W-Pos71 TEMPERATURE DEPENDENCE OF THE VESICLE-MICELLE TRANSITION OF EGG PHOSPHATIDYLCHOLINE (PC) AND OCTYLGLUCOSIDE (OG). M.G. Miguel, O. Eidelman, M. Ollivon+, R. Blumenthal and A. Walter*. National Institutes of Health, Bethesda, MD; +CNRS, Thais, France, and *Wright State University, Dayton, OH.

To develop a rational basis for reconstitution protocols, the temperature dependencies of the cmc of OG, the phase diagram of OG and egg PC, and aggregation of vesicular stomatitis virus-G protein (VSV-G) were determined. The OG cmc, as measured by ANS fluorescence, decreased from 31 mM at 5°C to 15.9 mM at 40°C. The behavior of the OG-PC-water system was monitored by the fluorescent lipid probes, NBD-PE and Rho-PE. Three regions were identified: lamellar (L), lamellar-micellar mixed phase (L&M), and micellar (M). The total [OG] at the two phase boundaries was linear with [PC] at all temperatures. The slopes of these lines are the OG:PC at the transitions, while the intercepts are the [OG] in the aqueous phase. The intercepts showed negative temperature dependencies which were less steep than that of the cmc. The L&M coexistence region is wider at 5°C (21.7-22.7 mM) than at 35°C (14.9-15.2 mM). The OG:PC ratio of the structures at L≒L&M decreased slightly from 1.88 to 1.75 as temperature rose from 5° to 35°C, whereas at the L&M≒L boundary, OG:PC increased significantly from 3.1 to 3.78 for the same temperature range. VSV-G solubilization, indicated by an increase in FITC-VSV-G fluorescence, was measured in the absence of lipid. VSV-G began to aggregate at [OG]~cmc for a given temperature and aggregation was complete just below the L&M≒M boundary. These data imply that temperature manipulation alone will not permit coexistence of monomeric VSV-G and micellar OG-PC structures, a condition considered necessary for successful protein reconstitution.

W-Pos72 MECHANISMS OF BACTERIORHODOPSIN INCORPORATION INTO LIPOSOMES USING DETERGENTS M. Paternostre and J.L. Rigaud, Laboratoire de Biophysique de Sytemes Membranaires Departement de Biologie, CEN/SACLAY, 91190 GIF/YVETTE FRANCE (Intr. by J.N. Weinstein)

To gain a better understanding of the mechanisms of protein insertion into liposomes, we examined the factors determining the solubilization by the detergents Triton X100 (TX00), Octylglucoside (OG), and Sodium Cholate (Na-Chol) of large unilamellar phospholipid vesicles prepared by reverse phase evaporation. Using the "Three-Stage" model describing the solubilizing process of phospholipid vesicles by detergents (Lichtenberg et al. ,BBA, 737, 285, 1983), we have determined for each detergent the "effective" detergent to lipid ratio for saturation and solubilization, and the maximal concentration of detergent monomers in solution in the presence of phospholipids. We then developed a method to determine at which step of the solubilizing process an integral membrane protein, bacteriorhodopsin (BR), could be inserted into liposomes. At each step of the solubilizing process the BR was added and the protein-phospholipid-detergent mixtures were subjected to SM2 Biobeads treatment to remove the detergent. The resulting vesicles were analyzed with respect to protein insertion and orientation in the membrane by freeze fracture electron microscopy, sucrose density gradients and proton pumping measurements. BR can be incorporated into preformed liposomes when previously saturated by OG. However, for proper BR incorporation, the lipid needs to be at least 30% solubilized if TX100 is used, and 100% solubilized Na-Chol is used. Interestingly, when incorporated into preformed vesicles using OG, the protein is 95% unidirectionally oriented within the membrane in comparison to 80-85% using TX100 and 70% using Na-Chol, demonstrating that the orientation of the protein in the resulting liposomes is critically dependent upon the mechanisms by which the protein is incorporated.

W-Pos73 A RAPID AND EASILY CONTROLLED METHOD FOR RECONSTITUTION OF VIRAL MEMBRANE PROTEINS FROM OCTYLGLUCOSIDE. T.M. Allen, Department of Pharmacology, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

In an effort to gain an understanding of the factors affecting the functional reconstitution of Sendai viral membrane proteins from octylglucoside (OG), we have developed a reconstitution method in which detergent can be removed in a controlled, incremental fashion. We were testing the hypothesis that optimum functional reconstitution of viral (and other) membrane proteins was critically dependent on protein and phospholipid forming simultaneously into bilayer vesicles, and that this ability was dependent on detergent to protein to phospholipid molar ratios, detergent concentration and rate of detergent removal. The reconstitution method involved small dilution and reconcentration steps in an Amicon filtration unit fitted with a YM-10 filter. Radiolabelled OG was used to follow detergent concentration at each dilution and reconcentration step and OG concentrations approached theoretical (calculated) levels at each step. Sendai virus envelopes reconstituted by this technique reproducibly demonstrated both fusion and hemagglutination activity, which was dependent on rate of detergent removal and molar ratios of detergent to phospholipid. This technique is a versatile one which may be more widely applicable to other membrane proteins and other detergents with high critical micelle concentrations.

W-Pos74 NON-RANDOM FLUCTUATIONS AND ATP-DEPENDENT DWELL TIME OF cAMP-GATED ION CHANNELS FROM OLFACTORY RECEPTOR FUNCTIONALLY RECONSTITUTED INTO BILAYERS. V. Vodyanoy and I. Vodyanoy, Dept. of Physiology & Biophysics, Univ. of California, Irvine, CA 92717.

We study the electrochemical properties of bimolecular lipid membranes (BLM's) treated with rat olfactory epithelial homogenates (ROH). The bare BlM was first formed by the successive transfer of two phospholipid monolayers upon the tip of a patch pipette, and rat olfactory homogenate was then added to the *cis*-side of the membrane. The membrane unitary currents were measured with a Yale Mk V Patch-clamp. We reported (V.Vodyanoy & I.Vodyanoy, Soc.Neurosci., Abstr.,Vol.13, Part 2, 389.8,1987) the existence of an ion channel with a conductance of about 70 pS in 30 mM KCl, 30 mM NaCl, 2 mM $CaCl_2$, activated by cAMP (without ATP or GTP). The mean open time was about 1 sec. Subsequent addition of ATP did not change the unitary amplitude of this channel but caused a significant decrease of the mean open time to 6 msec. This activity was completely antagonized by Porcine Heart Protein Kinase Inhibitor. We analyzed statistically the temporal distribution of a single ion channel fluctuations activated by cAMP and modulated by ATP. The histogram of the open state distribution shows a maximum ~ 5 msec. Study of the single-channel dwell time sequences revealed the existence of statistical dependency between adjacent open times. Further analysis of autocorrelation functions and Fourier spectra of the original and randomized open time sequences evidenced the positive correlation between open time events under presence of ATP. We propose a kinetic model with two open states and the irreversible steps which require an energy supply. Supported by U.S. Army Research Office grant DAAG29-85-K-01109, DAAL03-86-G-0131, and by NSF Research Grant BNS-8508495.

W-Pos75 DEPENDENCE OF PLASTOQUINONE LATERAL DIFFUSION COEFFICIENTS ON THE PROTEIN COMPOSITION OF MODEL MEMBRANES. Mary F. Blackwell and John Whitmarsh, USDA/ARS, Dept. of Plant Biology, University of Illinois, Urbana, Illinois 61801.

As the oxidation of plastoquinol (PQ) by the cytochrome b/f complex is the rate limiting step of linear electron flow in chloroplasts, it has been of interest to determine whether long-range (up to 100 nm) diffusion of PQ between the two protein complexes is ever rate limiting. PQ diffusion coefficients have been measured in phosphatidylcholine liposomes by the method of pyrene fluorescence quenching (Blackwell, M.F., K. Gounaris, S.J. Zara and J. Barber (1987) Biophys. J. 51:735); however, the effect that the high (50% or more) weight percentages of integral membrane proteins occurring in natural membranes would have on the diffusion rate of small hydrophobic molecules like PQ remains unknown. We therefore extended the measurements of PQ diffusion coefficients to phosphatidylcholine proteoliposomes containing the following integral membrane proteins: gramicidin D, spinach cytochrome b/f complex, reaction centers from Rb. Sphaeroides R-26 mutant, spinach cytochrome f or bovine heart cytochrome oxidase. PQ diffusion coefficients were smaller in proteoliposomes, falling to one-half the control value ($2 \times 10^{-7} cm^2 s^{-1}$) in the region of 30-50 weight % protein. The dependence of diffusion on the weight % of protein was consistent with the prediction of the effective medium or percolation theories (see Saxton, M.J. (1982) Biophys. J. 39:165) only if either the threshold for diffusion is higher than the theoretical prediction or the protein constitutes a domain that is somewhat permeable (10^{-9}-$10^{-8} cm^2 s^{-1}$) to PQ diffusion. The dependence of diffusion on protein weight % was not correlated with the molecular weight of the protein, inconsistent with protein effecting a subdivision into immobile (i.e. annular or trapped, see Pink, D.A., A. Georgallas and D. Chapman (1981) Biochem. 20:7152) and freely mobile lipid domains.

W-Pos76 AN EXAMINATION OF DETERGENT RECONSTITUTION OF SENDAI AND MUMPS VIRAL ENVELOPES. Christopher Di Simone and John D. Baldeschwieler, Division of Chemistry, 127-72, California Institute of Technology, Pasadena, CA, 91125.

In consideration of usage of Sendai and Mumps viral envelopes as part of a drug delivery scheme, the products given by octyl glucoside and Triton X-100 detergent dialysis reconstitution have been examined. Data on vesicle diameters and surface features were obtained by Electron microscopy and dynamic light scattering. Protein induced binding and fusion behavior were examined by octadecyl rhodamine and NBD-PE/Rd-PE fluorescent assays. With Sendai and Mumps, Octyl glucoside detergent reconstitution led to three products: 40-80 nm protein aggregates, 50-100 nm protein covered or 'spiked' vesicles, and 300-500 nmprotein free or 'bald' vesicles. Sendai virions showed protein dependent binding and fusion activity. With octyl glucoside, Mumps virions have displayed binding but no fusion activity. Triton X-100 reconstitution of Sendai led to two products: 80-140 nm protein spiked vesicles and 250-500 nm protein spiked vesicles. The virions showed protein dependent binding and fusion activity, the latter being monitored by the NBD/Rd fluorescent transfer assay. Experiments with Triton X-100 and $C_{12}E_8$ detergent reconstituted mumps virions are underway. This work was supported by grant no. DAAG-29-83-K-0128 from ARO and a gift from Monsanto.

W-Pos77 EFFECT OF BILAYER CURVATURE ON THE INCORPORATION OF BACTERIORHODOPSIN INTO PREFORMED VESICLES AND THE SUBSEQUENT GROWTH OF THE NASCENT PROTEOLIPOSOME.

Anthony W. Scotto (Intr. by A. Guffanti), Div. of Digestive Diseases, Cornell University Medical College, New York, NY 10021

The spontaneous incorporation of purified integral membrane proteins into small preformed unilamellar vesicles (ULV) in either the gel or liquid phase is speculated to be an inherent property of bilayers that is due to defects in the packing of the phospholipids which act as a foci for protein insertion. These packing defects are more prevalent in small vesicles prepared by sonication (~0.02μm) due to their small radii of curvature. To determine the possible role of this mechanism in cell organelles and cell membranes, two types of large phospholipid vesicles were prepared by extrusion: ULVs of 0.1 μm and "sized" multilamellar vesicles (MLV) ranging from 0.2-10.0 μm. Bacteriorhodopsin incorporates spontaneously into both ULVs $\leq$ 0.1 um and into sized MLVs $\leq$ 3.0 μm, but not into larger vesicles under either gel or liquid phase conditions. These results were achieved with vesicles of several lipid compositions, including dimyristoylphosphatidylcholine (DMPC), DMPC and cholesterol, dioleoylphosphatidylcholine (DOPC) and DOPC and cholesterol. All nascent proteoliposomes increase in lipid-to-protein (L/P) ratio with time in the presence of protein-free vesicles. Under fluid phase conditions the rate of incorporation and slow proteoliposome growth are independent of lipid concentration; in gel phase conditions, in vesicles containing DMPC, the fast increase in L/P ratio of the nascent proteoliposome is concentration dependent. Therefore, the spontaneous incorporation of at least one integral membrane protein can occur in membranes with curvatures equivalent to that of most cell membranes. (Supported by NIH grant GM 36651).

W-Pos78 PURIFICATION AND RECONSTITUTION OF AN UNCOUPLED MUTANT OF *LAC* PERMEASE: EVIDENCE FOR A DECREASED H^+/LACTOSE STOICHIOMETRY. Jonathan A. Lee, Lekha Patel and H. Ronald Kaback. Roche Institute of Molecular Biology, Nutley, NJ 07110.

The *lac* permease of *Escherichia coli* is a transmembrane protein, which couples the movement of H^+ down its electrochemical gradient ($\Delta\bar{\mu}_H{}^+$) with the transport of galactosides against the concentration gradient. In the absence of $\Delta\bar{\mu}_H{}^+$, the permease catalyzes reactions, which either involves net H^+ movement (efflux and facilitated diffusion) or occurs without net H^+ movement (counterflow and exchange. In order to investigate the mechanism of energy coupling, *lac* permease from *Escherichia coli* ML308-22 (a mutant uncoupled for lactose/H^+ symport) and ML308-225 (wild type) was purified to homogeneity and reconstituted into liposomes. Under active transport conditions driven by $\Delta\Psi$, the steady-state level of lactose accumulation in proteoliposomes containing permease ML308-22 is 20% of the level in proteoliposomes containing wild-type permease. The kinetics and extent of dansylgalactoside/lactose counterflow is comparable or slightly increased in ML308-22 proteoliposomes relative to ML308-225 proteoliposomes. The kinetics of lactose facilitated diffusion are comparable in proteoliposomes containing ML308-225 or ML308-22 *lac* permease. In contrast, the transport of H^+ during facilitated diffusion of lactose (as measured by the fluorescence of intravesicular pyranine) is undetectable in proteoliposomes containing ML308-22 permease. The results indicate that the H^+/lactose coupling of permease ML308-22 is significantly impaired.

W-Pos79 CHANNAL ACTIVITIES IN PATCHES CONTAINING MICROTUBULE PROTEINS, P.M. Vassilev, H.T. Tien, M.P. Kanazirska, Membrane Biophysics Laboratory, Giltner Hall, Department of Physiology, Michigan State University, East Lansing, MI 48824

There is evidence that microtubule proteins (MP) can assemble into membrane-like vesicular formations under defined conditions including the presence of minor amounts of lipids. We prepared a fraction containing MP (tubulin, MAPs) and associated lipids from a pellet obtained after two cycles of tubulin polymerization and depolymerization. Patches were formed from surface films containing these materials at the tips of patch-clamp micropipettes. The tips of patch-clamp micropipettes. The tip diameters were <1 μm. The patches were stable for 1-2 h and gigaohm seals (>4GΩ) formed readily. We studied single channel activity in these patches under the same conditions which we used in previous investigations on reconstituted Ca^{2+} channels in BLMs made from materials containing microtubule proteins and those in BLMs from brain microsomal components (BBA, 897, 324-330, 1987). The current-voltage relationships, single channel conductances and the time constants describing the channel kinetics in the two types of membrane were similar. One of the differences was related to the stimulating effects of nucleotides. In the patches containing microtubule components GTP (40μM) exerted a more pronounced stimulating effect on the Ca^{2+} channel activity than ATP which may be attributed to the specific role of GTP for the tubulin structure and functioning. A relatively large Mg^{2+} conductance was observed in patches containing microtubule proteins. An involvement of MP in ion transport in intracellular compartments is suggested. [Supported by NIH grant GM-14971 and ONR grant NOOO14-85-K0399]

W-Pos80 EXTRACTION AND RECONSTITUTION INTO PROTEOLIPOSOMES OF A MITOCHONDRIAL Na^+/H^+ ANTIPORTER. Sham S. Kakar and Keith D. Garlid (Intr. by A. Askari), Department of Pharmacology, Medical College of Ohio, Toledo, Ohio 43699.

Mitochondria contain two Na^+/H^+ antiporters, one of which transports K^+ as well as Na^+. The physiological role of this antiporter is to provide mitochondrial volume homeostasis. Its properties have been well documented in this laboratory, and K^+/H^+ antiport activity has been identified with an 82,000 dalton inner membrane protein (Martin, et al. [1984] J. Biol. Chem. 259, 2062 and [1986] J. Biol. Chem. 261, 12300). We now report that this carrier can be solubilized and reinserted into lipid vesicles, and that the reconstituted protein retains both transport activity and the characteristic inhibitor sensitivity profile. Proteins were extracted from inner membranes of rat liver mitochondria with Triton X-100 in the presence of cardiolipin and reconstituted into lipid vesicles by the freeze-thaw and sonication procedure. It proved to be necessary to remove most of the detergent before reconstitution, since otherwise the vesicles exhibited high non-specific $^{86}Rb^+$ transport. The conclusion that these procedures result in reconstitution of the K^+/H^+ antiporter is based on the following findings: [1] The reconstituted vesicles reproducibly exhibit $^{86}Rb^+$ transport which is inhibited by Mg^{++}, quinine and dicyclohexylcarbodiimide (DCCD). [2] Vesicles which were reconstituted from mitoplasts in which the K^+/H^+ antiporter was irreversibly inhibited by DCCD did not exhibit quinine-sensitive $^{86}Rb^+$ transport. [3] These protocols result in reconstitution of an 82,000 dalton [14]C-DCCD labelled protein into the proteoliposomes. This research was supported by N.I.H. grants HL 36573 and GM 31086.

W-Pos81 ATTENUATED TOTAL REFLECTANCE FOURIER TRANSFORM INFRARED STUDIES OF THE INTERACTION OF MELITTIN,TWO FRAGMENTS OF MELITTIN, AND TWO MODIFICATIONS OF MELITTIN WITH PHOSPHATIDYLCHOLINES. Josepsh W.Brauner, Richard Mendelsohn, and Franklyn G. Prendergast Department of Chemistry, Rutgers University, 73 Warren Street, Newark, New Jersey,USA 07102 (JWB and RM),and Department of Biochemistry and Molecular Biology, Mayo Medical School, Rochester Minnesota, USA 55905 (FGP). (Intr. by D.McCaslin)

ATR FT-IR has been used to monitor alterations in phospholipid organization in thin layers of DPPC and POPC, induced by the membrane lytic peptide melittin, its fragments 1-15 and 16-26,and two derivatives where trp residues have been introduced at positions 9 and 14 respectively. In addition, the secondary structures of the peptides and the orientation of helical fragments with respect to the bilayer plane were determined.

Insertion of melittin into POPC caused large perturbations in the order and increased rates of motion of the acyl chains, as monitored by band parameters of the CH_2 stretching modes, as well as ATR dichroic ratios. Changes in DPPC organization were less, and were consistent with peptide-induced static disordering of the acyl chains.Melittin adopted primary an alpha-helical structure. By considering the peptide as a bend rigid rod,a plausible model for its lytic properties has been developed.

The hydrophilic fragment 16-26 in DPPC showed a secondary structure with little helix present, while the hydrophobic fragment 1-15 gave Amide I patterns consistent with a mixture of predominantly antiparallel pleated sheet with a smaller fraction of helix. Some disordering of the acyl chains of the lipid was induced by this fragment.

W-Pos82 RECONSTITUTION OF CONNEXIN32 FROM GAP JUNCTIONS INTO VESICLES CORRELATES WITH SUCROSE PERMEABILITY. A.L. Harris, J. Park, K. Balakrishnan, C. Bevans, S. Rhee and D. Paul. Dept. Biophys., Johns Hopkins Univ., Baltimore and Dept. Anat. & Cell Bio., Harvard Med. Sch., Boston.

Incorporation of the major gap junction protein from rat liver (connexin32) into phospholipid vesicles was confirmed by a monoclonal antibody and correlated with high sucrose permeability, suggesting reconstitution of open channels from gap junctions. Connexin32 from isolated rat liver gap junctions was solubilized in octylglucoside and excess lipid and incorporated into vesicles formed on a gel-filtration column. We showed previously that when vesicles formed in a 459mM urea buffer are spun on iso-osmolar urea to sucrose density gradients, those permeable to urea and sucrose shift to a higher density (Soc. Neurosci. Abstr. 12:1191). A major fraction of vesicles formed in the presence of solubilized gap junction protein underwent a density shift. The shifted vesicles were shown to contain connexin32 by immunoblotting using a monoclonal antibody. Typically, the amount of connexin32 per vesicle could account for 3-6 hemichannels (hexamers). By calculation, this amount of protein is insufficient to cause a significant density shift of the vesicles. Moreover, vesicles formed with glycophorin, a non-channel-forming membrane protein, did not show a density shift. IgG fractions of rabbit sera raised against peptides corresponding to amino acid sequences predicted by cDNA for connexin32 were studied for effect on the density shift. Compared to pre-immune IgGs, IgGs from two sera aggregated the shifted vesicles, and one reduced the density shift. The results show that reconstitution of a junctional channel protein into vesicles correlates with sucrose permeability. Supported by NIH GM37751 to DP and GM36044 to ALH.

W-Pos83 TIME-RESOLVED ANISOTROPY OF MEMBRANE PROBES EXPLAINED IN TERMS OF HETEROGENEITY AND ROTATIONS GATED BY PACKING FLUCTUATIONS. Lesley Davenport, Jay R. Knutson and Ludwig Brand, Chemistry Dept., Brooklyn College of CUNY; NIH-NHLBI-LTD, and Biology Dept., The Johns Hopkins Univ., Baltimore, MD 21218

We have previously reported (Davenport et al., Biophys. J. 51:537a) that the emission anisotropy of coronene, a fluorescent membrane probe, is controlled by packing fluctuations in lipid bilayers. The long lifetime (>200 ns) and symmetry (D6h) of coronene make its rotation sensitive to chain disordering events that occur well after the decay of most other fluorescent probes. We previously used a compartmental gel-fluid equilibrium model to explain the time-resolved data. A more complete model, derived from Landau phase transition theory, has recently proven more appropriate. In it, we employ a "gating factor" that defines the number of lipid chains near coronene that achieve disorder, thus permitting coronene to rotate (out-of-plane). The model predicts a distribution of rotational correlation times (ϕ_i) that changes with temperature. Comparison of theoretical anisotropy decays with data taken from DPPC ULVs at several temperatures below T_m yields excellent agreement, not only at long times but also through the first few ns. Application of the model to DPH and other probes will also be discussed (e.g., r_∞ vs. ϕ_i), along with the effects of cholesterol and protein upon the observed fluctuation rates. (Supported in part by PSC CUNY award (L.D.), Petroleum Research Fund grant (L.D.) and NIH GM11632 (L.B.).

W-Pos84 **INTERMOLECULAR AND INTRAMOLECULAR EXCIMERIC PROBES FOR DETERMINING FLUIDITY OF VESICLES AND MEMBRANES** **DG Perry, M Sassaroli, P Somerharju* and J Eisinger, Department of Physiology and Biophysics, Mount Sinai School of Medicine, New York, NY 10029.** **The lateral mobility of 1'-pyrenedodecanoic acid (PDA) and 3-palmitoyl-2-(1'-pyrenedecanoyl)-phosphatitylcholine (pPC) in lecithin vesicles was determined by measuring the rate of excimer formation as a function of the probe/lipid molar ratio (y). The data for y between 0.0005 and 0.1 were analysed according to the milling crowd model (Eisinger *et al.*, Biophys. J. 49, 987, *1986*) which simulates the migration of the probes by a random walk in a planar array of lipids, with p_ε the probability of excimer formation between excited and ground state probes after becoming nearest neighbors. The lower limit (corresponding to $p_\varepsilon = 1$) of the lateral diffusion coefficient of PDA and pPC in extruded unilamellar egg lecithin vesicles was found to be 1 x 10^{-7} cm^2 s^{-1} at 24°C. It is about 3 times greater than that for PDA in the membranes of intact erythrocytes, presumably because of the absence of proteins in the bilayer. We have also investigated the properties of several phosphatidylcholines with pyrene moieties attached to each of the acyl chains in bilayer systems. These dipyrenyl-PC (dpPC) probes partition efficiently into erythrocyte membranes if the acyl chains are short (6 C) but otherwise require a PC-specific transport protein for insertion into membranes. In cholesterol-containing PC vesicles prepared with dpPC (12 C) at y = 0.001, the excimer/monomer emission ratio (E/M) doubles at a cholesterol/PC molar ratio of 0.8. Because E/M of dpPC's is independent of y and they may be used at very low probe ratios, they are promising membrane probes. The relationship between their E/M and lateral membrane fluidity was investigated.** **[* U of Helsinki, Helsinki, Finland]**

W-Pos85 MAPPING OF PHOSPHOLIPID PHASE DOMAINS BY PHASE-SPECIFIC FLUORESCENT DYE MEROCYANIN-540. T. Isac, K. Lui and S.W. Hui, Roswell Park Memorial Institute, Buffalo, NY 14263.

The application of the phase-specific dye merocyanin-540 (MC540) to label fluid phase domains in mixed phospholipid bilayers is discussed. In fluid phase bilayers, MC540 shows a pronounced fluorescence peak at 585 nm. In the presence of gel phase lipid alone, the weak fluorescence peak at 575 nm is indistinguishable from that in water. The fluorescence spectra of MC540-labeled mixed DPPC/egg PC or mixed DSPC/POPC vesicles, and vesicles of these lipid mixtures at various compositions are examined. At given temperatures, the fluorescence intensity of mixed vesicles at 585 nm is linear with the composition of the sample. Vesicles of mixed egg PC/DPPC shows highly variable intensity depending on the environment. The usefulness of MC540 in quantitation of phase ratios and microscopic mapping of phase-separated domains is illustrated.

W-Pos86 EVIDENCE FOR DIFFUSION-DEPENDENT FORMATION OF PYRENE EXCIMERS IN SMALL, UNILAMELLAR PHOSPHATIDYLCHOLINE VESICLES. G.P. L'Heureux and M. Fragata. Centre de recherche en photobiophysique, Université du Québec à Trois-Rivières, Québec, Canada.

Excimer formation of pyrene (PY) and 16-(1-pyrenyl)hexadecanoic acid (C_{16}PY) incorporated in small, unilamellar phosphatidylcholine vesicles (SUV-PC) has been studied on account of the fact that PY and its derivatives present a basic interest to studies of energy tranfer in natural and model membranes. With the use of absorption and fluorescence spectroscopy we demonstrated that up to 3.0 mol% of probe into SUV-PC membrane, no ground-state aggregates of PY or pyrenyl moieties of C_{16}PY were detected even if significant excimer/monomer ratios (I_E/I_M) were observed. We conclude that, in the conditions mentionned above, the formation of excimers occurs most likely via diffusion-controlled dynamic processes, instead of direct excitation of some ground-state aggregates as it was recently proposed in the literature (Kaneda *et al.*, (1985) *Photochem. Photobiol.* **41**, 519; Blackwell *et al.*, (1986) *Biochim. Biophys. Acta* **858**, 221). The apparent discrepancies referred to above may have their origin in differences of experimental materials as well as in preparative methodologies. We also conclude that PY excimer formation in membrane is most probably dependent on the physical properties of the bilayers, and distribution of PY and pyrene derivatives in phosphatidylcholine bilayers will be discussed. (This work was supported by grants from the N.S.E.R.C. Canada, and the Fonds F.C.A.R. du Québec).

W-Pos87 DYNAMIC AND THERMAL BEHAVIORS OF DEHYDROERGOSTEROL IN ASYMMETRIC MIXED-CHAIN PHOSPHATIDYLCHOLINES. Parkson Lee-Gau Chong, Department of Biochemistry, Meharry Medical College, Nashville, TN 37208.

Differential polarized phase and modulation fluorometry has been employed to characterize the rotational motions of dehydroergosterol (DHE), a naturally occurring fluorescent cholesterol analog, in asymmetric mixed-chain phosphatidylcholines (MCPs), which are known to form highly ordered interdigitated structures at temperatures below the main phase transition temperature. The fluorescence lifetime and anisotropy decay of 0.4 mol% DHE in C(18):C(10) have been measured as a function of temperature. In interdigitated structures the rotational rate of DHE, although lower than that in noninterdigitated structures, is still appreciable. The rotational hindrance, as inferred from the lower limiting anisotropy, does not vary significantly as the bulk lipid transforms from noninterdigitated to interdigitated structures. These results indicate that DHE undergoes appreciable rotations even in highly ordered environments. Differential scanning calorimetry has been used to characterize the thermal behavior of C(18):C(10) in the presence of DHE. 0.4 mol% DHE does not significantly alter the thermal behavior of C(18):C(10), however, the enthalpy change decreases remarkably with further increases in DHE concentration. The enthalpy change of C(18):C(10) decreases from 8.9 kcal/mol for 0 mol% DHE to 4.3 kcal/mol for 2.45 mol% DHE. This result suggests that the thermal behavior of MCP is extremely sensitive to sterol, a situation distinctly different from that of symmetric diacyl phosphatidylcholines.

W-Pos88 DIPHENYLHEXATRIENE FLUORESCENCE IN PHOSPHOLIPID VESICLES. D.W.Piston*, R.Fiorini+, G.Curatola+ and E.Gratton*. *Laboratory for Fluorescence Dynamics, Dept. of Physics, University of Illinois at Urbana-Champaign, Urbana 61801. +Facolta di Medicina, Universita di Ancona, Ancona, Italy.

Recent studies of 1,6-diphenyl-1,3,5-hexatriene (DPH) fluorescence in phospholipid bilayers have indicated the presence of a mixed phase state during the liquid crystalline to gel phase transition of the membrane (Parassasi, Conti, Glaser, Gratton, 1984: J.Biol.Chem. 259:14011-14017; Klausner, Kleinfeld, Hoover, Karnovsky, 1980: J.Biol.Chem. 255:1286-1295). In addition, a change in the fluorescence lifetime of the probe is observed across the phase transition. We propose a model of the phase transition with the coexistence of both solid and fluid domains and interconversions between phases along the domain boundaries. We have studied DPH fluorescence over the temperature range of the phase transition in unilamellar and multilamellar vesicles by the technique of multifrequency phase fluorometry. In our model, we consider a system which has interconversions between two lifetime species, one in the solid phase and the other in the fluid phase. The interconversion rates are temperature dependent. In addition to the temperature dependence of the fluorescence lifetime, we also measure the time-resolved fluorescence anisotropy decau using the differential phase method. Since the DPH molecules have less mobility in the gel phase, we can also analyze this rotational data by associating rotational species with the lifetime species obtained from the total intensity data. The data is analyzed globally for the best fit in both lifetime and rotational measurements. Supported by NIH RR03155 and CNR 87.00088.04.

W-Pos89 LATERAL DIFFUSION IN AN ARCHIPELAGO: THE EFFECT OF LARGE MOBILE OBSTACLES. Michael J. Saxton, Plant Growth Laboratory, University of California, Davis, California 95616; and Laboratory of Chemical Biodynamics, Lawrence Berkeley Laboratory, University of California, Berkeley, California 94720.

In order to provide a more accurate hard-disk model of diffusion of lipids in the presence of protein obstacles, Monte Carlo calculations were carried out for the diffusion of point tracers in the presence of mobile hexagonal obstacles on a triangular lattice. The obstacles diffused at a rate distinct from that of the tracers. These calculations yield the diffusion constant of the tracers as a function of the concentration of obstacles, the relative jump rates of obstacles and tracers, and the size of the obstacles. The diffusion constant does not depend strongly on the size of the obstacles, and the discrepancy [Saxton, Biophys. J., in press] between calculated and experimental values is not eliminated by changing the size of the obstacles. Evidence is presented that this discrepancy is a general feature of hard-disk models, suggesting that perturbation of lipid fluidity by integral proteins may be significant.

W-Pos90 THE EFFECT OF LOW CONCENTRATIONS OF DIETHYLSTILBESTROL ON L-α-DIMYRISTOYLPHOSPHATIDYLCHOLINE MULTILAMELLAR VESICLES AS DETERMINED BY d-EQUILENIN FLUORESCENCE. Donna Scott, Greg Smutzer, and Linda Chamberlin, Department of Urology, School of Medicine, State University of New York at Buffalo, Buffalo, New York 14215.

The phase behavior of L-α-dimyristolyphosphatidlycholine (DMPC) multilamellar vesicles was measured with the naturally occurring fluorescent estrogen d-3-hydroxyestera-1,3,5(10),6,8-pentaen-17-one (d-equilenin). In resuspended multilamellar vesicles containing 1.5 mole percent d-equilenin at pH 7.0, the steady-state fluorescence anisotropy decreased above the gel-to-liquid crystalline phase transition of DMPC, and steady-state fluorescence anisotropies differed from those reported for DMPC sonicated unilamellar vesicles. Fluorescence lifetime analysis of d-equilenin in multilamellar vesicles at pH 7.0 revealed a major lifetime component near 8 nanoseconds that underwent a small, repeatable increase in duration at the gel-to-liquid crystalline phase transition of DMPC near 23 °C. In DMPC multilamellar vesicles prepared with the non-steroid, estrogenically active lipophilic compound diethylstilbestrol, the steady-state fluorescence anisotropy pattern of d-equilenin was broadened at the phase transition temperature in membranes containing 5 mole percent diethylstilbestrol. Ten mole percent diethylstilbestrol essentially eliminated the DMPC phase transition in multilamellar vesicles. Under identical conditions, low concentrations of diethylstilbestrol slightly decreased the steady-state fluorescence anisotropy of d-equilenin in multilamellar vesicles. These results suggest that estrogenic compounds such as diethylstilbestrol can affect physical properties of membranes and may form membrane domains at these concentrations. The steady-state fluorometer was made available by a SUNYAB R&D Fund and NIH grant GM 24840, while fluorescence lifetime instrumentation was made available by NIH Regional Resource grant RR-01705-01.

W-Pos91 THE EFFECT OF POTENTIAL-SENSITIVE MOLECULAR PROBES ON THE NMR AND PHASE TRANSITION PROPERTIES OF MODEL MEMBRANES, B. P. Bammel, D. C. Evans, J. Fumero, G. Pritchett, H. P. Hopkins, J. C. Smith, and L. Strekowski, Dept. of Chemistry, Georgia State Univ., Atlanta, Ga; R. Haugland and W. Szalecki, Molecular Probes, Inc., Eugene, OR - The cyanines diS-C_3-(5) and diS-C_4-(5) broaden the DMPC vesicle ^{31}P resonance and reduce the T1 and T2 relaxation times due to an apparent dye-driven vesicle fusion process (BBA 896, 136, 1987). The effect of introducing negative charge into the DMPC system in the form of DMPG is to enhance the perturbation of the cyanine probes. The latter effect is being investigated as a function of medium ionic strength and of the mole percent DMPG present. Dye effects on the DMPC carbonyl ^{13}C resonance indicate that probe perturbations extend to the lipid backbone region of the bilayer. The ^{19}F spectrum of a DMPC vesicle-bound ^{19}F-labeled probe consists of two components one of which is eliminated by the paramagnetic shift reagent $Eu(FOD)_3$ at 35° C. The probe appears to occupy two distinct classes of sites perhaps one associated with each of the bilayer leaflets. The two sites are not in rapid exchange as judged by a lineshape analysis, indicating that the single resonance observed at 75° C is due to a differential temperature dependence of the two chemical shifts. Several probes reduce the T_m and broaden the heat capacity profile associated with both the pretransition and the main phase transition in multilamellar DMPC preparations. The ΔH of the main phase transition is modestly reduced, but the cooperative unit parameter (CU) is drastically lowered by the dyes. The opposite effect on the pretransition is observed. These data can be described by the ideal solution theory of Albon and Sturtevant. An apparent correlation between asymmetrical probes and the reduction in the main phase transition CU is being explored by the study of the effect of a homologous series of asymmetrical dyes on this parameter. Support NIH GM30552, NSF DMB-8500319.

W-Pos92 PHOSPHOLIPIDS EXCHANGE RAPIDLY BETWEEN PHOSPHATIDYLCHOLINE:TAUROCHOLATE MIXED MICELLES.
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The exchange of head-group-labeled (7-nitro-2,1,3-benzoxadiazol-4-yl)phosphatidylethanolamine (N-NBD-PE) between phosphatidylcholine (PC):taurocholate (TC) mixed micelles was measured by monitoring the increase in fluorescence as N-NBD-PE, initially in PC:TC micelles at self-quenching concentrations (20 mole % of total PC), transfers into excess unlabeled PC:TC micelles. At constant donor micelle concentration, the initial rate of transfer increases as a function of acceptor micelle concentration. The dependence of the initial rates on acceptor concentration could be accurately predicted by a kinetic model that assumes transfer occurs simultaneously by both monomer diffusion and collision-dependent mechanisms. Analysis of kinetic data for a series of N-NBD-PEs varying in acyl chain length indicated that the short-chain (diundecanoyl) N-NBD-PE transfers predominantly by monomer diffusion between micelles, whereas the longer-chain (dilauroyl to dipalmitoyl) N-NBD-PEs transfer predominantly as a result of micelle collisions. The transfer of these N-NBD-PEs between mixed PC:TC micelles is from two to six orders of magnitude faster than between PC vesicles, depending on the acyl chain length and micelle concentration. These studies demonstrate two previously undescribed properties of mixed micelles: 1) rapid collision-dependent transfer of long-chain phospholipids, and 2) rapid transfer of phospholipids from the micellar to water phase (increased dissociation rate constant) as compared to phospholipid transfer between PC vesicle bilayers. Supported by a grant from the American Heart Association, Georgia Affiliate.

W-Pos93 CONTINUOUS FLUORESCENCE LIFETIME DISTRIBUTION ANALYSIS OF MEMBRANE FLUOROPHORS IN THE STUDY OF MEMBRANE PERTURBATION.
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Fluorescence decay is traditionally analyzed in terms of weighted exponential series of discrete lifetime values. A recently developed alternative to this approach is to describe decay in terms of a continuous distribution of lifetimes. We have been investigating the potential of the latter approach in the analysis of membrane perturbation by hydrophobic compounds, using various fluorophores including DPH and TMA-DPH and multi-frequency phase fluorometry. One motivation for the continuous distribution approach has been that it may allow a more physically realistic representation of a fluorophor; however, this question is currently under discussion. Our position is that the important point is whether more information on the environment of a probe can be gained using continuous lifetime distributions. Membrane perturbation using fluorescent probes is usually assessed from changes in fluorescence anisotropy reflecting a disordering of the lipids. However, fluorescence lifetimes can also be affected, and perturbation may involve other effects besides the disordering of the membrane lipids. Using alcohols and other perturbants we found that the width of Gaussian or Lorentzian distributions, representing the decay of probes used, were particularly sensitive to the presence of the perturbants. In contrast, the center of gravity of the distribution and the lifetime values and pre-exponents obtained using the discrete lifetime approach were much less sensitive to the perturbation.

W-Pos94 THE PARTITIONING OF INDOCARBOCYANINE DYES BETWEEN GEL AND FLUID PHASES IN MULTILAMELLAR VESICLES.
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The quenching of fluorescence in indocarbocyanine dyes by nitroxy- spin-labelled phosphatidylcholine (7,6-PC) has been studied in two-phase gel-liquid crystal model membranes. The quenching data provide a means for quantitative evaluation of the partition coefficient of the dyes between liquid crystal and gel phases. The indocarbocyanine dyes, CndiI, where n is the alkyl chain length in the dye and varies from 12 to 22, were studied in mixtures of fluid phase 7,6-PC with dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC) and with the calcium phosphatidylserine ($CaPS_2$) gel phases. In the thermal gels (DPPC and DSPC) the dyes with longer alkyl chain lengths partition more strongly into the gel phase lipid. For example, in the DSPC case, values of the partition coefficients, Kp = conc. in fluid/conc. in gel, are 0.09 and 5.0 for the C_{18}- and C_{12}-diI probes, indicating a marked difference in partitioning behavior when the chain length in the probe is much shorter than the bilayer thickness of the gel. Also, when the alkyl chains of the dye are significantly longer than the bilayer thickness, there is a trend toward decreased stability in the gel phase. Similar results are obtained in the DPPC thermal gels. For the 7,6-PC mixtures with the $CaPS_2$ gel phase there is considerably less partitioning into the rigid gel phase than for the thermal gels.

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W-Pos95 FLUORESCENCE REDISTRIBUTION AFTER PHOTOBLEACHING-THE EFFECT OF DIFFUSION DURING BLEACHING

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In fluorescence photobleaching experiments with a focused laser spot, lateral diffusion coefficients (D) are determined as $D=w^2(0)/4\tau_{1/2}$ where $\tau_{1/2}$ is the half-time of fluorescence recovery after bleaching, and w(0) is the effective immediate post-bleach width of the bleached region. w(0) can be measured in each experiment by scanning the attenuated monitoring beam across the bleached region (Koppel, 1979, Biophys. J. 28: 281-292), but more often is estimated theoretically (Axelrod et al., 1976, Biophys. J. 16: 1055-1069) from w_o (the $1/e^2$ width of the laser spot, determined in separate measurements), for a given extent of bleaching, assuming: (i) a Gaussian beam profile, (ii) first-order bleaching kinetics and (iii) a bleaching time sufficiently short that no significant probe diffusion occurs during bleaching. To the extent that the last assumption is not or cannot be fulfilled, the values of D calculated in this approach will underestimate the true values. $\gamma_d(\equiv w^2(0)/w_o^2)$ has now been characterized experimentally under conditions of extended bleach. Bleaching experiments were performed on the fluorescent lipid probe NBD-PE incorporated into an artificial membrane system. The bleaching beam was chopped, allowing the periodic insertion of rapid scans of the fluorescence distribution with an attenuated monitoring beam. This enabled the measurement of both the shape of the fluorescence scan profiles and the amount bleached as functions of bleaching time and intensity. The scan profile widths were used to estimate γ_d, as a function of bleaching time and amount bleached. With these results, accurate values of D can now be determined from the experimental parameters $\tau_{1/2}$, the bleaching time and the amount bleached for an extended range of bleaching times. (Supported by NIH grant GM-23585).

W-Pos96 EVIDENCE FOR PHOTOINDUCED CHARGE TRANSFER BETWEEN METALLOPORPHYRINS AT THE LIPID BILAYER-WATER INTERFACE.

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Derivatives of Zn(deuteroporphyrin IX) were added to one aqueous phase of a planar bilayer system and illuminated with a 7 ns pulse of 560 nm light. Under saturating conditions, fast electrical measurements detect a negative photovoltage having an amplitude of ca. 0.5 mV and risetime $t_{1/2} \sim 0.2\mu s$, in the absence of exogenous electron acceptors. Photolysis is thought to induce an inter-porphyrin charge transfer to yield the charge-separated species (P^+P^-), similar to that observed in solution (J. Chem. Phys. 1980, 72 933). The signal polarity is consistent with anionic movement towards the aqueous phase. Use of stearoylamine to induce a (+) charge on the bilayer surface lowers the charge recombination time 15-fold from that observed with neutral phosphatidyl choline, whereas phosphatidyl serine(-) membranes exhibit a 15 times longer-lived photosignal. The reaction kinetics of the forward charge separation are invariant to pH, buffer concentration and replacement of H_2O by D_2O, indicating that protons are not moving into the lipid bilayer and that hydroxide or phosphate ions are not the predominant charge carriers. The enzymatic removal of O_2 from the aqueous phase also has no effect on the kinetics of this reaction. Thus it is believed that movement of the radical anion P^- is responsible for the observed photosignal.

W-Pos97 PSEUDOMONAS EXOTOXIN A FORMS pH-DEPENDENT CHANNELS IN LIPID BILAYER MEMBRANES.
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Exotoxin A of Pseudomonas aeruginosa (PsA, Mr 66,000) is a bacterial toxin which inhibits protein synthesis in eukaryotic cells by catalyzing the ADP-ribosylation of elongation factor 2. This enzymatic mechanism is identical to that of diphtheria toxin (DT, Mr 62,000), but the precise means by which these toxins (or their enzymatic portions) cross a lipid membrane to reach their cytosolic target remains uncertain. We report here that PsA efficiently forms channels in lipid bilayer membranes, and that these channels resemble DT channels in the following ways: 1) Low cis pH (<5.0) is required for channel formation. 2) A pH gradient across the membrane (trans>cis) stimulates channel formation. 3) Cis positive voltages cause increased rates of channel formation. 4) The single channel conductances are nearly identical in 1 M KCl. Although DT and PsA exhibit only minimal primary sequence homology, the similarity of the channels they form in lipid bilayers suggests that they share common structural and functional features. We suggest that the PsA channel, like the DT channel, may act as a "tunnel protein" to allow translocation of the toxic enzymatic fragment of PsA across a lipid membrane. Transmembrane pH gradients may be a common energy source for driving the translocation of proteins across membranes.

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W-Pos98 Ca INFLUX MEDIATED BY IONOMYCIN AND PHOSPHATIDATE IN LIPOSOMES WITH FURA 2 ENTRAPPED.
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A novel liposomal method permitted studies of Ca movements across the bilayers of multilamellar vesicles (MLV) which had entrapped the Ca-dependent, fluorescent indicator dye, Fura 2. Ionomycin-mediated Ca translocation across MLV of phosphatidycholine (PC) : dicetyl phosphate (DCP) 9:1 obeyed simple first order kinetics since log-log plots of initial rates versus ionomycin yielded slopes of approximately 1. Since Ca is translocated in a Ca-dependent fashion in course of stimulus-response coupling of cells which form diacylglycerol (DAG) and phosphatidate (PA) from polyphosphoinositides, we compared effects of PA with those of DAG. PA and DAG were incorporated in PC:DCP vesicles, in which trace amounts of ionomycin provided transmembrane potential (due to Ca^{2+}/H^{+} exchange). Significant increases in Ca movements were observed in presence of egg lecithin PA, dioleoyl PA and dipalmitoyl PA when compared with DCP or DAG containing MLV. DAGs such as 1-oleoyl-2-acetoylglycerol or 1,2-dioleoylglycerol in liposomes decreased rates of Ca translocation. Ca influx into PA containing MLV was dependent on the mol percent of the PA in bilayers; the complex kinetics of Ca influx were compatible with the formation of non-bilayer states. Incorporation of cholesterol into the liposomes inhibited initial rates of Ca uptake by MLV presumably by condensing the bilayers. Ca influx increased with increasing pH of the external medium from 6.9 to 7.9, in liposomes with internal pH of 7.4. The results indicate not only that transmembrane pH gradients affect rates of Ca fluxes but also demonstrate that in model systems PA but not DAG promotes Ca translocation, the rate of which is altered by lipid composition of the bilayer and the pH of the suspension.

W-Pos99 AMPHOTERICIN B INDUCES H+/OH- PERMEABILITY IN CHOLESTEROL-CONTAINING VESICLES.
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The effect of amphotericin B on the H+/OH- permeability of small unilamellar vesicles was investigated using kinetic spin probe techniques. Small amounts of amphotericin (*ca.* 1-10 molecules/vesicle) cause a slight increase in the H+/OH- permeability of egg phosphatidylcholine vesicles. Incorporation of ergosterol at 5 to 15 mole percent actually *decreases* this susceptibility. However, in the presence of cholesterol, amphotericin B causes a large increase in proton permeability: more than two orders of magnitude. Parallel experiments monitoring electrogenic K+ permeability and CD spectral changes indicate that a different amphotericin/sterol complex may be responsible for the K+ and H+/OH- permeability pathways. Potassium leakage combined with proton fluxes leads to a rapid collapse of pH gradients in cholesterol-containing vesicles, but not in vesicles containing ergosterol. Experiments using vesicles with altered dipole potentials suggest an H+/OH- or K+ carrier mechanism is not operative. The amphotericin b molecule contains a series of aligned -OH groups which raises the intriguing possibility that the molecule may act as a transmembrane "proton wire" when associated with cholesterol.

W-Pos100 PHOTOGATING OF LIPOPHILIC ION CURRENTS ACROSS BIMOLECULAR LIPID MEMBRANES. B. Christensen and D. Mauzerall, The Rockefeller University, NY, NY.

The photoinduced interfacial charge separation between Mg-Octaethylporphyrin (MgOEP) in a bilayer lipid membrane (BLM) and various aqueous electron acceptors (1) on both sides of a BLM is shown to influence both transient and stationary currents of lipophilic ions. In contrast, currents across ion channels (Amphotericin B) are much less sensitive to this charge. The positive charging increases the conductance caused by negatively charged lipophilic ions (eg. Tetraphenylborate or Dipicrylamine) by as much as 100%, whereas the steady-state conductance induced by the positively charged Tetraphenylphosphonium ion ($TPhP^+$) is decreased, maximally by 30%. We name this effect "photogating" since the change of translocated ionic charges (-3.5 nC for $TPhP^+$) is over 100 times greater than the photoinduced charge at the interface (30pC). For the first time, electrical relaxations of $TPhP^+$ are observed (0.5 ms). The gating is independent of the specific lipophilic ions, electron acceptors and lipids used. The gating lifetime can be adjusted between seconds and microseconds by addition of electron donors and its amplitude controlled by flash energy and acceptor concentration. Thus, the photogating is completely controllable and can be used to study the kinetics of ion transport in the BLM. (supported by NIH grant GM-25693).

1. Hong, F. and Mauzerall, D. Nature 240, 154-155 (1972).

W-Pos101 THE INFLUENCE OF ELECTROLYTE SHIELDING ON THE PERMEABILITY OF TRANSMEMBRANE ION CHANNELS. Peter C. Jordan, Russell J. Bacquet and J. Andrew McCammon, Dept. of Chemistry, Brandeis University, Waltham, MA 02254 and Dept. of Chemistry, University of Houston, Houston, TX 77004.

Changing ionic strength on either side of a membrane may significantly affect the electrical potential due to fixed charges within a pore or at a membrane-water interface. For a uniform, planar interface this is described by the well known Gouy-Chapman theory. We have developed an efficient method for solving the unlinearized Poisson-Boltzmann equation applicable to general cylindrically symmetric geometries. We can therefore assess the influence that altering ionic strength has on the electrical potential due to a wide range of physiologically significant electrical sources and consider the effect that ionic strength variation may have on the permeability of transmembrane ion channels. Preliminary results demonstrate that, for an axial electric potential generated by an ion within the pore, external electrolyte has little effect on the electrical potential within the pore itself; the potential differs only slightly from its value at zero ionic strength. However, at high ionic strength (> .5M) the potential exterior to the pore is strongly shielded by electrolyte. External electrolyte also has a significant influence on the potential profile due to an applied voltage, most notably at very high ionic strengths (> 1.0M).

W-Pos102 **AGGREGATION OF ALAMETHICIN MOLECULES IN THE PLANAR LIPID BILAYER**

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It has been shown (by measuring alamethicin induced voltage dependent capacitance) that alamethicin molecules interact with each other while being adsorbed (primarily in monomeric form) onto the planar bilayer (Biophys.J. 51, 563a, 1987). Here we report experiments in which the current-voltage relationship was continuously monitored after alamethicin addition. The results show that the alamethicin-induced transmembrane current increases to its maximum value in about 3-6 minutes after alamethicin addition and then declines to a steady-state value over the next 25-30 minutes. If we assume that alamethicin conduction is due to an ionic channel consisting of aggregated alamethicin n-mer then such kinetics are consistent with the idea that aggregation of alamethicin molecules proceeds as a bimolecular reaction. We suggest that the alamethicin molecules aggregate first into dimers and after a certain time additional monomers collide with these multimers. The multimers also collide amongst themselves. Thus the surface concentration of the conducting species appears to increase with time to a maximum and decrease toward its equilibrium value. The steep rising phase can be attributed to rapid formation of the n-mer conducting species, and the falling phase is due to the diminution of the concentration of this species as more and more alamethicin monomers becomes incorporated in higher than n order non conducting aggregates.

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W-Pos103 GLOBAL TRANSPORT COEFFICIENTS SYMMETRY BREAKING IN MATTER TRANSPORT THROUGH POROUS MEMBRANES. M. Bartoszkiewicz and S. Miękisz, Dept. of Biophysics, Academy of Medicine, Wrocław, Poland.

We have studied matter transport through a membrane having pores whose diameter is comparable with the "characteristic length" describing the range of molecular interactions in the system. We have taken into account both the interactions of the membrane with the permeating solution and the viscous effects.

Under those assumptions, using the continuum hypothesis and arguments from statistical physics, we derived expressions for the global transport coefficients which, in turn, were investigated by means of partial differential equations theory. We thus obtained exact symmetry conditions for the coefficients. The physical meaning of the conditions was shown to be the following : in the considered system the diffusion-viscous flow coupling is necessary and sufficient for breaking the symmetry.

W-Pos104 PHOTOINITIATED INTERFACIAL ELECTRON TRANSFER CATALYZED BY A LINKED CAROTENE-PORPHYRIN-QUINONE INCORPORATED INTO LIPOSOMES. Anna M. Joy, Thomas A. Moore, Devens Gust, Ana L. Moore, Peter Pessiki, and Paul Liddell. (Intr. by R.J. Lukas)

A molecule consisting of a covalently linked carotene-pyridyl porphyrin-quinone, called the pyridyl triad, was synthesized and found to undergo a photodriven separation of charge. The high energy charge separated state, with the electron residing on the quinone and the hole on the carotene, forms with a quantum yield of approximately 20% and lives for 400 ns in methylene chloride. This molecule mimics the initial charge separation steps of photosynthesis. Consequently, the pyridyl triad, or a molecule incorporating the same design, may be an effective sensitizer in photochemical solar energy conversion devices. For this reason the behavior and photochemical properties of the triad were investigated in the phospholipid bilayer of liposomes. Gel filtration studies of liposomes prepared from a lipid-pyridyl triad mixture demonstrated that all of the triad coelutes with the liposome fraction, indicating that the molecule was incorporated into the liposome bilayer. Nanosecond flash photolysis was used to detect and follow the decay of the charge separated state by monitoring the absorption band due to the carotenoid radical cation at 980 nm. Addition of oxidized cytochrome c, followed by ascorbic acid which reduces the cytochrome, to a suspension of liposomes incorporating the pyridyl triad resulted in a substantial quenching of the signal at 980 nm. This data can be explained by an electron transfer on the submicrosecond timescale from the cytochrome c to the carotenoid radical cation. Evidence is also presented which indicates that upon incorporation of the triad into the membrane it assumes a transmembrane orientation.

W-Pos105 KINETICS AND RADIATION-TARGET SIZING OF THE GLUCOSE TRANSPORTER IN CARDIAC SARCOLEMMAL VESICLES. W.E. Dale[1], Y.-S. Tsai[2], C.Y. Jung[3], C.C. Hale[4], M.J. Rovetto[1], and H.D. Kim[2], Depts. of Physiol.[1], Pharmacol.[2], & Vet Biomed. Sci.[3] Univ of MO-Columbia and Dept. Biophys. Sci.[4] S.U.N.Y.-Buffalo.

The glucose transporter is an integral membrane protein which catalyzes stereospecific facilitated diffusion of D-glucose. Previous studies have focused primarily on the glucose carrier from erythrocytes and adipocytes. Relatively little is known about the myocardial glucose transporter due to lack of an adequate vesicle system to study it. Sarcolemmal vesicles (SV) from bovine myocardium were used to characterize this glucose transporter. We assessed the apparent molecular size of D-glucose-sensitive cytochalasin B (CB) binding protein and D-glucose transport protein in SV by applying target theory to irradiation-inactivation data. Uptake of D-^{3}H-glucose and L-^{3}H-glucose were measured at 25°C, and was terminated by cold $HgCl_2$ and collection on glass fiber filters. Non-specific diffusion of L-glucose was less than 11% of total D-glucose transport. D-glucose accumulated to a maximum by 20 sec. CB (50 μM) totally inhibited specific transport of D-glucose. Transport in different SV preparations appeared to exist in one of two kinetic states. One was saturable and 90% of the preparations exhibited this characteristic (K_m=10.8 mM; V_{max}=0.9 nmol/mg/sec). In the other state, transport was non-saturable and could not be kinetically analyzed. The molecular size of the CB binding protein was 77 kDa and D-glucose transport protein 111 kDa. Respectively, these values are smaller than and nearly equal to, that estimated for erythrocyte membranes by radiation inactivation of CB binding. Supported by NIH-AM-3345, NIH-HL27336 and the Edward Mallinckrodt Foundation.

W-Pos106 **PURIFICATION OF DOG HEART GAP JUNCTIONS** Milton L. Pressler, Joseph A. Lash and David R. Hathaway, Krannert Inst. of Cardiology, Indiana Univ. Sch. of Medicine, Indianapolis, IN

Regulation of intercellular communication is thought to occur at gap junctions (GJ) but the proteins enabling intercellular passage are not fully characterized. To study the molecular basis for regulation in heart, we purified a membrane fraction enriched in GJ. Dog ventricular homogenates were extracted with alkali, KI, and detergents (Sarkosyl-NL; deoxycholate) then fractionated on sucrose step gradients. Septilaminar membranes characteristic of GJ were observed on electron micrographs of the material at the 35%/49% sucrose interface. Three major protein bands migrating at 214, 59 & 47 kilodaltons (kDa) were found on 5-7.5% SDS-PAGE of the enriched GJ fraction. The 47 kDa protein was purified to homogeneity by two approaches based on the greater solubility of the 59 kDa and other proteins in urea and guanidine. The 59 kDa protein was extracted from the GJ isolate in 8 M urea and then the 47 kDa protein electroeluted from excised gel segments. Net yield of pure 47 kDa protein was 280 μg/10.5 g wet wt. The NH_2-terminus of the 47 kDa protein was blocked after urea exposure so 2-4 M guanidine was used to extract the 59 kDa protein. Solubilization of the remaining pellet in 0.1% SDS allowed separation of the 47 kDa protein from proteins > 160 kDa by gel filtration. To date, we have observed phosphorylation of the 47 kDa protein to 0.15 mol ^{32}P/mol protein by the catalytic subunit of cAMP dependent protein kinase (PK). cGMP dependent PK also phosphorylated the 47 kDa protein but by a 5-fold lesser extent. The low stoichiometry of phosphorylation might suggest either a lesser role for cAMP-dependent PK in modulating GJ conductance in heart or negative cooperativity for phosphorylation of multiple subunits within the hexameric connexon. (Supported by grants from the NHLBI and IN Heart Assn)

W-Pos107 PATCH RECORDING OF SINGLE CHANNELS FROM THE CYTOPLASMIC SURFACE OF A GAP JUNCTION CONTAINING MEMBRANE. P.R. BRINK AND S.W. JASLOVE. Dept. of Anatomical Sciences, S.U.N.Y. Stony Brook.

The septal membranes of the median giant axon of the earthworm contain gap junctions. The axon is 100 um in diameter and each axonal segment is about 1mm in length. We have exposed the cytoplasmic surface of individual septal membranes and introduced patch electrodes onto the exposed surface. The exposed junctional membrane is bathed initially in saline with 2mM Ca++. After 15-30 minutes the preparation is perfused with a low Ca++, high K+ saline. The patch pipettes are filled with the same high K+ saline. Typical seal resistances are 0.5-5.0 gigaohms. Initial results indicate a channel with a conductance of 100 pS. It appears to lack potential sensitivity over a wide transjunctional voltage range. Records have been made from patches attached to the preparation and from excised (inside-out) patches. The channel activity is reduced when exposed to acid (pH=6.4). This approach in principal allows the observation of individual intact gap junction channels. The work was supported by NIH grant 31299.

W-Pos108 **SPECIFICITY, IONIC DEPENDENCE AND β-ADRENERGIC POTENTIATION OF ATP- INDUCED Ca^{2+}-TRANSIENTS IN CARDIAC VENTRICULAR MYOCYTES.** Mary Beth De Young and Antonio Scarpa, Dept. of Physiology and Biophysics, Case Western Reserve Univ., Cleveland, OH, 44106.

Addition of micromolar concentrations of ATP to isolated rat cardiac ventricular myocytes loaded with the fluorescent Ca^{2+} indicator fura2 results in a transient increase in intracellular Ca^{2+} which is comparable to Ca^{2+} changes induced by K^+-depolarization. Although ATP receptors on Ehrlich ascites tumor cells, hepatocytes, vas deferens and arterial smooth muscle cells also induce cytosolic Ca^{2+} transients, the ATP receptor-response of isolated ventricular myocytes has unique characteristics, including: (1) inhibition by nifedipine (1 μM) or verapamil (10 μM) which suggests ATP activation of an L-type Ca channel, (2) insensitivity to removal of Na^+ which indicates that Ca channel activation is not induced by depolarization from Na^+ influx, (3) inhibition by ATPγS, which is also a partial agonist, (4) inhibition by adenosine tetraphosphate, (5) stimulation by increased extracellular phosphate and (6) potentiation by β-adrenergic stimulation. A lack of response to the non-hydrolyzable ATP analogues αβ-methylene ATP, β,γ-methylene ATP and AMP-PNP at concentrations 10-fold higher than those required for the ATP response (10 μM) suggests that ATP hydrolysis is required for the response, which is also consistent with a partial effect of ATPγS and a lack of stimulation (and inhibition) by adenosine tetraphosphate. The unique agonist and antagonist specificities of the cardiac response suggest a difference in ATP receptor subtype; nifedipine-inhibition and β-adrenergic potentiation may indicate a novel mechanism of ATP-receptor regulation of cytosolic Ca^{2+} in cardiac cells. ATP is the only transmitter thus far described to elicit Ca^{2+} transients in ventricular myocytes. The availability of ATP from hypoxic cells, platelets, or either sympathetic or parasympathetic nerve terminals may render it a significant regulator in normal and pathological conditions. This work was supported by NIH grants HL 18708 and HL 07502.

W-Pos109 POTASSIUM INDUCED CALCIUM TRANSIENTS IN HYDROZOAN EMBRYOS.

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The oocytes, eggs, and embryos of several species of hydrozoans (*Phialidium*, *Mitrocomella*, and *Eutonina*) contain endogenous calcium sensitive photoproteins similar to aequorin. KCl treatment induces luminescence which has an ontogeny beginning at the first cleavage and growing to a fraction of roughly 40% of the total luminescent potential by the 32-cell stage. The luminescence triggered by KCl is thought to involve calcium channels because the response is blocked by external Co^{2+}, Mn^{2+} and dihydropyridines, and the magnitude of the response is dependent on the $[Ca^{2+}]_o$. In addition, calcium action potentials have an ontogeny that parallels the development of the KCl response. However, the response to KCl must involve more than the mere opening of calcium channels because 1) the amount of calcium entering during a single action potential is too small to account for the light produced, 2) simultaneous recordings of the calcium action potentials and the light flash show a considerable delay between the onset of the action potential and the peak of the calcium concentration, 3) measurement of the conduction of the response over the surface of 4-cell stage embryos shows a velocity of 200 to 400 microns per sec which is much slower than the propagation of the calcium action potential, and 4) repeated stimulation of 4-cell stage embryos by injected current fails to produce a second luminescent response. These results suggest that the KCl response requires an amplification step. Such a step could be due to the release of calcium from an internal store analogous to potassium contractures in skeletal muscle. The coupling might be through calcium induced calcium release or via a chemical messenger such as IP_3 or diacylglycerol and protein kinase C. This work was supported by NSF grant DCB-8602722, and NIH grants AM35597 and GM20024.

W-Pos110 THE BINDING OF NEOMYCIN TO PHOSPHATIDYLINOSITOL 4,5-BISPHOSPHATE (PIP_2).

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Schacht and coworkers have shown that neomycin binds strongly to PIP_2 [e.g. Biochem. Pharmacol. 29:597-601, 1980]. We studied this binding by measuring the effect of neomycin on the electrophoretic mobility of multilamellar vesicles formed from mixtures of phosphatidylcholine (PC) and PIP_2, and on the surface potential of PC:PIP_2 monolayers. Neomycin does not bind to PC. The adsorption of neomycin to PC:PIP_2 bilayers can be described by the Gouy-Chapman-Stern theory if we assume a tetravalent neomycin forms an electroneutral 1:1 complex with PIP_2. Specifically, the effect of neomycin on the zeta potential of PC:PIP_2 vesicles (molar ratios of 20:1, 10:1, and 5:1) formed in 0.1 M KCl at pH 7 can all be described by assuming neomycin binds to PIP_2 with an intrinsic association constant $2\ 10^5\ M^{-1}$. Our surface potential experiments with PC:PIP_2 (5:1) monolayers are qualitatively consistent with these results. Although micromolar concentrations of neomycin bind most of the PIP_2 in a liposome at pH 7, millimolar intracellular concentrations are required to affect a variety of biological processes that involve PIP_2 breakdown. One possible explanation is that most of the PIP_2 in biological membranes is bound to proteins and is not accessible to neomycin [e.g. Anderson & Marchesi, Nature 318:295-298, 1985]. Supported by NIH grant GM 24971 and NSF grant BNS 8501456.

W-Pos111 EFFECT OF MYOCARDIAL ISCHEMIA ON HEALING-OVER AND CELL COMMUNICATION.

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The influence of myocardial ischemia "in vitro" on the healing-over process and intercellular communication was investigated. Dogs were anesthetized with sodium pentobarbital (35 mg/Kg) and the heart immediately removed. After 45-60 min of total ischemia Purkinje fibers were dissected from left ventricle, transferred to a transparent chamber and superfused with oxygenated Tyrode solution. The depolarized fibers (resting potential - 60 mV (S.D. $\pm$ 5.4) were cut and the healing-over process was followed by recording the membrane potential near (500 μm) damage. Experiments made on 15 strands showed values of resting potential of - 28 mV (S.E. $\pm$ 4.3) near lesion 15 min after damage. Moreover, the input resistance (R_{in}) measured 15 min after lesion showed decreasing values towards the cut-end. The experimental results were compared with theoretical values of R_{in} estimated from cable equation for sealed and short-circuited fibers. The findings indicate that ischemic fibers loose the ability to heal-over. Surprisingly, the process of intercellular communication was well presented in these fibers. The space constant (1.85 mm $\pm$ 0.18; n = 16) and the intracellular longitudinal resistance (r_i) (1.31 MΩ/cm) (S.E. $\pm$ 0.24) were within the normal range. Intracellular Ca injection causes cell decoupling in about 300 sec. what indicates that the lack of healing-over is not due to inability of Ca ions to seal the gap junctions. The suppression of healing over is reversible in about 3 hours. These observations indicate that the healing-over process is highly dependent on cell metabolism. (Supported by Grant No. HL-34353 from the NIH).

W-Pos112 SEROTONIN (5HT) RESPONSE IN RNA - INJECTED XENOPUS OOCYTES: SHORT- AND LONG-LASTING DESENSITIZATION. Dafna Singer, Rony Boton, and Nathan Dascal. Department of Physiology and Pharmacology, Sackler School of Medicine, Tel Aviv University, Ramat Aviv, Israel

Xenopus oocytes injected with RNA from rat brain express the $5HT_{1c}$ serotonergic and acetylcholine muscarinic receptors. These were shown to couple to a second messenger system preexisting in the oocyte. Binding of an agonist to one of these receptors initiates the following chain of events: activation of a G-protein -- activation of phospholipase C -- production of IP_3 -- release of Ca from internal stores -- opening of Ca-dependent Cl channels -- inward current carried by Cl^- (Dascal et al., Mol.Brain Res. 1:201, 1986; Lubbert et al., J. Neurosci., 7:1159, 1987). Exposure of the oocyte treated with the ionophore A23187 to Ca leads to a similar response (the Ca-response). The 5HT response exhibits self-desensitization and cross-desensitization with acetylcholine; both last 3 to 5 hr (long-lasting desensitization). On the other hand, the Ca-response is also accompanied by a period of refractoriness during which a second Ca-response is diminished. This period lasts for up to 30 min (short-lasting desensitization). 5HT causes only short-lasting desensitization to responses evoked by injection of Ca or IP_3 (the injection is done at various times after the exposure to 5HT). Injection of Ca causes only short-lasting desensitization to subsequently applied 5HT. In contrast, the irreversible activator of G-proteins, GTP-γ-S, causes a long-lasting desensitization to subsequently applied 5HT. It is concluded that 5HT produces two types of desensitization: a short-term desensitization, which is most probably the result of a Ca-dependent inactivation of the Ca-operated Cl^- channel; and a long-lasting one, probably at the level of G-protein.

W-Pos113 REGULARIZATION OF BURSTING IN PANCREATIC BETA CELLS BY CHANNEL SHARING. A. Sherman, J. Rinzel & *J. Keizer, MRB, NIH, Bethesda, MD 20892 and *Dept. Chem. UC, Davis, CA 95616.

The Chay-Keizer theory describes the electrical activity of pancreatic beta cells. A calcium-activated potassium (K-Ca) conductance underlies bursting behavior of the model. Atwater, et al. [Cell Calcium 4:451-461] have proposed that the rarely open K-Ca channels are shared by several cells simultaneously. We have extended the theoretical model to show that such channel sharing could account for the difference in behavior between chaotically spiking isolated cells and bursting, electrically-coupled cells in a cluster. Because the K-Ca channel is large and only rarely open, single channel events can have a strong perturbing effect on an isolated cell. When the cells are coupled, the effect of the perturbations is reduced. We model the K-Ca channels stochastically, with Monte Carlo simulation of random channel events. Our present model of coupling is greatly oversimplified: instantaneous communication through zero resistance gap junctions. We have also updated the deterministic treatment of the spike-generating, V-gated (Ca++ and delayed-rectifier K+) channels to account for recent whole-cell voltage-clamp data by Rorsman and Trube. Our simulated single cell behavior looks strikingly like the irregular spiking seen experimentally. We find that as the total number of cells increases, the statistical variance of the fraction of K-Ca channels open decreases, and the activity shows a transition to temporally organized bursting.

W-Pos114 HOW BIG IS THE CARDIAC GAP JUNCTION CHANNEL? D.C. Spray, J.M. Burt†, and D.C. Brosius. Dept. Neuroscience, Albert Einstein College of Medicine, Bronx, NY. and †Dept. Physiology, University of Arizona, Tucson, AZ

The activity of gap junction channels between pairs of neonatal rat heart cells in culture was studied under control conditions and during uncoupling procedures using dual whole-cell voltage clamp techniques. Under control conditions (patch solutions well-buffered or weakly buffered for calcium ions and protons) macroscopic junctional conductance ranged from 0.05 - 30 nS. In low conductance pairs (<350 pS), single channel events with a mean unitary conductance of 51 ± 1 pS were apparent. The open time and probability of opening were estimated to be 0.95 seconds and 0.17, respectively. Single channel events of 50 pS amplitude were also evident under control conditions in high conductance pairs (>1 nS), although the incidence of coincident channel openings and closures was higher than in low conductance pairs. Analysis of transjunctional current fluctuations in high conductance (>10 nS) pairs revealed a major Lorentzian component with a cut off frequency of 0.5 ± 0.06 Hz (SEM, n=4), which corresponds to a mean open time of 0.3 sec. Unitary channel conductance was unaltered while channel open time was decreased during uncoupling induced by cytoplasmic acidification or application of heptanol. The constancy of unitary channel conductance under control conditions and during uncoupling procedures suggests that opening and closure of the gap junction channel are all-or-none processes, with no stable subconductance states formed. Supported by grants from PHS - HL 31008, the AZ AHA and the Az Disease Control Commission to J.M.B., and from PHS - NS 07512 and NS 16524, and the NY AHA to DCS.

W-Pos115 MODULATION OF CARDIAC GAP JUNCTION CONDUCTANCE BY THE MEMBRANE LIPID ENVIRONMENT.
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The influence of 16-doxyl stearic acid (16D-SA), 16-doxyl stearic acid methyl ester (16D-SAME), stearic acid (SA) and stearic acid methyl ester (SAME) on gap junction conductance, g_j, between pairs of neonatal rat heart cells was studied using dual whole-cell voltage clamp techniques. 16D-SA and 16D-SAME are spin probes which perturb the membrane (lipid and protein components) towards the center of the bilayer; 16D-SA is also negatively charged. Since these compounds are known to influence transmembranous calcium fluxes, all experiments were performed in the absence of extracellular calcium (Ca-free balanced salts solution with 0.5 mM EGTA) and with patch solutions containing 10 mM EGTA. 16D-SA reversibly decreased g_j to unmeasureable levels in a dose dependent manner, 50 μM typically within 1 minute of exposure (n=5). In contrast, 50 μM 16D-SAME was mildly stimulatory, causing junctional conductance to increase by 3 and 21% in two experiments. Neither SA or SAME had any measureable effect on g_j. Single channel events of approximately 50 pS amplitude were observed during uncoupling by 16D-SA or by cytoplasmic acidification of cells simultaneously exposed to 16D-SAME, SA or SAME. This value for unitary channel conductance is similar to that obtained under control conditions and during cytoplasmic acidification in the absence of these compounds (see abstract by D.C. Spray et al., this volume). These results suggest that perturbation of the lipid bilayer at a location towards the center of the bilayer (e.g. 16D-SAME) stimulates channel function; however, simultaneous perturbation of the membrane and increased negative surface charge (e.g. 16D-SA) inhibits channel function. Neither stimulation or inhibition of the channel by these agents appears to be mediated by an alteration of the unitary conductance of the channel. Supported by grants from PHS - HL 31008, the Ariz. Affiliate of the Amer. Heart Assoc, and the Ariz. Dis. Control Commission.

W-Pos116 DEPOLARIZATION-COUPLED CAPACITANCE CHANGES IN CULTURED BOVINE ADRENAL CHROMAFFIN CELLS.
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To quantify exocytotic activity in bovine adrenal chromaffin cells, we have monitored membrane area by admittance measurements. Changes in membrane capacitance and conductance were measured with a patch clamp amplifier and a 2-phase lock-in amplifier (Neher & Marty, P.N.A.S., 79,6712). Cells were internally perfused with a Cs and TEA solution and bathed in an external solution with TTX to block voltage-dependent Na and K currents. Stable readings of membrane capacitance (+/- 20fF for 10 min) were obtained with intracellular Ca buffered to $\leq$100 nM. Minimizing the capacitative and (non-Ca) ionic currents as well as adjusting the phase relation between the pulse and the reference sin wave allowed us to improve the bandwidth of our measurements. Depolarizing pulses of 5-50ms from a holding potential of -70mV produced fast 'jump' increases (2-30fF) in the capacitance signal. The magnitude depended in part on the amount of Ca that entered the cell during the pulse: the capacitance increases were largest for depolarizations to the peak of the Ca I-V relation. Both the Ca currents and the capacitance increases were reversibly eliminated by the substitution of Mg for Ca. A second slower phase of capacitance increase (1-5s) could be observed with either repetitive pulses or longer (100-200ms) pulses. Investigation of the cellular processes involved in these fast and slow capacitance increases should yield useful information on the mechanisms of depolarization-coupled secretion.

W-Pos117 APICAL MEMBRANE CHANNELS FROM SALAMANDER KIDNEY PROXIMAL TUBULE CELLS IN CULTURE. Rosemarie Drake-Baumann and Emile L. Boulpaep, Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, CT 06510.

We have used the patch clamp technique to examine the ionic permeability of the apical membrane cells of Ambystoma kidney proximal tubules in primary culture. These epithelial cells when grown as confluent monolayers on permeable collagen supports exhibit both the morphological and electrophysiological characteristics of proximal tubule cells in vivo: transepithelial potential of -3.63 mV and transepithelial resistance of 116.2 ohm.cm^2 as well as a depolarization of the apical membrane in response to addition of glucose to the mucosal solution. In cell-attached patches we have identified single K^+ channels in the apical membrane of cells in confluent monolayers. With 90 mM KCl and 5 mM NaCl in the patch pipette and HEPES-buffered nutrient solution with 95 mM NaCl and 2.4 mM KCl in the bath, the single channel conductance measured at $\pm$ 20 mV holding potential is ~ 18 pS. These channels show very fast flickering and short open times. At 0 mV holding potential the mean open time is 4.5 ms. The reversal potential falls in the range expected for a K^+ selective channel. In the cell-attached configuration we have also observed single Na^+ channels in the apical membrane, with characteristic long openings and multiple channels in a single patch. With 95 mM NaCl and 2.4 mM KCl in the patch pipette and HEPES-buffered nutrient solution in the bath, the single channel conductance was ~ 5.6 pS measured over a linear range of holding potentials of - 30 mV to +100 mV. Assuming a cell membrane potential of - 56 mV, the extrapolated reversal potential is compatible with that of a highly selective Na^+ channel.

W-Pos118 FUNCTIONAL ASSAY FOR THE VASOPRESSIN-INDUCIBLE WATER CHANNEL IN RENAL ENDOCYTIC VESICLES. A.S. Verkman, W. Lencer, D. Brown and D.A. Ausiello (sponsor G. Cabrini). CVRI, University California, San Francisco and Renal Unit, MGH, Harvard Medical School.

As a first step in isolating the renal vasopressin-inducible water channel, an assay of osmotic water transport in endocytic vesicles has been developed. Brattleboro rats with central diabetes insipidus were infused with fluorescein dextran or carboxyfluorescein (F) for 15 min with or without vasopressin. F was filtered into the kidney tubules and endocytosed from the tubule lumen. Vesicles isolated from renal cortex and papilla (containing F-labelled endosomes) were subjected to an inward osmotic gradient in a stopped-flow apparatus; F fluorescence decreased due to water efflux and F self-quenching. There was no signal change in the absence of an osmotic gradient. Cortical vesicles arising from proximal tubule gave a biexponential fluorescence decrease with time constants 0.035 and 0.6 s and relative amplitudes 1:0.7 which did not change with vasopressin. In contrast, papillary vesicles arising from collecting tubules gave a similar slower time constant (0.8 s), but a distinct fast time constant (0.030 s) only when vasopressin was infused. Similar results were obtained with fluorescein dextran infusion. The activation energies (E_a) for the fast and slow processes were 3.8 and 13.0 kcal/mol, respectively. Following horseradish peroxidase (HRP) infusion, HRP was taken up into a subpopulation of papillary vesicles with a diameter of 150±50 nm. These results indicate that a vasopressin-inducible population of papillary endosomes have rapid water transport with low E_a, suggesting the presence of water channels. This stopped-flow fluorescence assay is applicable to follow functional water transport activity for endosome purification studies.

W-Pos119 ESTIMATION OF INTRACELLULAR CHLORIDE ACTIVITY IN ISOLATED PERFUSED RABBIT PROXIMAL CONVOLUTED TUBULES USING A FLUORESCENT INDICATOR. R. Krapf, C.A. Berry, and A.S. Verkman. Cardiovascular Research Institute, University of California, San Francisco, CA 94143.

The chloride-sensitive fluorescent indicator 6-methoxy-N-(3-sulfopropyl)quinolinium (SPQ) has been used to study chloride transport mechanisms in isolated membrane vesicles by cuvette fluorometry (Biochemistry 26:1215-1219, 1987). We developed the methodology to measure cell chloride activity by fluorescence microscopy using SPQ. SPQ was loaded into cells of the in vitro microperfused rabbit proximal convoluted tubule (PCT, S_2 segments) by a 10 min luminal perfusion with 20 mM SPQ at 38°C. Fluorescence was excited with a broad band excitation filter (340-380 nm) and detected with a 435 nm cut-on filter. The signal to background (autofluorescence) ratio was 4.6 ± 0.6. The halftime for SPQ leakage from cells at 38°C was 8.6 ± 1.1 minutes. Incubation of suspended tubules with 20 mM SPQ for 15 minutes at 38°C did not affect O_2 consumption significantly. Intracellular SPQ calibration was performed using the ionophores nigericin and tributyltin, high external [K], and varying extracellular Cl activity. SPQ fluorescence was quenched by intracellular Cl with a Stern-Volmer constant of 12 M^{-1}. Cell chloride activity in tubules perfused with solutions characteristic for the late PCT was 27.5 ± 5 mM. The halftime of the transient in cell chloride activity upon change in bath [Cl] from 0 to 128 mM was 2.5 sec (38°C).

These results indicate that SPQ can be loaded into and calibrated in viable cells of the intact rabbit PCT where it can be used to measure steady-state values and transients in cell chloride activity. The findings that (1) cell chloride activity is above electrochemical equilibrium in the PCT and (2) the halftime of chloride influx into PCT cells is <3 seconds support a major role for active, transcellular chloride transport in transepithelial PCT chloride movement.

W-Pos120 NaCl REFLECTION COEFFICIENT OF RABBIT RENAL BRUSH BORDER MEMBRANE VESICLES (BBMV) IS NEAR UNITY. D. Pearce and A.S. Verkman. Cardiovascular Research Institute, University of California, San Francisco, CA 94143.

The mechanism of isosmotic volume reabsorption in the renal proximal tubule is controversial. Relative luminal hypotonicity due to a low reflection coefficient (σ) for NaCl has been proposed. The NaCl σ for rabbit proximal tubule brush border membrane was determined in BBMV using 3 independent approaches. BBMV were prepared by Mg precipitation and differential centrifugation. Experiments were done on a stop flow apparatus with a 1-2 ms dead time. "Null point" (np) was determined by the osmolality of an external NaCl solution which, when mixed with BBMV containing sucrose, caused no initial volume flux. The np was bracketed by external NaCl concentrations which limit σ to between 0.9 and 1. In a second method, the initial volume flux (J_vo) induced by the NaCl solution was compared with that induced by a sucrose solution of equivalent osmolality. Vesicles were subjected to inwardly directed osmolar gradients of 50, 100 or 150 mOsm of either sucrose or NaCl. The initial rate of vesicle shrinkage was determined by rate of change of scattered light intensity at t=0 (dI/dt_o). dI/dt_o was determined by the initial slope of a single exponential fit to the data over the first 200 msec. σ was determined for each gradient by the ratio $J_vo(NaCl)/J_vo(sucrose)$ assuming $\sigma(sucrose)=1$. $\sigma=1.08\pm.09$, $1.07\pm.1$ and $1.04\pm.07$ at 50, 100, and 150 mOsm gradients respectively. The third method employed the Cl sensitive fluorescent probe, SPQ (loaded intravesicularly), to detect solvent drag. We compared NaCl influx in the presence and absence of an outwardly directed osmotic gradient (driving water influx) from the time course of SPQ fluorescence. Solvent drag accounted for less than 5% of the total Cl influx placing σ at greater than 0.9. These results indicate that σ for rabbit BBMV is close to one.

W-Pos121 **K-SELECTIVE MICROELECTRODES BASED ON THE CROWN ETHER DIBENZO-18-CROWN-6**
Jennifer L. Mooney, Mercedes Acevedo, Vijay Lyall and William McD. Armstrong (Intro. by Ayus Corcia). Dept. Physiology and Biophysics, Indiana Univ. Sch. Med., Indianapolis. IN 46223.

Liquid ion-exchanger microelectrodes based on Corning code 477317 potassium ion-exchanger are widely used to measure intracellular K activity. Such electrodes are known to be much more sensitive to quaternary ammonium ions than to K and, in appropriate circumstances, may be used as specific probes for the former (e.g. Reuss, PNAS 82:6014, 1985). Under such conditions, the capability of measuring K activities with these electrodes may be seriously impaired or completely lost. This prompted us to develop a neutral carrier K-selective microelectrode based on the crown ether (CE) dibenzo-18-crown-6. The CE cocktail contained (w/w) 2.3% CE, 0.8% Na-tetraphenylborate, 30.1% 2-nitrophenyloctylether and 66.8% O-nitrotoluene Double-barreled CE and Corning 477317 microelectrodes were prepared by methods routinely used in our laboratory. Both kinds of microelectrodes had similar Na over K selectivities. Intracellular K activities measured in the same Necturus gallbladders with both kinds of electrodes did not differ significantly. However, CE based microelectrodes had immensely greater selectivities for K over quaternary ammonium ions than Corning 477317 electrodes. Selectivity coefficients of CE microelectrodes with respect to K for tetramethylammonium, choline, and acetylcholine were $1.4\pm0.3 \times 10^{-2}$, $1.1\pm0.1 \times 10^{-3}$ and $4.1\pm1.6 \times 10^{-2}$, respectively. Supported by USPHS DK 12715 and DK 36575.

W-Pos122 IDENTIFICATION AND PURIFICATION OF A STILBENE DISULFONATE BINDING GLYCOPROTEIN FROM KIDNEY BRUSH BORDER MEMBRANES. Reinhart A.F. Reithmeier and Sanjay W. Pimplikar, MRC Group in Membrane Biology, Department of Medicine and Department of Biochemistry, University of Toronto, Toronto, Ontario, Canada M5S 1A8. Renal brush border membrane proteins that bind stilbene disulfonate inhibitors of anion exchange were identified by affinity chromatography. A 130-kDa integral membrane glycoprotein from brush border membrane was shown to bind specifically to 4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonate immobilized on Affi-Gel 102 resin. The bound protein could be eluted effectively with 1 mM 4-benzamido-4'-aminostilbene-2,2'-disulfonate (BADS). The 130-kDa protein did not bind to the affinity resin in the presence of 1 mM BADS or when the solubilized extract was covalently labelled with 4,4'-diisothiocyanostilbene-2,2'-disulfonate (DIDS). This protein was labelled with [^{3}H]-H$_2$DIDS and the labelling was prevented by BADS. The 130-kDa protein did not cross-react with antibody raised against human or dog erythrocyte Band 3 protein. The 130-kDa protein was accessible to proteinase K and chymotrypsin digestion in vesicles but not to trypsin. The 130-kDa protein was sensitive to endo-β-N-acetylglucosaminidase F treatment both in the solubilized state and in brush border membrane vesicles showing that it was a glycoprotein and that the carbohydrate was on the exterior of the vesicles. This glycoprotein was resistant to endo-β-N-acetylglucosaminidase H treatment suggesting a complex-type carbohydrate structure. The protein bound concanavalin A, wheat germ agglutinin and Ricinus communis lectins and it could be purified using wheat germ agglutinin-Agarose. (Supported by the Medical Research Council of Canada and an Alberta Heritage Foundation for Medical Research Studentship [SWP]).

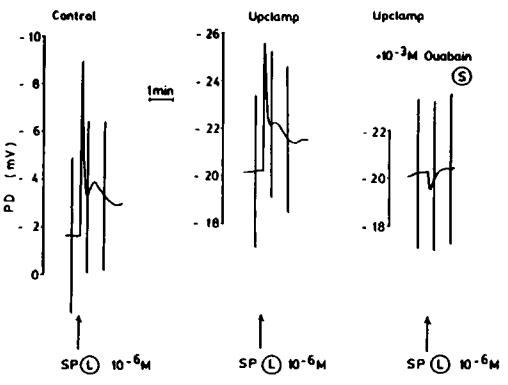
W-Pos123 EFFECTS OF CDPC ON SINGLE CHANNEL CURRENTS OF THE AMILORIDE-SENSITIVE NA CHANNEL FROM CULTURED RENAL CELLS. Y. Marunaka and D. C. Eaton, Dept.of Physiol., Emory Univ. School of Medicine, Atlanta, Georgia 30322. To investigate the properties of the amiloride-sensitive Na channel, single channel currents from cell-attached patches were measured in cultured renal cells (A6). Cells were cultured for 1-2 weeks on collagen supports prior to use . The composition of bath and pipette solutions was (mM): 129 NaCl, 3.4 KCl, 0.8 $CaCl_2$, 0.8 $MgCl_2$ and 11 HEPES (pH 7.4). 1 mM $BaCl_2$ was added to the pipette solution to block K^+ currents. The single channel conductance was 4pS. Interval histograms of open and closed states show that the Na channel has at least two open and two closed states. One open and one closed state is of long duration (seconds) while the remaining open and closed state are of short duration (milliseconds). Micromolar concentrations of the amiloride analogue, 6-chloro-3,5-diamino-pyrazine-2-carboxamide (CDPC), reduces the time spent in the long duration open state as well as the long duration closed state in a concentration dependent manner. On the other hand, CDPC has no effect on the single channel conductance and no effect on either the short duration open or closed events. Besides the effect on the long duration closed events, the closed interval histogram after CDPC has two additional exponential components compared with untreated patches. The mean dwell time of these closed-states is not affected by CDPC concentration. These results suggest that CDPC can interact with both open and closed channels. The distribution of open and closed intervals within the bursts of activity induced by CDPC suggests that the OPEN BLOCKED channel can occasionally close while CDPC remains associated with the channel. This implies that the kinetic model for CDPC interaction is cyclic with at least six states.

W-Pos124 WHOLE CELL CURRENTS IN CULTURED CANINE TRACHEAL CELLS. S.R. Shorofsky, N. Schoppa, D. J. Nelson and H.A. Fozzard. The University of Chicago, Chicago, Ill.

The epithelium lining the canine trachea actively secretes chloride ions. Chloride secretion can be stimulated by increasing either cAMP or Ca within the cell. Recent evidence indicates that the primary defect in cystic fibrosis is a defect in the regulation of chloride transport processes. We are investigating this problem by studying whole cell current from isolated canine tracheal cells using patch clamp techniques. Primary cultures of canine tracheal cells were made as previously described (Welsh MJ, J Membr. Biol. 88. 1985) with some slight modifications. Cells were studied between day 2 and day 5 in culture. Pipette solutions contained either 140 mM KCl or 140 mM CsCl with 5 mM EGTA, 0.5 mM $CaCl_2$, 10 mM Hepes and were buffered to a pH of 7.2. Holding potentials were -40 mV. The currents generated by the cells depended on the composition of the bathing solution. The addition of glucose and PO_4 to the bathing solution increased the steady state currents from 49.5 $\pm$ 7.1 to 86.0 $\pm$ 19 pA at a pulse potential of +50 mV and from -70.4 $\pm$ 15.6 to -157.8 $\pm$ 22 at -90 mV - ($p < 0.05$). Increasing the extracellular calcium from 2mM to 4.5 mM also increased the steady state currents (from 62.1 pA to 139.5 pA, + 50 mV). The steady state currents were increased by forskolin in 11 out of 24 cells and by A23187 in 9 out of 15 cells. An outward current could be identified which exhibited a time-dependent inactivation at depolarized potentials greater than 40 mV. The selectivity of this current is non-specific as determined from analyses of tail currents in bathing solutions of varying ionic composition. A method was also developed to isolate the Cl current from the nonspecific current. Using this method we hope to investigate the regulatory factors governing Cl secretion in canine and human tracheal cells.

W-Pos125 LUMINAL RESPONSES TO SUBSTANCE P ON RAT TRACHEA. P.K.Rangachari, D.McWade and B.Donoff. I.D.R.U., McMaster University, Hamilton, Ontario, Canada. L8N 3Z5

Luminal addition of tachykinins to the isolated canine tracheal epithelium produces a biphasic response--an early rapid transient decrease in P.D. (dip), followed by an increase (rise). The dip reflects a transient increase in the anion conductance of the paracellular path [Bioch.Biophys.Acta (1986) 863:305]. Rat tracheal responses to luminal tachykinins are different. A rapid transient increase in P.D. is seen ("FLASH") with a secondary rise (Fig.1). Like the dip, the "FLASH" is linearly related to the basal P.D., but, increasing P.D. attenuates, and reversing the P.D. exaggerates the response. An NK1 receptor is involved, since SP-O-methyl ester produces the same responses. Ouabain abolishes the flash and a dip is revealed. Replacing luminal Cl^- with isethionate abolishes the dip. Thus peptides increase anion conductances of the rat trachea as well, but in the presence of a functioning Na^+ pump, this is manifested as an increase in P.D., reflecting a movement of Cl^- into the luminal solution, perhaps from the intercellular spaces. (Supported by MRC Canada)



W-Pos126 EFFECT OF NUTRIENT HCO_3^- CONCENTRATION CHANGES ON PD AND RESISTANCE IN RESTING FROG FUNDUS IN Cl^- MEDIA. M. Schwartz, J. Wu, G. Carrasquer and W. S. Rehm. Departments of Physics and Medicine, University of Louisville, Louisville, KY 40292.

Previously for changes in nutrient (N) HCO_3^- (B) conc. in resting fundus, a linear relation was found between ΔPD and log [B] (BBA 819, 187-194, 1985). To account for linearity, we assumed that the resistance (R) of ion pathways including the B pathway varied inversely with B changes in N conc. In this study in resting fundus, B N conc. was changed from 25 to 5 mM and back to 25 mM first with N pCO_2 constant and second with N pH constant. In first part, decrease in N B gave ΔPD = -17.6 mV and ΔR = 292 ohm cm^2 while, in second part, decrease in N B gave a decrease in PD of 9.1 mV and an increase in R of 128 ohm cm^2. Thus changes in PD and R with N pH constant were about half changes in PD and R with N pCO_2 constant. Experiments were done to see if Δ% CO_2 could account for the differences in ΔPD between the two cases. With 25 mM N B, a decrease in N CO_2 from 5 to 1% gave no significant ΔPD and ΔR = 30 ohm cm^2. With 5 mM N B, R is high and the decrease in CO_2 from 5 to 1% now gave ΔPD = 6.0 mV and ΔR = -117 ohm cm^2 and the return to 5% CO_2 gave ΔPD = -9.4 mV and ΔR = 134 ohm cm^2. The ΔPD's corresponded well with the differences in ΔPD between pCO_2 constant and pH constant in going from 25 to 5 mM B and back to 25 mM. Attempts to attribute the differences to a H^+ and/or OH^- partial conductance are uncertain, in part because of the nature of the PD response with time due to a change in pH. The PD response may be affected by changes in resistance of other ions. Recent work with changes in K conc. for 25 and 5 mM B show that the partial K conductance is decreased at 5 mM N B compared to 25 mM N B. The latter results indicate that changes in N B cause changes in resistance of K pathway. (NSF support)

W-Pos127 CELLULAR pH (pH_i) AND APICAL Na-H EXCHANGE IN PRINCIPAL (P) CELLS OF R. PIPIENS. K. Drewnowska & T.U.L. Biber, Dept. of Physiology, Medical Coll. of Virginia, Richmond, VA 23298.

Measurements of pH_i and apical cell membrane potential (V_a) were made in P-cells of isolated frog skin epithelium with double barrel microelectrodes under open circuit conditions. The tissues were pretreated with 10^{-3} M DIDS, an anion exchange blocker, and bathed in HCO_3-free NaCl Ringer's solution that was buffered with 6 mM HEPES at pH 7.8. As reported previously (Biber & Drewnowska, Fed. Proc. 45:3, 1986) removal of extracellular Na (Na_o) on both sides of the epithelium caused cell acidification by 0.27 pH units. In the present studies the effect of Na_o restoration on pH_i was tested. Readdition of Na_o on (1) apical side and (2) both sides caused a pH_i recovery (alkalinization) of 0.24 $\pm$ 0.04 pH units (n=9) and 0.28 $\pm$ 0.02 pH units (n=7), respectively. At the same time V_a depolarized by 12 $\pm$ 1 mV and 14 $\pm$ 1 mV, respectively. No significant changes in pH_i and only a small depolarization of V_a by 3 $\pm$ 1 mV (n=8) occurred when Na_o was restored on the basolateral side alone. The initial rate of pH_i recovery (ΔpH_i/min) after restoration of Na_o on the apical side and on both sides was 0.59 $\pm$ 0.16 (n=9) and 0.71 $\pm$ 0.22 (n=7), respectively. In individual cells, the overall change in pH_i achieved with return of Na_o was correlated with initial rate of pH_i increase, i.e., a faster initial rate of pH_i increase was associated with a larger pH_i recovery. Cell alkalinization upon readdition of Na_o on the apical side was prevented by 10^{-5} M EIPA [5-(N-ethyl-N-isopropyl)-amiloride]. However, EIPA did not block the depolarization of V_a on adding Na_o to the apical side. In this case, V_a depolarized by 10 $\pm$ 3 mV (n=5). The results suggest the presence of a Na-H exchange mechanism located on the apical side of P-cells. Supported by NIH grant AM 26347.

W-Pos128 ACID FLUX AT THE BASOLATERAL MEMBRANE OF FROG SKIN. Thomas Cox and G. Ginsberg. (Intr. by R.L. Coulson) Dept. of Physiology, Southern Illinois University, Carbondale, IL 62901.
Acid flux at the basolateral membrane of skin of Rana pipiens was measured by a modification of the technique of Weinman and Reuss (J. Gen. Physiol. 80:299, 1982). Briefly, skins were bathed on both sides by 100 Na, 2 Ca, 2.4 K, 1mM HEPES chloride Ringer, pH 8.2. The solution was constantly mixed by aeration. This was abruptly replaced by a similar Ringer at pH 6.0. The rate of change in pH of the solution was measured for 12 minutes. The basolateral solution was then returned to pH 8.2 for 12 minutes. This was followed by an experimental 12 minute period at pH 6.0. Flux was calculated from the rate of change of pH and the buffering power of the solution. Rate is expressed in $\mu A/cm^2$ for comparative purposes and is not meant to imply a conductive mechanism. In 20 experiments at pH 6.0, the rate of alkalinization of the basolateral solution gave an average flux of $-4.8\pm0.5\mu A/cm^2$. This may be a proton influx or a hydroxyl efflux. When chloride was replaced by gluconate the flux was reversed going from -5.5 ± 1.2 to $+1.2\pm0.2\mu A/cm^2$ (n=5). Chloride replacement by sulfate partially blocked this flux. The blockers SITS or DIDS reduced the flux from -4.2 ± 1.3 to -3.1 ± 0.6 (n=5). Amiloride (1mM) decreased the flux from -6.5 ± 0.9 to -5.5 ± 0.6 (n=4). Replacement of Na by tetramethylammonium (TMA) increased the flux from -3.6 ± 0.7 to -4.5 ± 1.0 (n=6). The results of these studies indicate that that the majority of acid flux is chloride dependent. This may be via a Cl/HCO_3 exchange although it is not very sensitive to SITS or DIDS. The fact that it can be decreased by amiloride and increased by increasing the Na gradient suggests that a portion of the flux moves via Na/H exchange. (Support from AHA IA).

W-Pos129 DISSOCIATION AND CHARACTERIZATION OF CELLS OF FROG TADPOLE SKIN. Thomas C. Cox and Rebekah Woods. Dept. of Physiology, Southern Illinois University, Carbondale, IL 62901.

In contrast to the adult, the skin of the frog tadpole (Rana catesbeiana) transports Na at a very low rate. Nystatin added to the apical membrane increases short circuit current (Isc) to near adult levels. The channels in the apical membrane are relatively non-selective between monovalent cations and are stimulated (not blocked) by amiloride. With a 100mM Na, Ca-free Ringer on the apical side, 0.1mM amiloride caused a transient increase in Isc of up to 15μA. With 100mM K, Ca-free Ringer, the amiloride induced transient was as large as 16μA. We have developed techniques to dissociate the tadpole skin cells and identify the apical cells in order to study the apical membrane channels with the patch clamp. The apical side of the skin was first treated with FITC labeled wheat germ agglutinin. The skin was then treated with Ca-free, EDTA Ringer followed by mild trypsin (0.25%) treatment. Dissociated cells were plated on Concanavalin A coated cover slips. More than 90% of the cells obtained from the trypsin treatment were viable according to trypan blue exclusion. Examination of the cells with fluorescence microscopy showed that some of the cells were labeled. In some cases only a specific region of a cell was labeled. We interpret this to represent labeling of the apical domain of the apical cells. Preliminary patch clamp studies have indicated that gigaohm seals with channels can be obtained from these cells. This preparation should provide an opportunity to study the nonselective apical membrane channel.

W-Pos130 A 120 kDa PHOSPHOPROTEIN ASSOCIATED WITH THE APICAL MEMBRANE OF STIMULATED PARIETAL CELLS. T. Urushidani, D.K. Hanzel, and J.G. Forte. Dept. of Physiology-Anatomy, University of California, Berkeley, CA 94720

We have reported that stimulation of isolated rabbit gastric glands via cAMP pathway induces marked phosphorylation of 80 and 120 kDa regions in the apical membrane-rich fraction (B.B.A. 930:209, 1987). In the present study, we further examined the features of 120 kDa phosphoprotein. Isolated rabbit gastric glands were incubated with either 0.1 mM histamine plus 50 uM IBMX (stimulated) or 0.1 mM cimetidine (resting), homogenized, and fractionated. Subfractionation of the low speed pellet (4000 x g, 10 min) by density gradient produced an 18% Ficoll layer (D18), greatly enriched in H/K ATPase, derived from apical membranes of parietal cells. Autoradiography of SDS-PAGE revealed that ^{32}P in the 120 kDa region of D18 was markedly increased with stimulation. This was mainly due to the association of a small amount of 120 kDa acidic phosphoprotein (pI about 4.5) with D18. Among the fractions, cytosol (48,200 x g, 90 min sup) also contained 120 kDa protein whose radioactivity showed no change with stimulation. Although this M.W. region contained several peptides, one of these could be the same as the one in D18 since these shared the same M.W., pI, and peptide maps. This 120 kDa phosphoprotein has the same M.W. as vinculin, but they were revealed to be different: the 120 kDa protein showed different pI and peptide maps compared with rabbit vinculin. Furthermore, monoclonal anti-vinculin did not recognize the 120 kDa region of D18. We conclude that the 120 kDa phosphoprotein in the cytosol of the parietal cell could associate with apical membrane when the cell is stimulated, and in fact, may represent a functional component of the secretory cycle. (Supported by PHS Grant AM10141)

W-Pos131 LOWERING EXTRACELLULAR SODIUM RELEASES CALCIUM FROM HORMONE-SENSITIVE INTRACELLULAR STORES IN GASTRIC PARIETAL CELLS. Paul A. Negulescu and Terry E. Machen, Department of Physiology-Anatomy University of California at Berkeley, 94720.

Microspectrofluorimetry was used to monitor cytosolic free Ca, Ca_i, in single parietal cells of isolated rabbit gastric glands loaded with fura-2. Perfusion of the glands with solutions in which Na had been replaced with N-methyl-D-glucamine caused Ca_i to increase transiently from 100nM to 450nM. The increase began within about 25 sec and was complete within about 70 sec of exposure to the Na-free solution. Following the peak, Ca_i usually decreased back toward baseline. The transient increase occurred equally well in both Ca-free (.1mM EGTA) and Ca-containing solutions. If the intracellular Ca stores were first emptied with the cholinergic agonist, carbachol, treatment with Na-free solutions elicited no change in Ca_i. Conversely, initial exposure of cells to Na-free attenuated a subsequent carbachol response. We conclude that the increase of Ca_i in Na-free solutions is due to release of Ca from intracellular stores. The Na-free effect was independent of changes in membrane potential because ten-fold changes of K_o had no effect on Ca_i. The response was largely independent of changes in intracellular pH (monitored with BCECF) because Ca_i increased before the cell acidified significantly. In addition, acidification induced by a pulse of either NH_4Cl or CO_2 caused Ca_i to increase to only 160nM. The increase in Ca_i also showed a sharp dose dependency for extracellular [Na] (Na_o), with very few cells responding when $[Na]_o$ was above 30mM and most giving maximal responses at 20mM Na_o (K_m=22mM Na). We conclude that the effect of changing $[Na]_o$ on internal Ca stores is due to changes in intracellular [Na].

W-Pos132 ANIONS AND AMILORIDE MODULATE EPITHELIAL MEMBRANE IMPEDANCE. N.K. Wills, S.A. Lewis and C. Clausen, Univ. Texas Med. Br., Galveston, TX 77550 and S.U.N.Y. Stony Brook, NY 11794

In many tight epithelia, Na^+ transport is altered when mucosal or serosal chloride is replaced by other anions. Previous work indicates that this regulation involves modulation of the apical membrane Na^+ conductance and basolateral membrane K^+ conductance. At least two mechanisms can account for this regulation: 1) alteration of single channel properties or 2) insertion/withdrawal of lipid vesicles containing ionic channels. We used impedance analysis to determine individual membrane conductances and areas of toad urinary bladder. If vesicle fusion is the major mechanism of regulation, then changes in membrane conductance should be associated with changes in membrane area. The impedance data were best described by a model that distributes the basolateral membrane resistance along the lateral intracellular space in series with an extracellular parallel resistance and capacitance (RC). The apical membrane conductance (G_a) and capacitance (C_a) averaged 62±17 μS/cm² and 0.9±0.2 μF/cm² and the mean basolateral membrane conductance (G_b) and capacitance (C_b) was 2±0.3 mS/cm² and 4±0.5 μF/cm². Mucosal amiloride addition or symmetrical gluconate replacement of Cl^- decreased G_b by 28±6%, and 46±6%. G_a was also decreased by 72±3% and 42±3%, respectively. In contrast, acetate replacement of Cl^- had virtually no effects on G_b but increased the apical membrane amiloride-sensitive conductance ($G_{a\text{-amil}}$) by 18±8%. $G_{a\text{-amil}}$ was also increased by 42±17% (n=8) following a 10 minute incubation with amiloride. None of the above changes altered either C_a or C_b. We conclude that the net addition or withdrawal of large areas of membrane is not a major regulatory mechanism for short-term regulation of Na^+ transport for this epithelium. (Supported by N.I.H. Grants DK 29962 and DK 33243).

W-Pos133 EPITHELIAL CHLORIDE CHANNEL BLOCKADE BY 4,4'-DINITRO-2,2'-STILBENE-DISULFONIC ACID (DNDS). Robert J. Bridges, Dale J. Benos and Raymond A. Frizzell, Department of Physiology and Biophysics, University of Alabama at Birmingham, Birmingham, AL 35294.

Membrane vesicles derived from the rat colonic mucosa, a chloride secreting epithelium, were fused to planar lipid bilayers (PE:PS, 7:3). Chloride channels were observed 1-10 min after vesicle addition to the cis side when a 300 mM (cis) to 50 mM (trans) NaCl or KCl gradient was used. Properties of this chloride channel as previously reported by us (J. Membr. Biol. 95:47-54, 1987) included: one dominant open-state conductance, a weakly rectifying current-voltage relation with a cord conductance of 50 pS at -30mV in symmetric 200 mM Cl, and a halide selectivity sequence of I>Br>Cl>F. Addition of DNDS, a 4,4'-dinitro-substituted derivative of the anion transport inhibitor 4,4'-diisothiocyano-2,2'-stilbene-disulfonic acid (DIDS), to the cis side caused a concentration-dependent increase in current fluctuations between the open- and closed-states and a decrease in the open channel probability (Po). A detectable decrease in Po was observed at 0.1 μM DNDS, a half-maximal decrease at 2.5 μM DNDS, and complete channel blockade at 50 μM DNDS. At 2.5 μM, DNDS decreased mean open time from 166 ms to 15 ms and mean closed time from 78 to 30 ms. Amplitude distribution analysis demonstrated DNDS caused a decrease in the mean channel amplitude from 1.3 pA to 1.1 pA (recorded at +30 mV with a 300 to 150 mM cis to trans Cl gradient, at a bandwidth of 400 Hz) and skewed the amplitude distribution to the left. The results suggest that the on-off rate constants of DNDS block are relatively fast making it a "flickery" type blocker of the epithelial chloride channel. [Supported by NIH, DK38518].

W-Pos134 POTASSIUM CHANNELS IN THE APICAL MEMBRANES OF ISOLATED PARIETAL CELLS. Richard L. Shoemaker, Peter J. Veldkamp, and Gaetano Saccomani, Dept. of Physiology and Biophysics, University of Alabama at Birmingham, Birmingham, AL. 35294

Single channel currents were recorded from apical membranes of parietal cells obtained from rabbit gastric mucosa using cell attached (C/A) and inside out (I/O) modes of recording. Highly purified parietal cells were isolated by a combination of pronase and collagenase digestion and purification by linear Nycodenz gradient centrifugation (BBA. 888:116, 1986). Cells were enriched to 75 - 85% by cell count with a viability greater than 95%. Cells were plated on human placental collagen-coated plastic coverslips and fed DME/F12 medium with 2% penstrep, 1% gentamicin and 5% cbs serum. The cells were patched 18 - 48 hrs after plating. Five - 10 GΩ seal were obtained on the apical membranes. The pipette solution contained 75 mM KCl or 150 mM K-glutamate and the bath 150 mM KCl or K-glutamate (to help identify the K^+ channels) with 25 to 200 nM Ca^{2+}. K^+ channels were observed in 4 of 8 (C/A) and 4 of 5 (I/O) patches on cells from fed rabbit; channels were seen in 2 of 15 (C/A) and 9 of 12 (I/O) patches on cells obtained from fasted rabbits. 2 mM Ba^{2+} in the bath reversibily inhibited the channels in I/O patches. Percent open time increased as the $[Ca^{2+}]_i$ increased from 25 to 200 nM. A decrease in the pH from 6.0 to 2.5 activated the K^+ channels in 6 of 8 patches. These K^+ channels have a conductance of 65 pS and appear to be regulated by voltage, $[Ca^{2+}]_i$, and internal pH. (Supported by NIH, DK 37121).

W-Pos135 **ACETYLCHOLINE (ACh) AND ISOPROTERENOL (ISO) INCREASE SHORT CIRCUIT CURRENT (I_{scc}) IN TRACHEAL EPITHELIUM OF SWINE.** Greg Adderholt, Terry M. Dwyer* and Jerry M. Farley, Depts. of Pharmacology and *Physiology, Univ. of Miss. Med. Cnt., Jackson, MS 39216.

We have observed that $^{36}Cl^-$ efflux from isolated tracheal submucosal gland cells is enhanced by ACh. Therefore we examined the effects of ACh (and ISO) on the I_{scc} developed by isolated tracheal epithelium from swine to determine whether the observed ACh-induced increases in ^{36}Cl flux in isolated cells gave rise to a whole tissue response. Measurements were made using a standard Ussing chamber and voltage clamp electronics and at 37 °C in a standard bicarbonate buffer oxygenated by 95% O_2 + 5% CO_2. The resistance of the epithelium after pretreatment with indomethacin to block the formation of prostaglandins, was 106±6 ohms and the average I_{scc} was 69±6 μA. A dose dependent increase in I_{scc} was caused by serosal addition of ISO with the maximum change being 26.5±2.8 μA. The chloride channel blocker, diphenylamine-2-carboxylic acid (DPC, 10^{-4} M), when applied to the mucosal surface, decreased the maximal change in I_{scc} caused by ISO to 16.3±1.5 μA. ACh (serosal addition) increased I_{scc} in a dose-dependent manner. The change in I_{scc} was 18±1.3 μA at 10^{-5} M ACh. Serosal pirenzepine (10^{-6} M) caused a parallel shift to the right in the ACh dose-response curve of less than 1 log unit. This suggests that the ACh-induced increase in I_{scc} is not due to M_1 receptor activation. DPC (10^{-4} M, mucosal) reduced the change in I_{scc} caused by ACh (10^{-5} M) to 11.4±2.1 μA, implying that the increase is in part due to an increase in chloride permeability. These results demonstrate that the ACh-stimulated $^{36}Cl^-$ flux observed in isolated submucosal gland cells has a physiological correlate in the whole tissue. We propose that the ACh-induced chloride movement will be of significance in the movement of ions and water into the lumen of the submucosal glands and thence into the trachea. (Supported by the Cystic Fibrosis Foundation grants G120 and G106 and The Mississippi Lung Assoc.)

W-Pos136 IN VIVO AND IN VITRO PHOSPHORYLATION OF THE AMILORIDE-SENSITIVE Na^+ CHANNEL PROTEIN. S. Sariban-Sohraby[1], B.M. Brenner[1], and D.J. Benos[2], [1]Brigham & Women's Hospital, Boston, MA; [2]Dept. of Physiology and Biophysics, University of Alabama at Birmingham, Birmingham, AL 35294.

The amiloride-sensitive Na^+ channel of A6 epithelial cells has been isolated, purified, and found to contain six non-identical subunits of M_r of 300, 180-150, 110, 85, 65, and 35. These channels are known to be regulated by several factors, one of which is the antidiuretic peptide hormone, vasopressin. The mechanism by which vasopressin increases Na^+ transport through these channels is unknown, but adenylate cyclase is activated and intracellular cAMP levels are increased. In the present study, we have examined if the purified Na^+ channel protein can act as a substrate for phosphorylation and if vasopressin can induce the phosphorylation of the Na^+ channel protein when added to intact A6 monolayers. Cell monolayers were phosphate-depleted overnight, equilibrated with $^{32}PO_4^{-2}$ for 1 h, followed by the addition of 100 mU/ml arginine vasopressin to the basolateral surface. After 20 min (the peak Na^+ transport response), the monolayers were scraped, and the Na^+ channel purified. Compared to control, only the 300 kDa subunit was phosphorylated. This same subunit was rapidly and specifically phosphorylated in vitro, as assayed by SDS-PAGE/autoradiography, when the purified channel protein was incubated with γ-[^{32}P]ATP and purified catalytic subunit of cAMP-dependent protein kinase (provided by R.W. Wallace and E.A. Tallant). These results indicate that the epithelial Na^+ channel can be phosphorylated in vivo and in vitro, and suggest that the Na^+ transport effects of antidiuretic hormone may ultimately reside in the phosphorylation of the Na^+ channel protein. [Supported by NIH Grants DK37206 and DK37121].

W-Pos137 INTERACTION BETWEEN RED CELL DEOXYGENATION AND AGGREGATION. TL Fabry, Z Zhang and ED Chusid (Intr. by W Brodsky). Department of Medicine, Mt. Sinai School of Medicine, City University of New York, New York.

Hemoglobin binds to the cytoplasmic portion of Band 3 protein, the erythrocyte chloride-bicarbonate antiporter. On the cell surface, Band 3 is the receptor site for neutral or negatively charged macromolecules. Macromolecules of sufficient length form cross-links between erythrocytes forming spherical aggregates. Normal human erythrocytes were suspended at a hematocrit of 20% and pH 7.1 in NaCl 130 mM, KCl 5 mM and glucose 10 mM. Dextran (M.Wt. 77 000) was used to cross-link the erythrocytes. The size of the aggregates formed and the binding constant between the receptor site on the erythrocyte surface and the dextran molecule was measured as previously described. Results. The size of red cell aggregates decreased with decreasing pO2. In a 2% dextran solution and at atmospheric pO2 (150 mmHg) the aggregate size was 0.06 mm3 while at 9 mmHg pO2 it decreased to 0.035 mm3. As the pO2 decreased the size of the aggregates remained constant until the hemoglobin saturation reached 50%, then rapidly decreased. Aggregation decreased in 1.5, 2.5 and 3% dextran solutions to a similar extent, i.e. about twofold. The binding constant between the dextran and the dextran receptor on the erythrocyte surface stayed constant at 0.3 mM, the value we have previously observed. We conclude that the increased binding of deoxyhemoglobin to the cytoplasmic portion of Band 3 results in a transmembrane signal causing decreased erythrocyte aggregation.

W-Pos138 **ROTATIONAL MOTION OF ANION CHANNELS IN THE HUMAN ERYTHROCYTE MEMBRANE: NEW INSIGHTS USING ISOTOPICALLY SUBSTITUTED $^{15}N,^{2}H_{16}$-BSSDP.** A.H. Beth, P.S.R. Anjaneyulu, L.A. Faulkner, B.J. Sweetman, and J.V. Staros, Departments of Molecular Physiology and Biophysics, Biochemistry, and Pharmacology, Vanderbilt University, Nashville, TN 37232

The rotational dynamics and interactions of anion channels in *intact* human erythrocytes and ghost membranes have previously been investigated by saturation transfer EPR (ST-EPR) spectroscopy using the bifunctional spin label reagent bis(sulfosuccinimidyl)-4-doxyl pimelate (BSSDP; Beth et al., (1986) Biochemistry **25**, 3824-3832). We have now synthesized the $^{15}N,^{2}H_{16}$ isotopically substituted analogue of BSSDP and employed it to further characterize the rotational motion of anion channels. Isotopically substituted BSSDP, like its normal isotope counterpart, forms an intra-chain cross-link of the extracellular domain of the anion channel which can be blocked by pretreatment of *intact* erythrocytes with 4,4'-diisothiocyano-2.2'-stilbene disulfonate (Beth et al., (1987) in: "Membrane Proteins", (S.C. Goheen, ed.) pp. 371-382). The ST-EPR line shape of spin labeled anion channels in erythrocyte membranes exhibits characteristics of Y-axial motion of the spin label which is significantly different than experimental or computed line shapes for isotropic motion at any rotational rate. Rotational motion of anion channels is less restricted in ghost membranes in hypotonic buffer than in *intact* erythrocytes throughout the temperature range from 2 ° to 37 ° C. However, ghosts in isotonic buffer exhibit a decrease in motion of anion channels which approximates that observed in *intact* cells at the same temperature. These findings suggest that interactions which restrict the motion of anion channels are sensitive to ionic environment. Independent determination of labeling geometry by orienting erythroctyes in the EPR sample cell will permit extraction of $\tau_{\parallel}$ and $\tau_{\perp}$ for rotational motion of this transmembrane protein in the bilayer. These parameters, in turn, should show high sensitivity to interactions with other membrane proteins and to the physical state of membrane lipids. Supported by: NIH HL34737, DK31880, and RR01688.

W-Pos139 PROTON NMR STUDY OF THE HUMAN ERYTHROCYTE GLUCOSE TRANSPORTER. Scott A. Ross, Jin-Feng Wang, Joseph J. Falke, and Sunney I. Chan, Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125.

Glucose transport in the human erythrocyte has been studied for many years, yet an entirely suitable kinetic model has not been demonstrated. Results from different laboratories favor different mechanisms: recent evidence has been advanced in support of both a one-site, alternating conformer model and a two-site model with negative cooperativity. Exploiting the effect of rapid exchange of β-D-glucose between free and bound forms on the nuclear Overhauser effect between two glucose protons, we have observed glucose binding sites in erythrocyte ghost membrane preparations. This transferred NOE is fully eliminated in the presence of a saturating concentration of cytochalasin B, a known inhibitor of glucose binding to the transporter, supporting identification of the observed binding sites as transporter sites. We have drawn a relationship between the transferred NOE and the dissociation constant(s) of the binding site(s). Using right-side-out, inside-out and fragmented ghost preparations, the cytoplasmic and extracellular binding sites may be independently characterized. Preliminary results and their bearing on the transport mechanism will be discussed.

W-Pos140 CHLORIDE/BICARBONATE EXCHANGE IN HUMAN RED CELLS CHARACTERIZED BY A FLUORESCENT PROBE. Teresa Calafut, Michael Solomon, Robert Gelman and James A. Dix, Department of Chemistry, State University of New York, Binghamton, NY 13901.

6-Methoxy-N-(2-sulfopropyl)quinolinium (SPQ) is a fluorescent compound whose fluorescence is quenched selectively by chloride ions. SPQ has been proposed as a chloride-sensitive fluorescent probe useful in measuring transpsort kinetics in a variety of cell membrane systems (Illsley and Verkman, Biochemistry 26, 1215-1219 (1987)). We have used SPQ to characterize chloride/bicarbonate exchange through resealed human red cell ghost membranes. Chloride gradients were created by incubating ghosts, preloaded with 10 mM SPQ, with solutions of varying $[Cl]/[HCO_3]$, then mixing in a stopped-flow photometer with solutions of varying $[Cl]/[HCO_3]$. The resulting time courses were described well by a single exponential function convoluted with a function describing Stern-Volmer quenching. Experiments in which initial internal chloride concentration varied but final internal chloride concentration was held constant gave time constants of quenching (0.7 $\pm$ 0.2 sec, 25 ^{o}C, 75 mM final internal chloride) that were independent of chloride gradient, suggesting that chloride/bicarbonate exchange follows relaxation kinetics. Experiments in which the final internal chloride concentration was varied yielded a ratio of exchange rates of bicarbonate to chloride of 4-5. Supported by American Heart Association, Upstate New York Affiliate.

W-Pos141 **LOCALIZATION OF THE EOSIN-5-MALEIMIDE REACTION SITES ON HUMAN ERYTHROCYTE BAND 3.** Charles E. Cobb and Albert H. Beth, Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN 37232.

The rotational diffusion of the red blood cell (rbc) anion transport protein (Band 3) has been measured by saturation transfer electron paramagnetic resonance spectroscopy (ST-EPR; Beth et al., **Biochemistry 25**, 3824-3832, 1986) and by flash-induced transient dichroism (Nigg and Cherry, **Biochemistry 18**, 3457-3465, 1979). These measurements have permitted evaluation of the oligomeric structure of Band 3 and its interactions with other rbc membrane proteins. Both techniques require covalent attachment of an appropriate extrinsic probe for spectroscopic monitoring of rotational motion. We have developed a bifunctional spin labeling reagent, bis(sulfosuccinimidyl)-4-doxyl pimelate (BSSDP) which is employed in our ST-EPR studies. BSSDP forms an intrachain crosslink of the exofacial domain of Band 3 which spans the extracellular chymotrypsin cleavage site. Pretreatment of intact rbc's with the anion transport inhibitor 4,4'-diisothiocyano-2,2'-stilbene disulfonate (DIDS) completely blocks the reaction of BSSDP, suggesting that they are bound to the same, or overlapping, sites. Thus, BSSDP is monitoring rotational motion of anion channels at a locus which is accessible from the extracellular side of the membrane. The reaction site(s) of eosin-5-maleimide (EMA) on Band 3, the fluorescent probe used in transient dichroism studies, has not been fully characterized. Since knowing the location of the probes attached to Band 3 is crucial for comparisons of results obtained from the two techniques and for determining how motion detected at one locus is related to motion at other loci, identification of reaction sites of EMA have been undertaken. Pretreatment of intact rbc's with DIDS reduces the EMA labeling by approximately 50 - 70%, suggesting that labeling can occur at multiple sites on Band 3. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of the ghosts from chymotrypsin treated EMA-labeled intact rbc's has shown that all of the EMA comigrates with the 58000 M_r, N-terminal fragment of Band 3. Trypsin treatment further indicates that the EMA label is attached to the 17000 M_r membrane spanning segment of the 58000 M_r fragment. Localization of the EMA-labeled residues in this 17000 M_r polypeptide is underway. Supported by: NIH HL34737.

W-Pos142 SITES FOR p-CHLOROMERCURIBENZENESULFONATE (pCMBS) INHIBITION OF RED CELL WATER AND UREA TRANSPORT. David M. Ojcius and A. K. Solomon, Biophysical Laboratory, Harvard Medical School, Boston, MA 02115.

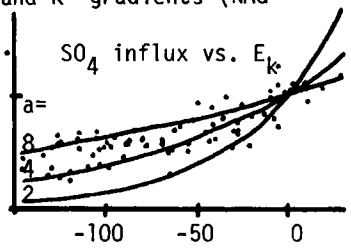
The mercurial sulfhydryl reagent, pCMBS, inhibits water and urea fluxes across the human red blood cell membrane. The kinetics and affinities for pCMBS binding to separate water and urea transport inhibition sites were previously determined by Toon and Solomon (Biochim. Biophys. Acta (1986) 860: 361-375) in red cells that had been treated with N-ethylmaleimide (NEM) to block five of the six sulfhydryls on the red cell anion exchange protein, band 3. We have used autoradiographs of gels from NEM-treated cells, labeled with ^{203}Hg-pCMBS, to localize the water and urea transport inhibition sites separately, and find that both are on band 3. Each site is saturable and the time course of each uptake can be fitted to the equations for a bimolecular association (with negligible dissociation) with time constants in agreement with those of Toon and Solomon. Determination of the binding stoichiometry shows one urea inhibition site and three water inhibition sites for every four band 3 molecules. These results indicate that band 3 plays a role in both urea and water transport and suggest that the functional unit may be a tetramer. (Supported in part by NIH grant GM 34099).

W-Pos143 FORMATION OF SICKLED CELLS DURING DEOXYGENATION-OXYGENATION CYCLES AND THE EFFECT ON OXYGEN BINDING. Kazumi Horiuchi, Sean Flynn and Toshio Asakura. The Children's Hosp. of Phila., Dept. of Pediatrics and Dept. of Biochem. and Biophys., Univ. of Penna., Phila., PA 19104.

There is hysteresis between the oxygen dissociation curve (ODC) and oxygen association curve (OAC) of sickle erythrocytes (SS cells). Since no such hysteresis is observed in normal red cells (AA cells), it is believed that the hysteresis is caused by the intracellular polymerization of deoxy-Hb S. We have investigated changes in the number and morphology of sickled cells at various PO_2 during deoxygenation-oxygenation cycles (d-o cycles between 0-120 mmHg,12 min/cycle) and the effects of these changes on oxygen binding properties of Hb S. SS cells were separated into two fractions by density. The less dense discocyte-rich fraction (density $\leq$ 1.11) was used in the experiments. SS cells were deoxygenated or oxygenated at a fixed flow rate (60 ml/min) of nitrogen or air during the d-o cycle experiments. When SS cells were subjected to d-o cycles, ODCs and OACs shifted to the right. This shift could be enhanced by lowering pH and by increasing the Ca^{2+} concentration of the suspension medium. SS cells became dehydrated during d-o cycles in the presence of Ca^{2+}, causing an increase in intracellular Hb S polymerization. We also found that the the number of typically sickled cells at PO_2 = 0 and 100 mmHg increased with repeated d-o cycles, while the number of maple leaf or star-shaped cells decreased. This tendency was greater at lower pH's and at higher Ca^{2+} concentrations. We conclude that the right shift of OACs and ODCs of SS cells during d-o cycles is caused by the formation of intracellular Hb S polymers. It appears that the degree of the shift is related to the amount of intracellular polymers. (NIH grant HL20750).

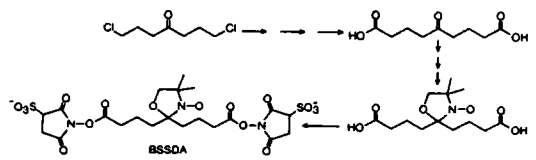
W-Pos144 USE OF MEMBRANE POTENTIAL TO DISSECT "ELEMENTARY" TRANSPORT EVENTS MEDIATED BY THE HUMAN RED BLOOD CELL ANION TRANSPORT SYSTEM. M.D. Frame and M.A. Milanick, Department of Physiology, University of Missouri School of Medicine, Columbia, MO 65212.

We have tested the notion that translocation of net charge through the red cell membrane can occur through a symmetric barrier in a single simple step. Unidirectional SO_4 influx should provide this information. The rate of SO_4 entry into red cells containing Cl^- is limited by the slowest step. This is probably the SO_4 half cycle. At pH_o=9, SO_4 influx probably involves $Cl^-/SO_4^=$ exchange and net charge transfer. Alternatively, SO_4 entry could be the result of net anion entry. At pH_o=9 SO_4 influx is potential sensitive. (AJP:247:C247,1984). For a symmetric Eyring/Lauger electrical barrier, the rate constant $k'=k_o\exp(zFV/aRT)$, (a=2). Using human red cells in media containing 125 mM K_2SO_4, at pH 8.8 to 9.0, the membrane potential was set by using valinomycin and K^+ gradients (Na^+ replacing K^+) and by using gramicidin and K^+ gradients (NMG^+ replacing K^+). As E_K varied from -100mV to 0mV the rate of SO_4 influx increased 2 fold and not 7 fold, as predicted by the equation (See Fig.). These data suggest that 4<a<8. Similar results were obtained at 5 mM SO_4 where influx was 10 fold less, so SO_4<Km. These results are consistent with the idea that charge movement occurs through an asymmetric Eyring/Lauger electrical barrier. Alternatively, in one of the slowest steps, part (or none) of the electrical field is sensed. For example, translocation may involve a series of steps or dissociation may be as slow as translocation. (Supported by NIH DK37512).



W-Pos145 **A NEW MEMBRANE-IMPERMEANT BIFUNCTIONAL SPIN LABEL: BIS(SULFOSUCCINIMIDYL)-5-DOXYL AZELATE.** P.S.R. Anjaneyulu, A.H. Beth, S.F. Juliao, B.J. Sweetman, and J.V. Staros, Departments of Biochemistry, Molecular Physiology and Biophysics, and Pharmacology, Vanderbilt University, Nashville, TN 37232

Recently, we introduced bis(sulfosuccinimidyl)-4-doxyl pimelate (BSSDP) as the first of a new class of bifunctional spin labels (Beth et al., (1986) Biochemistry 25, 3824-3832). Because in different proteins, reactive groups that must be spanned by such reagents are separated by different distances, we have now prepared the next longer homolog in this series, bis(sulfosuccinimidyl)-5-doxyl azelate (BSSDA) by the reaction scheme shown below:



Preliminary labeling experiments on the anion exchange channel in *intact* human erythrocytes confirm that the nine-carbon reagent BSSDA more readily spans the distance between a particular pair of amino groups than does the seven-carbon reagent BSSDP. Both reagents yield similar estimates of rotational mobility of the anion channel by saturation transfer EPR spectroscopy. Thus BSSDA, when reacted with appropriately separated groups, appears as tightly motionally coupled to the protein as BSSDP. Supported by: NIH DK31880, HL34737, and RR01688.

W-Pos146 KINETICS OF CO BINDING TO SICKLE CELL HEMOGLOBIN BY SINGLE CELL MICROSPECTROSCOPY. A.I. Alayash, Duke University Marine Laboratory, Beaufort, NC 28516 U.S.A.; Massimo Coletta and Maurizio Brunori, CNR Center for Mol. Biol., Dept. of Biochem. Sci., Univ. of Rome "La Sapienza," P. le A. Moro, 5 - 00185 Rome, Italy; M.T. Wilson, Dept. of Chemistry, Univ. of Essex, Colchester, U.K.; and P.A. Beneditti, Inst. of Biophys. of CNR, Pisa, Italy.

The kinetics of ligand release and uptake in red blood cells from SS patients has been followed by single cell microspectroscopy. The photodissociation of HbCO and the ensuing deoxygenation of intracellular hemoglobin brings about polymerization of HbS and deformation of cells, which is associated to a delayed optical change found to be very reproducible by repeating the cycle several times for the same cell. Comparison of visual observation and absorbance time course indicates that a sickled cell deforms always along the same preferential axis and thus retains a "memory" of its previous cycle(s), possibly via slow relaxations at the membrane. Moreover, CO uptake was found to be somewhat faster if rebinding is measured before sickling occurs, suggesting that the increase in intracellular viscosity, coupled to sickling, plays a significant role in modulating gas uptake in erythrocytes.

Experiments carried out in sickle erythrocytes containing varying amounts of Fetal Hemoglobin, showed that a critical threshold of Fetal Hemoglobin (> 10%), is possibly ameliorating the severity of this disease.

The "memory" of preferential deformation and the change in the dynamics of ligand uptake of sickled cells will be discussed with reference to the pathophysiological mechanism of sickling.

W-Pos147 A SIMPLE EXPERIMENTAL METHOD TO DISTINGUISH BETWEEN SIMPLE PORE AND SIMPLE CARRIER KINETICS APPLIED TO UREA TRANSPORT ACROSS THE ERYTHROCYTE MEMBRANE. Lenore W. Yousef and Robert I. Macey, Dept. of Biology, California State University, Fresno, CA, and Dept. of Physiology-Anatomy, University of California, Berkeley, CA.

Measurement of the permeability coefficient(P)at various penetrant concentrations(C) by the perturbation method(Farmer and Macey,1969,1971 and 1972)can be plotted to distinguish simple diffusion, simple pore kinetics and simple carrier kinetics as follows:for simple diffusion, $1/P=\text{constant}$;for a simple pore, $1/P=K_m/V_{max}+C/V_{max}$; for a simple carrier, $1/P=K_m/V_{max}+C/V_{max}+C^2/(V_{max}K)$where K_m and V_{max} are measured under equilibrium exchange conditions and K is the flux ratio constant. In human erythrocytes, permeability coefficients for diethylene glycol were constant indicating simple diffusion. For glucose, a plot of 1/P versus concentration was nonlinear indicating carrier kinetics. Plots of 1/P versus penetrant concentrations gave straight lines with positive slopes for urea in human and beef erythrocytes and for methylurea in human erythrocytes, indicating these penetrants follow simple pore kinetics or simple carrier kinetics in which K is very large. (Concentration ranges for urea in human erythrocytes were from 260mM to 1640mM, for methylurea from 135mM to 1350 mM and for urea in beef erythrocytes, from 880mM to 2260mM.) In human cells, using simple pore kinetics, we obtained an average V_{max} for urea of $3.3X10^{-7}m/cm^2$-sec (S.D.=$1.0X10^{-7}$,n=5)at pH7.8 and for methylurea $4.1X10^{-7}m/(cm^2sec)$(n=2) at pH7.0. Dimethylurea follows simple diffusion kinetics in human erythrocytes,(P=$1.7X10^{-5}$cm/sec,S.D.=$0.1X10^{-5}$,n=6), measured over a concentration range of 132 mM to 1317mM) and thus even though it competes effectively with urea, may not use the carrier to cross the membrane. (NIH GM-18819)

W-Pos148 TAURINE TRANSPORT IN RAT THYMIC LYMPHOCYTES. P. A. King (sponsored by R. B. Gunn, Department of Physiology, Emory University School of Medicine, Atlanta, GA 30322

We have investigated the rate of taurine influx into rat thymocytes as well as the rates of glycine, alanine, and α-aminoisobutyric acid (AIB) uptake. ^{14}C-Amino acid influx was measured at 37°C and pH 7.4 in Na-containing Ringer (138 mM Na) and Na-free Ringer (N-methyl-D-glucamine substituted) containing 0.1 mM amino acid (total osmolarity, 274 mOsm). In the presence of Na, the taurine influx averaged 0.81 ± 0.06 pmol/10^6 cells x min. (mean ± S.E., n=4) while the influx rates for alanine, glycine, and AIB were 28, 5.4, and 10 pmol/10^6 cells x min, respectively. In the absence of Na, taurine influx averaged 0.03 ± 0.01 pmol/10^6 cells x min. and the influx rates for alanine, glycine, and AIB were 3.5, 1.2, and 1.6 pmol/10^6 cells x min, respectively. The influx rates of taurine and AIB in control versus hypoosmotic (177 mOsm or 0.65 control osmolarity) medium were compared. The Na Ringer contained 69 mM Na and 69 mM N-methyl-D-glucamine (NMG) and osmolarity was changed by lowering NMG only. We found that taurine uptake by thymocytes was increased in hypoosmotic media; influx was increased 4-fold in hypoosmotic Na medium as compared to control Na medium and 14-fold in hypoosmotic Na-free medium as compared to control Na-free medium. AIB uptake, on the other hand, was decreased in the hypoosmotic media with influx in hypoosmotic Na medium equal to 54% that at control osmolarity and influx in hypoosmotic Na-free medium equal to 32% the rate at control osmolarity. Taurine efflux from ^{14}C-taurine loaded thymocytes was also measured; efflux into hypoosmotic medium (69 mM Na) was approximately 10-fold greater than the rate of efflux into control osmotic medium (69 or 138 mM Na). Supported by NIH grant HL 28647 to RBG and postdoctoral fellowship HL 07432.

W-Pos149 UREA AND THIOUREA TRANSPORT IN NORMAL AND Jk(a-b-) ERYTHROCYTES. O.Fröhlich, R.B. Gunn, J.J.Gargus and L.J.Rizzolo, Departments of Physiology and of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, GA 30322.

Erythrocytes possess a high permeability for urea such that placing the urea-free cells into saline-free (isosmotic or hyperosmotic) urea solutions causes rapid lysis. Thiourea transport is slower but still faster than passive diffusion through the bilayer. Jk(a-b-) blood type lyses only very slowly in 2 M urea (Heaton and MacLoughlin, Transfusion, 22:70,1982; A. Macknight, J. Moulds, pers. comm.). We measured urea and thiourea fluxes in these cells at 0°C and found them devoid of mediated urea transport: 1) Tracer thiourea efflux from Jk(a-b-) cells is independent of extracellular thiourea or the competitive inhibitor thionicotinamide (TN). 2) Its efflux rate constant (0.0018-0.0023 s^{-1}) is the same as that from normal cells in the presence of TN (0.0021 s^{-1}). 3) Tracer urea efflux from Jk(a-b-) cells is 2-fold slower (0.0011-0.0013 s^{-1}) than thiourea efflux, as expected from the bilayer permeabilities of urea and thiourea, and is the same in the presence and absence of the inhibitors pCMBS, phloretin and TN. Cl exchange in these cells is normal with respect to transport rate and DNDS inhibition. These experiments demonstrate that urea transport is not mediated by the anion transport protein. They also suggest that the protein responsible for the Kidd antigen is the urea transporter. Screening a cDNA expression library by Kidd antisera thus presents a novel approach to isolating the urea transport protein.

W-Pos150 EFFECTS OF EXTRACELLULAR SULFONATES ON CHLORIDE NET EFFLUX by V. K. Gottipaty and O. Fröhlich, Dept. Physiology, Emory University School of Medicine, Atlanta, GA 30322.

Cl net efflux was measured from gramicidin-treated intact erythrocytes at pH=7.0, T=20°C and K_o=4 mM, into different Cl_o concentrations and in the presence of several different organic sulfonate compounds. Efflux was measured by following either the rate of net K loss or by electronic cell sizing. The purpose of these experiments was to examine if extracellular sulfonates, when bound to the outward-facing anion binding site, inhibit net efflux of Cl by the tunneling mode. We clamped the anion transporter into the outward-facing state by using Cl-free efflux media. Under these conditions we made the following observations: 1) SO_{4-o}, up to 120 mM, inhibited Cl net efflux by less than 20%. 2) Other sulfonates inhibited with increasing potency (measured as ID_{50}) in the following order: sulfamate (>300 mM), methanesulfonate (53 mM), p-sulfanilate (48 mM), p-toluene-benzenesulfonate (8.5 mM), 5-nitro-2-methyl-benzenesulfonate (1.7 mM). For comparison, DNDS inhibited with a potency of 0.2 μM. 3) The extrapolated non-inhibitable fluxes were indistinguishable from the DNDS-insensitive flux. 4) 25 mM SO_{4-o} had no detectable effect on the ID_{50} with which Cl_o inhibited net efflux.

These findings are inconsistent with the notion that SO_{4-o}, when bound to the outward-facing anion exchange site, blocks the outward-tunneling of Cl. Under similar conditions, Milanick and Gunn (Am. J. Physiol. 247:C247-C259) had found that SO_{4-o} supports Cl_i-SO_{4-o} exchange with an affinity of 4 mM, suggesting that SO_4 binds to the transport site. It therefore appears that the SO_{4-o} binding site that participates in anion exchange does not block Cl net efflux. There exists, however, an additional binding site, as demonstrated by the inhibitory effect of the sulfonate analogues. (Supported in part by NIH grant GM 31269)

W-Pos151 DETERMINATION OF THE AREA EXPANSIVITY OF RED BLOOD CELL MEMBRANE BY LOW-IMPEDANCE PIPET-PORE ASPIRATION. Christopher Katnik and Richard E. Waugh. Department of Biophysics, University of Rochester School of Medicine, Rochester, NY 14642.

The resistance of red blood cell membrane to surface area dilation is characterized by the expansivity modulus, K. The value of K measured by micropipet aspiration has been found to depend on electric potentials applied between an electrode in the pipet and one in the chamber containing the cells. Using a current clamping microprobe system (WPI) and Ag-AgCl electrodes to apply this potential, the calculated K's ranged between 150 dyn/cm for -1000 mV and 1500 dyn/cm for 1000 mV applied potential. In an effort to understand the mechanism responsible for the dependence of the measured value of K on applied potential, we have developed a new approach for measuring the area expansivity of vesicular membranes. A cell is aspirated into a small circular opening in a thin (0.10 μm) silicon wafer cemented over the tip of a large (15.0 μm) micropipet. The osmotic strength of the solution is reduced (~130 mOsm) to make the cell more spherical, so that upon aspiration a small spherical projection is formed inside the large pipet. The membrane tension and the cell area and volume can be calculated from the applied pressure and the cellular dimensions. Changes in surface area or volume can be calculated from measurements of changes in the small, inner radius. The impedance of the pipet-pore (~4.0 MΩ) is significantly lower than the impedance of micropipets (50-200 MΩ), allowing more precise determinations of the potential drop across the cell itself. Using the pipet-pore we find a value for the expansivity modulus of the red cell membrane of approximately 1,200 dyn/cm, and we observe little dependence of the measured value on the potential applied across the cell (±100 mV). (Supported by PHS Grant No. HL31524).

W-Pos152 EVIDENCE THAT EXTERNAL NIP-TAURINE, NAP-TAURINE AND IODIDE INHIBIT RED BLOOD CELL ANION EXCHANGE BY BINDING TO THE SAME SITE ON BAND 3. Philip A. Knauf and Laurie J. Spinelli*, Dept. of Biophysics, Univ. of Rochester Sch. of Med., Rochester, NY 14642.

External NIP-taurine (N-(4-isothiocyano-2-nitrophenyl)-2-aminoethylsulfonate) binds to an external site where it inhibits red blood cell Cl exchange reversibly at 0°C or irreversibly after reaction at higher temperatures (Knauf et al., Am. J. Physiol. 253 (Cell Physiol), in press, Nov. 1987). Kinetic competition experiments indicate that NIP-taurine binds to the same site as does the photoaffinity reagent NAP-taurine (N-(4-azido-2-nitrophenyl)-2-aminoethylsulfonate). At high concentrations, external bromide and iodide have a noncompetitive inhibitory effect on anion exchange. To see whether or not the sites of iodide and NIP-taurine inhibition are the same, we measured the effects of NIP-taurine on Cl efflux from human red blood cells into media with no Cl and different concentrations of iodide (Cl-I exchange). Increasing external iodide from 10 to 150 mM inhibits Cl efflux and in parallel increases the concentration of NIP-taurine which 50% inhibits Cl efflux (the ID50) from 1.5 uM to 11.1 uM. This effect is not due to binding of iodide to the external-facing transport site, since the decrease in NIP-taurine affinity is much greater than that expected if all of band 3 were converted to the form with iodide bound to the outward-facing transport site. As expected (Jennings et al., J. Gen. Physiol. 86:653 (1985)), treatment with the cross-linking reagent BSSS (bis(sulfosuccinimidyl)-suberate) abolishes the external iodide inhibition of Cl efflux. BSSS also interferes with NAP-taurine inhibition of Cl exchange, raising the ID50 by about 5-fold. These data indicate that NAP-taurine and NIP-taurine bind to the same site as external iodide, so NIP-taurine may provide a useful label for this inhibitory site. (Supported by NIH Grant DK27495.)

W-Pos153 ERYTHROCYTE MEMBRANE AND SARCOPLASMIC RETICULUM MEMBRANE MAY BE CONTROLLED BY THE SAME GENE: A NON-INVASIVE DIAGNOSIS FOR MALIGNANT HYPERTHERMIA BY SPIN-LABELING TECHNIQUE. S. Tsuyoshi Ohnishi, Hiroaki Katagi, Tomoko Ohnishi and A. Keith Brownell. Membrane Research Institute, Phila. PA., Dept. of Biochemistry and Biophysics, University of Pennsylvania, Phila. PA and Dept. of Clinical Neurosciences, University of Calgary, Canada.

Malignant hyperthermia (MH) is a rare but serious complication to general anesthesia with a high risk of death. It is believed that abnormal calcium metabolism in skeletal muscle is involved in the pathogenesis of MH. Halothane may release an abnormal amount of calcium from the sarcoplasmic reticulum (SR), thereby triggering a vicious cycle of contracture and heat production. Halothane was shown to disorder the SR membranes of MH-susceptible pigs. In an attempt to develop a non-invasive diagnosis method for MH, we have found that erythrocyte membranes have a similar property to that of SR. The order parameter and rotational correlation time of erythrocyte membranes were measured using 12 and 16 doxylstearic acid, respectively. At 3 mM halothane, both the order parameter and rotational correlation time were greatly reduced, suggesting that these membranes had a similar abnormality. This suggests that both membranes may be regulated by the same gene, and that MH may be diagnosed non-invasively using the blood. References: ABB 247:294(1986); BBA 897:261(1987). Supported by NIH grants GM-35681 and GM-33025.

W-Pos154 INDUCED POTASSIUM FLUX BY A SCHISTOSOME HEMOLYTIC AGENT. M.R. Kasschau, D.L. Gentry, and D.F. Williams. Programs in Biological and Allied Health Sciences, University of Houston-Clear Lake, Houston, Tx 77058.

The blood parasite, Schistosoma mansoni, has been shown to have a unique hemolytic agent; the agent is membrane-bound and has a pH optimum of 5.0 (Kasschau and Dresden, Exp. Parisitol. 61:201). Prior to onset of rapid hemolysis, a substantial lag phase is always observed when assays are performed in isotonic sodium citrate, pH 5.1. The lag phase is reduced when the red blood cells (RBCs) are suspended in Hank's BSS (pH 5.1), but the slope of the lytic curve remains the same. To learn more about the lytic mechanism, we monitored K^+ and Na^+ fluxes prior to hemolysis. At 37° C complete K^+ efflux is rapid (30 minutes) at pH 5.1 and pH 7.5; with hemolysis occurring in 60 minutes to 90 minutes at pH 5.1, and no significant lysis after 4 hours at pH 7.5. There appears to be a small immediate Na^+ influx after the addition of the lytic agent at both pHs. At 25° C K^+ leakage was slowed, 3 hours were required for complete efflux, and there was no significant lysis in 4 hours (30% net hemolysis was observed after 20 hours). It appears that complete loss of K^+ from the RBCs is required before lysis occurs in either isotonic sodium citrate or Hank's BSS. Funded by NSF grant DCB-8517512.

W-Pos155 NON-ENZYMATIC GLYCOSYLATION OF THE GLUCOSE CARRIER OF HUMAN RED BLOOD CELLS (rbc) AND FUNCTIONAL CONSEQUENCES. Amira Klip, Ileana Kahan and Philip Bilan. The Hospital for Sick Children, Toronto, Ontario M5G 1X8, Canada.

Glycation, the non-enzymatic reaction of glucose with NH_2 groups of proteins, occurs both *in vivo* and *in vitro*. This reaction is responsible for the formation of the glycated hemoglobins like hemoglobin A_{1c}. The accumulation of hemoglobin A_{1c} depends on the concentration of glucose in the plasma during the preceding 2-3 months (the lifetime of the human rbc). This property is used to determine diabetic control. Other proteins that undergo glycation *in vivo* include lens crystallin, collagen, albumin, fibronectin and rbc membrane proteins. *In vitro* glycation can be reproduced by incubating tissues or isolated proteins with high concentrations of glucose over prolonged time periods. The reacting residues are the N-terminus and NH_2 groups of certain Lys residues. These reactions can affect significantly protein function. In the present study we demonstrate that the glucose carrier of human rbc becomes glycated *in vitro*, and concomitantly its function is altered. Rbc ghosts, depleted of extrinsic proteins, were incubated for 3 days with 80 mM D-glucose. Glycation was quantitated by the covalent incorporation of ^3H upon reduction with [^3H]$NaBH_4$. *In vitro* glycation yielded an incorporation of 0.3 mol glucose/mol Band 4.5 (containing predominantly the glucose carrier) as determined in SDS-PAGE. Control membranes incorporated only 0.09 mol glucose/mol Band 4.5. *In vitro* glycation was accompanied by a reduction in cytochalasin B binding, a specific inhibitor of glucose transport (increased K_d, unaltered B_{max}). Similarly, *in vitro* glycation of intact rbc resulted in a decrease in the rate of hexose uptake. The possibility that abnormal glycation of the glucose carrier occurs in diabetics and its possible role in exacerbating hyperglycemia will be discussed. Supported by the Medical Research Council of Canada.

W-Pos156 CALPROMOTIN A HIGH MOLECULAR WEIGHT OLIGOMER INVOLVED IN CALCIUM-DEPENDENT POTASSIUM TRANSPORT. G.A. Plishker, C.N. Pope and L.S. Seinsoth. Baylor College of Medicine, Houston, Texas 77030

Investigations of red blood cells indicate that a cytoplasmic protein, which we call calpromotin, is involved in the regulation of calcium-dependent potassium transport. We have identified calpromotin in the cytosol and the membrane fractions of red blood cells and brain extracts. In cultures of dissociated rat embryonic cerebral cortex, calpromotin is localized in neurons and not in glia. In the red blood cell, the amount of calpromotin in the membrane fraction increases with the cellular calcium (White and Plishker,, BBRC 114, 488-492,1983) and electron microscopy shows that the protein is bound to the cytoplasmic surface of the membrane. The carboxymethylation of calpromotin with iodoacetate (IAA) correlates directly with the inhibition of calcium-dependent potassium transport (Plishker, A J P 248,C419-C424,1985). Antibodies raised against calpromotin inhibit calcium-dependent potassium transport in resealed ghosts (Plishker et al., A J P 251,C535-C540, 1986). High speed gel filtration chromatography shows that calpromotin from red cell hemolysates migrates as both a dimer and as a high molecular weight oligomer. Column fractions corresponding to the 300,000 to 400,000 molecular weight region contain pure calpromotin. The examination of these samples by electron microscopy reveals oligomeric structures that form macromolecular aggregates. Treating purified calpromotin with IAA and DTT suggest that sulfhydryls and disulfide bonds are essential to the formation of oligomers. The binding of the protein to organomercurial agarose indicates that the accessibility of the sulfhydryls in calpromotin is influenced by calcium and magnesium. Supported by NIH grants NS-11535 and GM-30594.

W-Pos157 **EFFECTS OF PROTEOLYTIC ENZYMES ON CARDIAC SODIUM CHANNEL INACTIVATION AND BLOCK BY LOCAL ANESTHETICS.** Craig W. Clarkson, Department of Pharmacology, Tulane University Medical School, New Orleans, Louisiana.

The effects of α-chymotrypsin and trypsin on the gating behavior of cardiac Na channels and channel block by local anesthetics was investigated using the whole-cell patch clamp method. In single guinea pig ventricular myocytes (15-16°C, $[Na]_i$=10 mM $[Na]_o$=25 mM) intracellular application of 0.3-0.8 mg/ml chymotrypsin or trypsin through the pipette produced a marked slowing of the falling phase of the Na current during single depolarizing pulses until little if any inactivation was detectible during 20 ms pulses between -60 to +60 mV (V_h = -140 mV). However, complete inactivation could be evoked by pulses of 5-10 s duration. At -20 mV, Na current inactivated with τ = 1.1 ± 0.4 s (n=5). Recovery from inactivation at -140 mV was biphasic (τ_1 = 142 ± 52 ms & τ_2 = 6.0 ± 3.6 s; n=5). Both 100-200 μM lidocaine and 50 μM RAC109-I still produced marked use-dependent block during trains of 20 ms pulses following inactivation modification. Lidocaine induced a clear phasic block of Na current during 20 ms pulses, consistent with an open channel block mechanism. In contrast, RAC109 did not induce a definable time-dependent effect on Na current, suggesting that it may bind to an excited rested state. These data suggest that inactivation is not required for Na channel block by lidocaine and RAC109, and that chymotrypsin and trypsin may produce a different effect on Na channel inactivation in cardiac tissue than in nerve.

W-Pos158 MOLECULAR MECHANISM OF LOCAL ANESTHESIA. Chyuan-Yih Lee, Chemical Dynamics Corporation, 9560 Pennsylvania Avenue, Upper Marlboro, MD 20772.

According to the electron transfer (ET) model [C.Y. Lee, Bull. Math. Biology, 45, 759-780 (1983)], the Na conductance is controlled by electron hopping between two stable sites in each of a layer of ET complexes embedded in the membrane matrix. Site I is closer to the outer membrane surface than site II. The action of neutral local anesthetics (LA) is to bind at the site I so that the active electron has difficulty to jump to the site II even upon depolarization. This explains the importance of dipole formation in LA and why the positive end of the dipolar LA should be hydrophobic [c.f., B.I. Khodorov, Prog. Biophys. Molec. Biology, 37, 49-89 (1981)]. The 'receptor' of the cationic LA is at the site II, which becomes operative when the active electron is localized at this site, thereby attracting the positively charged LA. The positive field from LA, in turn, interferes the electron hopping between the two sites, thus reducing the gating current especially its 'off' component which results from the electron hopping from the site II to the site I. Since the site II is far from the outer membrane surface, the cationic LA is most effective when perfused internally. The frequency and voltage dependent block can also be explained.

W-Pos159 **TWO CLASSES OF BINDING SITES IMPLICATED FOR STX BLOCK OF NA CHANNELS IN SINGLE CARDIAC PURKINJE CELLS.**

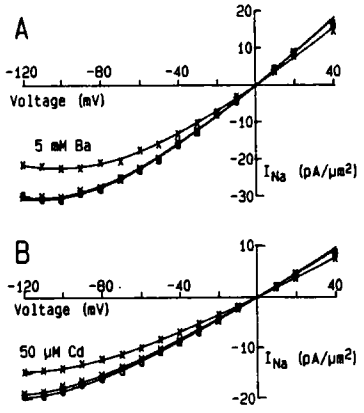
D.A. Hanck, M.F. Sheets, R. Rogart, D. Doyle, S. Lustig, & H. A. Fozzard (Intr. by A. Scanu). Cardiac Electrophysiology Labs, The University of Chicago and the Reingold ECG Center, Northwestern University, Chicago, IL.

Electrophysiological studies using TTX in mammalian cardiac preparations have failed to demonstrate more than one class of Na channels, whereas binding studies have consistently revealed two populations of [^{3}H]STX receptors in cardiac membranes. We studied the response of outward sodium current (I_{Na}) to extracellular STX in internally perfused single canine cardiac Purkinje cells, under conditions where there was no extracellular Na to interfere with STX binding. Cells were voltage clamped with a large suction pipette (20-30 μm) containing intracellular solution of (mM) 90 or 45 Na, 60 or 105 Cs, 150 F, 10 HEPES (pH 7.2), 10 EGTA. Extracellular solution contained (mM) 150 TMA, 2 Ca, 154 Cl, and 10 HEPES at 8°C. I_{Na} was measured after a test step to +20 mV from a holding potential that removed inactivation (-150 or -170 mV). Control measurements bracketed each of the measurements in STX (2-200 nM). Each measurement in STX was normalized to its control. In all four cells studied, the dose response data were poorly fit by a single site binding curve. When fit by a model of ligand binding to two independent sites, the k_d's were 13 ± 5 nM and 53 ± 13 nM (mean ± S.D.). Preliminary data from specific [^{3}H]STX binding to enzymatically dissociated preparations of single canine cardiac Purkinje cells, in which contamination by other cell types is minimized, also show two binding affinities. Supported by NIH HL20592 and HL01447, AHA 87-1117.

W-Pos160 Divalent Block of Sodium Current in Single Canine Cardiac Purkinje Cells

M.F. Sheets, D.A. Hanck, and H.A. Fozzard. Cardiac Electrophysiological Labs, The University of Chicago and the Reingold ECG Center, Northwestern University, Chicago, IL.

The instantaneous current-voltage relation in canine cardiac Purkinje cells exhibits prominent flattening at hyperpolarized potentials because of Ca block of I_{Na}. We investigated the ability of other extracellular divalent cations to produce similar block. Single cells were voltage clamped using a large suction pipette (20-30μm) with extracellular (mM) 45 Na, 105 Cs, 2 Ca, 152 Cl, 10 HEPES (pH 7.2) and intracellular (mM) 45 Na, 105 Cs, 150 F, 10 EGTA, and 10 HEPES (pH 7.2) at 12°C. Cells were held at -150mV, stepped to 20mV for 600-800μs, and then stepped to test potentials between -120 and 40mV. Currents was recorded at 400KHz with a 12-bit ad-converter (Masscomp 5520), leak corrected, and capacity corrected using a modified P-4 technique. I_{Na} was measured by averaging 4 points beginning 30-50 μs after the test step and was fit to a 4-barrier model based on Eyring rate theory. Previously reported model parameters (Sheets et al., *Biophys. J.* 52:13-22, 1987) were used except the depth of well G2 for Ca was iteratively determined by modified Gauss-Newton non-linear least squares algorithm (NAG) for each control data set (mean -2.97±0.27 RT units, n=9). Well depth of G2 for experimental divalents was determined using control parameters for Na and Ca. Data for two divalents are shown with experimental points (×), pre-control (O), and post-control (*). Model fits are drawn. Expressed as the fraction of the depth of well G2 for Ca, divalents were in rank order Ba 0.48, Mg 0.59, Ca 1.00, Co 1.15, Mn 1.27, and Cd 1.59. (NIH HL20592 and HL01447)



W-Pos161 GATING CURRENTS IN SINGLE CANINE CARDIAC PURKINJE CELLS

D.A. Hanck, M.F. Sheets, and H.A. Fozzard. Cardiac Electrophysiological Labs, The University of Chicago and the Reingold ECG Center, Northwestern University, Chicago, IL.

Na current and patch clamp data predict a high density of Na channels in canine cardiac Purkinje cells, a fact which predicts gating currents could be studied in these cells. Current responses from steps from -150mV to various test voltages were recorded with a 20-30μm suction pipette at 12°C in an extracellular solution of (mM) 150 CsAspartate, 2 $CaCl_2$, 10 HEPES (pH 7.2), and 100μM TTX and an intracellular solution of 150 CsF, 10 EGTA, and 10 HEPES (pH 7.2). Individual sweeps, filtered at 50 KHz (8-pole Bessel) and digitized by a 12-bit ad-converter (Masscomp 5520) at 100KHz, were leak corrected and capacity corrected using a modified P4 technique. Currents recorded under these conditions are outward, peak by 20μs, and decay more rapidly at very negative and at positive potentials. The Q-V relation is sigmoidal; the plateau predicts Na channel density in this cell to be 200 channels/μm^2 (assuming 6 e^-/channel).

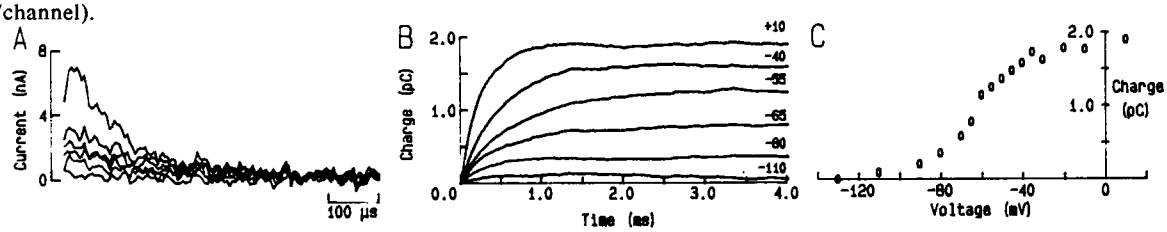


Figure: Gating currents in cell 64.02, 87pF. A) Currents recorded as described above and digitally filtered at 20KHz. B) Integrals of leak corrected currents. C) Q-V relation. Supported by NIH HL20592 and HL01447.

W-Pos162 MAGNETIC COUPLING BETWEEN CYCLIC GMP AND A SODIUM CHANNEL.

Roger E. Clapp, The MITRE Corporation, Bedford, MA 01730.

The zipper transition in an alpha-helix has been described earlier (J. Theor. Biol. **104**, 137-158, 1983; Biophys. J. **51**, 136a, 1987). In this conformational transition, charge migration along one or two of the three chains of hydrogen bonds leads to a bending of the alpha-helical protein rod, and to biological effects including the gating of the voltage-sensitive sodium channel. Note that the hydrogen-bond chains are themselves helical in arrangement, so that the charge migration is solenoidal and will be coupled to a magnetic flux line down the center of the alpha-helix. A similar magnetic flux line will be attached to a molecule of cyclic guanosine monophosphate (cGMP), if the energy stored in this molecule is in the form of an electronic excitation in which a positive charge circulates around a six-bond closed path containing a Möbius phase reversal. Such an orbit has L=1/2 as its orbital angular momentum and is metastable against radiative decay. The electronic kinetic energy in this case is approximately 11 kcal/mol, about the same as the measured energy released by hydrolysis. The magnetic flux associated with the continuing charge circulation can bind the cGMP to an alpha-helix, inducing a zipper transition that will be reversed when the cGMP unbinds. Experiment shows that three cGMP molecules must bind to the plasma membrane of the rod outer segment to open one cGMP-sensitive sodium channel. Similarly, three steps are needed to open a voltage-sensitive sodium channel in a nerve membrane, according to the Hodgkin-Huxley m^3h picture. It is proposed that zipper transitions in three alpha-helices bounding the ion channel cause the gating of each kind of sodium channel, and magnetic coupling is what drives each transition in the case of the cGMP-sensitive protein.

W-Pos163 POLYSIALIC ACID SUBSTITUENTS OF THE ELECTROPLAX Na CHANNEL CROSS REACT WITH ANTIBODIES RAISED AGAINST BACTERIAL POLYSIALIC ACID. W.M. James and W.S. Agnew, Department of Cellular and Molecular Physiology Yale University, New Haven, CT 06510.

Sialic acid can be cleaved from the large glycopeptide of the electroplax Na channel by a bacteriophage endosialidase, endo-N, suggesting the presence of linear α-2,8-linked polysialic acid chains (James & Agnew, in Press). Polysialic acid is antigenically distinct from monomers or short-chain oligomers, as demonstrated with well characterized antisera raised against bacterial polysialosyl determinants. We have found that monoclonal antibodies (IgG) raised against E. coli strain K1 polysaccharide and horse antibodies (IgM) against N. meningitidis group B coat polysaccharide, selectively recognize the eel Na channel glycopeptide. Horse antiserum against the N. meningitidis B-type coat antigen precipitated the detergent solubilized ^{3}H-TTX binding site. Addition of serum to 16% followed by centrifugation removed 50% of the Na channel from the supernatant, while 60% serum removed 97%. SDS-PAGE of the precipitate demonstrated the Na channel peptide. No precipitation was observed when antibodies were preblocked with pure B antigen or when antiserum against the Y-type coat antigen was used. Partially purified Na channel preparations were used for Western blot analysis. Peptides were first separated by SDS-PAGE, transferred to nitrocellulose paper and probed with the described antibodies. Immunostains showed that the polysialic acid specific antibodies, but not those against the Y coat antigen, appeared to label exclusively the Na channel peptide. These results suggest that the specific polysialic acid determinant is a most unusual post-translational modification occuring in the electroplax. Polysialic acid chains in the electroplax Na channel appear to be at least 10 tandem sialoysl residues, as this represents the smallest oligomer that mimics the determinants recognized by the horse serum IgM.

W-Pos164 O-DEMETHYL ENCAINIDE (ODE) INTERACTS WITH CARDIAC SODIUM CHANNELS IN A TEMPERATURE- AND VOLTAGE-DEPENDENT FASHION

Takafumi Anno, James Johns, Dirk Snyders, Paul Bennett and Luc Hondeghem, Vanderbilt University, Nashville, Tennessee

The metabolite ODE is responsible for most of the sodium channel block during treatment of arrhythmias with encainide. Its effects were studied in collagenase dispersed guinea pig cardiac myocytes that were voltage-clamped using the patch clamp technique. Peak I_{Na} (15 to 25°C) and $\dot{V}_{max}$ (25 to 37°C) were used as measures of sodium channel availability. ODE block was use-dependent, but since short and long pulses provided similar levels of block, block must primarily develop during activation and not during inactivation. Recovery from block was composed of at least two processes: a slow diastolic component and an activation-associated unblock (a phenomenon we recently described). The slow rate of recovery from block increased with temperature, but was always so slow that it could only minimally contribute to removal of block at normal heart rates. Activation-associated unblock increased with hyperpolarization: at 35°C, a plateau was reached at -120 mV while at 15°C it was not yet reached at -160 mV. However, the voltage-dependence of the availability for unblock curve was more negative and had a shallower slope than the availability curve for activation of drug free channels, and these differences were accentuated by cooling. As a result, at 15°C reduction of I_{Na} by ODE exhibited almost no recovery. At 25°C, activation-associated unblock could be induced by moderate hyperpolarization, and the results were similar for I_{Na} and $\dot{V}_{max}$. At 37°C, using $\dot{V}_{max}$ measurements, significant activation unblock occurs at normal resting potentials (-90 mV), but much less in depolarized cells. We conclude that ODE alters the voltage dependence of sodium channel gating in a temperature-dependent fashion.

W-Pos165 EFFECTS OF CALCIUM AND LIPID SURFACE CHARGE ON SODIUM CHANNEL GATING. Samuel Cukierman, William C. Zinkand, Robert J. French#, and Bruce K. Krueger. Department of Physiology, Univ. Maryland School of Medicine, Baltimore, Maryland 21201, and #Department of Medical Physiology, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

We have studied the voltage-dependent gating of batrachotoxin-activated Na channels from rat brain in planar bilayers composed of neutral and negatively-charged phospholipids. The midpoint ($V_{0.5}$) of the open probability, P_o, vs V_m relation was about -100 mV and the effective gating charge was about 3 in both negative (phosphatidylserine) and neutral (phosphatidylethanolamine) bilayers with Ca^{2+}-free 125 mM NaCl in the aqueous solutions. Addition of 7.5 mM Ca^{2+} to the external side of the membrane induced a depolarizing shift in $V_{0.5}$ of about 25 mV in negative bilayers and about 17 mV in neutral bilayers. Internal Ca^{2+} induced hyperpolarizing shifts in $V_{0.5}$ of 15 mV in negative and 8 mV in neutral membranes. We conclude that: 1) Ca^{2+} can cause shifts in $V_{0.5}$ by interacting with either the Na channel protein or with negative charges on the membrane lipids. 2) The effects of asymmetric Ca^{2+} on $V_{0.5}$ are greater for external than internal Ca^{2+}. We believe these differences in the magnitude of Ca^{2+}-induced shifts are due entirely to interactions with the channel protein. 3) The components of shifts in $V_{0.5}$ that we can attribute to Ca^{2+} interacting with membrane lipids are the same for external and internal Ca^{2+} suggesting that the charges on the membrane lipids are distributed symmetrically with respect to the channel's voltage sensor. Supported by NIH, U. S. Army Medical Research and Development Command, and the Alberta Heritage Foundation for Medical Research.

W-Pos166 LIDOCAINE AND BUPIVACAINE BLOCK OF SODIUM CHANNELS - RECOVERY KINETICS CORRELATE WITH POTENCY FOR PHASIC BLOCK. Daniel M. Chernoff and Gary R. Strichartz, Anesthesia Research Labs, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115 (Intr. by Thomas A. Rando).

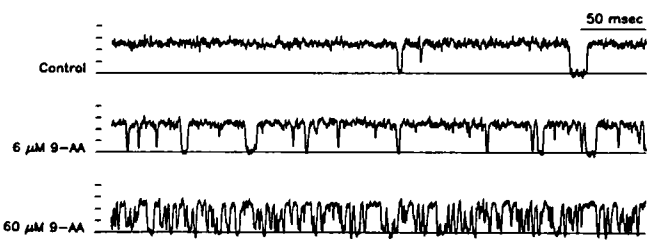
The local anesthetics lidocaine (Lid) and bupivacaine (Bup) block sodium channels in a use-dependent (phasic) manner, but differ in blocking potency and in channel recovery kinetics following repetitive depolarizations. Lid and Bup were applied outside the voltage-clamped node of Ranvier from toad (Bufo marinus) at concentrations sufficient to produce 30-60% resting block at -100 mV (25 uM Bup, 100 uM Lid). The dependence of phasic block on extracellular pH (pH_o) was evaluated at pH 6.2, 7.2, and 8.4, using stimulation frequencies of 1-10 Hz. For a given frequency of stimulation, the rate of approach to steady-state increased at higher pH_o. Steady-state phasic block at 10 Hz was also greater at higher pH_o. A three parameter kinetic model was used to fit the accumulation of phasic block during a pulse train, assuming a transient accessibility (or increase in affinity) of receptor during depolarization, and continuous unblocking during each recovery interval. Results of this parametric fit indicate that the kinetics of binding and (extrapolated) equilibrium block during depolarization are similar for Lid and Bup, while the rate of recovery from block during repolarization is 5-15x faster for Lid. This difference in recovery rate correlates with the differential potency of these drugs. Supported by NIH GM15904-21.

W-Pos167 MAPPING SODIUM CHANNEL PROTEOLYTIC CLEAVAGE FRAGMENTS WITH ANTIBODIES AGAINST DEFINED SEGMENTS OF THE 1° SEQUENCE. J. Yang, Y. Li, S. Zwerling, and R. Barchi. Institute of Neurological Sciences, University of Pennsylvania Medical School, Philadelphia, PA 19104.

The rat skeletal muscle sodium channel contains a 260 kDa α subunit which is cleaved by endogenous proteases during membrane preparation to yield an apparent single large subunit banding diffusely between 130-160kDa on SDS-PAGE. In this form, the purified protein retains the functional capabilities of the native channel. We have mapped this protease-sensitive site by probing Western blots of intact and endogenously nicked channel with antibodies raised against synthetic peptides corresponding to AA's 48-65 (L-14), 709-730 (L-16), 1265-1281 (L-15), 1288-1299 (R-12) and 1420-1432 (L-13) of the eel 1° sequence. These regions are highly conserved in other known Na channel sequences. Each Ab identifies only the 260 kDa subunit of eel, rat brain and muscle sodium channels under conditions in which proteolysis is prevented. In channel prepared from isolated rat skeletal muscle sarcolemma, L-14 labeled a broad band at 130 kDa while L-13-16 and R-12 recognized a diffuse band from 140-160 kDa, together coinciding with the broad 130-160 kDa band initially reported for the sarcolemmal channel. Based on MW calculations, the initial cleavage site for the sarcolemmal channel is near the distal end of the putative cytoplasmic region between homologous domains I and II. L-14 also identifies smaller fragments of ~80, 18, and 17kDa which may correspond to a peptide from the H_2N-terminus to the distal side of domain I, and to the H_2N-terminal peptide. Three groups of monoclonal antibodies raised to the purified channel exhibit labeling patterns identical to L-14; each recognizes epitopes in the 17K H_2N-terminal fragment of the the protein. Antibodies L-13-16 and R-12 segregate into two additional labeling patterns consistent with further limited cleavage of a small fraction of the sarcolemmal channel in the regions between domains II and III and at the -COOH terminus.

W-Pos168 BLOCK OF SINGLE BATRACHOTOXIN-ACTIVATED SODIUM CHANNELS BY 9-AMINOACRIDINE. W F Wonderlin, and R J French, Department of Medical Physiology, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Currents were recorded from single Na channels isolated from rat brain and incorporated into lipid bilayers (phosphatidylethanolamine:phosphatidylserine = 50:50 in decane) bathed in symmetric 200 mM Na acetate. Following activation by batrachotoxin, these channels are almost always open at voltages more positive than -70 mV. Addition, to the internal solution, of 9-AA (2-60 uM), a monovalent cation at physiologic pH, produced a "flickering" block of the single-channel current. Blocking rates were linearly dependent on [9-AA] while unblocking rates were almost independent of [9-AA]. Thus, each brief blocking event (average = 1.5-2.0 msec at +100mV) appears to result from the binding of a single 9-AA molecule within the pore. At 60 uM 9-AA and +100 mV, the channel was blocked about 50% of the time. We saw no evidence of block when 30 uM 9-AA was added externally. The unblocking rate was voltage-dependent with an approximately e-fold decrease per 50 mV depolarization over the range +40 to +100 mV. The blocking rate was less voltage-dependent. From the combined voltage dependence of the blocking and unblocking rates, we estimate that the binding site was about 70% of the way across the transmembrane voltage from the cytoplasmic end. In the future, analysis of the kinetics of 9-AA entry and block could enable detection of changes in functional properties of the inner section of the channel following chemical or genetic modifications of the channel structure.



Inset. Steady-state records at +100 mV.

This work was supported by the Alberta Heritage Foundation for Medical Research.

W-Pos169 **OSMOTIC EFFECT OF FORMAMIDE ON SODIUM CHANNEL GATING CURRENTS IN CRAYFISH GIANT AXON.** D. A. Alicata, M. D. Rayner and J. G. Starkus., Department of Physiology and Bekesy Laboratory of Neurobiology, University of Hawaii, 1993 East-West Road, Honolulu, HI 96822.

Schauf and Chuman (1986, "Ion Channels In Neural Membranes"), have reported 20% Formamide to be an effective pharmacological tool for removing the fast component of the sodium channel gating current in Myxicola giant axons. Changing the internal perfusate from the standard 430 milliosmole solution to 5% and 10% formamide (1300 milliosmole and 2600 milliosmole, respectively), reduces the fast component as noted by Schauf and Chuman. However, when we control for high internal osmolarity with 1300 and 2000 milliosmole sucrose, sucrose is equally effective in reducing the fast component of the gating current.

Stimers et al. (JGP 89:521, 1987), have noted uncovering of the fast component in squid axons associated with external hyperosmotic environments. These changes observed in the gating current were proposed to be associated with a decrease in series resistance (Rs). Internal hyperosmotic media might therefore be expected to increase Rs. We note that an imposed increase in Rs slows clamp speed in our system, while reducing clamp speed reversibly obscures the fast component of gating current. Thus we find no evidence to indicate a direct pharmacological effect of formamide on gating currents in the crayfish giant axon.

Supported by NIH grant NS21151-04, The Hawaii Heart Association, and BRSG.

W-Pos170 **SINGLE CHANNEL RECORDINGS IN CRAYFISH AXONS.** P. Ruben*, J.G. Starkus, and M.D. Rayner. (Intr. I.M. Cooke) Bekesy Lab. of Neurobiology, U. of Hawaii, Honolulu, HI 96822.

Two methods were used to record single channel activity from crayfish giant axons. Axons were either cut open to expose a large area of the internal surface (Bezanilla, Biophys. J. 51:195a), or a small opening was made and the pipette was inserted through the hole to the internal surface. In both cases outside-out patches were established with 1 um diameter micropipettes and seal resistances exceeding 10 gigaohms. The ability to obtain seals and the magnitude of the seal resistance were enhanced by the addition of 5 mM Ca to the bathing medium. Outside-out patches were excised from the internal surface of the axon with no decrease in seal resistance and no change in channel behavior. Single channel events were recorded from both attached and excised patches under steady state voltage conditions. In media that favored the recording of K currents (200 mM K in pipette; 20 mM K, 200 mM Na, 5 mM Ca in bath), single channel outward currents were recorded at voltages between -100 and 0 mV. Channel conductance was 60 pS and mean open time was about 1 ms. Preliminary experiments show that under ionic conditions that favored the recording of Na currents (0 Na, 200 mM Cs in pipette; 200 mM Na, 0 Ca in bath), inward currents were recorded at voltages between -150 and 0 mV. Channel conductance was 12 pS and mean open time was 1 ms. Single channel conductance was reversibly lowered when external Na was replaced by TMA. Low conductance channels (1 pS) were also recorded when 5 mM Ca was present in the bathing medium. In these conditions mean open time decreased to about 0.5 ms. These results might be due to a partial block of Na channels by calcium.

Supported by PHS grant #NS21151-04, Hawaii Heart Association, and UHBRSG.

W-Pos171 Ca CURRENTS IN XENOPUS OOCYTES INJECTED WITH RNA FROM RAT HEART: REGULATION BY ALPHA-ADRENERGIC AGONISTS. Eli Gershon, Yoram Lass, and Nathan Dascal. Department of Physiology and Pharmacology, Sackler School of Medicine, Tel Aviv University, Ramat Aviv 69978, Israel.

Xenopus oocytes express voltage-dependent Ca channels when injected with RNA from rat heart (Dascal et al., Science, 231:1147, 1986). 2 to 4 days after the injection, depolarization-actvated current was resolved with 40 mM external Ba^{2+}, using the two-electrode voltage clamp technique. The inward current displays two components that can be distinguished electrophysiologically and pharmacologically: a fast, transient current, and a slow, dihydropyridine (DHP)-sensitive current which corresponds to the L-channel. The fast current was selectively inhibited by Ni^{2+} (IC_{50}=40 uM) or by holding the mebrane at potentials positive to -20mV. The slow current was inhibited by Ni^{2+} with IC_{50}=500 uM and did not inactivate at -20mV. Only the slow current was affected by DHPs.

To study the effect of alpha-adrenergic agonists, the currents were evoked by voltage steps from -100 to 0 mV. The alpha agonists decreased the slow (L-type) current, without an appreciable effect on the fast one. Application of phenylephrine (1 uM) (alpha and beta agonist) together with 10 uM propranolol (beta-blocker) resulted in a 20% decrease of the peak amplitude of the Ba current. When phenylephrine was applied without propranolol, the current was enhanced, as expected from its beta-agonist activity. Similar results were obtained with epinephrine and methoxyamine. None of these agents had effects on the holding current. A further research is under way in order to distinguish between the alpha-1 and alpha-2 actions.

(Supported by grants from United States - Israel Binational Science Foundation and from the Israel Academy for Sciences and Humanities).

W-Pos172 PHOSPHORYLATION OF THE ELECTRIC EEL SODIUM CHANNEL GLYCOPEPTIDE. M. C. Emerick and W.S. Agnew (Intr. by M.B. Boyle), Dept. of Cell. and Molec. Physiol., Yale School of Medicine

The Na channel from *Electrophorus electricus* is phosphorylated *in vitro* by the catalytic subunit of bovine heart cyclic-AMP-dependent protein kinase (PK-A). The solubilized channel was rapidly labelled by low levels of kinase with γ^{32}P-ATP and, for short reaction times, was the principal phosphorylated protein in a partially-purified Na channel preparation. The eel Na channel primary amino acid sequence contains four consensus phosphorylation sites with serine and four with threonine as the target amino acid, all in putative cytoplasmic domains. It is not known which of these sites were labelled, but when maximally-labelled Na channel peptide was electroeluted from SDS gels, quantitative amino acid analysis revealed 1.5 to 2.5 pmol of phosphate per pmol peptide. When phosphoamino acids from a partial hydrolyzate of the electroeluted peptide were separated by thin layer electrophoresis, the major phosphoamino acid was found to be phosphoserine, which liquid scintillation counting showed to be in 3-fold excess over phosphothreonine. The initial rate of phosphorylation of kemptamide (H_2N-LRRASLG-$CONH_2$, one of the best substrates for PK-A) was only 4-fold greater than the initial rate for the Na channel under identical conditions, with kemptamide equal in concentration to the acceptor sites on the Na channel (0.3 μM). The Na channel is thus a potential physiological substrate for this enzyme. The Na channel reconstituted into liposomes was also labelled, to levels comparable to those of the solubilized channel. Protein kinase C, under conditions which produced heavy phosphorylation of histones (Sigma type III-S), produced no detectable labeling of the solubilized eel Na channel, in marked contrast to the Na channel α-subunit from rat brain, (Costa & Catterall, *Cell. Molec. Neurobiol.*, **4**, 291, 1984). It is possible that the phosphorylation sites for PK-C on the rat brain protein lie on the ~166-amino acid stretch that is absent in the eel protein. (Supported by NIH grant NS-17928 to WSA).

W-Pos173 **A PARTIAL CHARACTERIZATION OF MONOCLONAL ANTIBODIES SPECIFIC FOR EEL ELECTROPLAX SODIUM CHANNEL SUGAR-GROUPS** **Stan Ivey, William B. Thornhill, and Simon R. Levinson. Dept. of Physiology, Univ. of Colorado Medical School, Denver, CO 80262.**

Mouse monoclonal antibodies were raised against purified eel electroplax sodium channels. Of the 42 specific antibody-producing cell lines isolated, fifteen were chosen for epitope characterization via immunoblotting of SDS-PAGE separated sodium channel preparations. From this, ten of the monoclonals immunostained the mature channel (M_r = 260,000); the remaining five immunostained an apparent channel biosynthetic intermediate (M_r = 200,000). These monoclonals were then used to follow the biosynthesis of the channel in Xenopus oocytes and to immunostain the sialidase-treated mature channel.

Radiolabelled channel intermediates were synthesized in Xenopus oocytes injected with eel total RNA and incubated in the presence of ^{35}S-methionine. All ten of the monoclonals that recognized the mature channel precipitated glycosylated channel intermediates (as determined by various lectin precipitation protocols); the non-glycosylated channel polypeptide was not recognized. The mature channel was also enzymatically degraded by neuraminidase treatment, and Western Blotted to nylon membranes. The neuraminidase-treated channel's molecular weight shifted to 210,000, suggesting removal of its sialic acids. Nine of the ten monoclonals that recognized the mature channel also immunostained the neuraminidase-treated channel. These nine monoclonals probably recognize sugar groups on the channel that do not contain sialic acids; the tenth (non-reactive) monoclonal possibly recognizes an epitope containing sialic acids. One of the five monoclonals that did not recognize the mature channel (above) immunostained the neuraminidase-treated channel, suggesting that this antibody recognized a non-sialidated, immature form of the channel. These monoclonal antibodies to sodium channel carbohydrates should prove useful in the identification and isolation of channel intermediates in the sodium channel biosynthetic pathway. Supported by NIH NS-15879.

W-Pos174 **BLOCKADE OF SODIUM CURRENTS BY MODULATORS OF N-METHYL-D-ASPARTATE (NMDA) RECEPTOR/CHANNEL** M.L. Conder and J.R. McCullough, Research Dept., Pharma. Div., CIBA-GEIGY Corp., NJ 07901

Although many centrally acting antagonists are reported to be specific and potent antagonists for their respective receptors, these agents often possess other nonspecific actions. We have examined the question of selectivity and potency of a number of putative modulators of the NMDA receptor/channel complex by studying their sodium channel blocking actions. Whole cell voltage-clamp techniques were used to measure sodium current (INa) in freshly dissociated guinea pig ventricular myocytes and the actions of phencyclidine (PCP), Dextromethorphan (DM) and Tiletamine (TA) on INa were studied. Voltage clamp protocols consisted of either 1) 30 msec step potentials from a holding potential of -90 mV to determine the current voltage relationship of INa or 2) a series of 500 msec voltage step prepulses (varying from -120 to -30 mV) followed by a constant test pulse to -20 mV to determine steady state inactivation of INa. Compounds were dissolved and the cells were bathed in a low external sodium (20 mM) Tyrodes solution. A dose dependent block of INa was found with all three compounds. The 50 percent effective concentration (EC50) was 30-50 uM, 100 uM and 5 nM for PCP, DM, and TA, respectively. In addition, steady state inactivation (V1/2) was shifted by -13 mV and -9 mV by 100 uM PCP and 10 uM DM. PCP also showed use dependent block of INa. It is clear from these data that the potencies of these compounds at the sodium channel are in the same range as their binding affinities as modulators of the NMDA receptor/channel complex. We conclude that NMDA antagonists should be tested for local anesthetic properties and that this type of compound may have extraneuronal effects that would lead to a more complex spectrum of activity.

W-Pos175 AN EXTERNALLY ACCESSIBLE RECEPTOR FOR LIDOCAINE BLOCK OF SODIUM CURRENT IN CANINE CARDIAC PURKINJE CELLS

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Local anesthetic block of sodium current (I_{Na}) in nerve has been described as binding to a single receptor accessible from the cytoplasm, and this model has been accepted for heart. We tested this hypothesis in the voltage-clamped internally-perfused canine cardiac Purkinje cell. Lidocaine, a tertiary amine, and QX314, a charged quaternary amine, were added to either the outside solution with (mM) 45 Na, 105 Cs, 156 Cl, 2 Ca, 1 Mg, 10 HEPES (pH 7.2) or the inside solution with 150 Cs, 150 F, 10 EGTA, 10 HEPES (pH 7.2). Cells were held at -150 mV and stepped for 10 ms to -10 mV at 15°C. First pulse block (FPB) was determined by comparing control peak I_{Na} with that for the first depolarization after drug wash-in. Use-dependent block (UDB) was determined as the additional steady-state reduction of peak I_{Na} during trains of depolarizing steps. Lidocaine blocked I_{Na} more effectively from the outside for both FPB and UDB (table). QX314 blocked more effectively from the outside for FBP, but not for UDB. We conclude that unlike nerve the cardiac Na channel is more readily blocked from the outside by lidocaine. These findings have implications for the site(s) of local anesthetic action in heart and for the structure of the cardiac isoform of the Na channel.

Drug (μM)	FPB% (n)	UDB% (n)	Hz
$Lido_o$ 50	16±4 (4)	18±2 (6)	4
$Lido_i$ 50	1±2 (4)	5±1 (5)	4
$QX314_o$ 10	11±3 (4)	24±5 (4)	1
$QX314_i$ 10	1±2 (3)	76±4 (4)	1

(NIH grants HL-20592, K11HL01572 and T32HL07381; QX-314 gift of Astra Pharmaceuticals, Mass.)

W-Pos176 FUNCTIONAL α-SCORPION TOXIN SITES IN SINGLE SODIUM CHANNELS ARE EXPRESSED BY XENOPUS OOCYTES INJECTED WITH BRAIN RNA. J.R. Moorman, G.E. Kirsch, R.H. Joho, L.D. Possani and A.M. Brown. Dept Medicine, UTMB, Galveston; Dept Biochemistry, CIGB/UNAM, Mexico; Dept Physiology and Molecular Biophysics, Baylor College of Medicine, Houston, TX.

α-scorpion toxins bind specifically to sodium (Na^+) channels and delay development of inactivation. We compared the effects of TsIV-5, a peptide toxin purified from Tityus serrulatus venom, on single Na^+ channel currents in outside-out patches from neonatal rat cortical neurons (native channels) and from Xenopus oocytes injected with neonatal rat brain RNA (expressed channels). In both types of channels, the toxin at 0.5 to 2.0 uM produced long-lasting (10-140 ms) bursts of openings at all potentials and prolonged channel open times at strong depolarizations. At test potentials of -20 to -10 mV, mean open times in toxin-treated patches were prolonged approximately 6-fold in native and 4-fold in expressed channels. At test potentials of -60 to -50 mV, however, the mean open time in both was prolonged less than 2-fold. Single channel conductance was unaffected by toxin. A scheme in which open channels may close near threshold by inactivating or by re-entering a closed state but may close only by inactivating at stronger depolarizations could explain these results if the toxin selectively alters the inactivation gating region of the channel molecule. We conclude that TsIV-5 modifies the inactivation gating mechanism of brain Na^+ channels both in neurons and in RNA-injected Xenopus oocytes, and may thereby provide a useful probe of the structural basis of Na^+ channel inactivation. Supported by K01858 (JRM), AHA 87697 (GEK), HL36930 (AMB).

W-Pos177 BIOSYNTHESIS OF SODIUM CHANNELS FROM ELECTROPHORUS MUSCLE, HEART, AND BRAIN. W.B. Thornhill and S.R. Levinson. Dept. of Physiology, Univ. of Colorado, School of Medicine, Denver, CO 80262.

The voltage-sensitive sodium channel is a heavily post-translationally modified protein. For example, the eel electroplax channel (M_r 260,00) has been shown to be composed of 30% carbohydrate and 8% lipid by weight. These post-translationally acquired domains are responsible for the unusual behavior that the molecule exhibits on SDS-gels and may play a role in the distribution and operation of the channel in membranes.

We have isolated the mRNA from eel muscle, heart, and brain, and these different preparations were used to direct protein synthesis in both a cell-free and a frog oocyte system. Antisera raised against the electroplax sodium channel immunoprecipitated radiolabelled polypeptides that were synthesized as a result of the introduction of the different tissue specific mRNAs to either system. This suggested that the polyclonal sera to the electroplax sodium channel contained antibodies that recognized both carbohydrate and protein epitopes on the different tissue specific sodium channels. A partial characterization of these different sodium channel molecules was carried out. Support by NIH NS-15879 and NS-23509.

W-Pos178 HUMAN CORTICAL SODIUM CHANNELS IN PLANAR LIPID BILAYERS. D.S. Duch, E. Recio-Pinto and B.W. Urban, Depts. Anesthesiology and Physiology, Cornell U. Med. Coll., N.Y. 10021.

We report here the first recordings of single human cortical sodium channels, using planar lipid bilayer methodology. Human cortical samples were obtained from 4 patients undergoing routine craniotomies and pooled. This tissue was considered surgical waste; no tissue was removed specifically or only for these bilayer experiments. Synaptosomes were prepared as described by Cohen et al. (J. Cell Biol. 74:181, 1977). Sodium channels were incorporated into planar lipid bilayers in the presence of batrachotoxin and characterized as previously described (Recio-Pinto et al., J. Gen. Phys., 90:375, 1987). In symmetrical 500 mM NaCl solutions, the current-voltage relationship was symmetrical, and the single channel conductance was independent of membrane potential. The average slope conductance of the channels was 26.7 +/- 1.7 (s.d.;n=7) pS. The average steady state activation gating midpoint was -84.1mV +/- 13.8 (s.d.;n=3). Individual channels in multi-channel membranes could gate at discrete potentials. Hysteresis of channel activation midpoints was evident. The average gating charge valency was 2.1 +/- 0.1. The permeability ratio for Na:K (P_{Na}/P_K) was 3.3 when sodium was present on the outside of the channel and potassium on the inside, but was 5.7 under reverse conditions. Addition of submaximal concentrations of TTX to the extracellular side of the sodium channel resulted in channel closures without changing single channel conductance. This block was voltage-dependent, with block decreasing with depolarized potentials. The $K_{1/2}$ at 0 mV potential was 50 nM. These results indicate that single human sodium channels, and perhaps other human membrane proteins, can be successfully recorded and studied using planar bilayer techniques, making this a potentially powerful tool for molecular, structural and pharmaceutical studies of human ion channels.

W-Pos179 Single ionic channels induced by palytoxin. Matomo Nishio and Ikunobu Muramatsu. Dept. of Pharmacology, Fukui Med. Sch., Matsuoka, Fukui 910-11, Japan (Intr. by T. Narahashi)

Palytoxin, isolated from the zoanthid Palythoa species, is one of the most potent marine toxins and depolarizes the membrane in a Na^+-dependent but tetrodotoxin-resistant manner. We examined the mechanisms of palytoxin-induced ionic permeability by using the patach clamp technique and found that palytoxin induced a new type of ionic channel.

Cell-attached patch clamp was performed with single ventricular cells isolated from the guinea pig. Palytoxin ($1\text{-}2 \times 10^{-11}$ M, contained in the patch electrode) induced elementary currents flowing through the single channel. The amplitude of single channel current at resting potential was 0.65 ± 0.03 pA (n=6). The I-V relation of the current was linear, and the slope conductance was 9.5 ± 0.5 pS. The distribution of open times was fitted by a single exponential function (decay time constant: 235 ms), while that of closed times was fitted by the sum of two exponential functions (decay time constant: 3.9 ms and 2650 ms). Palytoxin-induced single channel current was resistant to tetrodotoxin (5×10^{-5} M) or cobalt ions (2×10^{-3} M), and was observed in Ca^{2+}-free conditions. However, no channel current was observed by palytoxin dissolved in Na^+-free, choline-Tyrode solution. Single channel currents were induced when palytoxin was dissolved in Na^+-free, NH_4^+-, Cs^+- or Li^+-Tyrode solution, and the slope conductances were 16.5 ± 1.6 pS, 11.0 ± 0.7 pS and 9.2 ± 0.7 pS, respectively. These results indicate that palytoxin induces a new type of channel with unique ionic selectivity and gating behavior differnt from those of the normal Na channel. The membrane depolarization caused by palytoxin seems to be due to an increase in Na^+ permeability through palytoxin-induced ionic channels.

W-Pos180 FUNCTIONAL PROPERTIES OF NEURAMINIDASE-TREATED RAT BRAIN SODIUM CHANNELS. T. Scheuer, L. McHugh, F. Tejedor & W. Catterall. (Intr. by F.F. Vincenzi) Dept. of Pharmacol., Univ. of Washington, Seattle, WA.

The rat brain sodium channel is a heterotrimeric glycoprotein with α (260 kD), β1 (36 kD), and β2 (33 kD) subunits. The functional relevance of the sialic acid residues of the attached carbohydrate was assessed by treatment of purified channels for 30 min with neuraminidase and reconstitution into PC/PE vesicles. Apparent size of the α subunit was reduced to 231 kD. Binding of ^{3}H STX and ^{125}I-labelled α-scorpion toxin was unaffected. Reconstituted vesicles were incorporated into planar lipid bilayers in the presence of batrachotoxin (1 μM). In symmetrical 0.1 M NaCl, control channels have a linear current-voltage relationship with an average conductance of 18 pS. Lower conductance substates are rarely seen. Neuraminidase-treated channels can attain a maximum conductance of 18 pS, but these channels are also characterized by multiple lower conductance states ranging down to 4 pS. Most commonly conductance is asymmetic with near control values for one orientation of the voltage gradient. A few channels had more complex behavior with currents in both directions differing from control. In some experiments, channels were first fused with the bilayer and subsequently treated with neuraminidase. The apparent size of α subunits in the recording chamber was similarly reduced under these conditions. Channels treated in this manner maintained control properties for more than 1 hr in the bilayer but then developed reduced conductance states similar to those seen with channels treated in the soluble condition. These results suggest that sialic acid stabilizes the normal conductance state of the BTX-activated sodium channel but is not required to achieve that state.

W-Pos181 EVIDENCE THAT cAMP-DEPENDENT PHOSPHORYLATION PROMOTES INACTIVATION IN EMBRYONIC RAT BRAIN CELLS IN PRIMARY CULTURE. J. Coombs, T. Scheuer, S. Rossie, and W. Catterall. Dept. Pharmacol. Univ. of Washington, Seattle, WA.

The α subunit of the rat brain Na channel can be phosphorylated by cAMP-dependent protein kinase with 3-4 moles phosphate/mole Na channel. After 3-4 weeks in culture, similar levels of phosphorylation can be attained in primary cultures of rat embryonic CNS neurons. We have used this culture system to study electrophysiological consequences of this phosphorylation. Cultures were triturated to dissociate cells and remove processes, allowing adequate voltage control. Cells, 14-20 μ in diameter, were voltage-clamped at a holding potential of -70 mV using the whole cell variant of the patch clamp technique. Bath solution contained (mM): 150 NaCl, 5 KCl, 1 $MgCl_2$, 1.5 $CaCl_2$, 6 3,4-diaminopyridine, 5 glucose and 5 Hepes, pH = 7.3, 20° C. Electrodes were filled with 90 CsAsp, 60 TEA-Cl, 8 NaCl, 2 $MgCl_2$, and 2 Na_2ATP, pH = 7.3. Experimental electrodes contained 5 mM cAMP to activate cAMP-dependent phosphorylation. Steady-state activation and inactivation of I_{Na} were determined with (n=23) and without (n=46) intracellular cAMP. Control inactivation curves could be approximated by Hodgkin-Huxley formalism, with a half-inactivation voltage ($V_{1/2}$) of -52 mV and steepness (k) of 7 mV. Steady-state activation was relatively unaffected by cAMP. In 52% of the experimental cells, inactivation was similar to control; in the remainder, the inactivation curve was biphasic with 20-70% of the inactivation occurring negative to -80 mV. These results raise the possibility that phosphorylation of sodium channel α subunits can modulate I_{Na} under physiological conditions. This effect may account for the biphasic voltage-dependence of inactivation that has been measured in some cell types. Studies to further characterize this effect are in progress.

W-Pos182 SIALIC ACID RESIDUES AND DELAYED RECTIFIER SURFACE CHARGE OF VENTRICULAR MYOCYTES. J. C. McDonagh and R. D. Nathan. Dept. of Physiology, Texas Tech Univ. HSC, Lubbock, TX 79430.

Sialic acid (NANA) constitutes a significant proportion of the sugar moieties associated with the sodium channel and produces much of the cationic staining of ventricular myocytes. In the present study, we tested the hypothesis that these residues are responsible for the negative surface charge of delayed rectifier potassium channels. Whole-cell current (I_K) was recorded from myocytes that had been isolated from 7-day embryonic chicks and cultured for 1-3 days. Inactivation or blockade (10^{-5}M $LaCl_3$) of calcium current failed to alter I_K, suggesting that I_K is not activated by Ca^{2+}. Steady-state (5-sec) activation-voltage relationships were determined in 1.8 mM external K^+ and from normalized current tails elicited at -60 mV. Within 7 min of rupturing the patch, the half-activation potential ($V_{\frac{1}{2}}$) had shifted to more negative potentials in 8/12 cells and to more positive potentials in 3/12 cells. Because of such "run down," I_K was measured, in different populations of cells for each condition, just after initiating the whole-cell clamp. When 8 cells were treated for 1 hr with 1.0 U/ml of neuraminidase, a concentration that removed more than 85% of the surface NANA, neither the threshold nor half-activation potential for I_K was changed significantly (-38.8 ± 1.8 mV and -0.8 ± 3.3 mV, respectively, versus -36.1 ± 1.8 mV and -0.2 ± 1.4 mV in 22 controls). In contrast, $V_{\frac{1}{2}}$ was shifted significantly (to -12.0 ± 3.4 mV; n=7; $P < 0.001$) by a tenfold reduction of external Ca^{2+}, confirming that surface charge was indeed present on the controls. Our results suggest that sialic acid residues do not constitute the surface charge that is functionally associated with these potassium channels. Supported by NIH grant HL 20708.

W-Pos183 FORSKOLIN BLOCKS K-CURRENTS IN SQUID AXONS INDEPENDENTLY OF cAMP/ATP LEVELS.

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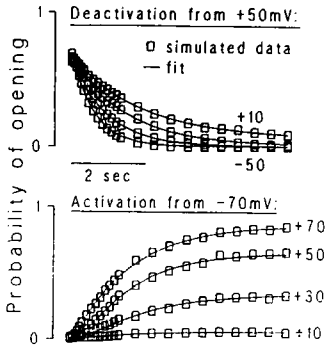
Forskolin is widely used as a stimulator of adenylate cyclase, a way to rise the intracellular concentration of cAMP in intact cells. Bath applications of Forskolin to intact or dialyzed squid axons under voltage-clamp conditions elicit a time dependent block of macroscopic outward currents, similar to the action of long chain quaternary ammonium compounds, in spite of their striking structural differences.Internally dialyzed axons under voltage clamp conditions were used. External solution was artificial sea water with 300 nM TTX and 1 mM NaCN. When dialyzed, the internal solution composition was (in mM): 310 K, 30 PO_4, 4 Mg, 110 Glycine and 1 EGTA with 0 Ca added. Internal pH was 7.3. Forskolin was dissolved in ethanol as stock solution and then diluted into the external solution to a final ethanol concentration never higher than 0.05 %. At that concentration, ethanol alone has no effect on K-current amplitude or kinetics. The $K_{1/2}$ for blockage is around 25 uM when Forskolin is applied externally, and the maximun blockage is achieved at 200 uM. Hill analysis of the dose-blockage data suggests an interaction of 1 Forskolin per channel blocked, with a Hill coeficient of 1.3. Forskolin blocks as effectively when in presence or in absence of internal cAMP. Besides, the efectiveness of the block does not depend on the presence of internal ATP, even though the current amplitude and kinetics are drastically affected by ATP (Biophys. J 47:222a 1985 and 49:215a 1986). These results suggest a direct local anesthetic effect of Forskolin on K-currents, totally independent of the stimulation of the adenylate cyclase.

In the presence of Forskolin, K-currents are smaller than their controls at any point during the voltage pulse, suggesting an interaction with the closed channel. The rate of blockage during the pulse increases in a voltage dependent way, reaching a plateau between 20-30 mV with a tau= 1.3 msec. The characteristics of this voltage dependent block indicates that Forskolin also blocks the open channel.(Supported by MDA and USPHS grant GM30376).

W-Pos184 A METHOD FOR DETERMINING MICROSCOPIC RATE CONSTANTS FROM WHOLE CELL CURRENTS. Jeffrey R. Balser, Dan M. Roden, Paul B. Bennett. Vanderbilt Univ., Nashville, TN.

Some ion channels, e.g. the cardiac delayed rectifier (I_K), are not present in sufficient density to permit routine recording of unitary events. We have therefore developed a maximum likelihood method to extract microscopic rate constants (k_i) from macroscopic currents. Recognizing that each k_i can be expressed in matrix form as a function of voltage ($[\mathbf{k}(V)] = \exp\{[A] \cdot [1,V,V^2]\}$; Stevens, 1978), our global fitting procedure simultaneously uses macroscopic records obtained at different potentials to extract the parameter matrix [A] and thereby determine [k(V)]. Two approaches have been used to validate the method. First, we analyzed macroscopic I_K obtained at a series of activating and deactivating potentials (-50 to +70 mV) from voltage-clamped guinea pig ventricular myocytes: fits to a 3-state Markov model ($C_2 \leftrightarrow C_1 \leftrightarrow$ Open) provided good simulation of the macroscopic currents. Second, using a known matrix [A] and assuming a 3-state model, we generated a series of 1000 simulated single channel events at each membrane potential. Fitting ensemble averages of these synthetic data (figure) showed that the method could extract the rate constants [k(V)]; the errors in determining [k(V)] were complicated functions of voltage, with greatest accuracy (error $\leq \pm 7\%$) at the mid-point of the voltage range. Hence, this procedure offers a method for determining kinetic parameters from whole cell currents and may be particularly useful under conditions unfavorable for single channel recording.



W-Pos185 **PHENCYCLIDINE: BLOCKADE OF K^+ CHANNELS AND NMDA-RECEPTOR COUPLED CATION CHANNELS OCCURS AT DISTINCT SITES IN HIPPOCAMPAL NEURONS.** J.M.H. ffrench-Mullen, S. Suzuki* ,J.L. Barker and M.A. Rogawski*, Laboratory of Neurophysiology and *Medical Neurology Branch, NINCDS, NIH, Bethesda, MD 20982.

The effects of phencyclidine (PCP) and related drugs on K^+ and *N*-Methyl-D-aspartate (NMDA)-receptor gated currents were investigated in cultured (embryonic rat) and enzymatically dissociated (adult guinea pig CA_1 region) hippocampal neurons. Whole cell voltage clamp recordings were carried out in the presence of 2 *u*M TTX. The slowly activating, minimally inactivating K^+ current, I_K, was elicited with depolarizing voltage steps to +60 mV from a holding potential of -60 mV. The fast transient K^+ current, I_A, was studied by depolarization to +60 mV after a 200 msec prepulse to -90 mV in the presence of 20 mM TEA to block I_K. PCP (0.5-300 *u*M) caused a dose-dependent block of I_K with IC_{50} of 17 *u*M; even at 300 *u*M, I_A was only reduced 47%. NMDA currents in cultured cells were examined in 0 Mg^{2+} solution. Acutely dissociated CA_1 cells showed no NMDA responses and could not be tested. PCP (0.1-5 *u*M) reversibly blocked NMDA inward current with an IC_{50} of 0.38 *u*M; there was no effect on kainate (KA) and quisqualate (QUIS) induced currents. Similarly, 20 *u*M D-2-amino-5-phosphonovalerate (D-APV), which had no effect on I_K or I_A, reversibly blocked the NMDA but not the KA and QUIS-induced currents. At 10 *u*M, dexoxadrol (DEX) and its behaviorally inactive enantiomer levoxadrol (LEV) both blocked I_K (10-20%), although DEX was slightly more potent. In contrast, at 10 *u*M, DEX produced near complete, but reversible, block of the NMDA current, whereas LEV up to 20 *u*M was inactive. We conclude that PCP is a potent and specific blocker of the NMDA-induced cation current in hippocampal neurons. At higher concentrations, PCP selectively blocks I_K. In addition to the concentration differences, the block of NMDA receptors shows a stronger stereoselectivity indicating that blockade of NMDA receptors and I_K channels occurs at pharmacologically distinct sites. These results suggest NMDA-receptor block is more relevant to the behavioral effects of PCP than is K^+ channel block. However, the effect on K^+ channels may account for some of the pharmacological effects of PCP, such as its convulsant activity.

W-Pos186 **K^+ CURRENTS IN PC12 PHEOCHROMOCYTOMA CELLS: EFFECTS OF PHENCYCLIDINE AND RELATED DRUGS.** M.A. Rogawski, M. Pieniek, S. Suzuki and J.M.H. ffrench-Mullen* (Intr. by L. Greene), Medical Neurology Branch and *Laboratory of Neurophysiology, NINCDS, NIH, Bethesda, MD 20892.

We investigated the effects of phencyclidine (PCP), a psychotomimetic dissociative anesthetic, and several related drugs on voltage-dependent K^+ currents in PC12 cells, a clonal cell line derived from a rat pheochromocytoma. Whole cell voltage clamp recordings demonstrated two kinetically distinct voltage-dependent K^+ current components: a rapidly activating and inactivating component, I_A, that was selectively eliminated by 4-aminopyridine (2 mM) and a slowly activating, minimally inactivating component, I_K, that was blocked by tetraethylammonium (20 mM). PCP (1-100 *u*M) produced a dose-dependent blockade of both I_K and I_A, however, at low doses the drug selectively reduced I_K with little effect on I_A; the IC_{50}s for blockade of I_K and I_A were 4 and 25 *u*M, respectively. The blockade of I_K was voltage-dependent so that the degree of block decreased with increasing depolarization, indicating that the blocking mechanism is likely one in which the positively charged PCP molecule is drawn into the channel pore. The block showed little use dependence, indicating that the I_K channel can be blocked in either the open or closed state. Several structural analogs of PCP also suppressed I_K. Thienyl-PCP (TCP), a drug that is behaviorally more potent than PCP, partially blocked I_K at low doses (31% at 1 *u*M), but even at high doses (25 *u*M) the degree of block was never as great as that produced by PCP. Like PCP, (+)-1-(1-phenylcyclohexyl)-3-methylpiperidine (PCMP) and dexoxadrol produced a dose-dependent block of I_K. However, in contrast to the stereospecificity these compounds exhibit in binding to PCP receptors and in eliciting PCP-like behavioral responses, their enantiomers (-)-PCMP and levoxadrol were equally potent in blocking I_K. These results demonstrate that PCP and related drugs are powerful, selective blockers of I_K. The structure-activity studies indicate that this effect occurs at a site that is pharmacologically distinct from the behaviorally relevant PCP receptor.

W-Pos187 BETA-ADRENERGIC STIMULATION MODULATES THE ACTIVATION OF THE VOLTAGE-DEPENDENT OUTWARD CURRENT IN HUMAN T8+ LYMPHOCYTES. J.C.R. Lee, B. Soliven, and D.J. Nelson.
The University of Chicago, Dept. of Neurology, Chicago, Ill. 60637.

Decreased intracellular levels of cAMP in lymphocytes are associated with a greater proliferation in vitro (DeRobertis et al., J. Immunol., 443: 151). A rise in intracellular cAMP inhibits the mitogenic lectin induced increase in intracellular Ca (Tsien et al, Nature, 295: 68) which is associated with early events in the proliferative response. A block of the delayed rectifier current is also associated with a decreased proliferative response in T-lymphocytes (DeCoursey et al., Nature, 307: 465; Matteson and Deutsch, Nature 307: 468). Therefore, we chose to study the effects of B-receptor activation on voltage-dependent outward current in subset selected T-cells. Cells were selected by rosetting freshly isolated mononuclear cells, labeling the T8+ cells with murine OKT8 antibodies, and attaching them to petri dishes using goat-anti-mouse IgG. Whole-cell voltage clamp currents were obtained prior to and following exposure of the cells to the B-agonist isoproterenol (100 nM). B-adrenergic stimulation resulted in a rapid decrease in the peak current amplitude with a concomitant decrease in the time constant describing current activation and inactivation similar to that observed for the murine B-cell (Choquet et al., Science, 235:1211). Peak current amplitude decreased at all potentials tested following isoproterenol perfusion (30-100% for a depolarization to 80 mV from holding potential of -80 mV, n=5) and was associated with a 40-95% decrease in the time constant describing current decay. In a representative experiment, the time constant of inactivation decreased from 245 to 69 msec. The addition of the adenylate cyclase activator forskolin (20 uM) to the bath produced a rapid decrease in the peak voltage-dependent outward current (10-20% decrease at 80 mV, n=3) with an associated decrease in the time constant of current inactivation. The B-adrenergic blocking propranolol (10 uM) completely inhibited the isoproterenol induced decrease in current amplitude. Thus, B-adrenergic stimulus may well serve as a feedback control mechanism limiting the extent of cellular proliferation.

W-Pos188 ALTERATION OF THE CLOSING RATE OF SINGLE DELAYED RECTIFIER K CHANNELS BY CATIONS. Fred N. Quandt. Dept. of Med. Physiology, University of Calgary, Fac. of Med., Calgary, Alberta, Canada T2N 4N1.

Voltage-clamp experiments applied to axons have shown that the closing reactions of delayed rectifier K channels are altered by the nature of the permeant ion as well as external Ca (Matteson and Swenson, Armstrong and Matteson, 1986, J. Gen. Physiol. 87). Single channel analysis was applied to neuroblastoma cells to extend these observations. Substitution of internal K with Rb using inside-out patches increased the open time of K channels at -20 mV by a factor of 5 and reduced the conductance of the channel to one-third of the normal value. These alterations were not unique to Rb, as substitution of internal K with NH_4 had similar effects. The effects of external ions were examined by measuring inward currents through K channels using high external, and low internal, K or Rb with outside-out patches. The inward currents measured with the reverse concentration gradients were generated by the opening of K channels, since they were blocked by the application of internal 4-aminopyridine (using inside-out patches). The closing rate of the K channel during a step depolarization was also reduced when external K was replaced with Rb, suggesting that Rb acts at a site associated with the permeation pathway. Closing reactions were also examined at -80 mV under these conditions by measuring currents following the termination of a step depolarization. Currents through K channels continued to reopen following repolarization. Slowing of tail currents by Rb was due to an increase in the probability that K channels would reopen. Reopening during tail currents carried by either K or Rb was antagonized by an increase in external Ca. Supported by the MRC (Canada).

W-Pos189 SOLUTE INACCESSIBLE AQUEOUS VOLUME CHANGES DURING OPENING OF THE POTASSIUM CHANNEL OF THE SQUID GIANT AXON. F. Bezanilla (UCLA), V. A. Parsegian (NIH), and J. Zimmerberg (NIH); MBL, Woods Hole, MA.

Some 20 molecules of water appear to enter the average potassium channel of the squid axon when it opens. This is our estimate from osmotic stress (Parsegian et al, Meth. Enzymol. 127:400-416, 1986) measurements on squid axons similar in concept to those made previously on VDAC incorporated into planar bilayers (Zimmerberg & Parsegian, Nature 323:36-39, 1986). Specifically, we apply solutions with varying osmotic pressures symmetrically to the inside and outside of a perfused, TTX-treated, giant axon and measure the potassium conductance as a function of time and voltage. We find a depression of conductance with increasing osmotic stress but no change in either the shape or the V_0 of the voltage-current curve. One must distinguish by controls three possible actions of the osmotic agent: stress, blocking, and lowered conductivity. We compared results working with pairs of solutions of either equal osmotic stress, equal conductivity, or the same blocking agent. Since there is the same depression of conductance irrespective of stressing species, sorbitol or sucrose, blocking is an unlikely mechanism. The conductivity of the external solution makes some difference but the primary determinant of conductance depression is the applied osmotic stress. Our estimate of a 600 $Å^3$ volume change is based then on an osmotically induced conductance depression modified by a correction for external conductance. This is the volume of approximately 4-6 amino acid residues. To account for our observations, we postulate a number of closed states under voltage control and a final opening state that is voltage-independent but osmotically sensitive.

W-Pos190 QUINIDINE BLOCKADE OF SINGLE POTASSIUM CHANNELS IN DISSOCIATED GASTRIC SMOOOTH MUSCLE CELLS. Brendan S. Wong, Department of Physiology, Baylor College of Dentistry, Dallas, TX 75246

The effects of quinidine, an antiarrhythmic alkaloid, on potassium-selective channels in enzymatically dissociated gastric smooth muscle cells from Rana pipiens and Bufo marinus were investigated using excised patches and the patch-clamp technique. The predominant potassium channel in these cells is the calcium- and voltage-activated maxi-K channel. Applications of quinidine (0.1 - 1 mM) resulted in resolvable rapid flickerings between the open and blocked states with a corresponding reduction in open channel amplitude and an increase in open channel noise. The quinidine blockade was relatively voltage independent over the membrane potential range studied. From data pooled from five different patches, a dissociation constant of 0.2 mM was observed. On the other hand, tetraethylammonium (TEA), another effective blocker of maxi-K channels, produced an apparent reduction in single-channel current amplitudes without significantly increasing open channel noise. Thus for maxi-K channels, both quinidine and TEA can be considered as fast open channel blockers, with TEA being faster than quinidine. Another potassium-selective channel with a single-channel conductance of 40 pS was completely blocked in the presence of 0.1 mM quinidine. However, a 15 pS potassium channel was not affected by quinidine but was reversibly blocked by TEA. Quinidine (0.5 mM) was also observed to decrease the opening probability of a 40 pS potassium channel from Bufo marinus without affecting its channel amplitude. Thus, quinidine appears to have diverse mechanisms of action on potassium-selective channels in smooth muscle cells, ranging from totally uneffective to highly selective, as a slow blocker for some and as intermediate and fast blockers for others. (Supported by BCD Research Fund.)

W-Pos191 POTASSIUM CURRENTS ON EMBRYONIC MUSCLE CELLS IN CULTURE. F. MOODY-CORBETT and R. GILBERT, Basic Medical Sciences, Memorial University of Nfld., St. John's, NF, Canada.

Whole cell current recordings were used to identify three potassium currents on muscle cells in culture. Cultures were prepared from the myotomal muscle of 1 day old embryos of Xenopus laevis. Whole cell current recordings were made using a patch clamp amplifier on small, individual cells in 1-2 day old cultures. The normal external recording solution contained (in mM) 140 NaCl, 5 KCl, 1 $CaCl_2$, 1.2 $MgCl_2$, 10 HEPES, 10 µg/ml tetrodotoxin, 10 µg/ml α-bungarotoxin (pH 7.4) and patch electrodes contained (in mM) 140 KCl, 10 EDTA, 5 $MgCl_2$, 10 HEPES (pH 7.4). Muscle cells were held at their resting membrane potentials and stepped in 10 mV increments from rest. Voltage steps in the hyperpolarizing direction resulted in an inward current that was activated at values negative to the equilibrium potential for potassium and the current was eliminated in the presence of CsCl (5 mM) suggesting it was mediated by potassium through the inward rectifier. Depolarization of the membrane from the resting level resulted in two outward currents. Both currents were altered in a predictable way by changing the external potassium concentration, suggesting they were mediated by current through potassium channels. One of the currents activated and inactivated rapidly (within 15 msec) and was blocked by $CoCl_2$ (5 mM) suggesting it may be a calcium activated fast transient current. The other outward current was activated at slightly less negative potentials, had a delayed onset and did not inactivate. This current was reduced by tetraethylammonium but not cobalt, suggesting it was mediated by potassium through the delayed rectifier. The presence of these currents during early development indicates their importance for membrane excitability at this age. (Supported by MRC, Canada)

W-Pos192 ADRENERGIC MODULATION OF THE K+ CURRENTS IN TONIC MUSCLE FIBERS OF THE FROG. J. Muñiz, J. Lomeli* & M. Huerta. Centro Universitario de Investigaciones Biomédicas. Universidad de Colima, Apdo. Postal 199, 28000 Colima, Col., México.

Potassium currents were recorded using three-microelectrode voltage-clamp technique. Experiments were performed on tonic bundle from cruralis muscle of Rana pipiens. The solution contained (mM): Na-CH_3SO_3, 117.5; K-CH_3SO_3, 2.5; $Ca(CH_3SO_3)_2$ 1.8 and sucrose 350. pH was adjusted to 7.4 with Imidazole-Cl. Experiments were performed at temperature of 20°C. Epinephrine, isoproterenol and propranolol were added from stock solutions (10^{-3} M). Muscle fibers were identified according to their passive electrical properties. Tonic fibers had a large effective resistance (Vo/Io) of 4.3 ± 0.9 MΩ (n = 11) while Vo/Io was of 0.7 ± 0.3 MΩ (n = 4) in twitch fibers. Application of epinephrine (10^{-6} M) to the bath enhanced delayed potassium current in approximately 40% (n = 5). Isoproterenol (10^{-6} M) (β-agonist) produced similar effects (n = 3). These effects were observed after 3 minutes of exposure and were more clearly observed when membrane was depolarized in the range of -20 to +60 mV. The kinetic of delayed potassium current remained unaffected after β-adrenergic stimulation. When tonic bundle was incubated for 30 minutes with the β-adrenergic blocker, propranolol (10^{-6} M), neither epinephrine nor isoproterenol (10^{-6} M) enhanced delayed potassium currents. These results demonstrated the existence of β-adrenergic receptors in tonic muscle fibers related to regulation of delayed potassium channels.

Supported by SEP-SESIC 84-01-0126/8, 84-01-0126/10 and Fondo R. J. Zevada 11-86 grants.
*CONACYT Fellowship.

W-Pos193 TOLUENE ACTIVATES THE 'S' K CURRENT IN APLYSIA NEURONS. V. Březina. Dept. of Biology, UCLA, Los Angeles, CA 90024.

In a recent study of the effects of arachidonic acid and its metabolites on Aplysia neurons (Piomelli et al. 1987, Nature 328, 38), these compounds were dissolved in toluene at one stage of the procedure, and residual toluene may have been present in the final mixture applied to cells. I have therefore investigated possible effects of toluene, recording voltage responses from pleural sensory neurons (studied also by Piomelli et al.) and currents, using two-electrode voltage-clamp techniques, from cells L2-L6 and R2 in the abdominal ganglion of Aplysia californica. Toluene, dispersed in bath solution and puffed or superfused, hyperpolarized the cells to limiting potentials between -75 and -85 mV, close to E_K. Under voltage clamp at -40 mV, toluene elicited corresponding outward currents; these reversed between -70 and -85 mV, and the reversal potential shifted with changes in extracellular K^+ concentration as predicted for a K-selective conductance. Large currents (>300 nA in cell R2) were activated with puffs of medium containing 0.01-0.1% toluene, and much lower concentrations are thus likely to be effective. Benzene appeared to be as effective as toluene, xylene and hexane less so. The toluene-induced K current was blocked by injection of TEA^+ or Cs^+ into the cell and by replacement of extracellular Ca^{2+} with Ba^{2+}, but not with Co^{2+}. I-V relations showed outward rectification and moderate voltage dependence for the K current, as well as an extra component of net inward toluene-sensitive current at positive potentials that disappeared in Ca-free solution, and may represent direct or indirect suppression of Ca-dependent K current. These results reproduce those of Piomelli et al., which these authors attributed to activation of 'S'-type K current by arachidonic acid metabolites. While the current is likely to be the 'S' current, the possibility cannot be ruled out that in the published experiments toluene, not arachidonic acid, was the active agent.

W-Pos194 PATCH-CLAMP AND WHOLE-CELL RECORDING FROM HUMAN OOCYTES. L. J. DeFelice, M. Mazzanti, J. Murnane, and J. Cohen, Emory Univ., Anatomy and Cell Biology, Atlanta, GA 30322

Recently we began to study the factors that control maturation of human oocytes by examining changes in the developing membrane of mature and immature cells. By comparing the membrane at different stages of development, we hope to understand the mechanisms behind maturation and to establish criteria for evaluating the success of hormone-controlled, in vitro maturation. The mature oocytes come from superovulated women undergoing treatment for infertility. The immature oocytes are aspirated from ovarian follicles during elective surgery. Oocytes are transported from the hospital in Dulbecco's balanced salt solution at pH 7.4 and 37°C. Prior to the experiments, we remove the cumulus mass, the follicular cells, and the zona pellucida either with pronase or manually; this results in a clean surface membrane suitable for patch clamping. Cell-attached patches were possible using either procedure, but the whole-cell experiments were more successful without the enzyme. Mature oocytes have a resting potential between -65 and -70 mV and a specific membrane resistance greater than 100,000 $\Omega \times cm^2$. In cell-attached patch experiments on mature oocytes, the most frequently observed channel is a 60 pS (1.3 mM external K), non-inactivating, K-selective pore which is activated by depolarization. This channel bears a strong resemblance to one of the cardiac delayed-rectifier channels (see DeFelice et al., this issue). We are in the process of cataloging the endogenous channels in the mature human oocyte. This catalog will serve as an atlas for evaluating the progression of events during development, and it will also serve as the baseline for determining the success of our in vitro maturation protocols. NIH HD-19770-03 supports this work.

W-Pos195 EFFECTS OF MAGNESIUM IONS ON ATP-SENSITIVE K-CHANNELS FROM RAT PANCREATIC B-CELLS. Frances M. Ashcroft and M. Kakei. University Laboratory of Physiology, Parks Rd., Oxford OX1 3PT, UK.

The effects of Mg ions on single ATP-sensitive K-channel currents were studied using inside-out membrane patches excised from rat pancreatic B-cells and standard patch clamp methods. Rundown of channel activity was prevented by alternating test solutions with a 30s exposure to a control solution (0.1mM ATP, 0.1mM ADP, 2mM Mg). Mg had 4 main effects: 1) it produced a voltage-dependent block of outward currents. 2) It decreased channel activity at all potentials. At -60mV, the dose-response curve was fitted with a Hill coefficient of 1.7 and a K_i of 5mM (free Mg). 3) Mg shifted the relationship between ATP and channel inhibition. When plotted as a function of total ATP, addition of 2mM Mg shifted the K_i from 4μM to 26μm. No shift was found when dose-response curves were plotted as a function of free ATP (K_i=2μM). Since addition of Mg will reduce free ATP this suggests free ATP is a more potent channel inhibitor than MgATP. 4) MgATP is required to reactivate rundown channels. Mg-free solutions and non-hydrolysable ATP analogues are ineffective, suggesting a phosphorylation step is involved in maintaining channel activity.

W-Pos196 AN INWARDLY RECTIFYING POTASSIUM CHANNEL IN CHICK LENS EPITHELIUM. J.L. Rae, Depts. of Physiol. and Biophys. and Ophthalmolgy, Mayo Foundation, Rochester, MN 55905

On-cell patches of apical membrane of chick lens epithelium contain an inwardly rectifying K+ channel which has several subconductance levels. Its main conductance level is a saturating function of the external [K+], reaching a maximum of 30-35pS at 150 mM. The steady state open probability increases monotonically with depolarization but no outward current can be seen at voltages positive to the reversal potential. At 9°C, voltage steps positive to E_K do not produce even transient outward currents in a 10 KHz bandwidth and so rapid channel closure cannot be demonstrated to be the cause of the rectification. When the transpatch voltage is stepped from values positive to E_K to values very minus to E_K, the channels are usually in the open state immediately following the step. The IV relationship shows obvious curvature beginning 10 to 15 mV negative to the reversal potential; the single channel conductance even over the limited range where it can be measured is not ohmic. Internal blockers have not yet been studied because the channel disappears rapidly from inside-out patches.

The channel is blocked by micromolar $[Cs+]_o$ in a highly voltage dependent way. This block can be characterized either by determining the mean open and mean closed times inside of bursts or by determining the difference spectrum between the open and closed channel currents. The histograms of open and closed times are each a single exponential. The difference spectrum is a single Lorentzian whose corner frequency is a function of voltage and $[Cs]_o$. A single binding site model for the block is inadequate to explain the voltage dependence of the power spectra and of the mean single channel currents.

W-Pos197 INWARD RECTIFYING K CHANNELS INDUCED BY IONOMYCIN IN CULTURED HUMAN MACROPHAGES. by E. K. Gallin, Physiology Dept.,AFRRI, Bethesda, Md.20814

Previous studies in this laboratory have described a 200 pS Ca-and voltage activated K channel and a 29 pS inward-rectifying K channel in macrophages. This study demonstrates that human peripheral blood monocytes cultured for 3-30 days exhibit a different K channel induced by the calcium ionophore ionomycin (10^{-6} M). When the patch electrode and bath contained (in mM) 148 KCl,10 NaCl, 1.1 $MgCl_2$,1.6 $CaCl_2$,10 HEPES, pH 7.3,and 148 NaCl,4.6 KCl,1.1 $MgCl_2$,1.6 $CaCl_2$, 10 HEPES, pH 7.3, respectively, 10/11 cell-attached patches exposed to ionomycin showed bursting inward single-channel currents (absent before ionomycin) at potentials more negative than -50 mV. Single-channel conductance (G) and extrapolated reversal potential (E_{rev}) for the bursting channel were 36 $\pm$ 4 pS and 58 $\pm$ 8 mV, respectively. Depolarization revealed 200 pS Ca-activated K currents but smaller outward current fluctuations were absent, indicating that the ionomycin-induced channel rectifies. Similar data was obtained with Kaspartate in the patch electrode. Decreasing K in the electrode to 75 mM by substitution with NaCl or isosmotic sucrose decreased the G to 25 pS and shifted E_{rev} to 44 mV. With 50 mM $[K]_o$ and 200 mM sucrose in the pipette SCG and E_{rev} were 16 pS and 31 mV, respectively. Thus, the ionomycin-induced channel is permeant to K and SCG = $c(K_o)^{1/2}$. As expected for a K channel, E_{rev} shifted to 0 mV for cells bathed in 148 mM KCl. 36 pS bursting inward channels were sometimes noted in patches not exposed to ionomycin immediately after obtaining a tight seal and then usually subsided. In summary, the 36 pS channel is distinct from the 29 pS inward-rectifying channel since 1) it is found both in patches with and without the 29 pS channel, 2) it has a larger conductance, 3)it has different kinetics and 4) it is activated by ionomycin.

W-Pos198 CALCIUM-ACTIVATED POTASSIUM-CHANNELS IN DOG COLON A.Carl and K.M Sanders Dept. of Physiology, University Nevada, School of Medicine, Reno, NV 89557

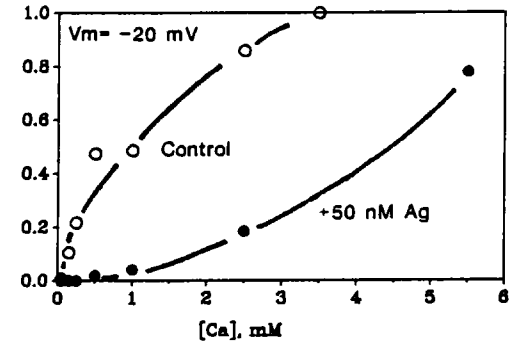
Ionic channels of canine colonic muscle cells were studied with the patch-clamp technique. A dominant population of spontaneously active channels was observed in nearly every patch. These channels were identified as K^+-channels by varying the K^+-gradient across off-cell patches. Channel activity was dependant on voltage and $[Ca^{++}]_i$ in a range of 10^{-4} to 10^{-9}. The slope-conductance of these channels in symmetric 140 mMol K^+-solution was 170 $\pm$ 30 pS. The slope-conductance in physiological K^+-gradients was 125 $\pm$ 30 pS and the reversal-potentials averaged -38 $\pm$ 13 mV. The Nernst potential calculated from this gradient was -82 mV. Thus we studied the selectivity of these channels. Replacing the bath-solution of symmetrical 140/140 $[K^+]_i/[K^+]_o$-solution with 140 mMol of a cation X the relative permeability P_X/P_K can be determined from the shift in reversal-potential according to the relationship of Hille (1975).

$$E_{rev} = \frac{R\,T}{F} \ln \frac{P_K \cdot [K^+]_o}{P_X \cdot [X^+]_i}$$

We found $K^+ > Rb^+ > Li^+ > Na^+$ as 1 : 0.5 : 0.07 : >0.07 . Thus the Na^+-conductance determined by this method was not sufficient to cause the deviation from the Nernst potential. Interaction between Na^+ and these channels was observed, however. When K^+ was replaced by Na^+ 2 types of blocking occurred: 1.) flicker-block (big increase in noise and decrease in channel-amplitude); 2.) change in channel-kinetics from long bursts to short openings (Supported by NIH AM 38717)

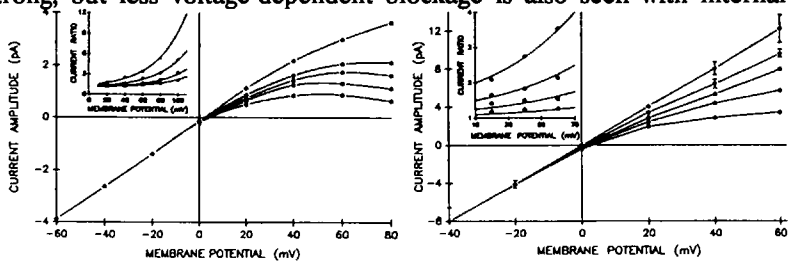
W-Pos199 NANOMOLAR SILVER ION IRREVERSIBLY MODIFIES GATING OF Ca-ACTIVATED K CHANNELS FROM RAT BRAIN IN PLANAR LIPID BILAYERS. Dieter K. Bartschat, Samuel Cukierman and Bruce K. Krueger, Department of Physiology, University of Maryland School of Medicine, Baltimore, MD 21201.

The effects of Ag^+ on single Ca-activated K channels (CAKC's) from rat brain were studied in planar bilayers. In symmetrical 250 mM KCl and 50 µM Ca^{2+}, the single channel conductance (γ) was 250 pS and the CAKC was blocked by extracellular charybdotoxin, indicating that this channel is similar to the "maxi" CAKC reported in a variety of tissues. Intracellular addition of 50 nM $AgNO_3$ (0.7 nM free Ag^+ based on solubility product), decreased the open probability (P_o), resulting in a depolarizing shift in the P_o vs V_m relation. Extracellular addition of $AgNO_3$ was without effect; Ag^+ never affected γ. The effect of Ag^+ was antagonized by raising Ca^{2+}_i from 50 µM to > 1 mM (see figure). High (> 1 mM) Ca^{2+}_i prevented the Ag^+-induced shift in P_o. The effect of Ag^+ was not reversed by perfusion, indicating that Ag^+ may cause an irreversible modification. The action of Ag^+ appears to be specific for the CAKC because intracellular 500 nM $AgNO_3$ had no effect on the gating of batrachotoxin-activated, voltage-dependent Na channels from rat brain. These results suggest that Ag^+ acts from the intracellular side of the CAKC, causing an irreversible decrease in sensitivity to Ca^{2+}. Supported by NIH and U. S. Army Med. Res. Dev. Comm.



W-Pos200 MAGNESIUM BLOCKAGE OF TWO K CHANNELS IN AN INSULIN-SECRETING CELL LINE. B. Ribalet, S. Ciani and G.T. Eddlestone. Dept. of Physiology; BRI; JLNRC. Univ. of Calif., Los Angeles, Ca 90024.

Two potassium channels have been identified in cell membranes of the insulin-secreting cell line RINm5F using the Patch Clamp technique. One is modulated by ATP, (K(ATP) channel), the other by Ca^{2+} and voltage (K(Ca,V) channel). Both play a role in the membrane potential response to glucose. The figure to the left displays I-V curves for the K(ATP) channel in an excised inside-out patch in symmetrical 140 mM K^+ solutions, Mg^{2+} bathing only the intracellular side of the membrane. In the direction of increasing blockage, the Mg^{2+} levels were (mM): 0, 2.2, 5, 10, 20. The inset to the figure shows ratios of the currents in the absence of Mg^{2+} to those in the presence of the cation. Data fitting with an Eyring-type model suggests competition beween Mg^{2+} and K^+ for a binding site within the channel (K_D(Mg)=34 mM; K_D(K)=290mM), located at one third of the pore-length away from the inner mouth. Strong, but less voltage-dependent blockage is also seen with internal Na^+ (K_D(Na)=59mM). The figure to the right shows that Mg^{2+} has similar effects upon the K(Ca,V) channel (K_D(Mg)=41mM) under the ionic conditions described above. Channel blockage by internal Mg^{2+} and Na^+ may be important mechanisms to prevent loss of K^+ in conditions of depressed metabolism.



W-Pos201 REGULATION OF HIGH CONDUCTANCE Ca-ACTIVATED K (P_{KCa}) CHANNELS BY GUANOSINE NUCLEOTIDES. D. Williams, G. Katz, D. Himmel, and J.P. Reuben, Merck Institute, West Point, PA 19486 and Rahway, NJ 07065

P_{KCa} channels within on-cell patches on cultured bovine aorta smooth muscle cells can be potentiated (increased open time and number of openings) by dibutyryl cGMP (1-2 mM) and by vascular smooth muscle dilatory agents that increase cytoplasmic levels of cGMP. The latter include nitroprusside (10 uM), adenosine (10 uM), and ANF (10^{-7} M). In light of these findings, a series of guanosine and adenosine nucleotides were tested on inside out excised patches in bathing solutions containing (in mM) 7 EGTA, 5 Ca, 135 KCl, 1 Mg, and 5 Hepes (pH 7.3). The electrode solution contained 150 KCl, 1 Mg, 5 Hepes (pH 7.3). While cAMP, AMP, ADP, and ATP were ineffective, all of the guanosine nucleotides potentiated P_{KCa} channel activity (up to 500 uM). However, only GMP consistently enhanced channel activity in the 10-100 uM range. Channel conductance was not modified by GMP. The phosphate moeity is essential for channel modulation, since guanine and gaunosine (500 uM) were ineffective. GMP increased the % open time over a wide range of holding potentials (E_H's), and the increase in this percentage can be sizeable. For example, at 50 uM GMP and E_H=-20 mV, the % open time increased 20 X control value. These data suggest that GMP could be a modulator of P_{KCa} channels under physiological conditions, but it is not clear whether GMP is the critical second messenger for agents that increase cytosolic cGMP levels. The latter would require a step to convert cGMP to GMP.

W-Pos202 THE DETERMINATION OF INTRACHANNEL CHARGE DENSITY FROM PERMEABILITY, T.L. Schwartz, Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT 06268.

New theoretical expressions having a very wide range of applicability, derived from very general thermodynamic expressions have been utilized to calculate the intrachannel charge density of a potassium selective cholinergic channel in Aplysia californica directly from measurements of the channel's permeability under a variety of conditions.

This calculation depends on the relationship between the intrachannel electric field and the permeability. This relationship is laid bare by these new theoretical expressions. Older theoretical treatments of ionic permeability which depend on the rather arbitrary assumption of a constant intrachannel electric field are incapable of this insight. This failure is due to the fact that the constant field assumption "sweeps under the rug" precisely that information required for the investigation.

Qualitative results correspond well with those yielded by physical intuition regarding these phenomena. Thus, increasing permeability corresponds to increasing negativity of intrachannel charge. Decreasing permeability corresponds to increasing positivity of intrachannel charge.

W-Pos203 TWO TYPES OF POTASSIUM CHANNELS IN A LYMPHOMA CELL LINE. by M.S. Shapiro & T.E. DeCoursey, Dept of Physiology, Rush Medical Center, Chicago, IL 60612.

The gigohm-seal technique was used to study ion channels in the Louckes (a non-EBV American Burkitt's lymphoma) cell line. Studied in the whole-cell configuration with pipettes containing (in *mM*) 130 Cs-aspartate, 5 MgATP, 2 $MgCl_2$, and 10 HEPES, pH 7.2, and "Ringer" in the bath (160 NaCl, 4.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 5 HEPES, pH 7.4), many cells displayed inward rectification, with inactivation or block of inward currents at potentials negative to about -100 mV. Bathed in 160 K^+ Ringer, the inward currents increased >10-fold, and the apparent reversal potential shifted to more positive potentials by about 55 mV, indicating a largely K-selective conductance. In a few cells, the outward currents seen turned off at positive potentials, giving rise to a region of negative slope conductance. Adding 5 mM Ba^{++} to K^+ Ringer induced voltage-dependent block of inward currents. Observed reversal potentials could be accounted for using the Goldman-Hodgkin-Katz equation, by setting P-Cs/P-K at about 0.3 and P-Na/P-K at about 0.05.

In on-cell patches, a variety of unitary currents were seen. Many patches had a strongly inwardly rectifying channel, with a slope conductance of 33-55 pS negative to the resting potential with K^+ in the pipette, or 12-15 pS with Cs^+. This channel showed no obvious voltage-dependence. In on-cell patches with 5 Ba^{++} K^+ Ringer in the pipette, voltage-dependent block of inward currents was observed. This channel appears to be responsible for the whole-cell currents described above.

In addition, most patches and cells had type 'l' K^+ channels, identified by their distinctive voltage-dependent gating and TEA sensitivity (*J. Gen. Physiol.* 89:379). To our knowledge this is the first observation of type 'l' channels in cells of human origin. (Supported by N.I.H.)

W-Pos204 POTASSIUM CURRENTS IN HAIR CELLS ISOLATED FROM THE COCHLEA OF THE CHICK. P.A. Fuchs and M.G. Evans. Dept. Physiol. U. Colorado Health Sciences Center, Denver, Colorado. 80262.

We have used tight-seal, whole-cell voltage clamp recordings to identify a variety of K currents in solitary chick cochlear hair cells. Tall hair cells from the middle third of the cochlea ('basal cells') were dominated by a Ca-activated K current (I_{Kc}) in the voltage range positive to -50 mV. I_{Kc} reversed at E_K, required Ca in the external saline and was half blocked by 0.5 mM tetra-ethyl ammonium (TEA). I_{Kc} activated within 1 to 5 msec in different cells, and the rate of tail current decay increased e-fold with 22 mV hyperpolarization. Virtually no other K current was seen in basal cells in the voltage range -100 mV to 0 mV. In contrast, tall hair cells from the apical tip of the chick cochlea (apical cells) expressed a variety of K currents. While some I_{Kc} was present in these cells, significant outward current was carried through K channels which were solely voltage-activated. This Ca-independent K current (I_{Kv}) was slow, with half rise times near 20 msec. Tail currents reversed at E_K and their relaxations showed little voltage dependence, changing e-fold over 100 mV. I_{Kv} was not affected by 10 mM TEA, which completely blocked I_{Kc}. K_v channels appeared to have a smaller conductance than K_c channels, as judged by the marked difference in current noise. Finally, only apical cells had an inward rectifier current (I_{IR}). I_{IR} was activated near -85 mV, reversed at E_K, and was blocked by external barium (0.1 mM) or cesium (5 mM) ion. Supported by NIH grants NS01007 and NS21454.

W-Pos205 EFFECTS OF AMMONIUM CHLORIDE (NH_4Cl) AND SODIUM PROPRIONATE (NaPr) ON THE ACTIVITY OF A METABOLITE REGULATED K^+ CHANNEL IN RAT PANCREATIC ISLET AND RIN INSULINOMA CELLS. K. Gillis, J. Tabcharani, A. Hammoud, and S. Misler. The Jewish Hospital, St. Louis, MO.

Metabolite-induced electrial activity of B cells is depressed by bath application of weak bases, but is augmented by weak acids. Changes in membrane P_K are implicated in these effects (e.g. 20 mM NH_4Cl hyperpolarizes B cells and decreases membrane resistance within 1-2 min as it alkalinzes). These manuevers affect the mean activity (I/i) of the inward rectifying, metabolically regulated K^+ channel, K^+ (MR) Cell Attached Patch Recording: (1) NH_4Cl exposure at constant pH_0=7.25. Addition of 15-20 mM NH_4Cl to Ringers produces a 2-3 fold increase in I/i usually peaking within 1-2 min. Typically I/i falls within 5 min to baseline levels. Washout of NH_4Cl produces an immediate dramatic decrease in I/i to levels below baseline. Recovery occurs within 5-10 min but is slowed by amiloride (200 μM) or replacement of Na^+ with N-Me-glucamine. Similar results were obtained with RINm5F cells. (2) NaPr exposure. Addition of 20 mM NaPr produces a transient decrease in I/i which is sometimes enough to provoke spikes in the absence of glucose. Recovery occurs as fast as 2 min. These results are consistent with K^+(MR) being directly sensitive to ΔpHi or some process directly affected by ΔpHi. Excised Patch Recording. I/i of K^+(MR) is directly sensitive to pHi, being greater at alkaline than acid pHi's. Changing pHi from 8.0 (in HEPES) to 6.2 (in PIPES buffer) produces a Δ%(I/i) roughly comparable to that shown in cell attached patches between peak activity 1-2 min after NH_4Cl exposure and trough activity 1-2 min after washout. Measurement of pHi during NH_4Cl, NaPr and glucose exposure and washout should help determine the contribution of pHi gating to the physiological regulation of K^+(MR) activity.

W-Pos206 CYCLIC CHANGES IN MEMBRANE SURFACE AREA AND POTASSIUM INWARD RECTIFIER DURING CELL DIVISION IN EARLY BLASTOMERES FROM BOLTENIA VILLOSA EMBRYOS. M.L. Block and W.J. Moody, Dept. Zoology, NJ-15, University of Washington, Seattle Washington 98195

Many changes occur in cell membrane and cytoplasm as oocytes mature and as blastomeres differentiate during development. Some of these changes are due to the requirements of cell division. The 2-electrode voltage-clamp and whole-cell patch clamp techniques have been used to investigate these cyclic changes during development in individual blastomeres from embryos of the ascidian Boltenia villosa,and in these same cells cleavage-arrested with cytochalasin B. A rise in membrane capacitance (C_m) is seen in cronic voltage-clamp recordings of normal 1-cell embryos from after fertilization until the time of first cleavage. This surface area increase is presumably due to a cell's addition of membrane in preparation for first cleavage. The magnitude and kinetics of the potassium inward rectifier (IR) also change with time during cronic voltage-clamp recordings. In cytochalasin B (4ug/ml) treated cells, over the time required for three nuclear divisions, C_m rose just before and during each nuclear division and fell after nuclear division was complete. The IR also changed in a cyclic manner. Just before and during nuclear division the magnitude of the current increased and the kinetics changed, both effects being very dramatic. After nuclear division was complete the magnitude and kinetics reverted to their values before division began. The changes observed in the potassium inward rectifier do not appear to be a result of either channel protein additions to the membrane or to changes in E_k. The time courses of the two effects are very similar, which suggests that both the C_m and IR changes may be mediated by a common cytoplasmic event. Supported by grant NS07775 to MLB and HD17486 & Res. Career Dev. Award to WJM

W-Pos207 PATCH-CLAMP ANALYSIS OF POTASSIUM CURRENTS IN CULTURED INSECT NEURONES. Y.LARMET, B.N.CHRISTENSEN, Y.PICHON. Dept. Biophys., C.N.R.S. Lab. Neurobiol. Cell. Mol. Gif-sur-Yvette,France.

Neurones isolated from embryonic cockroach brains were maintained in culture for several weeks. The present study was designed as an attempt to characterize the single channel events which underlie the voltage-dependent potassium conductance. The cell-attached, cell-free inside-out and outside-out configurations of the patch-clamp techniques were used to analyse the effects of membrane depolarization. Long recordings (several seconds or tens of seconds) were taken for each potential level and analyzed off line. The polarity of the unitary currents which were recorded under those conditions was consistent with that expected from the whole cell recordings of the potassium current although their reversal potential was in some cases more positive than the calculated K+ equilibrium potential. Three types of channels were observed. The most common type consisted into very brief openings which were too short to be properly analyzed properly with our experimental set-up. A second class of channels was characterized by open times that did not change with the membrane potential. This class contained two categories of openings with conductances of around 15 pS and 30 pS and mean open times of 4.7 ± 0.6 ms and 5.4 ms ± 1.1 ms . The third type of single channel activity consisted of opening that were larger (110 pS) and occurred in longer burst of discrete steps. In most cases, the open time probability was almost independent of the membrane potential. The results suggest that, in cultured insect neurones, the voltage dependent potassium conductance of is due to an increase in intracellular calcium.

W-Pos208 NON-SELECTIVE MONOVALENT CATION CHANNELS IN FROG SKELETAL MUSCLE. R. Godinez, F. Estrada and J.A. Sanchez. Department of Pharmcology, CINVESTAV, Mexico City, A.P. 14-740, 07000, Mexico.

The presence of non-specific monovalent channels has been documented in other preparations. Our experiments examine its presence in skeletal muscle. Methods: The triple vaseline gap voltage clamp technique for cut muscle fibers (Hille & Campbell, J G P (1976) 67:265-293) and conventional intracellular techniques in intact fibers were used. Membrane currents were continuously recorded by a VCR at 16 bits and were also sampled at 12 bits by a microcomputer every 60 µs during 100 ms command pulses. Solutions (mM) External: C^+=117, Cs^+=2.5, Ca^{2+}=1.8, TTX=0.1, where C^+=monovalent cation, pH=7.2, $CH_3SO_3^-$ was used as anion: Internal: Cs -aspartate =110, Mg $(CH_3SO_3)_2$=6.5, $Ca(CH_3SO_3)_2$=0.069, Na_2ATP=5 and EGTA=0.2, pH=7.2, T=20-22°C. Results: When the impermeant cation N-methylglucamine was replaced by Na^+ at Eh=-90mV an inward current became apparent, the effect was fully reversible. Inward currents with different cations were as follows: (µA/cm²) Na^+=1.7±0.3(5), TEA=1.8±0.2(5), K^+=0.7±0.5(4), Cs^+=3.4±0.4(4), NH_4^+=9.5±0.8(7), Li^+=2.2±0.3(5), Rb^+=2.8(2), Ca^{2+} was not measurably permeant. The sequence in the amplitude of inward currents was: $NH_4^+ > Cs^+ > Rb^+ \geq Li^+ > TEA^+ \geq Na^+ > K^+ >> Ca^{2+}$. IC did not show any strong voltage dependence and the Na^+ measured at different Eh was linear with a slope of 6.6 µS/cm²(1) and a reversal potential ca. 0 mV. Amiloride (0.4 mM) partially blocked IC^+. The presence of IC^+ at -90 mV suggests a role in the resting potential. Accordingly, in intact fibers, resting potentials were -89.9±0.4(9) and -109.8±0.9(9) after amiloride.

W-Pos209 K^+ Channels from yeast plasma membranes incorporated in black lipid membranes. F. Gómez-Lagunas*, A. Peña*, A. Liévano**, & A. Darszon**. *Instituto de Fisiología Celular, Universidad Nacional Autónoma de México and ** Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, México.

Yeast plasma membranes present a high K^+-permeability which apparently exerts a regulatory role on the resting membrane potential set by the H^+-ATPase. Recently Gustin, et al., (Science (1986) 233:1195) recorded K^+-Channels in the plasma membrane of these cells. With the purpose of extending the study of yeast channels, we have begun to look for their properties in model membranes. Plasma membrane fragments, from both commercially obtained yeast (La Azteca, S.A.) and the wild type Xt3000.3A strain, were purified according to Calahorra et al., (B.B.A. (1987) 899:229). The isolated plasma membranes were fused with black lipid membranes made of PE/PS. Using a gradient of KCl, 300/100 mM (Cis/Trans), pH 8.0, across the bilayer, we have most frequently detected two types of ionic channels with unitary conductances of 65 and 112 pS. Both showed reversal potentials consistent with a K^+-selectivity. The channels were blocked by 10 mM TEA or 10 mM $BaCl_2$. The 65 pS channel is voltage-dependent. This work was partially supported by grants of CONACyT (Fellowships to F.G.L. and A.L.) and the Organization of American States.

W-Pos210 A SODIUM-ACTIVATED POTASSIUM CURRENT IN BRAINSTEM NEURONS. Dryer, S. E.*, Fujii, J. T.*, and Martin, A. R., Dept. of Physiol. University of Colorado, Denver, CO.

We have used patch-clamp recording techniques to examine a sodium-activated potassium current in cultured chick brainstem neurons. Under conditions of whole-cell clamp, depolarizing commands from a holding potential of -60 mV evoked a transient outward current the amplitude of which was dependent on the size of the preceding inward current. This transient outward current decayed completely within 10 msec and was abolished by bath application of 1 μM TTX. The current was not detected when all external Na+ was replaced with Li+. In the presence of Li+, TTX-sensitive inward currents of close to control amplitudes were observed. Tail-current measurements revealed that the TTX-sensitive outward currents reversed close to the calculated potassium equilibrium potential (-79 mV). These results together demonstrate the presence of a sodium-activated potassium current in brainstem neurons. We have also examined the properties of single sodium-activated potassium channels in inside-out patches. These channels were quiescent in bath solutions containing 150 mM Li+, but were open nearly continuously in 150 mM Na+. The channels remained active as long as Na+ was present, and reversed at the calculated potassium equilibrium potential. Channel openings were observed at a lower frequency in 50 mM Na+. Under these conditions openings occurred in bursts interrupted by many brief closures. Individual bursts were separated by relatively prolonged closed periods. The main channel conductance was 50 pS when [K]o = 150 mM and [K]i = 5 mM. In addition a subconductance state of 16 pS was observed in association with bursts to the main state. Subconductance states were only rarely observed in isolation. Direct transitions between closed, subconductance, and main states were observed.

W-Pos211 CHANGES IN CALCIUM CURRENTS OF STRIATED MUSCLE DURING TADPOLE METAMORPHOSIS. García, J. and Stefani, E.; Dept. Physiology, Biophysics and Neurosciences, CINVESTAV-IPN, México, and Baylor College of Medicine, Dept. Physiology and Molecular Biophysics, One Baylor Plaza, Houston, Texas 77030.

We have studied some properties of calcium currents in tail cut fibers of tadpoles at premetamorphic and prometamorphic stages using the double vaseline gap voltage-clamp technique. Two calcium currents were elicited differentially in both stages: in most of the premetamorphic fibers we detected a fast activated current (I_f) and, only in a small percentage (33%), a slow activated one (I_s); as maturation progresses (prometamorphic stage), I_s was more frequently found (70%). I_f was present in all. The I-V curves were similar in both stages. Inward currents increased in amplitude when Ca^{2+} was replaced by Ba^{2+} in the recording solution (Ba/Ca ratio ~3.5 for I_f and ~2.8 for I_s), decreased by the lowering of Ca^{2+}, and were reversible blocked by 10 mM-Mg and 2 mM-Cd. When Ca^{2+} was replaced by Ba^{2+}, the curve was well described by the Boltzmann equation, with a mid-point potential (V1/2) of -44 mV and a steepness (k) of 9 mV. The inactivation curve was bell-shaped for I_f, reaching the maximum at -50 mV in premetamorphic tadpoles, while in prometamorphic it was displaced by about 20 mV to the left, suggesting a potentiation of calcium current by previous calcium entry. The inactivation curve for I_s had a V1/2 = -39 mV and k = 5.7 mV for premetamorphic tadpoles and -51 mV and 9 mV for prometamorphic. These results indicate that I_f develops earlier than I_s in the metamorphosing tadpoles and both show posterior changes during the course of maturation. Supported by NIH Grant AM35085.

W-Pos212 CALCIUM CURRENTS IN SINGLE UTERINE SMOOTH MUSCLE CELLS. E. Stefani, L. Toro and S.D. Erulkar.* Dept. Fisiologia, Biofisica y Neurociencias. CINVESTAV-IPN, Apdo. Postal 14-740, México; Dept. Physiology and Molecular Biophysics, Baylor College of Medicine, Houston, Texas 77030; *Dept. Pharmacology & Mahoney Institute of Neuroscience, University of Pennsylvania, Philadelphia, PA 19104.

Calcium currents I_{Ca}, were recorded from single myometrial cells of non-pregnant rats (≃200 g), in culture (2-5 day old). The whole cell configuration of the patch clamp technique was used. External solution contained (mM): 120 TEA $(CH_3SO_3)_2$, 5 HEPES-TEA, 1 3,4-diaminopyridine, 65 sucrose. Pipette solution contained Cs^+ as the main cation plus 20 mM TEA. I_{Ca} had an activation potential, V_a = -40 ± 7 mV (n = 5) (± S.D., n = number of cells); time to peak, t_p = 13 ± 3 ms (n = 7); maximum current potential, V_{Imax} = 18 ± 9 mV (n = 4); and a maximum slope conductance, G = 18 ± 6 $\mu S/cm^2$ (n = 4). This I_{Ca} was also seen when Ringer-Krebs was used as the external solution and K^+ was the main cation in the pipette. Depolarizations above -30 mV elicited outward currents that overlapped I_{Ca}, modifying its time course. In conclusion, myometrial cells possess Ca^{2+} channels whose activation gives rise to an inward current that predominates during the first ms, over the large K^+ outward currents. This behavior suggests that these Ca^{2+} channels have a meaningful role in the excitation of uterine smooth muscle. Supported by grants NS12211, 5ROIAR, AM3508503 (NIH).

W-Pos213 THE DECAY OF THE SLOW CALCIUM CURRENT IN TWITCH MUSCLE FIBRES OF THE FROG (Rana Pipiens). F. Francini and E. Stefani, Dept. Physiology, CINVESTAV-IPN, México, and Baylor College of Medicine, Dept. Physiology and Molecular Biophysics, Houston, TX, 77030, U.S.A.

The decay of the slow Ca^{2+} current was studied, at 17°C, in single cut fibres from semitendinosus muscle by using the double vaseline gap technique. External solutions (mM): (ES1) TEA-CH_3SO_3 105; 3,4 DAP 1, TEA-HEPES 5 and Ca-$(CH_3SO_3)_2$ 10; (ES2) Ca^{2+}-buffered: $(TEA)_2$-malate 15, Ca-malate 126, TEA-HEPES 10. Internal solutions (mM): (IS1) NaF 20, Cs_2-EGTA 20, $MgCl_2$ 6.9, Cs-glutamate 50, Na-pyruvate 10, Cs-HEPES 10, Na_2-ATP 5 and dextrose 5; (IS2) $(TEA)_2$-EGTA 70, Na_2-HEPES 10, Na-pyruvate 10, Na_2-ATP 5 and dextrose 5. I_{Ca} decay (τd) had a constant value (1.4 sec) in ES1 and IS1, during depolarizing pulses (-20 mV to 50 mV from -90 mV), in inactivated I_{Ca} and after replacing ES1 with ES2 (Ca-buffered solution). With similar pulses but with IS2 (EGTA: 70 mM) and ES1, τ_d was also constant but three times faster (0.54 sec). In two pulse experiments, with 300 msec prepulses to +80 mV, with ES1 and IS1, I_{Ca} decayed with a single exponential and a constant τ_d during the second pulse at various potentials (-20 to +40 mV). On the other hand with high intracellular EGTA (IS2), I_{Ca} decayed with two exponentials, one slow (τ_{d2}), similar to the one observed during long depolarizing pulses (0.53 sec), and a first one three times faster (τ_{d1}: 0.16 sec). τ_{d2} had a constant value at different potentials and τ_{d1} becames faster with larger current amplitudes. With high EGTA (IS2) and Ca^{2+}-buffered solution (ES2) between -20 to 40 mV, τ_{d1} was not detected and τ_{d2} was slower and constant. These data support the idea that tubular Ca depletion depends on the composition of intracellular solution, being more evident with high intracellular EGTA. Supported by Grant NIH AM 35085.

W-Pos214 CALCIUM CURRENTS FROM ISOLATED BOVINE CAROTID ARTERY SMOOTH MUSCLE CELLS.

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Whole-cell Ca^{++} currents were studied from enzymatically isolated Bovine carotid artery smooth muscle cells which were relaxed (length = 150±8 x 10^{-6} m) and contracted in response to both hormonal agonists and high K^+ depolarization. Ca^{++} currents were measured using single microelectrode, whole-cell voltage clamp methods with 110 mM $BaCl_2$ as the charge carrier. K^+ currents were blocked by replacing KCl with 130 mM CsCl in the pipette.

Two types of Ca^{++} currents were identified which exhibited properties similar to those previously characterized in rabbit ear artery (Benham et al., Circ. Res, 61(supplI):10-16,1987) and dog saphenous vein (Yatani et al., Circ. Res, 60:523-533, 1987). I_{low} (HP = -80 mV) activated at -40 mV and peaked at potentials near 10 mV. I_{high} (HP = -30 mV) peaked at pulse potentials near 40 mV. Kinetic analyses showed I_{low} to inactivate with a tau near 30 msec at 10 mV and recover with a tau near 70 msec at -80 mV. I_{high} had an inactivation tau near 80 msec at 30 mV and a recovery tau near 400 msec at -30 mV. Although several cells exhibited both current types, others exhibited only I_{low} or I_{high}. We have developed procedures to enzymatically isolate relaxed vascular smooth muscle cells which are suitable for studying calcium currents. (supported by NIH HL 35682,14388 to RCB and AHA IAG-41 to EFS)

W-Pos215 INACTIVATION OF T TYPE CALCIUM CURRENT IN CARDIAC PURKINJE CELLS.

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In canine cardiac Purkinje cells, two types of Ca^{2+} currents (T and L) can be separated by their voltage-dependence of availability. We used the whole cell voltage clamp technique to study inactivation and repriming of the T current. During depolarizing steps, T type current decayed with a single exponential time course. The time constant had a U-shaped voltage dependence with a minimum value of about 10 msec near -10 mV (20 mM $[Ca^{2+}]_o$, 30°C). Unlike L type current, the inactivation of T type current seemed to be purely voltage-dependent. Consistent with surface charge effects, changes in $[Ca^{2+}]_o$ caused a shift along the voltage axis of the inactivation curve and the time constants for inactivation, altering the peak currents. Replacement of Ca^{2+} by Ba^{2+} or Sr^{2+} showed the same effect. Recovery from inactivation was voltage dependent and was affected very little by the ionic species of the charge carrier. The time course of repriming, however, was critically dependent on the protocol used to measure it. Using a standard 2-pulse protocol with a holding potential of -80 mV, recovery of the T type current after a 200 msec long prepulse to -30 or -10 mV had a time constant of 166 ± 17 msec (mean ± S.D., n = 4) when fit with a single exponential. From a depolarized holding potential of -30 mV, hyperpolarizing conditioning steps of variable duration to -80 mV yielded a markedly slower time course of recovery. The time constant was 837 ± 126 msec (n = 3) when fit with a single exponential. Prolongation of the time course of recovery from inactivation was also observed using a 2-pulse protocol where the duration of the prepulse was increased. The results suggest that different inactivated states may exist for T type calcium channels in cardiac Purkinje cells.

W-Pos216 CADMIUM BLOCK OF CALCIUM CURRENTS IN SQUID NEURONS. R.H. Chow and C.M. Armstrong. Dept. of Physiology, Univ. of Pennsylvania, Philadelphia, PA 19104.(Intr. by A.L. Obaid)

Whole cell patch clamp was used to study cadmium block of Ca currents in squid GFL neurons. These cells probably possess a single class of Ca channels, resembling the FD or "L" channel. A constant-flow bath exchange system allowed change of solutions in under 60 s. Currents were recorded in 50 mM external $CaCl_2$, using a potassium-free internal solution. 125 µM Cd reversibly reduced the steady state current by 50% for a step to 0 mV from an HP of -80 mV. Current was reduced more with larger depolarizations: in 62.5 µM Cd, the steady state block (by 10 ms) was 25% at -5 mV and 75% at +40 mV. The inward current "inactivated" for depolarizations to $V_m > +5$ mV; e.g. for a step to +20 mV in 125 µM Cd, the inward current peaked by 1 ms and declined to steady state by 4 ms. In the absence of Cd, the Ca current inactivated only slightly even for depolarizations up to 100 ms long. Internal Cd up to about 2 mM did not produce the inactivating time course. Tail currents in Cd had a "hook": following a 10 ms step to +20 mV in 62.5 µM Cd, tail currents at -40 mV did not reach a maximum amplitude for 140 µs as compared with 40 µs in the absence of Cd, and this maximum was only 60% of that in the absence of Cd. The tail current thereafter relaxed with the same time course as that of tails in control solutions. RHC is a trainee of the Medical Scientist Training Program, grant 5-T32-GM07170.

W-Pos217 CALCIUM CURRENT BLOCK BY HYDROGEN PEROXIDE IN LIGATED NEURONS OF THE LOBSTER CARDIAC GANGLION. D.Livengood, Department of Physiology, Armed Forces Radiobiology Research Institute, Bethesda Maryland, 20814.

Free radicals are thought to be involved in cellular damage resulting from a number of pathological insults, including toxic agents, ionizing radiation and ischemia followed by reperfusion. H_2O_2 is produced as a by-product of the elimination of hydroxyl free radicals. The action of H_2O_2 was examined on the voltage sensitive Ca^{++} currents in the large neurons of the lobster cardiac ganglion. These cells have been shown to have I_{Ca}, I_A, I_K and I_C (Tazaki and Cooke J. of Neurophys.56:1739-1762, 1986). H_2O_2 in the range from 0.35 mM to 3.5 mM was added to the bathing sea water. The I_{Ca} was reduced by 50% to 100% of the control value. The action of H_2O_2 in cells with complete block of I_{Ca} was essentially irreversible. The effect on partial block was reversible. No change in I_K, I_A or in I_{leak} was observed. Twin pulse experiments at a holding potential of -50 mV indicate that inactivation of I_{Ca} is considerably prolonged in H_2O_2. The late outward current in the voltage range from -35 mV to 0 mV was "N" shaped in the presence of 30 mM TEA. This component of the late outward current may reflect the late calcium dependent K current as it was decreased following exposure to Mn. This outward current was greater after exposure to H_2O_2 even though the I_{Ca} was decreased. The increase in the late outward current in the presence of H_2O_2 supports the view that the H_2O_2 induced decrease in I_{Ca} is due to an increase in $[Ca^{++}]_i$ which inactivates the voltage sensitive Ca^{++} current.

W-Pos218 EVIDENCE THAT CALCIUM IONS ENTER CHROMAFFIN CELLS THROUGH A VOLTAGE-DEPENDENT PATHWAY WHICH IS INSENSITIVE TO DIHYDROPYRIDINES AND ω-CONOTOXIN

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The increase in intracellular free calcium concentration ($[Ca^{2+}]_i$) that follows depolarization of the chromaffin cell is thought to play an important role in the exocytotic release of catecholamines. We have studied the modulation of $[Ca^{2+}]_i$ by membrane potential in bovine adrenal medulla chromaffin cells by monitoring the fluorescence of cells loaded with the Ca^{2+} indicator FURA II. Following stimulation with high K^+ concentrations (10-65 mM), $[Ca^{2+}]_i$ first rapidly increased to a peak value and then decreased to a "plateau" level with a much slower time course.The relationship between the rapid $[Ca^{2+}]_i$ increase ($\Delta[Ca^{2+}]_i$) and the calculated membrane potential could be well described by the Boltzmann distribution function for two state transitions(half-maximal activation at -23 mV). $\Delta[Ca^{2+}]_i$ was abolished by EGTA and increased with $[Ca^{2+}]_o$ (0.1-2 mM) in a dose-dependent fashion. Preincubating the cells with the Ca channel antagonist La^{3+} (4-40 μM) suppressed $\Delta[Ca^{2+}]_i$, but La^{3+} was ineffective in reducing $[Ca^{2+}]_i$ when added after K^+ stimulation. While the dihydropyridine(DHP) agonist +202-791 increased $[Ca^{2+}]_i$ in the presence of 30 mM K^+,thus indicating the presence of a DHP receptor in these cells, DHP antagonists (1-5 μM nifedipine or nitrendipine) reduced $\Delta[Ca^{2+}]_i$ by only ca. 20%, regardless of the membrane potential in the range -25/-5 mV. ω-conotoxin (0.1-1 μM) did not reduce $\Delta[Ca^{2+}]_i$. It is proposed that depolarization activates a DHP-insensitive Ca channel in chromaffin cells, and that Ca^{2+} influx brought about by depolarization generates Ca^{2+} release from intracellular stores.

W-Pos219 PHARMACOLOGICAL PROFILE OF SKELETAL MUSCLE CALCIUM CHANNELS IN PLANAR LIPID BILAYERS.

Hector Valdivia and Roberto Coronado, Department of Physiology and Molecular Biophysics, Baylor College of Medicine, Houston, Texas 77030.

Ten dihydropyridines, d-cis-diltiazem and four phenylalkylamines were tested at HP 0 mV in the T-tubule Ca^{2+} channel incorporated into PE/PS planar bilayers and activated by 1 μM racemic Bay K 8644. i) Racemic nitrendipine, PN200-110, nimodipine or ii) pure enantiomers (+)-nimodipine, (-)-nimodipine, (+)-Bay K 8644 or iii) nifedipine, were found to block the channel in the 0.01 to 10 micromolar range but 2-10 times more effectively when added to the external solution than when added to the internal solution. The same holds for d-cis-diltiazem and tertiary phenylalkylamine D600. Verapamil has no preferential side of block. Quaternary D575, D890 block internally only. Threshold for channel activation by (-)Bay K 8644 is at 10 nM (external side) and 0.5 μM (internal side). At low external concentrations (10-100 nM), (-)Bay K 8644 increases channel lifetime while at higher concentrations (>100 nM), there is also a significant increase in frequency of openings; however, neither channel lifetime nor frequency of openings were enhanced with increasing concentrations of racemic Bay K 8644. The apparent affinity of blockade by the quaternary DHP 207-180 was found to increase 5-10 fold when the bilayer lipid composition was changed from 50% PE/PS to 25% PE-75% PS. The results indicate that i) the T-tubule Ca^{2+} channel studied in bilayers is coupled to the high affinity dihydropyridine receptor, and ii) the receptor is buried in the lipid phase adjacent to the external end of the channel. Supported by AHA, MDA, NIH GM36852, HL 37044.

W-Pos220 INTERACTION OF CALCIUM CHANNELS OF SKELETAL MUSCLE WITH MONOCLONAL ANTIBODIES SPECIFIC FOR ITS DIHYDROPYRIDINE RECEPTOR. Janeen Vilven, Albert T. Leung, Toshiaki Imagawa, Alan H. Sharp, Kevin P. Campbell and Roberto Coronado. Department of Physiology and Molecular Biophysics, Baylor College of Medicine, Houston, TX 77030, and Department of Physiology and Biophysics, The University of Iowa, Iowa City, Iowa 52242.

Mab IIC12 and Mab IIF7, two monoclonal antibodies specific for the 170,000 Da dihydropyridine-binding subunit of the skeletal muscle dihydropyridine receptor (Leung, Imagawa, and Campbell, 1987. JBC. 262:7943-7946), and Mab $VD2_1$, a monoclonal specific for the 52,000 Da subunit (Leung et al., 1987. JBC, in press) were tested for functional effects on dihydropyridine-sensitive calcium channels of skeletal muscle incorporated into planar lipid bilayers (Affolter and Coronado, 1985. BJ 48:341-347). Open probability was measured during 200 sec control period and 200-600 sec following external addition of 0.1-1.0 µM Mab. Control and test were compared in a plot of cumulative np product vs time. Mab $VD2_1$ activated the channel >10 fold above control levels in the absence of agonist Bay K 8644. Mab $VD2_1$-activated channels were unresponsive to inhibition by nitrendipine up to a concentration of 20 µM. Control channels activated by 1 µM Bay K 8644 are blocked by nitrendipine with an apparent Kd of 5 µM. Mab IIC12 and IIF7 did not activate nor did they interfere with nitrendipine blockade. In the presence of Bay K 8644 Mab IIC12 reduced control levels of activity and decreased channel conductance approximately 2-fold. We suggest that the 52,000 Da and 170,000 receptor subunits are integral components of the dihydropyridine-sensitive Ca channel of skeletal muscle. Supported by MDA, AHA, and NIH GM36852, HL 37044, HL 37187.

W-Pos221 CONDUCTANCE-ACTIVITY, CURRENT-VOLTAGE, AND MOLE FRACTION RELATIONSHIPS FOR CALCIUM, BARIUM AND SODIUM IN THE T-TUBULE CALCIUM CHANNEL. Jianjie Ma and Roberto Coronado, Department of Physiology and Molecular Biophysics, Baylor College of Medicine, Houston, TX 77030.

We describe the conductance behavior as a function of voltage and concentration of the skeletal muscle T-tubule calcium channel in symmetrical solutions of calcium, barium, and sodium. Measurements were made in planar bilayers using the agonist Bay K 8644. Sodium current could be measured only under conditions of low internal and external divalents. At HP 60 mV (cellular convention), the Kd for Ca^{2+} blockade of sodium current was 300 µM on the external side and 5 µM on the internal side. At HP -60 mV the Kd was 6 µM on the external side and 200 µM on the internal side. At HP 0 mV, the extrapolated Kd for internal or external blockade was approximately 35 µM. This value is in excellent agreement with estimates based on well depths for the channel's free energy profile while conducting Ca^{2+} ions. Block by internal or external Ca^{2+} could be relieved by large positive and negative potentials, respectively, generating current-voltage curves that were highly non-linear. Block by external 40 µM Ca^{2+} at HP 0 mV could be relieved by increasing internal Na^{+}. These results suggest strongly that Na^{+} and Ca^{2+} compete for internally-located binding sites. We find, however, that the magnitude of the ion-ion repulsive interactions inside the channel are significantly lower than in previous reports. This is a conclusion forced upon us by the unique set of parameters (energy peaks, wells, and repulsion factors) that simultaneously fit conductance-concentration, current-voltage, and mole fraction relationships for calcium, barium and sodium. Supported by AHA, MDA, and NIH GM 36852, HL 37044.

W-Pos222 INCUBATION IN PHORBOL ESTER DOWN-REGULATES T-TYPE Ca CHANNELS IN RAT DRG NEURONS. Jean E. Schroeder, Peter Fischbach, E.W. McCleskey (Intr. by R. Wilkinson) Washington University, 660 S. Euclid Ave., St. Louis, MO 63110.

Sensory neurons of the dorsal root ganglion transduce and encode a wide variety of sensations and, therefore, might be expected to display distinct electrophysiological properties among subpopulations of neurons. Consistent with this expectation, we find a wide variation in the density of T-type Ca current in whole cell patch clamp recordings from neonate rat DRG neurons grown in tissue culture. 35% of cells (16 of 46) have no detectable T current, despite having substantial L-type. The remaining cells may have T current amplitudes approaching 0.4nA, nearly as large as the L-type currents in the same cell.

We find that pre-incubation of cultures for 15 minutes or more in TPA, a phorbol ester which activates protein kinase C, radically diminishes the proportion of cells with T currents. Only one cell out of 13 treated with 100 nM TPA had detectable T current. 4-alpha-phorbol, a phorbol ester which does not activate protein kinase C, failed to eliminate T currents (5 of 7 cells had T current). Acute application of TPA during a patch clamp recording did not affect T current; we do not know if this indicates that prolonged incubations are necessary or if a crucial compound is washed from the cell during intracellular perfusion.

Our studies suggest that there is a varying spectrum of Ca channel types among different subpopulations of rat DRG neurons and that activation of protein kinase C can shift the spectrum by down-regulating T channels.

W-Pos223 EFFECTS OF ATRIAL NATRIURETIC FACTOR ON CALCIUM CHANNEL CURRENTS IN BOVINE ADRENAL GLOMERULOSA CELLS. R.T. McCarthy+, H. Rasmussen* and P.Q. Barrett*, Miles Inst. Preclin. Pharmacol.+ and Depts. Cell Biology+ and Medicine*, Yale University, New Haven, CT 06510 USA.

Atrial natriuretic factor (ANF) is a polypeptide (4-28 human) hormone which blocks Angiotensin II (AII) stimulated increases in aldosterone secretion by both cyclic nucleotide-dependent and - independent pathways. Voltage clamp studies have been compared with measured rates of aldosterone secretion to determine the mechanism of action of ANF at the cellular level. The whole cell voltage clamp technique has been used to evaluate the effects of ANF on two populations of voltage-dependent calcium channels (transient and slowly-inactivating) in freshly dispersed bovine adrenal glomerulosa cells. Consistent with T-type channels in other tissues, the transient calcium channels activate at more negative potentials, deactivate slowly, are not susceptible to application of omega conotoxin (.01 or 1 μM), and are blocked by cadmium. Activation of the transient calcium channels underlies, at least in part, the stimulatory action of AII and K^+ on aldosterone secretion. Consistent with the ANF induced block of AII or K^+ stimulated increases in aldosterone secretion, transient calcium channel current is inhibited by ANF (3 nM, 3 min. pretreatment) with a corresponding shift of the steady-state availability curve to more negative potentials (≅-12.0 mV) and a slowing of the rate of transient channel deactivation. This shift is not accompanied by a similar shift in the voltage dependence of activation of these channels. Higher concentrations of ANF (10 nM or 100 nM) increase a component of calcium channel tail current (possibly from L-type channels). These results suggest that ANF acts via separate effects on two populations of voltage dependent calcium channels.

W-Pos224 INTERACTION OF ω-CONOTOXIN WITH NEURONAL Ca^{2+} CHANNELS. P. Feigenbaum, M.L. Garcia, and G.J. Kaczorowski, Dept. of Biochemistry, Merck Institute, Rahway, NJ 07065.

ω-conotoxin, a 27 amino acid peptide derived from the sea snail Conus Geographus has been shown to be a potent irreversible blocker of neuronal Ca^{2+} channels. Using [^{125}I] ω-conotoxin (GVIA), we have demonstrated the existence of a single class of extremely high affinity saturable binding sites in purified synaptic plasma membrane vesicles derived from rat brain (K_d=0.7 pM; B_{max}=1.1 pmole/mg protein). This high affinity site is unique to neuronal tissue since it is not present in highly purified plasma membrane vesicles derived from cardiac, vascular smooth muscle or GH_3 anterior pituitary cells. Although binding of ω-conotoxin is irreversible, the kinetic constants of the bi-molecular binding reaction can be determined from a plot of K_{obs} of ligand association vs. toxin concentration (K_{+1}=0.013 min^{-1} pM^{-1}; K_{-1}=0.011 min^{-1}) and these yield a K_d=0.86 pM. Binding of ω-conotoxin is not affected by members of different classes of Ca^{2+} entry blockers, except organic cations which block the pore of Ca^{2+} channels (eg. amiloride analogs, aminoglycosides) and various metal ions ($La^{3+} > Ni^{2+} > Cd^{2+} \sim Co^{2+} >> Ca^{2+} \sim Ba^{2+} \sim Sr^{2+} > Mg^{2+}$). The site density for ω-conotoxin receptors in synaptic plasma membrane vesicles is ca. 4-fold greater than the number of sites for L-type channels in this preparation and ω-conotoxin (1 uM) had no effect on either nitrendipine or diltiazem binding in brain. Receptors for ω-conotoxin have been solubilized from vesicles using digitonin. These results indicate that high affinity receptors for ω-conotoxin are functionally associated with neuronal Ca^{2+} channels and that this toxin can be a selective probe to delineate the biochemical, pharmacological and physiological properties of this channel.

W-Pos225 INTERACTION OF FLUSPIRILENE WITH CARDIAC L-TYPE Ca^{2+} CHANNELS. V.F. King, M.L. Garcia, and G.J. Kaczorowski, Dept. of Biochemistry, Merck Institute, Rahway, NJ 07065.

Neuroleptics of the diphenylbutylpiperidine series have previously been shown to interact in a potent fashion with L-type Ca^{2+} channels from skeletal muscle, but not with the cardiac channel (Galizzi et al., PNAS 83, 7513, 1986). Using [^{3}H]fluspirilene (Flu), we have studied the interaction of this class of compounds with the Ca^{2+} entry blocker receptor complex in highly purified porcine cardiac sarcolemmal membrane vesicles. By carefully controlling the experimental conditions, [^{3}H]Flu was shown to bind in a saturable fashion to a single class of high affinity binding sites in these membranes (K_d=0.6 nM, B_{max}=1.5 pmoles/mg protein). The K_d of Flu was confirmed by analysis of ligand association and dissociation kinetics (K_{+1}=0.033 $min^{-1}nM^{-1}$, K_{-1}=0.025 min^{-1}). The stoichiometry of Flu sites is 1:1 with separate receptors for dihydropyridine, aralkyl amine, and benzothiazepine classes of channel modulating agents which also exist in the Ca^{2+} entry blocker receptor complex. Flu binding is modulated in an allosteric fashion by members of all other structural classes of Ca^{2+} entry blockers, consistent with the idea that Flu binds at a distinct site within the receptor complex. In addition, Flu binding is affected by various di- and trivalent cations which are either substrates or blockers of L-type Ca^{2+} channels; all blockers (eg. Cd^{2+}) are found to stimulate Flu binding, while permeant ions (eg. Ca^{2+}) inhibit the binding reaction. These results suggest that Flu binds in a potent fashion to a unique, previously undescribed site, in the cardiac Ca^{2+} entry blocker receptor complex and that an interaction at this site may account for some of the pharmacological properties of this chemical class of agents.

W-Pos226 A LONG-LASTING CALCIUM CURRENT IN CELLS OF THE ZONA GLOMERULOSA OF RATS' ADRENAL GLANDS.
Durroux, T., Gallo-Payet, N.* and Payet, M.D., Dpt of Physiology & Biophysics and *Dpt of Endocrinology, Faculty of Medicine, University of Sherbrooke, Sherbrooke, Québec, Canada J1H 5N4

Previous studies have shown the existence of Ca^{2+} channels in cells of the Zona Glomerulosa from rats' adrenal glands. We describe here a new component of calcium current which is a long-lasting one. The recordings of experiments made with a CsCl-filled pipette on cells bathing in a solution containing 20 mM of Ca^{2+} and kept at a HP of -80 mV to remove steady state inactivation, show that the threshold potential is around -20 mV and that the maximum current is reached for potentials close to +60 mV. The substitution of 20 mM of Ba^{2+} for 20 mM of Ca^{2+} was accompanied by a shift toward more negative potentials. In these conditions, step depolarizations more positive than 40 mV from a holding potential of -80 mV enhance the inward current. The h_∞ curve shows that the midpoint-voltage is around -55 mV and that the slope factor is around 5.5 mV. A rundown was also detected, after 15 min, there existed only a very small inward current. No bigger current could be recorded later, even after a resting period of several minutes nor by hyperpolarization, nor by the addition of an agonist such as Bay K8644. The sensitivity of the channel to different drugs was analyzed. The addition of 5.10^{-7} Cd^{2+} is sufficient to block the calcium current. The addition of 1 µM of Bay K8644 enhances the current. A decrease is observed when more than 5 µM of Bay is added. Finally nifedipine 10^{-7} allows a strong blockage of the Ca^{2+} channels which is difficult to reverse except when Bay K8644 is added. The characteristics of this type of current are very close to those found by Nowycky et al.* for type L Ca^{2+} current. Supported by MRC to N.G.-P. and M.D.P.

*Nature (1985), 316: 440-443.

W-Pos227 EFFECTS OF INTRACELLULAR FREE MAGNESIUM ON CALCIUM CURRENT IN ISOLATED CARDIAC MYOCYTES.
Richard E. White* and H. Criss Hartzell, Department of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, GA 30322.

Magnesium ions play a fundamental role in cellular function, but the effects of changes in intracellular ionized magnesium ($[Mg^{2+}]_i$) on cell physiology are incompletely understood. We have employed the whole-cell configuration of the patch clamp technique to examine the effect of changing $[Mg^{2+}]_i$ on the voltage-gated calcium current (I_{Ca}) in cardiac cells isolated from frog ventricle. Our results indicate that increasing $[Mg^{2+}]_i$ from 0.3 to 3.0 mM by internal perfusion has little effect on basal I_{Ca} or I_{Ca} elevated by dihydropyridine calcium channel agonists. In contrast, I_{Ca} elevated by cAMP-dependent phosphorylation is decreased > 50%. $[Mg^{2+}]_i$ exerted a similar inhibitory influence upon I_{Ca} elevated by isoproterenol, 8-bromo-cAMP, and the catalytic subunit of cAMP-dependent protein kinase; this ruled out possible effects of $[Mg^{2+}]_i$ on β-adrenoceptors or phosphodiesterases. Therefore, the inhibitory effect of $[Mg^{2+}]_i$ is not due to changes in [cAMP] or the velocity of phosphorylation, but rather appears to be a direct effect upon the phosphorylated channel or on channel dephosphorylation. Given these results, the composition of the intracellular solution should be considered when comparing different experiments. (Supported by grants from the Georgia Heart Association and NIH (HL21195 and HL27385).

W-Pos228 VOLTAGE-DEPENDENT Ca CURRENT IS MODIFIED BY REMOVAL OF CELL SURFACE SIALIC ACID FROM VENTRICULAR MYOCYTES.
Hal F. Yee, Jr. Cardiovasc. Res. Labs, UCLA-CHS, Los Angeles, CA 90024.

The control of Ca movement across the plasmalemma (PL) is of paramount importance for cell viability and function, particularly in the heart where Ca entry is critical for contractility. As with other cells, the myocardial PL has an abundance of carbohydrate on its surface, including sialic acid (SA) a ubiquitous anionic surface sugar known to bind Ca. As previously reported (Science 193: 1013 (1976);Biophys. J. 51:112a (1987)), removal of cell surface SA from cultured rat cardiocytes leads to an increased cell Ca content as measured by radioisotope exchange techniques. Recently, I have found that this increase can be blocked by cations in the order La>Cd>Mn>Mg, the same as that for block of Ca channels. Nifedipine was observed to inhibit this increased cell Ca in a dose related manner. These results suggested a role for Ca channels in this phenomenon. Hence, voltage-dependent Ca current was measured in isolated guinea pig ventriculocytes at 23°C, using the whole-cell variation of patch clamp technique. (Bath:(mM)140CsCl,5$CaCl_2$,0.33NaH_2PO_4,10Hepes,10glucose, 10^{-3}TTX,pH7.3;Pipette:150CsCl,1$CaCl_2$,1$MgCl_2$,10NaH_2PO_4,14EGTA,20Hepes,1KADP,1NAD,2F-1,6-P,1K_2HPO_4,2 NaPyruvate,pH7.1) Current-Voltage relationships were determined with holding potential -80mV. Peak ICa increased (69+/-10%) following removal of SA with neuraminidase. This increase was completely blocked by Cd (0.5mM). After SA release, the voltage-dependent ICa also became more rapidly activated and inactivated. There was no apparent shift in I-V curves following loss of SA. These increases in peak ICa may be related to the phenomenon we have reported whereby removal of cell surface SA leads to an increase in cell Ca content. (Supported by NIH MSTP grant GM08042.)

W-Pos229 Ca^{2+} CHANNELS FROM SEA URCHIN SPERM PLASMA MEMBRANES INCORPORATED INTO PLANAR BILAYERS. A. Liévano, E. Vega*, L. de De la Torre, I. Vargas and A. Darszon. Dept. of Biochemistry, CINVESTAV-IPN, Apdo. Postal 14-740, 07000 México City and *Dept. of Physiology, UAP, Puebla.

The uptake of Ca^{2+} across the sea urchin sperm plasma membrane plays a fundamental role in sperm physiology. Pharmacological evidence suggests the involvement of Ca^{2+} channels in the egg jelly-induced sperm acrosome reaction, but the direct demonstration of Ca^{2+} channels has not been achieved. We are studying the sperm Ca^{2+} channels by fusing *Strongylocentrotus purpuratus* sperm plasma membranes into planar bilayers. With a $Ba(HEPES)_2$ gradient across the membrane, we reported the incorporation of high conductance cationic channels with bilayers made of PE/PS (Biophys. J. 51:433a). Here we show that the channels display a complex voltage-dependent kinetic behavior, having mainly a high conducting state at potentials from -25 to +100 mV, and several conducting states, with the smaller ones having a higher open probability, at potentials more negative than -25 mV. These channels are permeable to Ca^{2+}, Ba^{2+} and Sr^{2+} ions, with maximal conductance values of 171, 220 and 266 pS respectively, in 50 mM divalent cation-chloride, 10 mM HEPES-TMA pH 8.0. The channels are blocked by La^{3+}, Co^{2+} and Cd^{2+} which blocks the egg jelly induced acrosome reaction at similar concentrations. In bionic experiments made with the channels incorporated in diphytanoilphosphatidylcholine bilayers, 100 mM monovalent cation *cis* and 50 mM divalent *trans*, we obtained PC^{2+}/PC^{+} values of 3 (Ca^{2+}/Na^{+}) and 2 (Ca^{2+}/K^{+}). These results indicate that these channels appear to be different from Ca^{2+} channels found in other preparations. This work was partially supported by grants from R.J. Zevada Foundation, CONACyT (fellowship to A.L.) and the Organization of American States.

W-Pos230 AN EGG JELLY INDUCED HYPERPOLARIZATION MAY BE NECESSARY TO ACTIVATE VOLTAGE DEPENDENT Ca^{2+} CHANNELS AND TRIGGER THE ACROSOME REACTION IN SEA URCHIN SPERM. A. Darszon, L. de De la Torre, M.T. González, A. Guerrero, A. Liévano, E. Morales and I. Vargas. Dept. of Biochemistry, CINVESTAV-IPN, Apartado Postal 14-740, 07000, México City.

As in many species, sea urchin sperm must undergo the acrosome reaction (AR) to fertilize eggs. The outer investment of the egg "the jelly" triggers Na^{+} and Ca^{2+} influxes and H^{+} and K^{+} effluxes, an increase in intracellular pH (pHi) and a membrane potential depolarization, which are necessary for the AR to occur. Ca^{2+} channel antagonists like nisoldipine block Ca^{2+} uptake and the AR induced by egg jelly suggesting the involvement of voltage sensitive Ca^{2+} channels (VSCC) in this process. However, increasing external K^{+} in sea water does not induce Ca^{2+} uptake and actually blocks the jelly induced AR. Recently, we found a jelly induced K^{+} dependent hyperpolarization, probably mediated by K^{+} channels that preceeds the depolarization (FEBS Lett 218:247). This hyperpolarization may be required to activate VSCC and also a voltage dependent Na^{+}/H^{+} exchanger. This would imply that depolarizing with K^{+} should induce Ca^{2+} uptake and the AR if preceeded by a hyperpolarization. To test this hypothesis *Lytechinus pictus* sea urchin sperm were hyperpolarized in K^{+} free sea water adding valinomycin (1-2 μM). The changes in membrane potential were followed using diS-C_3-(5) and pHi with dimethyl carboxyfluorescein. Under these hyperpolarizing conditions pHi increased, and the addition of KCl (30 mM) to sperm induced a depolarization, a decrease in pHi, Ca^{2+} uptake measured with Quin 2 and the AR. These results indicate that the jelly induced opening of K^{+} channels may be required to hyperpolarize sperm and activate VSCC, increase pHi and induce the AR. This work was partially supported by grants from R.J. Zevada Foundation, CONACyt, COSNET and OEA.

W-Pos231 **ELECTROPHYSIOLOGICAL PROPERTIES OF CALCITONIN-SECRETING TUMOR CELLS.** David R. Van Wagoner and Edward F. Nemeth, Dept. of Physiol. and Biophys., Case Western Reserve Univ., Cleveland, OH, 44106.

Small changes in $[Ca]_o$ regulate the secretion of calcitonin and parathyroid hormone, and cause parallel changes in $[Ca^{2+}]_i$. We have characterized the regulation of $[Ca^{2+}]_i$ in a calcitonin secreting cell line derived from a rat medullary thyroid carcinoma (r-MTC 6-23). Fura-2 fluorescence measurements of $[Ca^{2+}]_i$ in these cells show that either small increases in $[Ca]_o$, or the addition of depolarizing concentrations of K_o, evoke rapid increases in $[Ca^{2+}]_i$. The increase of $[Ca^{2+}]_i$ by either stimulus was enhanced by the addition of 0.1 μM Bay K 8644, and blocked by the addition of 0.1 μM nifedipine. While these studies suggest the presence of a dihydropyridine sensitive calcium channel, no electrophysiological studies of isolated ionic currents in these cells have been reported.

We have begun to characterize the voltage sensitive currents in these cells using the whole cell patch clamp technique. Cells voltage clamped with K^{+} containing pipettes and bathed in a normal saline (1 mM $[Ca]_o$) displayed a rapidly inactivating sodium current followed by a slowly activating potassium current. Raising the $[Ca]_o$ from 1 to 5 mM caused a small inhibition of the peak I_{Na}, and a 5-10 mV left shift in the current voltage curve of the peak potassium current, possibly indicating the presence of Ca-activated K-channels. When both Na and K currents were eliminated (by substituting N-methylglucamine for monovalent ions inside and out), two distinct classes (analogous to the "L" and "T" type channels) of voltage sensitive calcium currents were detected. From a holding potential of -60 mV a noninactivating current, maximal at +10 mV, was recorded; from a holding potential of -80 mV an additional, inactivating component was observed. This current was isolated by measuring the difference between pulses to the same test potential from the two holding potentials; it was maximal at -30 mV, with a peak current amplitude half that of the non-inactivating current. Inactivation rate varied as a function of the test pulse amplitude, with a τ of 30 msec at zero mV. (Supported by NIH DK-37878-02 and a N.E. Ohio AHA Postdoctoral Fellowship (D.V.W.)).

W-Pos232 REGULATION OF PURIFIED SKELETAL MUSCLE DIHYDROPYRIDINE RECEPTOR/Ca^{2+} CHANNEL IN PLANAR BILAYERS BY PHOSPHORYLATION, DIHYDROPYRIDINES, VOLTAGE, AND OLIGOMERIC STATE. Lin Hymel[1], Ibeg Striessnig[2], Hartmut Glossmann[2], and Hansgeorg Schindler[1] (Intr. by J. Oliver McIntyre). [1]Institute for Biophysics, University of Linz, A-4040 Linz, Austria and [2]Institute for Biochemical Pharmacology, University of Innsbruck, A-6020 Innsbruck, Austria.

The purified 1,4-dihydropyridine (DHP) receptor from skeletal muscle has been incorporated into planar bilayers and the effects of modulatory agents on channel characteristics investigated. The DHP agonist BAY K 8644 (5 uM) was without effect on spontaneous activity. It also failed to activate silent channels which could be highly activated by phosphorylation using cAMP-dependent protein kinase. The DHP antagonist (+)-PN200-110 did not inhibit Ca^{2+} channel activity at 10 uM. Added to prephosphorylated channels in the bilayer, (+)-PN200-110 induced a dramatic prolongation of the open state and stabilized conductance substates, making possible the analysis of channel oligomeric state. Single receptor molecules form channels of 0.9 pS (100 uM Ba^{2+}) and show no voltage-dependent gating. Upon association, both voltage dependent gating and higher conductance events are recovered. Even multiples of 0.9 pS were observed, predominantly 7.5 and 15 pS, and multiples of these values up to 60 pS. We conclude that the L-type Ca^{2+} channel exists *in situ* as a functionally-synchronized array of DHP receptor molecules. Similarly-sized oligomers (16 subunits) of purified ryanodine receptor/Ca^{2+} release channels observed in planar bilayers suggest a model of directly-coupled Ca^{2+} channels at the triad junction, constituting the molecular machinery of excitation-contraction coupling.

W-Pos233 RECONSTITUTION OF THE PURIFIED SARCOPLASMIC RETICULUM CA^{2+} RELEASE CHANNEL INTO PLANAR LIPID BILAYERS. QI-YI LIU, ERIC ROUSSEAU, F. ANTHONY LAI, AND GERHARD MEISSNER. (Intr. by D.C. Richardson) Department of Biochemistry, University of North Carolina, Chapel Hill, NC 27599.

The purified 30S Ca^{2+} release channel complex from rabbit skeletal muscle sarcoplasmic reticulum (SR) comprising M_r 360,000 polypeptides, has been reconstituted into planar lipid bilayers. The conductance for Ca^{2+} ions was 90 pS with 50 mM $Ca(OH)_2$/250 mM HEPES, pH 7.4 buffer in the trans chamber, and μM free Ca^{2+}, 250 mM HEPES/Tris, pH 7.4 cis. Three subconductance states with 65, 40 and 20 pS were observed. The fraction of channel open time (P_o) of the 90 pS channel was reduced by decreasing the free Ca^{2+} concentration to less than 0.1 μM cis, the side to which the purified protein was added. The P_o increased upon addition of mM ATP cis, and was decreased by adding mM Mg^{2+} cis. 20 μM ruthenium red (RR) cis, inhibited the channel's activity. Sometimes 20 μM RR trans was required for full channel closing, suggesting that some channels might have been inserted into the bilayer with an opposite orientation. A maximum single channel conductance of 600 pS was observed when the Ca^{2+} release channel was reconstituted in symmetric 500 mM NaCl and 2 μM free Ca^{2+}, 20 mM Na-Pipes, pH 7.0 buffer. Na^+ and not Cl^- was found to be the major conducting ion. The Na^+- conducting channel displayed 300 pS and 200 pS subconducting states. The Na^+ conducting channel had the same pharmacological properties as the native SR Ca^{2+} release channel. 10 μM ryanodine induced the formation of a fully open, subconducting channel state, while addition of mM Ca^{2+} to the trans chamber reduced the Na^+ conductance. These results suggest that reconstitution of the 30S protein complex, previously identified as the junctional feet-ryanodine receptor complex, into planar lipid bilayers, induces a calcium conducting pathway with characteristics of the native SR Ca^{2+} release channel.

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W-Pos234 A CA^{2+} TRANSIENT IS ASSOCIATED WITH HIPPOCAMPAL SLICE EXCITATION. John S. George and John C.. Fowler. Los Alamos National Laboratory, Life Sciences Div. M/S M-882, Los Alamos, New Mexico 87545

A variety of evidence suggests that cytoplasmic Ca^{2+} may regulate the sensitivity of neurons in the hippocampus and other central neural structures. We have employed Fura-2 to detect and characterize a cytoplasmic Ca^{2+} transient associated with electrical stimulation of rat hippocampal slice neurons. Cells were loaded by preincubation in a physiological saline containing 5-25 μM Fura-2 AM and small amounts of DMSO and Pluronic F-127. Preincubation was typically at 30-33 °c but most experiments were conducted at room temperature. Bipolar electrical stimulation of the slice with a pair of fine wire electrodes produced a rise in cytoplasmic Ca^{2+} in hippocampal slice cells detected by a decrease in flourescence excited at 380 nm. This flourescence transient was not observed with excitation at 360 nm, an isosbestic point in the family of Ca^{2+}-dependent excitation spectra. Rise time for the response was 10-40 msec depending on stimulus parameters and other experimental conditions. This was typically longer than the duration of the slice population spike recorded with an extracellular glass microelectrode, but shorter than the EPSP. Ca^{2+} activity approached baseline in 100 to several hundred msec following stimulation. The rate of Ca^{2+} rise, time to peak, and amplitude of the response were correlated with the strength of electrical stimulation and the size of the EPSP and population spike. Subthreshold stimulation did not produce a detectable Ca^{2+} transient. By appropriate filtering of the photodiode signal and improvements in system mechanics and electronics, it was possible to observe responses in single passes and obtain reliable signals in averages of 4-8 trials. Future experimental strategies include application of an intensified video system. The kinetics of the Ca^{2+} response suggest that it might be involved in medium to long term facilitation of neural sensitivity associated with bursts of high frequency stimulation.

W-Pos235 CHICK AND RAT PINEAL CELLS EXPRESS DIFFERENT MEMBRANE CONDUCTANCES N.L.Harrison, N.Lambert, J.L.Barker and M.Zatz (Intr. by R.Jernigan). LNP, NINCDS, NIH and LCB, NIMH, Bethesda, MD 20892.
The pineal gland of rat and chick make and release melatonin (MEL) at night. In culture, chick pineal (CP) cells show a 'spontaneous' nocturnal rise in MEL production, inhibited by light or α_2-adrenoceptor activation. Rat pineal (RP) cells are not photosensitive, but produce MEL in response to β-adrenoceptor stimulation. Pineal cells obtained from adult rats or 1 day old chicks were dissociated into primary culture; recordings were made (whole-cell patch) after 1-4 days. Both cell types have input resistances in the GΩ range; neither generates TTX-sensitive action potentials. CP cells have two small Ca^{2+} currents (N-type and L-type). MEL output from CP (but not RP) cells is reduced by nitrendipine and increased by Bay K 8644, indicating a role for 'L-type' Ca^{2+} channels in regulation of melatonin output from CP but not RP cells. CP cells express a sustained outward current, (I_K?), but no transient outward current. In contrast, RP cells express I_K and a transient outward current similar to I_A. CP cell membrane potential often fluctuates rapidly over a 10-30mV range; this type of activity is not seen in RP cells, raising the possibility that it is related to CP cell photosensitivity. Under voltage-clamp this activity is seen as the opening and closing of channels that reverse polarity near to 0mV and have a unit conductance of ~30pS; ion substitutions suggest these channels are permeable to small cations. These differences in membrane conductances may be related to differences in mechanisms regulating MEL output from avian and mammalian pineal cells.

W-Pos236 INACTIVATION OF CALCIUM CURRENT IN INTERMEDIATE LOBE CELLS. E.F. Stanley*, J.T. Russell$. Lab. Biophys. NINCDS (*) and Lab. Developmental Neurobiol. NICHHD ($), NIH, Bethesda Md. 20892

The intermediate lobe (IL) cells of the pituitary secrete α-MSH and β-endorphin by a Ca dependent process. Prolonged depolarization of these and other secretory cells by high external K leads to an inactivation of secretion, presumably by the closure of Ca channels (Tomiko et al. Neurosci. 6:2259, Baker and Rink J.P. 253:593). We have examined the voltage-dependent inactivation of Ca currents during prolonged depolarizations in primary cultured rat IL cells.

In patch clamped IL cells in 6 mM Ba, two inward currents were identified, a transient (I-t) and a sustained (I-s) component. The I-t current decayed rapidly with a single time constant of 10 ms at -10 mV that decreased e-fold with an 18 mV depolarization of the membrane potential. The I-s current amplitude was found to be steeply dependent on the holding potential. Changing the holding potential from -120 to -60 led to a 50 % decline in I-s. I-s was shown to decay with two time constants of 1.8 and 25 seconds at 0 mV, that decreased e-fold with 15 and 27 mV depolarizations respectively. 10 % of the total inward current at 0 mV showed no appreceable decay.

The simplest interpretation of our results are that the calcium current in IL cells corresponds to two ion channel types (see Cota, JGP 88:83), one with a single inactivation rate (I-t) and the other with two inactivation rates (I-s), although the alternative that the latter can be further subdivided into two ion channel types (Taleb et al. NLet 66:55) can not be ruled out. Our results confirm that Ca currents in the IL cell undergo pronounced inactivation with prolonged depolarization. In addition we show that the Ca current in these cells, and hence secretion, can be regulated by relatively small changes in the resting potential.

W-Pos237 INTRACELLULAR Ca RELEASE ACTIVATES Ca-PERMEABLE ION CHANNELS IN CORONARY ARTERY SMOOTH MUSCLE CELLS. M Sturek, SA Thayer, and RJ Miller. Department of Pharmacological and Physiological Sciences, University of Chicago, Chicago IL 60637

Coronary artery smooth muscle cell (SMC) contraction by some neurotransmitters involves Ca release from the sarcoplasmic reticulum followed by Ca influx, but no depolarization. We determined if Ca influx might be due to intracellular free Ca activation of Ca-permeable ion channels. During whole-cell voltage-clamp of single bovine coronary artery SMC bathed in physiological saline, release of Ca by caffeine (CAF, 10 mM) activated inward cation currents at a holding potential of -80 mV (also the K equilibrium potential). The currents were absent when 10 mM EGTA was included in the pipette. Ca-activated single-channel currents of 12 pA (conductance 150 pS) were resolved and when external NaCl was replaced by TEA Cl the unitary current decreased to 3 pA, which was probably Ca influx. A second Ca-activated current was not resolved as single-channels in whole-cell recordings, but the amplitude ranged from 20-300 pA. This current was repeatedly activated by brief (15 s) CAF exposures and was decreased 35% during the first CAF exposure in Ca-free solution. Similarly, intracellular free Ca transients measured by fura-2 were also elicited repeatedly by CAF exposures and the transient decreased 25% during the first CAF exposure in Ca-free solution. These data suggest that intracellular free Ca activates ion channels that might be sufficiently Ca-permeable to further elevate free Ca. Typical L-type voltage-dependent Ca channels could not have this role at resting membrane potentials.

W-Pos238 **INACTIVATION OF CALCIUM CURRENTS IN BULLFROG SYMPATHETIC NEURONS.** Theodore N. Marks and Stephen W. Jones, Department of Physiology and Biophysics, Case Western Reserve University, Cleveland, Ohio 44106.

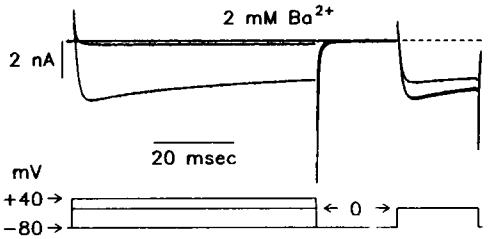
Ca currents in dissociated frog sympathetic neurons inactive partially during depolarizations (tens of msec), change slowly but reversibly with the holding potential (minutes), and run down (tens of minutes).

Whole-cell recording (Axopatch or List EPC7) used external Ba or Ca, internal and external monovalent ions replaced by N-methyl-D-glucamine, and internal MgATP. 60 msec pulses from -80 mV inactivated maximally (10-40%) near the point of peak inward current, whereas currents at more depolarized potentials inactivated only slightly, either during the pulse or as measured by postpulses (see Figure). This suggests that inactivation was Ca-dependent. However, preliminary results indicate that inactivation does not obviously change with the charge carrier (Ca or Ba), the amplitude of the current (by changing [Ca] or [Ba]), or the concentration of EGTA in the pipet (0.1-10 mM).

Steps from -40 mV inactivated less. This, plus the incomplete inactivation from -80 mV, suggest that multiple currents may be present, possibly analogous to the "L" and "N" currents of chick dorsal root ganglia. If so, inactivation of the N-current might be either voltage- or Ca-dependent.

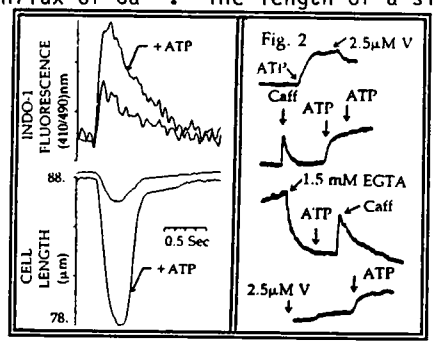
Muscarine and ATP have small and inconsistent effects on Ca currents, typically 10-30% inhibition. During larger responses, the current remaining often shows less inactivation.

Supported by NIH grant NS 24471.



W-Pos239 EXTRACELLULAR ATP ENHANCES CONTRACTILITY OF ISOLATED CARDIAC MYOCYTES AND POTENTIATES TRANSSARCOLEMMAL INFLUX OF Ca^{2+}. R. S. Danziger, R. Moreno-Sánchez, S. Raffaeli, M. C. Capogrossi, H. A. Spurgeon, R. G. Hansford, and E. G. Lakatta. Gerontology Res. Ctr., NIA, Baltimore, MD 21224

Extracellular ATP activates P_2 purinergic receptors and has been shown to raise cytosol free Ca^{2+} ($[Ca^{2+}]_c$) in suspensions of cardiac myocytes (Sharma and Sheu, Biophys. J. 49, 351a, 1986). We now show that ATP is a potent positive inotropic agent in heart and stimulates trans-sarcolemma influx of Ca^{2+}. The length of a single isolated rat cardiac myocyte loaded with Indo-1 was measured with a video edge detector and inverted microscope: simultaneously, $[Ca^{2+}]_c$ was derived from the ratio of fluorescence emission at 410 nm/490 nm. ATP (1 μM) resulted in a > 2X increase in velocity and extent of shortening, when the cell was stimulated at 1 Hz in 1 mM Ca^{2+}: contraction duration was unaffected. The systolic $[Ca^{2+}]_c$ transient was markedly enhanced (Fig. 1). Although this implies an increased sarcoplasmic reticulum (SR) pool, the primary site of action of ATP is at the sarcolemma. Thus, caffeine (10 mM), which discharges SR Ca^{2+}, does not prevent the subsequent increase of $[Ca^{2+}]_c$ due to ATP (100 μM) in suspensions of myocytes containing quin 2 (Fig. 2). Further, lowering of extracellular Ca^{2+} to < 1 μM with EGTA abolishes the response of $[Ca^{2+}]_c$ to ATP, though not the response to caffeine. The effect of ATP is partially sensitive to verapamil (V) (Fig. 2).



W-Pos240 **INOSITOL 1,4,5-TRISPHOSPHATE OPENS CALCIUM CHANNELS FROM AORTIC SARCOPLASMIC RETICULUM IN VESICLES AND IN PLANAR BILAYERS.** Barbara E. Ehrlich*and James Watras#. Departments of Medicine*# and Physiology*, University of Connecticut, Farmington, CT.

Inositol 1,4,5-trisphosphate (IP_3) induces the release of calcium (Ca) from the sarcoplasmic reticulum (SR) of smooth muscle by opening Ca permeable channels. Ca release from SR vesicles made from canine aortic smooth muscle was examined using the extracellular Ca indicator antipyrylazo III and the intracellular Ca indicator chlorotetracycline. With antipyrylazo III, the vesicles were found to accumulate Ca in the presence of ATP, and then release Ca upon addition of IP_3 with a $k_{0.5}$ of ~1 uM IP_3. The half-time of this release was ~3 sec, and corresponded to 25% of the accumulated Ca. Similar data were obtained using the fluorescent Ca indicator chlorotetracycline. To test the hypothesis that Ca release is channel mediated, the aortic SR vesicles were incorporated into either tip-dip bilayers or black lipid membranes. In both types of bilayers, we observed IP_3-stimulated Ca channel openings with a single channel conductance of 20 pS. ATP (100 uM) alone did not open bilayer-incorporated Ca channels, whereas ATP increased the sensitivity of the channels to IP_3 ~5-fold. These results indicate that the IP_3-induced Ca release from aortic SR is mediated by an IP_3-gated channel. The conductance of this IP_3-gated channel is lower than the Ca-sensitive Ca channels in skeletal muscle SR which may, in part, explain the relatively slow rate of IP_3-induced Ca release from aortic SR vesicles.

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W-Pos241 INTERCELLULAR COMMUNICATION IN COELENTERATE CELLS AS VISUALIZED BY ENDOGENOUS CALCIUM DEPENDENT BIOLUMINESCENCE. J Lechleiter, P Brehm, S Smith* and K Dunlap, Physiol Tufts Univ, Boston MA *Mol Neurobiol, Howard Hughes, Yale, New Haven CT.

We have previously suggested that chemical signalling through gap junctions controls Ca dependent bioluminescence in *Obelia geniculata* (Nature 325:60). We found that photocytes, which contain an endogenous Ca activated photoprotein, will not luminescence in isolation and that light emission required contact with a neighboring support cell. The chemical signal between cells was hypothesized to be Ca or a Ca dependent signal since Ca channel blockers and the removal of extracellular Ca blocked light emission. Importantly, light emission was blocked when gap junctions between the photocyte and the support cell were uncoupled. If bioluminescence is triggered by chemical signalling through the gap junction, then light emission in photocytes would be predicted to initiate at the sites of contact with the support cells. We have tested this hypothesis by examining the source of light emission using video microscopy. Bioluminescence in photocytes was stimulated with a high K solution. In support of chemical signalling through gap junctions, we found that light emission in photocytes spread from the points of contact with support cells in **all** cases where cellular morphology was clearly defined (n=13). Analysis of these data further showed that luminescence spread as a wave across the cell and frequently exhibited a brighter emission near the plasma membrane. To test if Ca entry through the gap junction and subsequent Ca diffusion could account for these observations, we injected Ca directly into an isolated photocyte. Under these conditions, we found that luminescence spread from the point of injection throughout the cell. Interestingly, the wave of luminescence did **not** invade the photocyte when extracellular Ca was removed from the bath but remained focal at the site of Ca injection. We conclude that the chemical signalling through the gap junction initiates luminscence and that a likely candidate for this signal is Ca. In addition, a secondary process which is dependent on extracellular Ca is responsible for the 'wavelike' spread of luminescence across the cell and likely involves the activation of voltage independent channels in the photocyte plasma membrane. *Supported by NSF grant 8503159. JL was supported by a Grass Fellowship.*