbitol for the osmotic agent with internal solutions containing 200mM K and external solutions of Na-free (tris-replacement) artificial sea water.) Suppression and enhancement of K conductance correspond nicely to the response one expects to OS, but might also reflect some channel blocking or lowering of solution conductivity. The data do not fit a simple blocking model that assumes a probability of blockage going as the activity of the blocking agent. Also, blocking by sucrose and sorbitol at a given osmotic stress should be different, but it is not. The data suggest to us a set of voltage-dependent closed states and an open state whose occurrence depends primarily on the osmotic stress always acting on a channel, an unavoidable consequence of channel selectivity. An unmistakable increase in K channel conductance under lower than normal osmotic stress suggests that channels in situ are closed some significant fraction of the time. This lack of full expression is also seen from the g_K vs. V curve under osmotic stress where we observe no shift in voltage dependence but find suppression of conductance by an almost constant factor over the full range of applied voltage. The channel volume inferred from hypertonic stress has an upper bound of about 1300 A^3, a number that is large if one expects a cork or turnstile gating mechanism but is quite reasonable if one imagines a rearrangement involving the entire ionic path.

M-Pos278 VOLUME-REGULATION IN CLONED T-LYMPHOCYTES. C. Deutsch, J. Patterson, M. Price, S. Lee & M. Prystowsky. University of Pennsylvania, Philadelphia, PA 19104.

We have studied the volume-response of a murine non-cytolytic T-cell clone, L2, to hypotonic shock. Using electronic cell sizing we have found that non-cycling L2 cells swell in response to a decrease in the tonicity of the suspending solution to 0.67 isotonic, but then exhibit little or no regulatory volume decrease (RVD) towards their initial volume. Gramicidin (1 uM), in a medium in which choline had been substituted for Na, induced rapid RVD in response to the same decrease in tonicity, indicating that the lack of RVD in the absence of gramicidin is due to a deficiency in cation permeability. Recombinant human interleukin 2 (rIL2, Cetus Corp.) causes L2 cells to increase in size and initiate DNA synthesis. We have previously demonstrated that L2 K^+ conductance is increased approximately 4-fold at 24-48 hr after rIL2 stimulation. (Lee et al., JCB, 1986). L2 cells stimulated with rIL2 for 24 and 48 hr regulate their volume in response to hypotonic shock. This response can be accelerated with gramicidin and blocked by the K channel blocker quinine (100 uM). Verapamil blocks the K conductance in IL2-stimulated cells with a K_i of 7 uM and also blocks the hypotonically-induced RVD. When stimulated L2 cells are suspended in 0.67 isotonic solution containing 90 mM KCl, they continue to swell after the initial increase in size. This behavior is similar to that described for human peripheral blood lymphocytes (Grinstein et al., AJP 246:C204). Our results are consistent with the conclusion that the volume-regulatory cation channel is the K-conductive channel seen in whole-cell patch clamp: unstimulated L2 cells are deficient in voltage-gated K channels, as are human B cells, and neither exhibits appreciable RVD in response to hypotonic shock. Stimulated L2 cells have a K-conductance approaching that of human T cells, and both are capable of RVD. (NIH AM 27595).

M-Pos279 GLUCOSE SENSITIVITY OF POTASSIUM CHANNELS IN OBESE MOUSE BETA CELL MEMBRANES.

J.L. Schwartz*, G.A.R. Mealing*, Y.M. Siddiqui+ and J.T. Braaten+ from * the National Research Council of Canada, Ottawa, Canada and + the University of Ottawa, Ottawa, Canada.

Single channel currents were recorded from 5 to 15 day old cultured insulin secreting cells from obese mice (C57BL/6J, Jackson Laboratory, Bar Harbor, Maine) using the cell-attached patch-clamp technique. At least two types of channels were active under a pipette filled with 140 mM KCl, when the cells were perfused in glucose-free medium. The density of the small channels (10 pS or less) in most patches was three times larger than that of the large channels (50 pS or more). The small channelswere insensitive to glucose (22.4 mM) or glybenclamide# (4 uM) in the bath; they were reversibly blocked by TEA (tetraethylammonium, 20 mM). The large channels, when glucose concentration was changed from 0 to 22.4 mM and back to 0, either were irreversibly blocked or remained active, showing occasional bursts of activity and/or current spikes, even in glucose-free medium; they often displayed a sharp voltage dependency around -20 mV in the pipette, the opening probability shifting from over 50% to less than 5% for a 20 mV depolarizing step; their currents reversed at a pipette potential of -40 mV; they were insensitive to TEA.

This work shows that ob/ob mouse beta cell potassium channels behave differently from those of normal neonatal rat (Schwartz et al, Biophys. J., 1985, 47(2), 446a) and supports results from measurements of ob/ob mouse (Norwich colony) membrane potentials showing altered glucose sensitivity attributed to modified potassium permeability (Rosario et al, Quat. J. Exp. Physiol., 1985, 70, 137-150).

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M-Pos280 AMMONIUM CHLORIDE INCREASES CYTOPLASMIC pH, ACTIVATES K^+-PERMEABILITY, AND INHIBITS GLUCOSE-INDUCED ELECTRICAL ACTIVITY AND INSULIN RELEASE IN SINGLE MOUSE ISLETS OF LANGERHANS

L.M. Rosario, I. Atwater and E. Rojas N.I.H., Bethesda, U.S.A.

It has been reported that the Ca^{2+}-activated K^+ channel in the rat pancreatic B-cell is pH-sensitive. To clarify the role of cytoplasmic pH (pH_i) on K^+-permeability, glucose-induced electrical activity was recorded from B-cells in microdissected mouse islets of Langerhans using high-resistance microelectrodes, and pH_i was measured from single collagenase-isolated islets pre-loaded with an intracellularly trapped fluorescent pH probe (5(6) sulfofluorescein diacetate). Application of NH_4Cl (15 mM) in the presence of glucose (11 mM) promptly hyperpolarized the B-cell membrane, reduced the input resistance by 60% and suppressed electrical activity. These changes were paralleled by an increase in islet fluorescence, corresponding to a pH_i increase of about 0.2. The NH_4Cl-induced hyperpolarization was impaired by the K^+ channel blockers quinine and glibenclamide. When glucose (22 mM)-induced electrical activity was measured in single islets simultaneously with insulin release, application of NH_4Cl (5 mM) reduced electrical activity and strongly inhibited insulin release. The results suggest that a rise in the B-cell pH_i is followed by activation of K^+-permeability, which in turn reduces Ca^{2+} influx through voltage-sensitive Ca channels,resulting in a marked inhibition of release of the hormone.

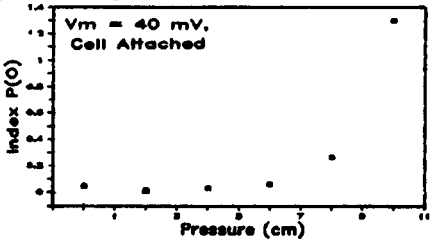
M-Pos281 A METABOLICALLY REGULATED K CHANNEL IN PANCREATIC B CELLS. L. Falke , K. Gillis , and S. Misler. Jewish Hospital and Washington University, St. Louis, MO 63110.

A current hypothesis for stimulus-secretion coupling in B cells is that nutrient metabolism → decreased K permeability (P_K) → membrane depolarization → insulin granule exocytosis. In dispersed rat islet cells, we have identified a K channel which is the major channel open at the cells' resting potential; its behavior parallels the metabolically regulated P_K. In cell attached patches formed with 138 mM K pipettes and the cell exposed to 0 glucose, Na Ringers, there are channels with a slope conductance (γ) of ~65pS and a zero current potential (E_{rev}) of Vpipette = -70 mV (bath ground). γ decreases to ~12pS and E_{rev} shifts positively to Vpipette = 0 when pipette [K] is reduced to 5.5 mM. The average number of channels open in a patch (A), as well as, single channel duration (τ) and opening frequency (υ) are not affected by the clamping voltage. A and τ are drastically reduced when a nutrient [glucose, mannose, leucine, or glyceraldehyde (5-25 mM)] is added to the cell's bath but are unaffected by non-metabolizable 3-O-methyl-glucose, galactose or ß-OH-butyrate (each at 20 mM). In 0-25 mM glucose bathing solutions, addition of a mitochondrial inhibitor [FCCP (1-5 µM), or Na azide, Na arsenate, or KCN (1-3 mM)], or glycolysis inhibitors [Na iodoacetate (3-5 mM) or mannoheptulose (20 mM)] restores channel activity. This channel is closed by adding 10-100 µM ATP to the cytoplasmic bath. Two other K channels, one 130pS and voltage dependent, the other 12pS and non-voltage dependent are not affected by bath metabolites. A 55pS K channel (P_K:P_{Na}>25) is seen in patches excised, inside-out, into 138 mM K. This suggests that metabolic regulation of resting P_K may occur through a specific K channel gated by the intracellular concentration of ATP.

M-Pos282 AN ATP-SENSITIVE K^+ CHANNEL IN INSIDE-OUT MEMBRANE PATCHES FROM PANCREATIC B CELLS. K. Gillis, L. Falke, and S. Misler (Intro. by A. Mauro). Jewish Hosp. St. Louis, MO

Cell metabolism couples glucose stimulation to insulin secretion in B cells; the actual intracellular intermediate(s) remains uncertain. We have reexamined gating, by various substances, of an ATP-sensitive 55 pS K^+ channel, seen in inside-out excised patches, whose kinetics and conductance resemble that of a metabolically regulated K^+ channel in cell attached patches. In inside-out excised patches, symmetrically bathed in 138 mM K^+ and $\leq$ 20 µM Ca^{2+}, the average number of channels open (A) is reduced to half by addition of 10-20 µM ATP to the cytoplasmic bath. The non-metabolizable ATP analogue ß-γ, methylene ATP is equally effective. NAD(H) and NADP(H) mimic this effect at 200-500 µM while ADP is only effective at 2 mM. AMP and cAMP (up to 2 mM) are ineffective, with cAMP occasionally increasing A. Altering cytoplasmic solution pH from 6.6 to 7.3 does not appear to alter A or the ATP-induced reduction in A. A is also unaffected by addition of glucose, glucose 6-P or glyceraldehyde (up to 25 mM) or Na azide (up to 3 mM). When ATP is washed out, channels reopen, often with a larger A then after initial excision into a solution containing 0 ATP. This "refreshment effect" on A wanes over the next 10-20 min can be seen over several cycles of ATP introduction and washout. No "refreshment effect" was seen with ß-γ methylene ATP. The oral hypoglycemic sulfonylurea tolbutamide also reduced A at concentrations of 0.1-1 mM but did not effect the activity of the 220 pS Ca^{2+} activated K^+ channels. Tolbutamide closes down the 65 pS K^+ channel in cell attached patches when applied to the bath at 2-5 µM. Hence nucleotides, especially ATP, and secretogogue drugs may gate the same potassium channel which mediates B cell depolarization. (Supported by DRTC at Washington Univ.).

M-Pos283 STRETCH-ACTIVATION OF A K-CHANNEL IN SNAIL HEART CELLS. Wade J.Sigurdson and Cathy E.Morris, Biology Dept., U. of Ottawa, Ottawa, Ont. Can. K1N6N5. Single channel recording from isolated Lymnaea stagnalis heart cells has revealed a stretch activated K selective (SAK) channel (Brezden, Gardner and Morris, J.exp.Biol. in press). To characterize the stretch sensitivity of the SAK channel we apply suction through the recording pipette, monitoring the negative pressure by an in-series manometer. Pipettes (Corning 7052) fashioned to have relatively parallel sides near the tip consistently form gigaseals. These patches generally withstand about 15 cm Hg before breaking. Examination of inside-out patches (400X Nomarski) shows that the patches are not at the tip, but are situated at least 10 µm inside. Such patches consistently contain >3 SAK channels; it is usually not possible to determine the number. The figure illustrates pressure sensitivity measured from a cell attached patch. An index of the probability of being open (P(O)) is obtained as the time-averaged current divided by the single channel current. Typically, patches show threshold behaviour, with stretch-sensitivity becoming evident in the range of 3-6 cm Hg. Threshold behaviour may not be intrinsic to the channel. To help test this, different pipette geometries are being tested. With our present geometry, application of pressure causes the patch to round up and distend. For these experiments, a high K (30mM) saline was used. The reversal potential was -40 mV and the conductance was 45.5pS. Supported by grants from Canadian Muscular Dystrophy Assoc.and NSERC, Canada.



M-Pos284 PHARMACOLOGICAL ISOLATION AND CHARACTERIZATION OF A VOLTAGE-DEPENDENT K CHANNEL IN GH3 CELLS. Kay W. May* and Gerry S. Oxford, Physiology Dept., Univ. of N.C., Chapel Hill, NC 27514.

The GH3 pituitary tumor cell exhibits two general classes of K currents, a Ca^{++}-activated current (IKCa) and a voltage-dependent current (IKV) which inactivates. We have combined patch clamp measurements of macroscopic and single K channel currents with pharmacological techniques to isolate and discriminate features of voltage-dependent K channels in these cells. In whole-cell clamp, external TEA^+ blocks 20% of peak K current, however following a depolarizing prepulse to enhance IKCa and inactivate IKV, TEA^+ blocks the remaining current during a test pulse. Internal EGTA greatly reduces IKCa and subsequent addition of 5mM TEA^+ eliminates the remaining steady-state current leaving "pure" IKV. In some cases a small residual current (4% of total) remains after prepulse inactivation of IKV. The channels responsible for IKV have a high selectivity for K^+ over Na^+ or Cs^+ as revealed by exchange of the patch electrode solutions during whole-cell experiments. External K^+ is required for expression of IKV. IKV is reversibly reduced by external Co^{++} and Cd^{++} but not Mg^{++}. External 4AP reversibly blocked IKV in whole cells, but was more potent when added internally by exchange of the electrode solution. Frequency-dependent relief of 4AP block was not observed. Single K channel currents were recorded in inside-out patches using the same solutions as for macroscopic experiments. These single channels were reversibly blocked by internal 4AP. Activation of IKV is voltage-dependent with half-activation occuring at 0mV. Inactivation of IKV proceeds with 2 exponential components exhibiting voltage-independent time constants of 25 and 100 ms. Each component is half-inactivated at a potential of -40mV suggesting that they represent two states of the same channel rather than separate channel types. (Supported by NIH grant NS18788).

M-Pos285 PHORBOL ESTER BLOCK OF VOLTAGE-DEPENDENT K^+ CURRENT IN MONOCYTE-DERIVED MACROPHAGES. D.J. Nelson, L. Rufer, T. Nakayama, and J.M. Zeller. The University of Chicago, Dept. of Medicine, Chicago, Il. 60637.

Mononuclear phagocytes (circulating monocytes and tissue macrophages) are capable of increasing oxygen uptake more than 50 fold when exposed to soluble and particulate stimuli. The sharp increase in oxygen uptake associated with what is termed the respiratory burst generates the microbicidal oxidants of phagocytes. The present study employed a suspension culture system to examine changes in respiratory burst activity coincident with changes in voltage dependent current activation during human monocyte differentiation in vitro. Monocytes which were cultured for up to 10 days in teflon vials, elicited less that 30% of the luminol-enhanced chemiluminescence (CL) produced by fresh monocytes following phorbol myristate acetate (PMA, 100 ng/ml) stimulation. Whole-cell voltage clamp experiments which were performed on freshly isolated monocytes showed only a high resistance linear membrane, however, after one day in culture cells exhibited a voltage- and time-dependent outward current which was K^+-dependent, 4-AP sensitive, and activated upon membrane depolarization above approximately -30 mV. To determine whether those stimuli capable of eliciting the respiratory burst do so through the modulation of voltage dependent current activation, whole-cell current recordings were made from a holding potential of -70 mV in the presence and absence of 2 ng/ml PMA. Voltage clamp analysis revealed an 80-95% reduction in the long-term component of the outward K^+ current. In a typical cell, the outward current amplitude at 5 sec was reduced from 165 to 24 pA at 110 mV with no obvious changes in inactivation kinetics. These results suggest that PMA may induce protein kinase C activation and ultimately respiratory burst activity while effecting a reduction in the voltage-sensitive K^+ current. Supported by NIH-NS18587.

M-Pos286 TRANSIENT AND DELAYED POTASSIUM CURRENTS IN THE RETZIUS CELL OF THE LEECH, MACROBDELLA J. Johansen and A. L. Kleinhaus Dept. of Neurology, Yale Univ. School of Med. New Haven, Ct. 06510.

It has been previously reported that leech neurons can support Na-and Ca-dependent action potentials through separate inward conductance mechanisms (Kleinhaus & Prichard, J. Physiol. 1975; Comp. Biochem. Physiol. 1983). However, information about the nature and properties of outward time- and voltage-dependent currents in these neurons has been completely lacking. Using two-electrode voltage clamp techniques, we have identified two distinct K-currents in the Retzius cell of the leech Macrobdella with the properties of transient (I_A) and delayed (I_K) K-currents. The time-courses of both currents were fitted by Hodgkin-Huxley type equations and their kinetic parameters analyzed. The transient current I_A had rapid voltage-dependent activation kinetics with a time constant which varied from 6 to 2 msec for command potentials between -20 and 10 mV at 22-24°C. The inactivation process was slower, voltage-independent and had a time constant of about 90 -110 msec. The steady-state inactivation of I_A varied with holding potential and was complete at potentials more positive than -30 mV. I_A was completely blocked by 3 mM 4-AP but was insensitive to external TEA. The delayed K-current I_K had slower activation and inactivation kinetics than I_A was nearly completely blocked by 25 mM TEA, but persisted in 4-AP. The time constants for activation and inactivation were voltage-dependent. τ_m varied from 12-4.5 msec and τ_h from 1500-700 msec for potential steps to 0-30 mV. The data represent the first kinetic analysis of K-currents in hirudiniid neurons under controlled voltage and apart from adding to the knowledge of leech physiology such information about different ion conductances is of importance for phylogenetical considerations of ion channel evolution.

M-Pos287 THE COMPLEX CONDUCTANCE OF THE INWARD, K-CURRENT RECTIFICATION PROCESS IN APLYSIA NEURONS. H. Hayashi* and H.M. Fishman, Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas, 77550.

The complex admittance of single neurons in the abdominal ganglion of Aplysia was determined by application of Fourier synthesized signals to a two-microelectrode voltage clamp system and by Fourier computations. Complex conductances were obtained by complex vector subtraction of the admittance near zero-mean holding current and in zero $[K_o]$ from admittances in non-zero $[K_o]$ and at all other voltages (-90 to -20mV). The resulting complex conductance has a capacitive component that dominates above 50Hz, is unaffected by potential changes and may be due to $[K_o]$-induced admittance changes in the attached axon or morphological changes in clefts. Two prominent conductance relaxations also occur; one near 10Hz, the other near 0.1Hz. Both relaxations are voltage sensitive, but the slower one occurs in K-free media whereas the faster one disappears in zero $[K_o]$ and is suppressed in Ba media. Curve fits of a linear, complex conductance model to the complex conductance data from neurons yielded times for the faster component that increased from 4 to 8msec in the potential range of -80 to -40mV and that were sensitive to $[K_o]$. Thus, the faster relaxation (msec) appears to be due to inward-current carried by K_o and the slower relaxation (sec) due to inward-current carried by Cl (Chenoy-Marchais, 1983, J. Physiol., 342:277).

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M-Pos288 LATE OUTWARD CURRENT IN CULTURED, LYMPHOKINE-ACTIVATED HUMAN LYMPHOCYTES. Paul A. Sheehy, Elizabeth A. Grimm* and Jeffery L. Barker. (SPON. E. K. Gallin) Laboratory of Neurophysiology and * Surgical Neurology Branch, NINCDS, NIH, Bethesda, MD 20892.

Resting human peripheral blood-derived lymphocytes were grown in vitro in the presence of 100 units(Cetus)/ml Interleukin-2 to induce solid tumor-directed killing activity. Under such conditions cytolytic activity is detectable after 2-3 days and is associated with a subpopulation of large granular lymphocytes. After 1 to 9 days in culture cells were voltage-clamped using the whole-cell variation of the patch-clamp technique. Electrodes contained (in mM) 140 KF, 5 NaCl, 0.1 $CaCl_2$, 1.1 EGTA, 0.6 $MgCl_2$, 10 Hepes, pH 7.25. All cells exhibited time- and voltage-dependent outward K^+ currents similar to those previously described in freshly isolated T lymphocytes. After 2-3 days of culture a subpopulation of larger lymphocytes expressed outward current at voltages positive to 0 mV which developed after the transient K^+ current had largely inactivated (>500 msec). The reversal potential determined from tail currents ranged between 0 and -10 mV and was unaltered by exchanging external NaCl with TEACl, by raising $[K^+]_o$ from 4.6 mM to 20 mM or by adding 1 mM $CdCl_2$. Substitution of CsF for KF inside the pipette blocked the transient K^+ current but not the later current response. These results suggest that specific ionic currents may be associated with the development of effector activities during lymphocyte differentiation.

M-Pos289 POTASSIUM CHANNELS IN PROTOPLASTS FROM SAMANEA SAMAN. N. Moran[1], G. Ehrenstein[1] K. Iwasa[1], C. Bare[2], C. Mischke[2], and R. Satter[3]. [1]Lab. of Biophysics, NINCDS, NIH, Bethesda, MD 20892; [2]Weed Science Lab., ARS, AEQI, Beltsville, MD 20705; [3]Dept. of Mol. and Cell Biology, Univ. of Conn., Storrs, CT 06268.

In the pursuit of a role for ion channels in plant cells, we have recently expanded patch clamp studies to membranes of protoplasts from cells of the motor organ of Samanea saman. Previous experiments have shown that the leaflet movement of this plant results from cell volume changes associated with large ion fluxes (primarily of K^+ and Cl^-) through cell membranes in the motor organ. In the "whole cell" configuration and voltage clamp mode, depolarizing pulses elicited outward membrane currents with time constants of seconds. Since K^+ was the predominant cation and since 40 mM TEA applied externally abolished most of the current, it is likely that K^+ is the carrier of this current. For a pulse to +20 mV, the membrane conductance of the cell rose up to about 1000 pS. In the "cell attached" mode, we observed single channels with a voltage-dependent conductance ranging from 15 to 40 pS. The open probability of these channels increased upon depolarization and decreased within several seconds upon hyperpolarization. 30 mM TEA in the external solution, perfused within the recording pipet, blocked most of the single-channel current.

We conclude that the plasmalemma of Samanea saman protoplasts contains depolarization-activated potassium channels. These channels may be the pathway for passive K^+ fluxes, leading to cell volume changes.

M-Pos290 K^+ SELECTIVE ION CHANNELS IN HIT CELLS. Douglas B. Light and David G. Levitt, Dept. of Physiology, Univ. of Minnesota, Minneapolis, MN 55455

Glucose metabolism by pancreatic β-cells is required for insulin secretion, and has been linked to an initial depolarization of the cell via a decrease in K^+ permeability. Single channel current records were obtained from inside-out patches from HIT-T15 cells (a Simian virus transformed hamster beta cell line that secretes insulin). Seven different K^+-selective ion channels ranging from 15pS to 215pS (in symmetric 140mM KCl solutions) were distinguished along with one nonselective channel of 370pS. A K^+ channel of 215pS was voltage and pH activated but Ca^{++} insensitive. At -20mv this channel was inactive at pH 6.6, partially active at pH 7.4, and fully active at pH 8.0; at -20mv and pH 7.4, 1mM concentrations of ATP, ADP, AMP, GTP, AMPCPP, or AMPPCP were sufficient to fully activate the channel, whereas, 3mM concentrations of cAMP, cGMP, or AMPPNP were ineffective. Another K^+ channel of 200pS was Ca^{++}-activated and voltage dependent, but pH and nucleotide insensitive. At -30mv it was inactive in 5 x 10^{-8} M free Ca^{++}, partially active in 10^{-7} M free Ca^{++}, and fully active in 5 x 10^{-7} free Ca^{++}. A third K^+ channel of 15pS was insensitive to voltage, Ca^{++}, pH, and nucleotides. Two other K^+ channels, of 50 and 60pS, were insensitive to voltage, Ca and pH. The former was blocked by 5mM TEA but not .1mM quinine, whereas the latter was blocked by 1mM quinine but not 5mM TEA. An additional K^+ channel of 200pS was voltage and 1mM TEA sensitive but insensitive to Ca^{++}, quinine, pH and nucleotides. Finally, another K^+ channel of 190pS was voltage, 5mM TEA and pH sensitive, but insensitive to Ca^{++} and nucleotides. The nonselective channel was blocked by 1mM TEA. The physiological role of these channels is unclear and remains to be elucidated (supported in part by NIH grant AM 30519-03).

M-Pos291 Potassium current in cultured glomerulosa cells from adrenals rats.Effect of ACTH and TEA.

PAYET,M.D.,GALLO-PAYET,N. and SAUVE,R. Dept Biophysics,Dept Medicine,Univ.SHERBROOKE,and Dept Physiology,Univ MONTREAL,QUEBEC,CANADA.

Ionic currents from cultured glomerulosa cells were studied with the patch clamp technique in whole cell configuration.Depolarising pulses elicited from HP-60 mV trigger a strong outward current which activates rapidly and inactivates almost completly over 4 to 5 s.The IV curve shows that the zero current voltage is found at -40 mV and that the channel rectifies in the outward direction.The peak-conductance voltage relationship reaches a steady state level(13 nS) at potentials positive to -5 mV.The activation phase is S-shaped and the inactivation phase could be described by the sumof 2 exponentials.The time constant,Tau n,decreases from 11 ms(-24 mV) to 2.7 ms (14 mV) if the membrane is depolarised.Tau jfast,for the fast inactivation component,is rather voltage insensitive (mean value 500 ms) but Tau jslow,for the slow component,decreases from 3.1 s to 1.7 s (-24 to 14 mV).ACTH,a natural secretagogue was added in the bath at a final concentration of 10^{-8} M.The outward K current was rapidly reduced by 84% after 5 min.TEA (20 mM),a known K current blocker,was also found to reduce the same K current as ACTH do.

Based on these results,a secretagogue effect effect of TEA was postulated.Indeed,we found that TEA increases therate of aldosterone and corticosterone secretion in a dose dependant way and that a too high TEA concentration decreases the secretion.ACTH and TEA induced-secretions are blocked by Cobalt and TEA increases the ACTH response for low ACTH concentrations only.

Supported by MRCC to MDP and NGP.

M-Pos292 THE RATE OF ACTIVATION OF M CURRENT AT POSITIVE POTENTIALS IN BULLFROG SYMPATHETIC GANGLION CELLS. P. Pennefather, Faculty of Pharmacy, U. of T., Toronto, Ontario M5S 1A1.

In the range of membrane potentials between -20 and -100 mV, both the steady state and kinetic voltage dependence of the M-currents (I_M) can be described by a Boltzman distribution predicted for a model where I_M is regulated by a gating particle with a valency of 2.5. At -35 mV, I_M is half maximally activated and has a time constant of 140 ms (Adams et al., 1982, J. Physiol., 330: 537-572). The model predicts a time constant of 1.9 ms at +40 mV and that an appreciable fraction of I_M can be activated by an action potential. In order to confirm this, we have attempted to measure the rate of activation of I_M at positive potentials. A two electrode voltage clamp was used. Experiments were carried out in 25 mM K to reduce distortion due to K accumulation. Calcium was removed and Cd (0.2 mM) was added to minimize distortion introduced by calcium dependent currents. Octanol (1 mM) was added to inhibit the delayed rectifier current. This medium had no effect on the time and voltage sensitivity of I_M at potentials below -20 mV. At potentials above -20 mV, the level of outward current other than I_M was reduced to only 2-3 times that of I_M. Under these conditions an I_M component of the tail current following a command to a positive potential could be identified by its time constant and its sensitivity to muscarine. Commands of 10 or 20 ms duration, to positive potentials, evoked much less I_M than expected. The data suggests that at +40 mV, I_M will activate with a time constant greater than 16 ms and that very little I_M will be activated during a single action potential.

P.P. is a Career Scientist of the Ontario Ministry of Health; supported by NS 18579 to P.R. Adams

M-Pos293 SINGLE CHANNEL RECORDS FROM THE MURINE MACROPHAGE CELL LINE J774.1. L.C. McKinney and E.K. Gallin., Dept. of Physiology, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145.

The macrophage cell line J774.1 expresses two K conductances, an inward rectifying conductance present in long-term (>4 hr) adherent cultures and an outward rectifier expressed only transiently (2-6 hr) after plating (Gallin and Sheehy, J. Physiol., 1985). We have obtained single channel records using the cell-attached patch configuration whose properties correspond to those of the inward rectifying whole-cell current. When cells were bathed in Na Hanks (KCl = 4.5 mM, NaCl = 145 mM) and the electrode contained K Hanks (KCl = 145 mM, NaCl = 10 mM) discrete inward current fluctuations were noted at the zero current potential (ZCP, ~-70 mV). The current amplitude increased as the patch potential became more negative, decreased at potentials more positive than -70 mV, and was not observed to reverse up to 125 mV. Thus, the voltage range over which the channel was active was the same as that over which the inward rectifier was observed. The channel conductance (G) was 29 ± 1 pS (n = 12) which increased to 40 ± 2 pS (n = 3) with 300 mM K in the electrode. The increase in G was proportional to $\sqrt{K}$ corresponding to the behavior of the whole-cell current. The channel was open about 50% of the time at the ZCP, became more active at more negative potentials, then showed long duration closures as the patch potential declined further. The extrapolated reversal potential (Erev) of the channel was -10 ± 6 mV (n = 6; Ek = 0 mV). Placing the cell in K Hanks did not significantly change Erev assuming Vm shifted from -70 to 0 mV. Using the whole-cell G obtained from cells in K Hanks together with the 29 pS channel G, we estimate that J774 cells contain at least 1300 ch/cell. This channel was only observed in cells which exhibited the inward rectifier current. These data suggest that this channel underlies the inwardly rectifying current observed in J774.1 cells.

M-Pos294 SINGLE-CHANNEL RECORDING IN PARAMECIUM. Boris Martinac, Yoshiro Saimi, Michael C. Gustin and Ching Kung, Laboratory of Molecular Biology, University of Wisconsin, Madison, WI 53706

The cell membrane of Paramecium has a variety of ion channels. The ion currents through the channels in both wild type and mutants have been studied in detail using two-electrode whole-cell voltage clamp. We present here the first report on single-channel recording in Paramecium by use of the patch-clamp technique. Single-channel currents were recorded in plasma membrane blisters which were induced by exposing cells to a high-salt solution (100 mM NaCl). Two types of channels were studied in more detail using inside-out excised patches. Both were identified as K^+ channels due to their strong selectivity for K^+ over Cs^+. Their characteristics are as follows:

I. Ca^{2+}-dependent K^+ channel: a) Conductance was approximately 150 pS. b) Opening probability increased with increasing depolarizing voltage and Ca^{2+} concentration. c) Plot of P_o vs. Ca^{2+} concentration suggested binding of one Ca^{2+} ion. d) Kinetically, this channel could be described by a minimal number of three closed and one open state.

II. A Second K^+ Channel: a) Conductance was 42 pS. b) Opening probability showed almost no voltage dependence and varied from channel to channel between 60 and 98%. c) It showed no dependence on Ca^{2+}. d) Kinetically, this channel could be described by a minimal number of two closed and two open states.

Future experiments on single-channel activities may reveal how different activities contribute toward the macroscopic currents recorded from whole cells and how mutationally or chemically altered channels behave. (Supported by NSF BNS-8216149 and NIH GM-22714.)

M-Pos295 K-CHANNEL BLOCKERS AND T-LYMPHOCYTE PROLIFERATION. S. Lee, M. Price and C. Deutsch. University of Pennsylvania, Department of Physiology G4, Philadelphia, PA 19104. (Intr.by D.Wilson)

Previous work has demonstrated voltage-gated K channels in human and murine T-lymphocytes (Matteson & Deutsch, Nature 307:468; DeCoursey et al., Nature 307:465; Fukushima et al., JP 351:645), which can be blocked with pharmacological agents that will also inhibit mitogen-stimulated proliferation (Chandy et al., JEM 160:369). However, considerably higher concentrations of channel blockers are required to inhibit proliferation than to block the channels under voltage clamp. In serum-free medium, we found a significant decrease in the K_i's for half-maximal inhibition of phytohaemagglutinin-stimulated proliferation of human peripheral blood T-lymphocytes with the channel blockers quinine (K_i = 82.0 uM when cultured with 10% human serum to 39.5 uM in serum-free), 4-aminopyridine (3.8 mM to 0.9 mM), tetraethylammonium (13.5 mM to 8.3 mM) and Cs (13.8 mM to 4.8 mM), whereas the K_i's for channel block by quinine (22 uM) and 4-AP (0.25 mM) were shifted by less than 50% in whole-cell patch clamp at 25°C in the presence of 10% serum. Therefore, serum binding alone cannot explain either the K_i discrepancy between inhibition of mitogenesis and conductance or the relative inefficiency of 4-AP block of proliferation. Extracellular Cs (140 mM) produces little block of outward K current, while high intracellular Cs (130 mM + 25 mM K) blocks completely. Blockade at lower Cs concentrations (50 mM + 105 mM K) is strongly voltage dependent and not significant at negative potentials. A suggested role for the K channel is to mediate the rise in intracellular Ca which occurs on mitogen binding; however, inhibition of proliferation due to any of the above blockers is not overcome by raising extracellular Ca or adding the Ca ionophore ionomycin. (NIH AM 27595).

M-Pos296 GENE-DOSAGE EFFECTS ON A K^+ CURRENT IN DROSOPHILA. F.N. Haugland and C.-F. Wu, Depts. of Physiol. and Biophys. and Biology, University of Iowa, Iowa City, IA 52242.

Mutations of the Shaker (Sh) locus of Drosophila specifically affect the transient K^+ current I_A. Voltage clamp of larval muscle has shown that various alleles eliminate or alter I_A. However, the number of gene products expressed by the Sh locus and their roles in the synthesis or regulation of I_A channels are not known. We examined the effect of combining different alleles in heterozygotes for clues of interactions among different Sh gene products.

I_A in heterozygotes possessing Sh^M (or 6 others which eliminate I_A) and one normal allele (Sh^+) is reduced to about 50% of normal. These muscles may possess 50% fewer normal channels. This simple gene dosage effect holds true for other alleles with residual I_A (e.g. Sh^{E62}, Sh^5 and Sh^{rK0120}). Combinations between these alleles or with above alleles produce an I_A which is about the average of the two. Even the altered voltage dependence characteristic of Sh^5 was manifested according to gene dosage in Sh^5 heterozygotes (Sh^5/Sh^+, Sh^5/Sh^M, etc). This suggests that the I_A channel(s) contains a single copy of the gene product(s) defined by these alleles.

Sh^{KS133}, which itself eliminates I_A, violates the simple gene-dosage effect. For example, I_A in Sh^{KS133}/Sh^+, Sh^{KS133}/Sh^5 and Sh^{KS133}/Sh^{rK0120} is reduced to less than 10% of that expected. This highly nonlinear interaction could result from a diffusable gene product which disrupts the synthesis or function of the channel component(s) defined by the other alleles. The Sh^{KS133} region may thus involve a different genetic mechanism, producing a gene product distinct from that perturbed by the other mutations. Supported by NIH and NIMH grants NS00675, NS18500 and MH15172.

M-Pos297 THE MODULATION OF A K^+ CHANNEL BY NERVE GROWTH FACTOR AND INTERFERON IN DIFFERENTIATING F9 EMBRYONAL CARCINOMA CELLS. John Ives Halperin, Naval Medical Research Institute, Bethesda, MD. (Introduced by S. Yeandle)

Retinoic acid and dibutyryl-cAMP induces differentiation in the F9 embryonal carcinoma cell line. Patch clamp techniques were used to characterize an ionic channel induced by this differentiation procedure.

The conductance on-cell was about 130 pS under deplorizing voltages. These channels were specific to K^+ over Na^+. Channel kinetics were voltage-dependent while conductance was voltage-independent (between +10 and +120 mV from rest). Kinetics were also influenced by $[Ca^{2+}]$ on the cytoplasmic face. This channel is probably the calcium-activated potassium channel described for many excitable cells.

Nerve growth factor (NGF) induced an increase in the average opened times and a decrease in the average closed times of these channels. The experiments were done on-cell and the effect was rapid (reaching a maximum in less than 1 minute) when a concentration of 10 ng/ml or more was used. Apparent dose dependence was indicated by a delay in the onset of the effect when 5 ng/ml was used. When NGF was used in the differentiation protocol, action potential-like spikes were detected in some cells after 10 days in culture.

Interferon was also tested for its affects on these high-conductance potassium channels. Murine α and β interferon and human A interferon induced a decrease in the average closed times but had no apparent effect on the opened times of these channels. Work was supported by Department of Biology, Georgetown University, Washington, D.C.

M-Pos298 EFFECTS OF PROSTAGLANDINS ON 2 POTASSIUM CHANNELS OF ISOLATED GASTRIC SMOOTH MUSCLE CELLS. T.E. Ary and K.M. Sanders, U. of Nevada School of Medicine, Reno, NV 89557 USA.

The patch clamp technique of Hamill et.al. (Pflug.Arch. 391:85-100,1981) was employed to examine and identify the ionic basis for prostaglandin modulation of the electrical events responsible for gastric motility (Sanders and Szurszewski, Am.J.Physiol. 241:G191-G195,1981). Strips of gastric muscle were excised from the corpus region of stomachs of Bufo marinus. These were minced and digested in trypsin and collagenase to disperse single isolated smooth muscle cells. Patch pipets formed gigaseals (2-4 gigaohms) readily with the muscle membranes. In the cell-attached, inside-out patch configuration two ion channels could be distinguished which had slope conductances of 110 and 35 pS (recorded with 110mM K^+, 1.08mM Ca^{2+}, and 20mM HEPES in the pipet). When the pipet was filled with a solution containing 50 mM K^+, the shift in the zero-current potential was consistent with a channel selective for K^+-ions. Tetraethylammonium (TEA,20mM) in the pipet abolished activity of the large conductance channel and reduced the open-time probability of the small conductance channel. PGE_2 and $PGF_2\alpha$ (pipet concentration = 10^{-7}M) decreased the open-time probability of both channels. At 10^{-6}M both prostaglandins abolished activity of the 110 pS channel. The effects of these prostaglandins occur in the same concentration range as the effects on tissue electrical events. It is possible that the effects of prostaglandins on electrical activity may result from the effects observed on potassium channels. (Supported by NIAAA grant 05883 and Nevada Affiliate of the American Heart Association.)

M-Pos299 SINGLE CHANNEL STUDY OF A CALCIUM-ACTIVATED POTASSIUM CHANNEL OF A SMALL UNITARY CONDUCTANCE IN HELA CANCER CELLS. R. Sauvé, C. Simoneau and G. Roy*. Département de physiologie, département de physique* & Groupe de recherche en transport membranaire, Université de Montréal, Montréal, Québec H3C 3T8.

A single channel study based on the patch clamp method described by Hamill et al. (1981) was undertaken in order to characterize the molecular mechanisms responsible for the calcium dependent potassium permeability observed on HeLa cancer cells (Roy and Sauvé, 1983)**. Our results essentially indicate that the HeLa cell external membrane contains potassium channels whose activity can be triggered within an internal calcium concentration range of 0.1 to 1 μM. This particular channel was found to behave as an inward rectifier in symmetrical 200 mM KCl with a conductance of 50 and 10 pS at large negative and large positive membrane potentials respectively. I-V curves were also measured in 10, 20, 75, 200 and 300 mM KCl and the data interpreted in terms of a one site two barrier model. In addition, the channel activity appeared to be moderately voltage dependent, an increase in open channel probability, P_o, being associated with more negative potential values. The observed voltage sensitivity corresponded to an e-fold change of P_o per 110 mV. Furthermore, an analysis of relationship between P_o and the cytoplasmic free calcium concentration has revealed that at least two calcium ions are required in order to open the channel. We found in this regard that an increase of the internal free calcium level affects more the number of channel openings per second than the actual channel mean lifetime. Finally, it was concluded following a time interval distribution analysis, that this particular channel has at least three closed states and one open state. ** Can. J. Physiol. Pharmacol. 61: 144-148, 1983.

M-Pos300 CALCIUM-REGULATED CHANNELS IN A SMOOTH MUSCLE CELL LINE (A10). M.E. Friedman, G.M. Katz, K. Kusano, M.L. Garcia, G.J. Kaczorowski and J.P. Reuben, Dept. of Biochemistry, Merck Institute for Therapeutic Research, Rahway, New Jersey 07065.

In characterizing the receptor- and voltage-regulated channels in A10 cells by whole-cell and isolated patch clamping, propranolol (1-50 μM) was noted to have a unique effect. It, only in the absence of intracellular EGTA, reversibly hyperpolarized the membrane by as much as 50 mV (under current clamp) and enhanced outward currents. Under identical conditions, no hyperpolarization was observed and both inward and outward currents were attenuated by propranolol in GH_3 pituitary cells as has previously been reported by Akaike *et al*. Br. J. Pharmacol. 73;431 (1981) for neuronal cells. Although A10 cells have β-receptors, this effect of propranolol is not β-receptor mediated since β-antagonists (timolol, alprenolol, tolamolol) as well as agonists (isoproterenol) neither mimic nor modify the propranolol-induced responses. In view of the apparent dependence of the responses on Ca_i, we examined isolated patches exposed to Ca (1-2 mM) in both the electrode and bathing solution. To date, we have focused on currents generated by a multistate 400 pS_{max} channel with a unitary state of 100 pS. Since the linear I *vs*. V curve is shifted 10-20 mV positive to zero (inside-out patch) by halving Cl in the bathing solution (normally $Cl_i = Cl_o$), it is assumed to be a chloride channel. This channel portrays a high level of flickering between states and this behavior was enhanced by propranolol. Also observed routinely was a 30 pS channel whose ion selectivity and possible modification by propranolol has yet to be examined. It remains to be determined whether the effects of propranolol on chloride channel activity in isolated membrane patches will solely account for the hyperpolarization and enhancement of outward currents observed in intact A10 smooth muscle cells.

M-Pos301 ION BLOCKADE OF CALCIUM-ACTIVATED POTASSIUM CHANNELS IN AMPHIBIAN GASTRIC SMOOTH MUSCLE CELLS. Brendan S. Wong, Department of Physiology, Baylor College of Dentistry, Dallas, TX 75246

Single smooth muscle cells dissociated from the stomach of *Rana pipiens* by repeated enzymatic digestion with 0.1% trypsin and 0.1% collagenase in a calcium-free Ringer solution were studied using the patch-clamp technique. The predominant outward current in these cells passes through a calcium- and voltage-activated potassium-selective channel with a single-channel conductance of approximately 200 pS in symmetrical 116 mM K solutions. The effects of tetraethylammonium (TEA) and internal Na ions on the movement of K ions through these channels have been studied. Either extracellularly or intracellularly applied TEA ions reversibly blocked the channel, resulting in a decrease in the amplitude of the single-channel current. The TEA blockade was essentially voltage-independent over the membrane potential range studied, with extracellularly applied TEA being the more effective blocker. Dissociation constants of 0.15 mM and 20 mM was observed for external and internal TEA, respectively. Internal Na ions were found to cause an increase in the number of flickering bewtween the open and closed states for outward-going currents while having little effect on inward-going currents. At high internal Na concentrations, the single-channel current-voltage curve displayed a negative slope at large depolarized membrane potentials, indicating a voltage dependence for internal Na block. These results suggest that both TEA and internal Na interfere with the movement of K ions through calcium-activated potassium channels in smooth muscle cells, but acting at different time scales.

(Supported by BCD Research Grant and the American Heart Association, Texas Affiliate.)

M-Pos302 NEUROLEPTICS BLOCK THE CALCIUM-ACTIVATED POTASSIUM CHANNEL IN AIRWAY SMOOTH MUSCLE.
John D. McCann & Michael J. Welsh, Dept. of Int. Med., Univ. of Iowa, Iowa City, IA.

We examined the effect of neuroleptics on Ca-activated K channels because these agents can inhibit a variety of other Ca-mediated processes. Excised, inside-out patches of membrane that contained one or two channels were bathed with symmetrical 140 KCl solutions and 1mM internal [Ca] to maximize the open probability and to obtain the most reproducible kinetics. Several neuroleptics potently and reversibly blocked the K channel from the internal but not the external surface. Measurement of the effect on the probability of the channel being open indicates dissociation constants for the drug-channel complex of Haloperidol (HALDO) 1.0±0.1 μM, Trifluoperazine 1.6±0.2 μM, Thioridazine 2.3±0.2 μM, and chlorpromazine (CHLOR) 12±3 μM. The rank-order potency of these neuroleptics in blocking the K channel is different from their potency as calmodulin inhibitors. Under these conditions, the open-state durations could be fit by a single exponential; 1 μM HALDO decreased the open-state time constant from 42 mS to 6 mS. Under control conditions the closed-state durations could not be fit by a single exponential. However, with increasing concentrations of HALDO the mean closed time increased and with 1 μM HALDO the closed distribution was fit with a single exponential with a time constant of 10 mS. Greater concentrations of HALDO produced no further increase in closed times. The data support a simple kinetic model in which the neuroleptics reversibly bind to and block the open channel. Block by HALDO and CHLOR was weakly voltage-dependent, both blockers were less potent at hyperpolarizing voltages. However, the charged neuroleptic, Methochlorpromazine, had the same voltage-dependence as the uncharged compounds. These agents may prove valuable in attempts to isolate and identify Ca-activated K channels.

M-Pos303 **Effects of a Unique Ca-Channel Inhibitor, Diacetyl Monoxime (DAM), on Patch-Clamped K^+ Current in Embryonic Chick Hearts.** **Hideaki Sada, Ghassan Bkaily, and Nicholas Sperelakis, Department of Physiology and Biophysics, University of Cincinnati, Cincinnati, OH 45267-0576**

Diacetyl monoxime (DAM) was reported to reduce the Ca^{++} influx (I_{Ca}), presumably through its dephosphorylating action on the Ca^{++} channel proteins (J. Pharmacol. Exp. Ther., 212:217, 1980; Europ. J. Pharmacol., 71:307, 1981). In the previous study (Europ. J. Pharmacol. 112:145, 1985), one action of DAM was suggested to be depression of I_{Ca}. Since DAM greatly shortened the duration of APs in embryonic chick hearts and rabbit ventricular muscle, the enhancement of the potassium current(s) (I_K) by DAM was also suggested. In the present study, a cell-attached patch clamp was used to determine whether DAM also affected I_K. Ventricular muscle dissected from old (16-17-day) chick embryos was dispersed by trypsinization, and the cells were placed into culture for 1-10 days. The K^+ concentration inside the patch pipettes was either 5 or 150 mM. In the control, the current signals recorded showed a marked inward-going rectifying property at pipette potentials between 0 and -70 mV (with 5.4 mM K^+). In the current/voltage relation, the current magnitude was minimal at 0 mV, and small at pipette potentials between 0 and -60 mV (reversal potential; near 0 mV). When the K^+ concentration inside pipettes was raised to 150 mM, the reversal potential was shifted to a more negative potential range, and following a nearly pure K^+ electrode. In the presence of DAM (10-20 mM), both the incidence and duration of the channel openings were greatly increased, regardless of [K] inside the pipette. The amplitude of these single channel currents was little affected by DAM. The peak effects were attained within 30-60 sec after drug addition. Hence, it is concluded that DAM enhanced I_K and does this mainly by increasing the probability of opening and open time of the channels. This would account for the shortening of AP duration produced by DAM. (Supported by grant HL-31942.)

M-Pos304 ELECTRICAL MEMBRANE PROPERTIES AND Ca-ACTIVATED K-CHANNELS IN NORMAL HUMAN SENSORY NEURONS IN CULTURE. K. Nieminen, B.A. Suarez-Isla and S.I. Rapoport. Laboratory of Neurosciences, NIA, NIH, Bethesda, Maryland 20892.

Down syndrome expresses an abnormality in the electrical membrane properties (EMPs) of cultured human dorsal root ganglia(DRG) neurons(1). In particular, neurons show a decreased hyperpolarizing afterpotential (HAP) which could be related to a decreased potassium permeability. In order to generate a basis for comparison with Down DRG neurons, we examined the EMPs of normal human DRG neurons with conventional intracellular recording, and studied Ca-activated K-channels and whole cell currents with the patch clamp technique. DRG neurons were obtained from tissue from abortuses 14 to 17 weeks old. Neurons were dissociated with trypsin, plated on collagen-coated Petri dishes and kept in Eagle's Minimum Essential Medium, 10% fetal calf serum, at 37°C, 5% CO_2. Cells were tested at regular intervals after 6 to 30 days in culture. The mean resting potential was -50 ± 1.6 mV (n=29; all values are means ± s.e.m.) in neurons from 6 dissociations. For 6 representative neurons, the mean input resistance was 30.8 ± 7.4 Mohm and action potential parameters were: dVmax/dt= 58.2 ± 10.3 V/s; AP amplitude= 75.6 ± 4.4 mV; HAP amplitude= 7.9 ± 2.0 mV and HAP duration at half amplitude= 9.7 ± 2.2 ms. In the cell-attached patch mode, 2 types of outward channels of 110 and 30 pS were observed (normal Tyrode in the bath and pipet). The 30 pS channel was less frequent but both could be adscribed to Ca-sensitive K-conductances. These findings establish baseline values for electrical properties of normal human sensory neurons in culture. (1) Scott, B. S., Develop. Brain Res. 2, 257-270 (1982).

M-Pos305 Ca^{2+}-ACTIVATED K^+ CHANNELS IN 1321N1 HUMAN ASTROCYTOMA ARE ACTIVATED BY EXTRACELLULAR CARBACHOL. Barry S. Pallotta and Scott A. Oglesby, Dept. Pharmacology and Curriculum in Neurobiology, Univ. North Carolina School of Medicine, Chapel Hill, NC 27514

Using single channel recording techniques, a Ca^{2+}- and voltage-sensitive K^+-selective channel was found in surface membrane patches from 1321N1 astrocytoma cells grown in culture. Membrane patches typically contained 10-20 channels. Single channel conductances ranged from 290-330 pS in symmetric solutions of the following composition (mM): KCl, 144; $MgCl_2$, 2; EGTA, 1; TES, 2; pH 7.2. Intracellular surface Ca^{2+} was varied over a range of 0.01 to 10 μM and did not affect the conductance. Extracellular surface Ca^{2+} was 0.01 μM in these experiments.

Channel open probability increased with both depolarization and Ca^{2+} concentration. At +50 mV, for example, channel % open time increased from approximately 0.1 to 95% as Ca^{2+} was varied from 0.01 to 10 μM. When Ca^{2+} was fixed at 1 μM, % open time increased from approximately 3 to 95% over the voltage range -60 to +60 mV.

On-cell channel activity from K^+-depolarized 132-cells showed at least 0.1% open time at +50 mV (n=5). Bath-application of 1 μM carbachol + 2mM Ca^{2+} caused a rapid (sec) increase in channel activity, with peak % open times ranging from 38-61%. In three experiments channel activity was maintained throughout the carbachol perfusion (5-14 minutes) and for several minutes after halting carbachol perfusion. In two experiments channel activity declined approximately 16 seconds and 2 minutes, respectively, after initiating carbachol perfusion. These results suggest that extracellular carbachol application caused an increase in intracellular Ca^{2+}. Supported by NIH grant GM32211.

M-Pos306 THE LARGE CONDUCTANCE CA-ACTIVATED K CHANNEL: ACCOUNTING FOR THE CA SENSITIVITY. Owen B. McManus and Karl L. Magleby, Department of Physiology and Biophysics, University of Miami School of Medicine, Miami, FL 33101

Currents from single large conductance Ca-activated K channels in excised membrane patches from rat myotubes were recorded and analyzed to develop a kinetic scheme to account for the effects of $[Ca]_i$ on channel activity. Maximum likelihood methods were used to determine the most probable rate constants for a given model by simultaneously fitting distributions of open and shut intervals obtained at three different $[Ca]_i$. Analyzed data was stable and missed events were accounted for. Most likely models allowed binding of four or more Ca ions and had six shut and three open states. Openings occurred directly from three different shut states. The models could describe the distributions of open and shut intervals over a range of Ca concentrations that produced a sixty fold change in open probability. The rate constants for binding of Ca to the shut states of the channel increased as more Ca ions bound to the channel, except for the binding of the final Ca to the shut state. The rate of binding of Ca to the open states increased as more Ca ions were bound. As more Ca ions bound to the channel, the relative heights of the energy barriers to opening from the shut states decreased and the relative energy barriers to closing from the open states increased. In addition to accounting for the Ca-dependence of the distributions of intervals and open probability within single experiments, the model could account for the Ca-dependence of a 700 fold change in open probability for data pooled from 12 experiments. Supported by NIH grants AM 32085 and NS 0707044 and a grant from the Muscular Dystrophy Association. OBM was a recipient of a Muscular Dystrophy Association Fellowship.

M-Pos307 FAST CHLORIDE CHANNELS FROM RAT SKELETAL MUSCLE SHIFT BETWEEN NORMAL AND BUZZ MODES OF ACTIVITY. A. L. Blatz and K. L. Magleby. Dept. of Physiology and Biophysics, University of Miami School of Medicine, Miami, FL 33101.

The stability of single chloride channel kinetics was studied using voltage clamped excised inside-out surface membrane patches from rat myotubes. Durations of 100,000 successive open or shut intervals were averaged, 50 at a time, and the mean durations plotted against interval number. The fluctuations of the moving mean duration about the observed mean open and shut intervals could be predicted for 99% of the intervals by a model with five shut and two open states. However, about eight times in 100,000 intervals the mean open and shut interval durations fell to markedly lower values for fifty to several hundred successive intervals. In one example of such a period, the mean open interval fell from 0.97 to 0.12 ms and the mean shut fell from 0.89 to 0.22 ms. The single channel current during this period consisted of a burst of about 300 consecutive very brief open and shut intervals. These results suggest two modes of activity of the fast chloride channel: normal mode, which contributes about 99% of the open and shut intervals, and a buzz mode, which contributes about 1%. Buzz mode activity was consistently observed in all experiments. Normal mode activity is described by five shut and two open conformational states. Activity during the buzz mode is approximated by a simple two state open-shut model with a closing rate constant of 17,200/s and an opening rate constant of 9700/s. With increased filtering or higher temperatures, transitions to the buzz mode appear as partially conducting states due to the time averaging of the brief intervals. Supported by NIH grant AM 32085 and a grant from the Muscular Dystrophy Association.

M-Pos308 CORRECTING SINGLE CHANNEL DATA FOR MISSED EVENTS. K. L. Magleby and A. L. Blatz, Dept. of Physiology and Biophysics, University of Miami School of Medicine, Miami FL 33101.

Interpretation of currents recorded from single ion channels is complicated by the necessarily limited time resolution of the recording and detection systems. Brief open and shut intervals go undetected leading to increased durations of the observed intervals. A method is presented to correct for these missed events for models with two or more states. The method can be used to predict the distributions of open and shut intervals that would be observed for a given model, rate constants, and time resolution. The fractions of missed events for the various open and shut states are first calculated for the kinetic scheme and time resolution. These fractions are then used to obtain effective rate constants which are used with the Q-Matrix method of Colquhoun & Hawkes (1981) to calculate the distributions of open and shut intervals that would be observed experimentally. Iterative techniques allow the true underlying rate constants to be obtained from experimentally observed distributions. Missed events can add additional exponential components and/or lead to missed exponential components, depending on the kinetic scheme and time resolution, as indicated by Roux & Sauvé (1985). Even two state models and models without loops are found to have at least two sets of rate constants which give identical descriptions of the data. Incorrect sets resulting from missed events can be eliminated by analyzing the data at different time resolutions, as suggested by Colquhoun & Sigworth (1983). The effect of decreasing time resolution on observed data for a five state model for the large conductance Ca-activated K channel is presented in detail. Supported by NIH grant AM 32085 and a grant from the Muscular Dystrophy Association.

M-Pos309 VESICLES EXTRACTED FROM SKELETAL MUSCLE FIBERS VIA SUCTION: POSSIBLE IMPLICATIONS FOR LOOSE AND TIGHT PATCH VOLTAGE CLAMPING. R.L. Milton and J.H. Caldwell, Dept. of Molecular and Cellular Biology, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206 and Dept. of Physiology, Univ. of Colorado Medical School, Denver, CO 80262.

Observations we have made using the loose patch voltage clamp technique on enzyme dissociated flexor digitorum brevis muscle fibers of the rat suggest that caution be observed when interpreting results from loose patch and possibly also tight patch voltage clamp measurements. We have observed the formation of large vesicles (up to 45 μm in dia.) following the application of suction to loose patch pipettes (4 to 20 μm in dia.) pressed against the fiber membrane. These vesicles arise suddenly from small localized regions of membrane and remain connected to the fiber by a tether <2 μm in diameter. Since these vesicles can contain an order of magnitude more membrane than was initially under the pipette, large errors are possible when relating total current to specific membrane current. Membrane Na^+ current does not exhibit a sudden increase either during or shortly after vesicle formation. We suspect that the vesicular membrane may not be representative of normal surface membrane, but may largely consist of those membrane components with the highest mobility in the bilayer, i.e., lipids and freely mobile proteins. Proteins that form large aggregates or are tied to the cytoskeleton may be excluded. If a similar process of vesicle formation is occurring during the formation of tight patch seals, it could explain the surprisingly large amount of membrane drawn up into the pipette during seal formation. Also if this membrane is, in fact, not truly representative of normal surface membrane, then the properties of channels in this membrane may be abnormal.

M-Pos310 INCORPORATION OF CALCIUM CHANNELS FROM CARDIAC SARCOLEMMAL MEMBRANE VESICLES INTO PLANAR LIPID BILAYERS. B.E. Ehrlich, M.L. Garcia* and G.J. Kaczorowski*, Dept. of Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, New York 10461, and *Dept. of Biochemistry, Merck Institute for Therapeutic Research, Rahway, New Jersey 07065.

Incorporation of purified porcine cardiac sarcolemmal vesicles into planar lipid bilayers formed at the tip of patch style pipettes from 1:1 PE:PS results in the appearance of divalent cation channel activity. Single channel measurements in Ba medium indicate that this activity is voltage-dependent, is noninactivating during prolonged depolarization and is markedly selective for divalent cations over anions. Determination of divalent cation selectivity from single channel reversal potentials indicates a relative permeability ratio for Ba:Ca·Mg of 1:.45:.08. Mean channel conductance in 0.1 M Ba, 0.01 M Mg is 8 pS. Channel activity is reversibly inactivated in Ca medium. This vesicle preparation possesses distinct high affinity receptors for 3 classes of organic Ca entry blockers; dihydropyridines, aralkyl amines and benzothiazepines. Reconstituted channel activity is increased in the presence of Bay K8644 (prolonged open times), reversibly blocked by nitrendipine, and dihydropyridine inhibition is competitively antagonized by Bay K8644. Receptor studies demonstrate that the aralkyl amine site displays stereoselectivity with (-) D-600 binding 25-fold more avidly than (+) D-600. In accordance with these results, (-) D-600 (1.6 μM) reversibly blocks channel activity while (+) D-600 (1.6 μM) does not. In addition, d-cis-diltiazem (2 μM) also reversibly inhibits reconstituted channels. These results demonstrate that the divalent cation channel reconstituted from sarcolemmal membranes possesses many of the characteristics of voltage-regulated Ca channels in heart and that the sites responsible for pharmacological sensitivity to organic Ca entry blockers are functionally associated with this channel.

M-Pos311 **REGULATION OF CA CHANNELS CONDUCTING NA IN THE ABSENCE OF EXTERNAL CALCIUM INVOLVES FACTORS OTHER THAN VOLTAGE.** M. Mazzanti and L. J. DeFelice, Dept. of Anatomy and Cell Biology, Emory University, Atlanta, Ga. 30322

We studied the slow Na-conducting channel during spontaneous APs in cell-attached patches on ventricular cells from 7-day chick embryo. In the absence of Ca (less than 10^{-7} M) in the patch pipette (133 Na, 3 EGTA), we see a slow inward current carried by Na through calcium channels (Hess and Tsien, Nature 309: 453-456, 1984; Levi and DeFelice, IUPAB, p. 280, Bristol, 1984.) Patching a non-beating cell, and clamping the pipette potential to simulate the voltage during spontaneous activity, causes average channel current to occur earlier during the AP. Since the voltage experienced by both the beating and non-beating cells is the same, we conclude that the Ca channels (conducting Na in the absence of external Ca) are not simply voltage-dependent, but are influenced during the spontaneous beating either by the influx of ions across the cell membrane or the release of Ca from the reticulum. Figure: Comparison between whole-cell, spontaneous stimulation of the slow inward current (top) and patch stimulation of a non-beating cell. Bath solution (in mM): 2 K, 133 Na, 1.5 Ca.(Supported by NIH HL27385)

M-Pos312 EFFECT OF MEMBRANE POTENTIAL ON [^{3}H]NITRENDIPINE (NIT) BINDING DETERMINED IN AN ISOLATED CARDIAC SARCOLEMMA PREPARATION (SL PREP). W.P. Schilling and J.A. Drewe, Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX, 77550.

To test the hypothesis that NIT binding affinity changes with membrane potential, potassium Nernst potentials were developed in the presence of valinomycin, by establishment of potassium gradients across the vesicular membrane of a highly enriched SL prep isolated from canine ventricle. Aliquots of SL prep loaded with 140 mM KCl, 10 mM TRIS-Cl (pH 7.4 for 20°C) were equilibrated with [^{3}H]-NIT for 75-90 min at 20°C. A large dilution of this prep (> 200-fold) was made into a medium containing (final concentrations) 0.1 μM valinomycin, 10 mM TRIS-Cl, pH 7.4, sufficient KCl to yield potassium Nernst potentials of -95 and 0 mV and sufficient N-methyl-D-glucamine to maintain ionic strength equal to that of the loading medium. Dissociation of NIT with time (5 sec to 90 min) was monoexponential at both -95 and 0 mV ($k_{-1} = 9.67 \times 10^{-4} \pm 4.7 \times 10^{-5}$ S^{-1}). However, extrapolation of the dissociation time course to zero time revealed that a component of binding was rapidly lost (i.e., before 5 sec) upon hyperpolarization of the membrane. As potential varied from 0 to -60 mV approx. 17% of the total NIT bound was lost within 15 sec. An additional 8-10% loss was observed between -80 and -134 mV. In parallel experiments with LiCl as the salt in the polarization media, the rapid loss of binding at negative potential was only observed in the presence of valinomycin. The magnitude of the valinomycin-sensitive component of NIT loss was ∿20% and occurred between -80 and -134 mV. These findings are consistent with the hypothesis that NIT binding affinity decreases upon membrane polarization.

M-Pos313 SPECIFIC OUABAIN (OB) AND NITRENDIPINE (NIT) BINDING IN AN ISOLATED CARDIAC SARCOLEMMA PREPARATION (SL PREP): EFFECT OF DIVALENT CATION CHELATION. W.P. Schilling and J.A. Drewe, Dept. of Physiol. and Biophys., Univ. of Texas Med. Branch, Galveston, Texas, 77550.

The effect of removal of divalent cations on OB and NIT binding has been determined in a highly enriched SL prep isolated from canine ventricle. Maximal binding of OB determined in the presence of Mg and Pi (control conditions) averaged 340±7.4 pmol/mg. Pretreatment of the prep with SDS increased OB binding to 471±27 pmol/mg. Thus, these SL preps consist of 27.8% sealed inside-out vesicles. Addition of EDTA, in the absence of added Mg reduced OB binding to 204±7.7 and 11.7±3.5 pmol/mg in control and SDS-treated preps respectively. The simultaneous presence of EDTA and the divalent ionophore A23187 reduced OB binding to 13±3.2 and 4.0±0.6 pmol/mg in control and SDS-treated preps, respectively. These findings suggest that this SL prep consists of 43.6% sealed right-side-out vesicles which contain sufficient endogenous divalent cation trapped in the intravesicular space to support OB binding. Maximal high affinity NIT binding measured in the absence of added Ca or Mg was 968±89 fmol/mg. NIT binding in the presence of EDTA decreased to 380±51 fmol/mg which equates to 39.3% of control. The simultaneous presence of EDTA and A23187 in the binding buffer resulted in a decrease in NIT binding to below detectable levels. These results suggest that divalent cations trapped within vesicles can support high affinity NIT binding. The correspondence between the percentage of OB binding that remains in the presence of EDTA and the percentage of NIT binding under the same conditions suggests that 1) NIT and OB binding sites reside in the same vesicles (i.e., the NIT binding site is of sarcolemma origin), and 2) divalent cations interact with sites on the cytoplasmic membrane surface to support NIT binding.

M-Pos314 COMPARISON OF DIHYDROPYRIDINE ACTIONS ON CALCIUM CURRENTS OF CARDIAC AND SMOOTH MUSCLE CELLS. J. M. Caffrey, A. Yatani and A. M. Brown (Intr. by Susan L. Hamilton). Department of Physiology, Baylor College of Medicine, Houston, Texas 77030.

Pharmacological studies have shown that dihydropyridines (DHPs) such as nitrendipine (Nit) have higher affinities for smooth muscle than for cardiac muscle. This has been attributed to voltage-dependent binding of DHPs to Ca channels with smooth muscle normally more depolarized than cardiac muscle. We have compared the actions of Nit and Bay K 8644 which are racemates and the optical isomers (S and R) of the DHP 202-791 on Ca channels of cardiac and smooth muscle cells under voltage clamp. Single cells were isolated from guinea pig ventricle and Amphiuma stomach. Dose-response curves having at least three measurements per point were constructed. At holding potentials (hp) of -80 mV the IC_{50}s for Nit and R in smooth muscle were between 1/2 and 1/10 the values in cardiac muscle. Similar smooth muscle: cardiac muscle ratios were observed at hp's of -30 mV. In both tissues the IC_{50}s were between 30 and 100 times smaller at the depolarized hp's. We also found at equivalent hp's that the ED_{50}'s for Bay K 8644 and the S enantiomer were lower in smooth muscle. Thus the differences in potency of DHPs for cardiac and smooth muscle cannot be explained by differences in resting potential. We conclude that the DHP receptors are different in the two tissues. Supported by NIH HL25145 and American Heart Association 85 1159.

M-Pos315 ATROTOXIN INCREASES PROBABILITY OF OPENING OF SINGLE CALCIUM CHANNELS IN CULTURED NEONATAL RAT VENTRICULAR CELLS. Antonio E. Lacerda and A. M. Brown (Intr. by Michael C. Andresen). Department of Physiology, Baylor College of Medicine, Houston, Texas 77030.

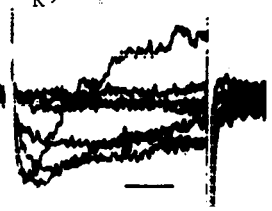
Atrotoxin (ATRX) has been shown in whole cell patch clamp experiments to increase specifically the calcium current of adult guinea pig ventricular cells (Hamilton et al., Science 229:182, 1985). To characterize the effect of ATRX on single cardiac calcium channels experiments were done using outside-out patches from cultured neonatal rat ventricular cells. 115 mM Ba was used in the bathing solutions so that whole cell and single channel results were directly comparable. Two populations of single Ca channels were observed in the outside-out patches corresponding to the low and high threshold Ca channels described by Nilius et al. (Nature 316:443, 1985). Whole cell currents and summed single channel records from neonatal ventricular cells showed two components of inward current; a transient low threshold current and a maintained high threshold current. A partially purified fraction of ATRX increased calcium current in whole cells by approximately 20%. Analysis of single channel data from outside-out patches using this fraction showed that unitary current amplitudes were unchanged. ATRX does not affect the low threshold channels but does increase the probability of opening of the high threshold channels. This effect is due mainly to an increase in the number of openings per record. Supported by NRSA HL07348 and NIH HL25145.

M-Pos316 GTP-BINDING PROTEINS MEDIATE TRANSMITTER INHIBITION OF VOLTAGE-DEPENDENT CALCIUM CHANNELS IN EMBRYONIC CHICK SENSORY NEURONS. George G. Holz, Stanely G. Rane, and Kathleen Dunlap. Department of Physiology, Tufts University School of Medicine, Boston, MA 02111.

Exposure of chick dorsal root ganglion (DRG) cells to noradrenaline (NA) or gamma aminobutyric acid (GABA) leads to a decrease in voltage-dependent calcium current, a decrease in action potential duration (APD) and an inhibition of neurosecretion. The present study examined whether GTP-binding proteins (G-proteins) mediate these responses. Microelectrode and whole cell patch clamp recordings obtained from primary cultures of DRG cells (Dunlap and Fischbach, Nature 276 837, 1978; Rane and Dunlap, PNAS, in press) demonstrated that the 50 uM NA and GABA-induced decrease in APD or calcium current was blocked by pertussis toxin (PTX, 140 ng/ml) in a time and temperature-dependent fashion. A progressive decrease in the fraction of cells responding to NA was observed after: 1) incubation in PTX for 30, 50 or 70 min at 37 °C, or 2) incubation in PTX for 4 hr at 5, 23 or 37 °C. These changes were paralleled by a progressive decrease in the magnitude of the response to NA. The actions of PTX suggest that transmitter responses are mediated by a G-protein similar in stucture to N_i (Gilman, Cell 36 577, 1984). Studies of DRG cells voltage clamped and internally dialyzed with guanosine 5'-O-[2-thiodiphosphate] (GDP-β-S) support this conclusion. The 10 uM NA-induced decrease in voltage-dependent calcium current was blocked by GDP-β-S (100-500 uM) in a dose-dependent fashion. The actions of PTX and GDP-β-S are specific for receptor-mediated inhibition of calcium channels: neither blocks the decrease in APD or calcium current in response to 60 uM 1,2-oleoyl acetylglycerol. These findings constitute the first direct demonstration that G-proteins mediate the transmitter modulation of voltage-dependent calcium channels.

M-Pos317 Ca^{2+} AND K^+ CURRENTS IN SINGLE VASCULAR SMOOTH MUSCLE CELLS FROM RAT AORTA. L. Toro* and E. Stefani. Dept. Physiology, Biophysics and Neurosciences. Centro de Investigación y de Estudios Avanzados del IPN. Apdo. Postal 14-740, 07000 México, D.F.

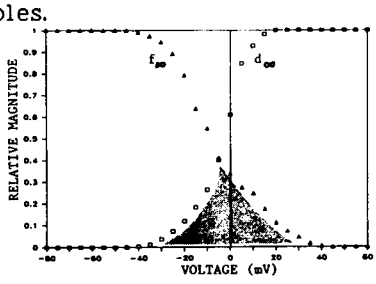
We report Ca^{2+} (I_{Ca}) and K^+ (I_K) currents in single isolated vascular smooth muscle cells (VSMC). Cells from thoracic aortas from 20 days rats, were maintained for 2-3 days in culture. Whole cell voltage clamp technique was used. Membrane properties were as follows: C_m=17±3 pF (n=16), R_{in}=3±1 GΩ(n=19), Area:(20±3)10^{-6} cm^2 (n=10) and C_m/cm^2=0.9±0.09 μF/cm^2 (n=10). Two cell populations were encountered: one had both, I_{Ca} and I_K (70%) (Fig. 1); and the other, had prominent I_K and no clear I_{Ca} (30%). I_{Ca} was recorded in 0 Na-10 μM TTX external solution, with internal Cs^+ substituted for K^+. From a V_h=-70 mV, I_{Ca} was detected at -30 to -50 mV. For a pulse to +36 mV, the peak time was 10 msec, the activation constant (τ_m) ≈ 1.5 msec and the decay time constant (τ) ≈ 35 msec. The maximal current in the I-V curves occur between -10 and +30 mV and the maximum slope conductance (g_{Ca}) ranges from -20 to -60 μS/cm^2. On the other hand, in cells with prominent I_K, depolarizations beyond -10 mV produced this type of current, which exhibited practically no inactivation. From I-V curves, g_K=350±10 μS/cm^2 (n=8); I_K was eliminated when Cs^+ was used instead of K^+ in the patch pipette. These results show in a direct manner the existence of Ca^{2+} and K^+ voltage operated channels in VSMC of rat aorta in culture. Fig. 1. Ca^{2+} and K^+ currents in rat aortic SMC. V_h= -97 mV, V_c from -87 to -7 mV in 10 mV steps. Calibration bar: 20 pA, 10 msec.



Supported by grants 1R01 AM35085 (NIH, USA), PCCBBEU-022519 (CONACyT,MEXICO) and Fundación Zevada. *Doctoral Training Program, UAM-I.

M-Pos318 STEADY-STATE KINETIC PARAMETERS OF THE CALCIUM CURRENT (I_{Ca}) IN NEONATAL RAT SINGLE CARDIAC MYOCYTES. Neri M. Cohen and W. J. Lederer. Dept. of Physiology, University of Maryland Medical School, 660 W. Redwood St., Baltimore, MD 21201

Kinetic parameters of calcium currents (I_{Ca}) were studied in single cardiac myocytes isolated from neonatal (2-7 day old) rat hearts and grown in primary culture. Whole-cell currents were recorded using the GΩ seal whole-cell voltage-clamp method in the presence of 20 mM CsCl in the pipette and 10 μM TTX in the bath at 22-23 °C. I_{Ca} is completely blocked by 0.1 mM Cd or by 1 μM D600. The figure shows the data obtained when appropriate voltage protocols are used to measure the steady-state activation (d_∞) and inactivation (f_∞) variables. With 1 mM Ca^{2+} in the superfusion solution the data are best fit by the equations $d_\infty=(1+\exp[-(V-(-4))/5.5])^{-1}$ and $f_\infty=(1+\exp[(V-(-8))/10.5])^{-1}$. The overlap of the two curves (shaded area) implies that there is a large component of steady-state I_{Ca} contributing to calcium influx during the action potential plateau. In neonatal rat heart, modulation of I_{Ca} will have important effects on action potential shape and duration as well as on tension.



Supported by a PMA grant to NMC and NIH grant HL 25675 to WJL.

M-Pos319 DIFFERENT TYPES OF CARDIAC CALCIUM CHANNELS INCORPORATED INTO PLANAR LIPID BILAYERS. J.F. Worley III, L.A. Fieber, L. Jones and M.T. Nelson. Department of Pharmacology, University of Miami School of Medicine, Miami, Fl. 33101 and Department of Pharmacology and Medicine, University of Indiana School of Medicine, Indianapolis, IN.

A membrane vesicle preparation from cardiac muscle containing both junctional sarcoplasmic reticulum and transverse tubule membranes was incorporated into planar lipid bilayers. The specific nitrendipine binding of the membrane fraction was about 1 pmol/mg. Planar lipid bilayers composed of PE and PS were formed across a 150-250 μm hole separating two aqueous solutions. The solution on the side of membrane vesicle addition (defined as the cis side) was typically 80-250 mM $BaCl_2$, pH 7.0 and on the opposite side (trans side) was 0-250 mM $BaCl_2$, 0-150 mM NaCl, 0.5-2.5 mM EGTA, pH 7.0. To increase the open times of the calcium channels, the calcium channel agonist, Bay K 8644 (5 μM), was routinely included in the bathing solutions. After addition of the heart membrane preparation (final concentration 1-10 μg/ml) to the cis side, three different sizes of single channel currents were observed. The single channel conductances were 7-10 pS, 20-25 pS, and 120-150 pS and all three channel types select for barium ions over sodium and chloride. The two lower conductance channels may be related to the two types of calcium channels observed in intact single cells from heart (cf. Nilius et al., Nature, 1985). The large conductance channel appears to be similar to a recently reported calcium channel from skeletal muscle sarcoplasmic reticulum (cf. Smith et al., Nature, 1985).

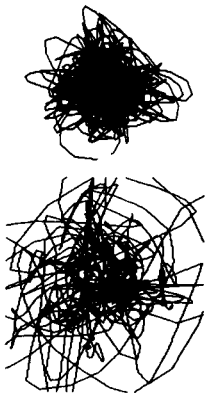
M-Pos320 THE CALCIUM CURRENTS OF ANTERIOR PITUITARY CELLS. C.F. Marchetti(*), G.V. Childs(**) and A.M. Brown(*), (Intr. by Arthur M. Brown), (*)Department of Physiology, Baylor College of Medicine, Houston, Texas 77030 and (**)Department of Anatomy, UTMB, Galveston, Texas 77550.

The relation of calcium currents to function in pituitary cells has not yet been established. Calcium currents were investigated in dissociated culture cells from adult rat anterior pituitary using the patch clamp technique. The preparation is heterogeneous and contains five different types of pituitary cells. Ninety per cent of the cells had two components of the calcium current as reported in GH_3 cells. These are a high voltage activated (threshold, -10 mV in 20 Ca) persistent component (HVA) and a low voltage activated (-50 mV in 2 Ca, -40 mV in 20 Ca) transient component (LVA). The contribution of LVA was almost completely abolished when the holding potential (HP) was -50 mV or more whereas the contribution of HVA was much less affected at HP -50mV. There were pharmacological differences as well. LVA seemed insensitive to the agonist effect of BAY K 8644 (1 μM), was not blocked by Verapamil (100 μM) and was not completely abolished by a high concentration 2 mM $CaCl_2$. The relative amplitude of the two components varied from cell to cell and was independent of the presence of (Mg) ATP (2mM) and/or cAMP (0.1 mM) in the internal solution. In Tyrode solution, another fast transient inward current was present in half of the cells. This was identified as a Na current from kinetics and TTX sensitivity. The activation voltage was more positive than that of LVA calcium current. This may be related to the fact that even cells which did not show significant Na current in voltage clamp had spontaneous fluctuations of up to 40 mV amplitude in current clamp. The frequency of these fluctuations was in one case increased by the addition of 30 nM GRF.

M-Pos321 SINGLE CHANNEL CALCIUM AND BARIUM CURRENTS IN THE GH_3 CELL LINE. D.L. Kunze and A.K. Ritchie, Baylor Coll. Med., Houston, TX 77030 & Univ. Texas Med. Br., Galveston, TX 77550.

The permeability of Ca channels to Ba and Ca were compared in the GH_3 pituitary cell line. GH_3 cells possess 2 types of Ca currents (Matteson and Armstrong, JGP 83:371-394, 1984) that are distinguished by different activation thresholds and inactivation characteristics. Both whole cell and single channel experiments were performed using a bath solution containing (mM) 10 $CaCl_2$ or $BaCl_2$, 130 TEACl, 1 $MgCl_2$, 5.6 KCl, 10 HEPES, 5 4AP, 10 Glucose and a pipette solution containing (mM) 130 $CsCl_2$, 11 EGTA, 1 $CaCl_2$, 2 $MgCl_2$ and 10 HEPES. The I-V relationship for the rapidly inactivating low threshold Ca current was obtained by subtracting the noninactivating component from the peak early current. Maximum amplitude of the low threshold current in Ca and Ba were similar. Single channel conductances were calculated for 10 outside-out patches from measurements of long duration openings over the potential range of -30 to 0 mV. The low threshold channel conductance was 5.0 pS in Ba and 5.3 pS in Ca. Thus the permeability of the low threshold channel for Ba is similar to that of Ca. Mean open times at -30 mV were also similar, 1.27±0.41 ms ($\bar{x}$±S.D., N=3) for Ba and 1.25±0.32 ms for Ca. The whole cell high threshold current measured as the amplitude of the noninactivating component was five times larger in Ba as compared to Ca. The unit conductance for the high threshold channel was 9 pS in Ba and 3.5 pS in Ca. The greater permeability for Ba than Ca accounts in part for the larger whole cell currents in Ba. The mean open time at -20 mV for this channel was not significantly different in Ba (1.05±0.21 ms) as compared to Ca (0.96±0.17 ms). An increase in opening probability may account for the remainder of the difference. Supported by DHHS HL27116 and DHHS AM33898.

M-Pos322 PHASE SPACE DISPLAY AND DIMENSIONAL ANALYSIS OF HUMAN E.E.G. DURING GENERAL ANESTHESIA,
R.C. Watt & S.R.Hameroff, Dept. of Anesthesiology, Univ. of Arizona, Tucson, AZ 85724



Introduction: Electroencephalography (EEG) measures scalp potentials emanating from cerebral cortex and is useful for neurological diagnosis and monitoring of brain integrity and anesthetic depth during surgical procedures. Raw EEG waveforms may be difficult to assimilate and interpret, so new display modes such as Fourier transform and spectral analysis have emerged. We describe another method in which digitized EEG data is plotted in the phase space and dimensional analysis used to correlate the degree of order and chaos with level of consciousness.

Method: With Human Subjects' Committee approval, EEG was recorded from the C3P3 electrode pair during surgery under general anesthesia. EEG data was digitized and plotted as [x(t) vs x(t+T)] in which x is the voltage, t is time and T is a fixed time interval. Phase space trajectories were computer plotted, and dimensional analysis done according to the method of Grassberger and Procaccia.

Results: EEG data appear as dynamic trajectories which are visually robust (Figure at left, Top: awake patient, Bottom: anesthetized). As patients become more anesthetized, the trajectories "unravel" with larger circumference and decreased density. Dimensional analysis shows lower dimensions as patients become more deeply anesthetized.

Discussion: Phase space display and dimensional analysis may have useful features. Our study suggests the EEG data moves reversibly from "chaos" to "order" with general anesthesia.

M-Pos323 CHANGES OF LOOSE PATCH CLAMP SEAL RESISTANCE ASSOCIATED WITH EXOCYTOSIS OF CORTICAL GRANULES IN RELATION TO CHANGES OF PATCH MEMBRANE CONDUCTANCE IN EGGS OF THE SEA URCHIN Lytechinus variegatus. David H. McCulloh and Edward L. Chambers, (Intro. by Birgit Rose), Department of Physiology and Biophysics, University of Miami School of Medicine, Miami, FL 33101.

A major increase of membrane conductance starts from the site of sperm attachment and sweeps around the egg propagated by a wave of stimulation which travels through the cytoplasm of the whole sea urchin egg during fertilization (J. Cell Biol. 101 in press). Measurements of local membrane conductance are sensitive to changes of the resistance of the "seal" between the plasma membrane and the loose patch clamp pipette. The purpose of this study was to determine if changes of seal resistance could be responsible for the measured increase of local membrane conductance. The seal resistance was found to increase transiently during fertilization from its initial value (roughly 10X the pipette resistance) and then to decrease to a value much less than the initial seal resistance. The seal resistance increased after the conductance of the membrane patch increased. Therefore, the biphasic change of seal resistance cannot be responsible for the increased conductance of the membrane patch. The time when seal resistance increased was correlated with the distance between the site of sperm attachment and the tip of the patch pipette, indicating a similarity to propagation of the increased patch conductance and the exocytosis of cortical granules. The causes of the seal resistance increase and decrease may involve an increased area of pipette-membrane contact during exocytosis, followed by a decreased closeness of contact after the fertilization envelope separates from the egg plasma membrane. (Supported by NIH F32-HD06505, NIH R01-HD19126 and NSF DCB-8316864.)

M-Pos324 EXOCYTOSIS IN MAST CELLS ELICITED BY DIACYLGLYCEROL AND PHOSPHATIDYLSERINE. E.F. Nemeth and K.M. Hoyle, Intr. by B.R. Masters, Dept. Biochem/Biophysics, Univ. of Penna., Philadelphia, PA 19104.

Exocytotic secretion of histamine from rat mast cells elicited by immunologic stimuli is potentiated specifically by phosphatidylserine (PS). Secretion elicited by concanavalin A (Con A) is absolutely dependent on the presence of PS, although the action underlying this adjuvant effect of PS is uncertain. We have found that cell permeant analogs of diacylglycerol, such as sn-1,2-dioctanoylglycerol (DiC_8, 3 μM) and 1-oleoyl-2-acetylglycerol (OAG, 2 μM), potentiated maximal and submaximal secretory responses to Con A + PS. These effects were also obtained with phorbol esters that activate protein kinase C (phorbol myristate acetate (PMA, 1 ng/ml) and 4ß-phorbol-12,13-dibutyrate (PDB, 10 ng/ml)), but not with inactive analogs (4α-phorbol (4αP) and 4α-phorbol 12,13-didecanoate (4αPDD), 100 ng/ml each)). PMA, DiC_8, and OAG were also found to substitute for PS in Con A-induced secretion, although slightly greater responses were obtained with the later adjuvant. The omission of extracellular Ca inhibited secretion elicited by Con A + PS by 83% but was without effect on secretion elicited by Con A + PMA. The combinations of PS plus PMA, PDB, DiC_8, or OAG, but not PS plus 4αP or 4αPDD, elicited exocytosis in the absence of Con A, and such secretory responses were partially dependent on the presence of extracellular Ca. The results suggest that the adjuvant effect of PS for Con A-induced exocytosis may involve an action on protein kinase C. The ability of PS plus phorbol esters or diacylglycerol analogs to evoke exocytosis in the absence of any conventional secretagogue suggests that activation of protein kinase C may be sufficient to initiate secretion in mast cells. Supported by NIH grant AM-33928.

M-Pos325 ROLE OF SODIUM IN NEUROTRANSMITTER RELEASE. D.T. Lee, D.R. Mosier, S. Molchan, and J.E. Zengel, Dept. of Neuroscience, Univ. of Fla. Coll. of Med. and Veterans Admin. Med. Ctr., Gainesville, FL 32610.

At least four processes can act to increase transmitter release during and following repetitive stimulation at the frog neuromuscular junction: two components of facilitation which decay with time constants of about 60 and 400 msec; augmentation which decays with a time constant of about 7 sec; and potentiation which decays with a time constant of tens of seconds to minutes (Zengel & Magleby, J. Gen. Physiol. 80:583-611, 1982). It has been suggested that stimulation-induced changes in intra-terminal Na^+ may be involved in one or more of these processes. To further examine the role of Na^+ in stimulation-induced changes in release, we looked at the effect of changes in extracellular Na^+ concentration on release during and following repetitive stimulation.

End-plate potentials (EPPs) were recorded from frog sartorius muscles under conditions of reduced quantal content (0.4-0.5 mM Ca^{++}, 5 mM Mg^{++}). The increase in EPP amplitude during 10 impulse (20/sec) conditioning trains was 50-70% greater when extracellular Na^+ was reduced by 50% (sucrose added to maintain osmolarity). Substitution of Li^+ for 50% of the Na^+ produced a smaller (~20%) effect. Reduction of extracellular Na^+ produced little or no change in the magnitude or time course of augmentation and potentiation following longer conditioning trains (400 impulses at 20/sec). These results suggest that reduction of extracellular Na^+ may selectively increase facilitation of transmitter release.

M-Pos326 EXTRACELLULAR CALCIUM AND "COOPERATIVITY" OF TRANSMITTER RELEASE AT THE SQUID GIANT SYNAPSE. George J. Augustine and Milton P. Charlton, Dept. of Biological Sciences, Univ. Southern California, Dept. Physiology, Univ. Toronto, and Marine Biological Laboratory, Woods Hole.

Transmission at many synapses is a steep, non-linear function of the extracellular Ca concentration ($[Ca]_o$). This has been interpreted as indicating that Ca ions may "cooperate" at some unknown step in transmitter release. Some experiments examining transmission at the squid giant synapse (at a constant $[Ca]_o$) have found a linear relationship between presynaptic Ca current and postsynaptic response. This implies that the non-linear relationship between $[Ca]_o$ and release reflects a non-linear dependence of the presynaptic Ca current upon $[Ca]_o$. We have tested this possibility at the squid giant synapse by measuring the Ca current of voltage clamped presynaptic terminals (under conditions which eliminate Na and K currents) while varying $[Ca]_o$ from 1 to 50 mM. The combined results of 12 experiments indicated that Ca current was a linear function of $[Ca]_o$, tending to saturate at the highest $[Ca]_o$ examined (K_D approximately 30 mM). Further, postsynaptic currents measured by simultaneously voltage clamping the postsynaptic cell had a steep non-linear dependence upon $[Ca]_o$ over the same range. These results indicate that the apparent "cooperativity" of Ca action occurs beyond the presynaptic Ca channel. It is possible that multiple Ca ions are required for one or more reactions, within the presynaptic terminal, which are responsible for transmitter release. Supported by NIH Grant NS 21624 to GJA and an MRC (Canada) Grant to MPC.

M-Pos327 PROTEIN KINASE C ACTIVATION ENHANCES TRANSMISSION AT THE SQUID GIANT SYNAPSE. L. Osses, S. Barry, G. Augustine and M. Charlton (Intr. by C. Lumsden). Dept. Biological Sciences, Univ. Southern California, Dept. Physiology, Univ. Michigan, and Dept. Physiology, Univ. Toronto.

Protein kinase C is a Ca/phospholipid-dependent kinase which is present in nervous tissue. We have tested the possible role of kinase C in synaptic function by examining the effects of agents which activate kinase C upon transmission at the squid giant synapse. Electrophysiological techniques were used to measure postsynaptic potentials (PSPs) or currents (PSCs) evoked by electrical stimulation of the 'giant' presynaptic terminal. Drugs were applied to the synapse by arterial perfusion. 12-deoxyphorbol 13-isobutyrate 20-acetate (DPBA), a phorbol ester which activates kinase C, produced dose-dependent increases in PSP amplitude (n = 8). DPBA also enhanced PSC amplitude (n = 10), which indicates that it does not increase PSPs by increasing postsynaptic input resistance. PSCs were 21% larger in 50 nM DPBA, 59% larger in 500 nM DPBA and 400% larger in 2 uM DPBA. These effects are apparently due to kinase C activation, rather than non-specific actions of DPBA, because structural analogs which do not activate kinase C (phorbol, 500 nM and 4α-phorbol 12,13-didecanoate, 1 uM) did not enhance transmission. Further, 12-O-tetradecanoylphorbol 13-acetate (1 uM), another phorbol ester which activates kinase C, also enhanced transmission. We conclude that kinase C activation affects transmission at the squid giant synapse. It is therefore possible that kinase C plays a role in mediating or modulating transmission at chemical synapses. Supported by a Grass Fellowship to LO, MBL (Woods Hole) Summer Fellowships to SB and GA, NIH Grant NS 21624 to GA and a MRC (Canada) Grant to MC.

M-Pos328 Elemental Composition of Bovine Adrenal Chromaffin Granules *in situ* and *in vitro*. R.L. Ornberg and R.D. Leapman, Lab. of Cell Biology and Genetics, NIADDK, and Biomedical Engineering and Instrumentation Branch, DRS, NIH, Bethesda, MD 20892

To better understand the formation, osmotic stability, and exocytotic release of secretory granules, we have measured the elemental composition of isolated secretory granules and secretory granules and the surrounding cytoplasm *in situ* in freeze-dried sections of unfixed, rapidly frozen isolated chromaffin granules and cultured chromaffin cells by x-ray microanalysis. Analysis of salt solution standards provided the basis for quantitative measurements of phosphorus (P), potassium (K), chloride (Cl) and calcium (Ca) as millimoles / kg dry weight. These were converted to millimolar concentrations assuming a 60% water content for the granule core. Our measure of granule phosphorus, *in situ* and *in vitro*, 650 mM, is in reasonable agreement with previous reports(1) in that granule P is in the form of ATP and that the ATP concentration is 150 - 200 mM. The granule potassium ranged from 120 to 150 mM *in situ* and dropped to 35 mM (80 % decrease) in isolated granules. However even this lower value for potassium is 5 to 10 times that previously reported. Since our method measures total K and previous work measured free K^+, our results suggest that potassium may be bound in the core. *In situ* granule calcium, 30 - 45 mM, was higher than that measured in isolated granules, 10 - 15 mM. This indicates that granules are a major store for calcium and may have the ability to release calcium in certain conditions, i.e. exocytosis. Chloride, which partitions across the granule membrane according to membrane potential, was more abundant in the granule than in the cytoplasm making the granules inside positive. References: (1) Winkler and Carmichel (1982) in *The Secretory Granule* A.M. Poisner and J.M. Trifaro (eds.) Elsevier-North Holland pp 2- 81.