Tu-Pos59 FURTHER CHARACTERIZATION OF ABP-50, A 50KD ACTIN BUNDLING PROTEIN FROM DICTYOSTELIUM DISCOIDEUM

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As previously reported, ABP-50 is a monomeric protein which has the ability to bundle actin filaments and sediment actin bundles at low g force (50,000xg, 1min). Here, we report on the further characterization of the protein's properties, in particular its binding activity, relationship to other bundling proteins, and the morphology of the bundles it forms with actin. The protein was isolated by a modified procedure of Hock, R.S., et.al. J. Cell Biol. 1986.103:411a., which yields 0.28 mg of protein per ml of packed cells. In whole cells, ABP-50 is 6% of total cellular protein, as determined by scanning densitometry. Binding affinity of ABP-50 to F-actin was determined by cosedimentation at 100,000xg, with varying concentrations of ABP-50 and 4.5 uM actin. Supernatent and pellet samples were analyzed by SDS-PAGE, with the quanity of ABP-50 bound to F-actin determined by scanning densitometry. ABP-50 has a $K_d = 2X10^{-6}M$ for F-actin, and a molar ratio at saturation of one ABP-50 per one actin monomer. Morphological analysis of ABP-50/actin bundles by negative stain electrom microscopy indicates center to center spacing between filaments in the bundles of 8nm, with a high degree of alignment of the actin subunits in the bundle. To determine its relationship to the 30K actin bundling protein isolated by both Fecheimer (Fechheimer,M. 1987 J. Cell Biol.104:1539-1552) and Pardee (Johns,J.A.,et.al.. 1988. Cell Motil. and Cytosk.9: 205-218) from Dictyostelium amoebae. Western blot analyses were done using a polyclonal monospecific antibody to ABP-50, and an antibody to purified 30K protein, supplied by Pardee. The antibody to ABP-50 reacted only with ABP-50, but not to 30K, while the antibody to 30K reacted only with 30K, but not with ABP-50. This indicattes that these proteins are unrelated, although both bundle actin filaments in vitro. (Supported by NIH grant 28513 and the Hirschl Trust).

Tu-Pos60 MOLECULAR SIZE AND CELLULAR LOCALIZATION OF A 40 kD MYOFIBRILLAR PROTEIN WHICH BINDS TO AN ACTIN CAPPING PROTEIN. Zhican Qu and Shin Lin. Department of Biophysics, The Johns Hopkins University, Baltimore, MD 21218.

Chicken muscle capactin, an actin filament capping protein with subunits of 32 and 36kD (Mullmann and Lin, J. Cell Biol. 103, 391a, 1986), has been shown to bind to a 40 kD band of chicken breast muscle protein transferred from SDS gel to nitrocellulose. To study its molecular size under non-denaturing conditions, we measured the rate of sedimentation of highly purified 40 kD protein in 5-20% sucrose density gradients in 10 mM Tris-HCl buffer with 50 mM KCl at pH 8.0. We found that the protein migrated as a discrete peak of 8.6 S. Similar results were obtained at pH 6.0 and 10.0 and when the concentration of KCl was increased to 250 mM. For cellular localization studies, we raised rabbit antibodies against highly purified 40kD protein in non-denaturing form and affinity-purified the antibodies on a nitrocellulose strip containing the 40 kD band transferred from an SDS gel. Using indirect immunofluorescence techniques, we found that the antibodies stained the I-bands of myofibrils prepared from chicken breast muscle. The results of this study indicate that the capactin binding protein is a component of the muscle sarcomere and that it forms high molecular weight multimeres/aggregates under non-denaturing conditions in vitro. (supported by NIH grant GM-22289)

Tu-Pos61 Kaptin, a 43 kd protein isolated from platelets by F-actin affinity columns is present and active at sites of actin polymerization. E.L. Bearer. Univ. of California, San Francisco, CA 94143. Intr, by Wojtech Licko.

Using F-actin affinity chromatography of activated versus resting platelet extracts novel actin-associated proteins were identified. By Coomassie-stained standard SDS gels, ten of these proteins were more enriched in eluates from F-actin columns loaded with activated cell extracts than from parallel columns loaded with resting cell extracts. Polyclonal mouse sera raised against each of these ten bands cut from polyacrylamide gels were screened against platelets for localization to the tips of pseudopodia during activation. Monoclonals were made to the three antigens that had this distribution. One of these, a protein of apparent 43 kd, was found to be present at the leading edge of migrating fibroblasts and leukocytes as well as at the tips of the stereocilia in the hair cells of the chick cochlea, a site of known actin polymerization where no known actin-binding protein has yet been identified. This antigen, named "kaptin" because of its location, elutes from F-actin columns with micromolar ATP and magnesium, but not with sodium pyrophosphate or magnesium alone, and hence might be an ATPase. There is both a cytosolic and a membrane-bound pool, and by immunofluoresence appears membrane-associated. It does not separate from actin in standard gels, but can be separated in acid urea gels. Monoclonal antibodies to it do not blot to purified skeletal muscel actin, nor do they give an actin-like immunofluoresence pattern. Despite this, the protein was difficult to separate from actin. In preliminary experiments, when antibodies were injected into migrating fibroblasts, there was an apparent decrease in activity at the leading edge as observed by time lapse video microscopy. These findings suggest that kaptin plays a role in the site-specific formation of F-actin filaments which occurs at the membrane surface in migrating cells. Supported by NIH GM31286.

Tu-Pos62 Diffusion of Tracer Particles in Reconstituted Actin/Filamin Mixtures. Li Hou, Katherine Luby-Phelps, and Frederick Lanni. Center for Fluorescence Research in Biomedical Sciences. Carnegie Mellon University, Pittsburgh, PA 15213.

The Brownian dynamics of tracer macromolecules in F-actin solutions can be studied by a combination of fluorescence recovery after photobleaching (FRAP), high-resolution fluorescence microscopy, and differential interference contrast (DIC) microscopy. We have used size-fractionated FTC-ficoll, FTC-dextran, and fluorescent and nonfluorescent polystyrene latex (PSL) particles of 5 to 900 nm (diameter). In F-actin solutions up to 5mg/ml, the relative diffusion coefficient of the tracer is a decreasing function of both tracer size and F-actin concentration (Hou, Thompson, and Luby-Phelps, J. Cell Biol. 107 (1988) abstr.). The inclusion of filamin during actin polymerization results in increased tracer mobility relative to the tracer in F-actin alone. This result is most likely due to the bundling of actin filaments by filamin, increasing the void volume of the system accessible to the tracer. To make these reconstituted self-assembling systems more amenable to measurement we have constructed a dialysis optical cell in which actin polymerization can be initiated during observation. In 2mg/ml actin, 0.6 micron PSL tracer particles in rapid Brownian motion undergo reduced range and rapidity of motion as actin polymerization proceeds, to complete immobilization in about 15 minutes. If we can eliminate binding of PSL to F-actin as a cause of this immobilization, we will have a direct test for the percolation cutoff or mesh of the void spaces in the actin network (Hou and Lanni, Biophys: J. 53 (1988) abstr.). When actin/filamin mixtures are polymerized in the optical cell, the filament network structure can be observed directly by DIC microscopy. Supported by NIH grant GM34639.

Tu-Pos63 ORIENTATION OF THE ACTIN MONOMER IN ACTIN FILAMENTS EXAMINED WITH MONOCLONAL ANTIBODIES. Carole L. Moncman and Donald A. Winkelmann, Department of Pathology, Robert Wood Johnson Medical School, Piscataway, New Jersey 08854

Structural evidence suggests the actin monomer has two globular domains of approximately equal size; however, the orientation of these domains in the actin filament is unknown. We have characterized the interaction of two anti-actin monoclonal antibodies (mcAb), 2G11.4 and 4E3.ad1, with F-actin to examine the accessibility of the epitopes defined by these mcAbs and to determine the orientation of the actin monomer in the filament. Both mcAbs are of the IgM immunoglobulin class and react specifically with actin in ELISA and western blot assays. The location of the epitopes defined by these mcAbs within the primary structure of actin was determined using limited proteolytic digests of actin and western blots. Both epitopes map between residue 67 and the C-terminus of actin. A 6 kD peptide containing the 2G11.4 epitope was isolated from a V8 protease digest of actin by ion-exchange chromatography and has been partially sequenced. Two other V8 peptides which determine the location of the 4E3.ad1 epitope to within 1 kD were partially sequenced directly from an electroblotted membrane. Based on the N-terminal sequence and size of these three peptides, the 2G11.4 epitope lies between residues 67-83 of actin, and the 4E3.ad1 epitope lies between residues 195-240. The accessibilty of these epitopes to antibody in native actin filaments was determined with a sedimentation binding assay. The mcAb 4E3.ad1 as well as an Fab fragment derived from the antibody bind strongly to the actin filaments. In contrast, very little of the mcAb 2G11.4 or its Fab fragment bound to actin filaments. These results indicate the epitope defined by 4E3.ad1 (residues 195-240) is accessible in actin filaments, and the epitope defined by 2G11.4 (residues 67-83) is inaccessible. This places constraints on the orientation of the actin monomer in the filament, which will be more fully appreciated when the relationship between the domain structure of the actin monomer and the primary structure of the protein is defined by x-ray crystallography. We are extending these results by examining competition between the mcAbs and other actin binding proteins. (Supported by grants from the NIH and the MDA)

Tu-Pos64 SPATIAL RELATIONSHIP BETWEEN POINTS IN THE ACTIN MONOMER. Reiji Takashi. CVRI, University of California, San Francisco, San Francisco, CA 94143

Fluorescence resonance energy transfer was employed to investigate the spatial relationship between probes attached to Gln-41, Cys-374 and the nucleotide binding site of G-actin. Labeled actin was prepared by the methods described earlier (Miki and Wahl, Biochim. Biophys. Acta., *786:* 188, 1984; Takashi, Biochemistry, *27:* 938, 1988; Kasprzak, et al., Biochemistry, *27:* 4512, 1988) and purified on Sephadex G-150. The distance between the bimane donor attached to Cys-374 and the cadaverine derivative (fluorescein or lucifer yellow) attached to Gln-41 was 4.6-4.8 nm or 4.1-4.4 nm. The distance between the 1,N^6-ethenoadenosine 5'-triphosphate donor at the nucleotide binding site and the fluorescein acceptor at Gln-41 was 4.1-4.8 nm. A similar distance was obtained using a lucifer yellow acceptor at Gln-41. The foregoing results provide some data required to orient the actin monomer in the F-actin filament, as described in our recent paper on the radial coordinate of actin points (Kasprzak, et al., Biochemistry, *27:4512, 1988).* (Research supported by HL-16683)

Tu-Pos65 SUBTILISIN CLEAVAGE OF ACTIN INHIBITS ITS IN VITRO MOVEMENT. Y.Y. Toyoshima, D.H. Schwyter, S.J. Kron, J.A. Spudich, and E. Reisler, Dept. of Cell Biol., Stanford Sch. of Med., Stanford, CA 94305, Dept. of Chem. and Biochem., and the Mol. Biol. Inst., Univ. of California, Los Angeles, CA 90024.

The protease subtilisin has been used to cleave actin between Met-47 and Gly-48 [Schwyter and Reisler, Biophys. J. 53:575a (1988)]. The cleaved protein is shown here to retain the main properties of intact actin including the binding of HMM, the dissociation from HMM in the presence of ATP, and the activation of HMM ATPase activity. The maximal activation of ATPase by cleaved actin depended strongly on salt conditions, and while equal to that of intact actin in 2.5 mM KCl, it was about 5 times lower than control activation in 25 mM KCl. In 2.5 mM KCl, the ATPase activity of copolymers of intact and cleaved actin decreased linearly with a decreased fraction of intact actin in the copolymer. At 25 mM KCl this relationship was nonlinear. The in vitro movement of actin filaments was assayed as before [Toyoshima, et al., Nature 328:536 (1987)]. In intact actin all filaments were mobile and moved at an average rate of 6-7 μm/sec. In cleaved actin, however, only about 30% of the observed filaments appeared mobile. These filaments moved at an average velocity of 3-4 μm/sec. Ionic strength (2.5 or 25 mM KCl) had no effect on either the number of filaments moving or the rate at which they moved. Generally, copolymers containing >40% control actin were mobile. Below that content of intact actin in the copolymers, the fraction of mobile filaments decreased with the increase in the percentage of cleaved actin. These results suggest that the motility of actin filaments depends on a step which is impaired by subtilisin cleavage and is separate from activation of myosin ATPase.

Tu-Pos66 LENGTHS OF ACTIN FILAMENTS CAPPED WITH CYTOCHALASIN D. Jose Luis Arauz-Lara and Bennie R. Ware. Graduate Biophysics Program and Department of Chemistry, Syracuse University, Syracuse NY 13244.

Direct physical measurement of the lengths of actin filaments in solution is a difficult challenge that has led to significant controversy. Filaments formed from gel-filtered actin without added shortening agents may achieve lengths that are too long to be measured by any available techniques. We have attempted to determine the lengths of filaments formed in the presence of cytochalasin D. The diffusion coefficients of fluorescence-labeled actin filaments were measured for various actin concentrations using the fluorescence photobleaching recovery technique. The apparent filament lengths have been estimated from the measured diffusion coefficient values. Additionally, the lengths of the filaments were obtained by measuring the macroscopic viscosity of the actin filament solutions at different shear rates for a range of actin concentration. The filament lengths estimated from the diffusion coefficient and viscosity measurements are compared for a range of molar ratios of cytochalasin to actin in order to compare the two methods and to determine the dose dependence of the activity of cytochalasin D in shortening actin filaments.

Tu-Pos67 THE EFFECTS OF GELSOLIN ON PROBE DIFFUSION IN ACTIN SOLUTIONS. Jay Newman, Kenneth L. Schick and Gerlinde Gukelberger, Physics Department, Union College, Schenectady, NY 12308.

The actin capping protein gelsolin was added to column-purified 0.65 mg/ml monomeric actin solutions prior to polymerization with 100 mM KCl in the presence of micromolar levels of Ca^{2+} in order to regulate the lengths of the actin filaments. Dynamic light scattering experiments show the effective mean length, at various molar ratios of gelsolin to actin, to be in agreement with published electron microscope data (Janmey et al., J. Biol. Chem. (1986) 261:8357-8362). Experiments with added 500 nm monodisperse polystyrene latex sphere (PLS) probes monitored the diffusion coefficients, D, and degree of mobility of the probes as functions of both the inverse scattering vector, $1/q$, (40-400 nm, dependent on the scattering angle) and the ratio of actin concentration, c, to c^*, the semi-dilute overlap concentration ($0.02<c/c^*<50$, calculated on the basis of the number average filament length which is dependent on the amount of gelsolin added. With no added gelsolin, a large fraction of the PLS are immobilized at the longer $1/q$ distances and those mobile PLS have D values of 1/10 that in water. As the molar ratio of added gelsolin increases, the fraction of immobilized PLS decreases until, for $c<c^*$, the PLS are fully mobile on distance scales of 40 to 400 nm, with angle independent D values which approach the probe D value in water for $c=0.02c^*$. We find that the probe D values scale linearly with $\log(c/c^*)$, or with $\log(L)$, where L is the number average filament length, over 3 decades of c/c^*. We thank J.E. Estes & L.A. Selden for purified actin, K. Zaner for gelsolin, and NSF for grant DMB-8607031.

Tu-Pos68 DYNAMIC LIGHT SCATTERING STUDY OF PROBE DIFFUSION THROUGH CROSS-LINKED ACTIN GELS. Jay Newman, Kenneth L. Schick and Gerlinde Gukelbergr, Physics Department, Union College, Schenectady, NY 12308.

The diffusion of monodisperse polystyrene latex spheres (PLS) in column-purified 0.65 mg/ml actin solutions, polymerized with 100 mM KCl in the absence and presence of a cross-linker, actin-binding protein (ABP), has been studied using dynamic light scattering. Measurements over a wide range of scattering angles from 90° to 8°, corresponding to inverse scattering vector probing distances of about 40 to 400 nm, respectively, give a measure of both the fraction of PLS mobile over the probing distance (from the normalized time autocorrelation function amplitude) and the average diffusion coefficient of the mobile PLS. Both 100 nm and 500 nm PLS are fully mobile in polymerized actin solutions over distances of less than 100 nm, as reported previously (Biophys. J. 53, 573a and Biopolymers, to appear). At increasing probing distances, or when ABP is added at molar ratios of 1:750 or 1:150, greater fractions of the PLS are immobilized, up to almost 99% at the conditions of a 400 nm probing distance with 500 nm probes and at a ratio of 1:150 added ABP to actin. The degree of immobilization correlates well with the amount of added ABP, the size of the PLS, and with the probing distance. At increasing probe distances, as the degree of immobilization increases, the remaining mobile fraction of PLS has an increasing average diffusion coefficient. These results suggest a somewhat broad range of pore sizes in the actin gels with a mean size of a few hundred nm. We thank J.E. Estes & L.A. Selden for purified actin, K. Zaner for ABP, and NSF for grant DMB-8607031.

Tu-Pos69 ON THE INTERACTION OF CYTOCHALASIN D WITH MG-ATP-ACTIN J.E. Estes, L.A. Selden, D.B. Dietschy and L.C.Gershman Research and Medical Services, Veterans Administration Medical Center, Albany,NY and Depts. of Physiology and Medicine, Albany Medical College, Albany, NY 12208.

In previous work we measured a burst of ATP hydrolysis upon addition of cytochalasin D (CD) and neutral salt to Mg-ATP-actin (Biophys. J. 53:572a, 1988). This initial burst is followed by steady-state ATP hydrolysis which was previously attributed to a monomeric actin ATPase activity (Brenner and Korn, J.Biol.Chem. 256:8663-8670, 1981). In the present study we examined the early burst and steady-state hydrolysis of ATP, the polymerization of actin, and the actin-bound nucleotide as a function of CD concentration. Both the initial burst and steady-state ATP hydrolysis rate depended on the CD concentration, and the amount of polymer formed was decreased from control at all CD concentrations . Interestingly, measurements of the actin-bound nucleotide during steady-state ATP hydrolysis revealed an increase in ADP-actin as the CD concentration was increased even in the presence of excess ATP. Conventional light scattering and the fluorescence intensity of pyrene-labeled actin during steady-state indicated no polymer was present at higher CD concentrations, but the use of intensity fluctuation spectroscopy showed the actin population was a heterogeneous mixture of monomers and n-mers. This evidence is consistent with a model in which CD increases the concentration of filaments, m, in solution such that the product ($m \cdot k_{\bar{A}DP}$) exceeds the rate of exchange of actin-bound ADP for ATP. The limiting exchange rate of ATP for $\bar{A}DP$ on actin accounts for the observed first order kinetics of steady-state ATP hydrolysis and the resultant build-up of ADP-actin monomer results in an increase in the critical actin concentration to a value intermediate between that for ATP-actin and ADP-actin. Supported by Veterans Administration and NIH grant #GM 32007.

Tu-Pos70 COMPARISON OF COMPETITIVE EXCHANGE MODEL VS. ISOMERIZATION MODEL FOR DIVALENT CATION EXCHANGE ON ACTIN L.C. Gershman, L.A. Selden and J.E. Estes Research and Medical Services, Veterans Administration Medical Center, Albany, NY and Department of Physiology and Medicine, Albany Medical College, Albany, NY 12208.

On the basis of fluorescence measurements on 1,5-I-AEDANS actin, Frieden proposed a model for the exchange of tightly-bound Ca^{++} for Mg^{++} on actin which incorporated a slow isomerization step (isomerization model) (J. Biol. Chem. 257:2882-2886,1982). We have proposed instead a simple competitive exchange model in which the slow exchange results from the low rate constant for dissociation of divalent cations from actin (J.Biol.Chem. 262:4952-4957,1987). Measurements of k_{app} for divalent cation exchange in our laboratory and others agree with the values for k_{app} predicted by either the competitive exchange or isomerization model. Thus, measurements of k_{app} do not help to choose which model is correct. Here we argue that in the case of actin, at least three lines of evidence favor the competitive exchange model : 1) From measurements using fluorescent chelators, the rapid pre-equilibration required by the isomerization model does not occur; 2) The characteristics of the Mg^{++} aquo ion explain the differences between the kinetics of binding of Mg^{++} and Ca^{++}; 3) Preliminary measurements of the association rate constants k_{Ca} and k_{Mg} are far removed from the values assumed in the isomerization model and agree with the values estimated from the competitive exchange model. Competitive exchange models for divalent cation binding such as this and the characteristics of the Mg^{++} aquo ion may have still wider applicability in cell biochemistry. Supported by Veterans Administration and NIH grant #GM 32007.

Tu-Pos71 THERMODYNAMICS OF ACTIN POLYMERIZATION: INFLUENCE OF ACTIN-BOUND CATION AND BOUND NUCLEOTIDE L.A. Selden, H.J.Kinosian, J.E. Estes and L.C. Gershman Research and Medical Services, Veterans Administration Medical Center, Albany, NY and Depts. of Physiology and Medicine, Albany Medical College, Albany, NY 12208.

We previously published apparent thermodynamic values for the polymerization of ATP-actin containing Mg^{++} or Ca^{++} as tightly-bound divalent cation (J. Musc. Res. Cell Motility 7:215-224, 1986). To understand the effect(s) of both the actin-bound cation and nucleotide on the actin polymerization reaction, we recently measured the critical concentration (C_c) values for Mg-ADP-actin and Ca-ADP-aactin in 0.1 M KCl over the temperature range 5 - 35°C. Equilibrium C_c values were found to be similar whether starting with ADP-actin polymer and inducing depolymerization by dilution and sonication or by adding 0.1 M KCl to monomeric Mg- or Ca-ADP-actin. Values for ΔG^0 at 25 °C were calculated and found to be similar for both Mg-ADP and Ca-ADP-actin and slightly smaller than the values for Mg-ATP-and Ca-ATP-actin. The temperature dependence of the C_c values for the ADP-actins were non-linear. This non-linearity suggests the simultaneous occurence of another process besides polymerization, probably denaturation, and makes calculation of ΔH^0 problematical. The similarity for the data for Mg-ADP and Ca-ADP-actin suggest that the thermodynamics of polymerization are the same for both actins. This similarity in the apparent thermodynamic values is consistent with our recent report that the kinetics of polymerization are similar for the two ADP-actins (Biochim.Biophys. Acta, in press.) This contrasts with the differences between the polymerization characteristics of Mg-ATP-actin and Ca-ATP-actin which we reported earlier and suggests that the bound nucleotide influences the effect of the bound cation on actin properties. Supported by Veterans Administration and NIH grant #GM 32007.

Tu-Pos72 OPTICAL MEASUREMENT OF INTRACELLULAR pH IN A SINGLE CELL. R.Y. Wong, M.J. Delay and W.R. Giles, Faculty of Medicine, University of Calgary, Calgary, Canada.

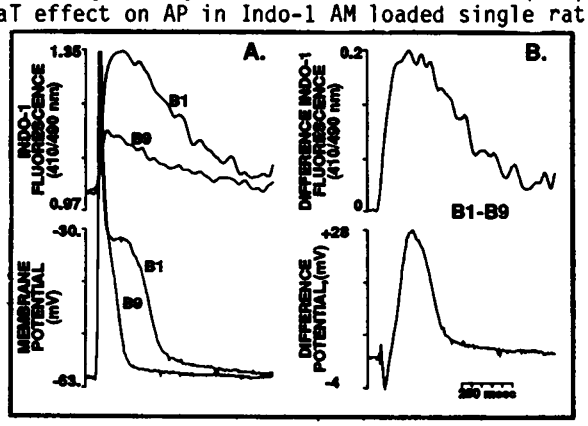
We have employed an optical method to measure the intracellular pH (pH_i) of a single cell. The method uses a completely *in vivo* calibration procedure whose results are more reliable than *in vitro* measurements. The pH_i of single, isolated cardiac cells was measured using the fluorescent, pH-sensitive dye BCECF. Isolated cells were exposed to 3 uM BCECF-AM for 10 minutes and placed in a microfluorimeter to allow measurement of pH_i over a selected region of a single cell. Two excitation wavelengths were used: 500 nm, at which BCECF is pH-sensitive, and 450 nm, at which relatively little pH sensitivity was found. Fluorescence F was measured at an emission wavelength of 530 nm, and the ratio R=F(500 nm)/F(450 nm) was obtained. *In vivo* calibration of fluorescence signals was achieved by measuring changes in R resulting from alternate additions of a weak acid (butyrate) and a weak base (trimethylamine) to the bathing solution. By extension of the methods of Szatkowski and Thomas (*Pflügers Arch.* 407:59, 1986), changes in R are used to calculate the resting pH_i and calibration parameters of intracellular BCECF. The resting pH_i of a frog atrial cell in CO_2/bicarbonate buffered solutions was found to be 7.07. However, cells which had been transferred from CO_2/bicarbonate to HEPES buffered solutions showed an initial resting pH_i of 7.35. Since the Na^+/H^+ antiporter is believed to be relatively inactive at such high pH_i, our observation is consistent with the hypothesis that the bicarbonate/Cl^- antiporter, a cell acidifying mechanism, is important for physiological pH_i regulation in cardiac cells. The pH_i of rabbit ventricular cells was found to equilibrate with extracellular pH in solutions containing 50 mM sodium acetate, which exceeds the cell's pH buffering ability. Our method therefore allows us to investigate mechanisms which regulate pH_i in a single cell. Research supported by the A.H.F.M.R. and A.H.S.F.

Tu-Pos73 $[Ca^{2+}]i$ MEASURED IN HUMAN VENTRICULAR CELLS OBTAINED BY MEANS OF ENDOMYOCARDIAL BIOPSIES. J. R. López[1], R. Espinoza[2], V. Parthé[1], M. Penso[2], G. Cordovez[1]. [1]Centro de Biofísica y Bioquímica, Instituto Venezolano de Investigaciones Científicas (IVIC), Caracas, Venezuela. [2]Unidad de Cardiología, Hospital Miguel Pérez Carreño, Caracas, Venezuela.

We have measured the intracellular free calcium concentration $[Ca^{2+}]_i$ by means of calcium selective microelectrodes in human right ventricular cells. Right ventricular endomyocardial biopsies using primarily the Stanford Caves Schultz bioptome were obtained from 3 subjects with the purpose of studying essentially normal myocardium. The muscle biopsies were dissected free of connective tissue and then divided in two small segments, for electrophysiological measurements and light microscopy. The resting $[Ca^{2+}]_i$ was 120 $\pm$ 20 nM (M $\pm$ SEM, n = 18). Increasing the external [Ca] from 1.8 up to 10 mM increased $[Ca^{2+}]_i$ to 190 $\pm$ 22 nM (n = 16). Increasing external $[K^+]$ from 5 to 20 mM induced an increase in $[Ca^{2+}]_i$ up to 260 $\pm$ 26 nM, (n = 12) which was unaffected by D600. Exposure to strophantidin (10 µM) increased $[Ca^{2+}]_i$ to 405 $\pm$ 60 nM (n=18). The results show that in Na-containing solutions either increasing external $[Ca^{2+}]$ or depolarization can increase $[Ca^{2+}]_i$. The fact that these increases of $[Ca^{2+}]_i$ occur when Ca channels are blocked supports the idea that they result, at least in part, from the Na/Ca exchange, operating in the reverse mode. (Supported by Conicit S1-1277 and Laboratorios Elmor de Venezuela).

Tu-Pos74 DIRECT MEASUREMENT OF CYTOSOLIC CALCIUM MODULATION OF THE ACTION POTENTIAL OF RAT CARDIAC VENTRICULAR MYOCYTES. William duBell, Harold A. Spurgeon, Mark Boyett, Maurizio C. Capogrossi, Edward G. Lakatta. Gerontology Research Center, NIA, Baltimore, MD 21224

The modulation of action potential (AP) configuration by the cystolic calcium transient (CaT) remains speculative. We have directly measured the CaT effect on AP in Indo-1 AM loaded single rat myocytes, bathed in Hepes buffer ([Ca] = 1.0 mM) at 23°C, in the first and ninth beats following rest, which differ in the amplitude and time course of CaT. Panel A shows AP and CaT, measured as the 410/490 nm Indo-1 fluorescence. Panel B shows the difference (Δ) tracings (electronic subtraction) of AP and CaT. During the initial rise in CaT, the ΔAP (beat 1 minus beat 9) is negative; subsequently the ΔCaT and ΔAP transients follow a similar positive trajectory. This indicates that the CaT modulation of AP (1) occurs early following AP peak and produces a net outward current, and (2) subsequently causes a net inward current throughout the remainder of AP duration. The latter effect is compatible with Ca^{2+} extrusion via an electrogenic Na/Ca exchanger.



Tu-Pos75 EFFECTS OF INTRACELLULAR CALCIUM ON CARDIAC CALCIUM CHANNELS
AM Gurney, P Charnet[1,2] & J Nargeot[1], Department of Pharmacology, UMDS, St. Thomas's, London, UK, [1]CNRS, Centre de Recherches de Biochimie Macromoleculaire, Montpellier, France & [2]Div. Biology, Caltech, Pasadena, CA.

Intracellular Ca is known to inactivate Ca currents during membrane depolarisation. It is also suggested that, at low concentrations, Ca may potentiate the current in cardiac cells (Marban, E & Tsien, RW. J. Physiol. 329:589-614, 1982). More recently, intracellular Ca has been suggested to regulate restitution of cardiac L-type channels (Tseng,G. Circ. Res. 63:468-482, 1988) by a similar mechanism. Much of the evidence supporting a facillitatory role for intracellular Ca is, however, indirect. We have taken a more direct approach to studying how Ca affects Ca channels in frog atrial cells, by applying intracellular Ca "jumps" with flash photolysis of the photosensitive Ca chelator nitr-5. Currents through L-type Ca channels were recorded with the whole-cell patch-clamp technique. Ba carried the current to prevent effects from Ca entering through channels. Presenting a light flash, which raised the intracellular Ca concentration to around 1 M, to cells containing nitr-5 (2mM, 50% Ca-complexed), increased current amplitude by up to 3-fold and prolonged current decay. The current increased slowly, taking several seconds to develop fully. Current enhancement followed a similar time course to that produced by the -adrenergic agonist isoprenaline.

Tu-Pos76 INTRACELLULAR Ca AND H INTERACTION IN CULTURED CHICK HEART CELLS.
S. Liu and M. Lieberman. Division of Physiology, Department of Cell Biology, Duke University Medical Center, Durham, NC. 27710

In cardiac muscle cells, perturbations that result in intracellular acidosis usually also increase intracellular Ca (Ca_i). The link between an increase in intracellular proton (H_i) and Ca_i may be attributable to either the resultant interaction of Na/Ca and Na/H exchange and/or competition between Ca_i and H_i for protein binding sites. In this study, we have demonstrated a direct interaction of Ca_i and H_i by using neutral carrier pH-sensitive microelectrodes to monitor pH_i of cultured chick heart cells. To eliminate the possible involvement of Na-dependent transport mechanisms, cells were exposed to Na-free HEPES/Tris buffered modified Earle's salt solution to deplete Na_i. In the presence of 1 mM $[Ca]_o$, removal of Na_o (tetramethylammonium substitution) caused a rapid (in 3 min) increase in H_i (pH_i decreased from 7.29±0.02 to 6.81±0.03, n=3). Subsequent removal of Ca_o (+ 1 mM EGTA) promptly resulted in a decrease in H_i (pH_i increased to 7.07±0.07); reintroduction of Ca_o (1 mM) in Na_o-free solution then caused a rapid increase in H_i. It seems unlikely that sarcolemmal proton transport was responsible for the Ca-dependent, Na-independent loss of H_i because the movement of protons would be against the proton electrochemical gradient. Therefore, the results support the interaction of Ca-H at intracellular binding sites in cultured chick heart cells.

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Tu-Pos77 EFFECTS OF CARBACHOL ON L-TYPE CALCIUM CURRENT IN ATRIAL MYOCYTES.
Chr. Romanin, K. Groschner*, W.R. Kukovetz*, H. Schindler. (Intr. by Charles E. Cobb). Inst. f. Biophysics, Linz, Inst. o. Pharmacodynamics and Toxicology*, Graz, Austria.

Direct cholinergic inhibition of calcium currents in mammalian cardiac cells is still a matter of controversy since estimation of the effects is complicated by an additionally induced, superimposed, time-dependent outward K-current and by run-down of the calcium current. We investigated the effects of the muscarinic agonist carbachol on basal calcium inward currents in atrial myocytes of the guinea pig by single electrode voltage clamp. Generally outward currents were minimized by substituting Cs^+ for K^+ and experiments in which recovery of calcium current was not obtained were excluded. The effects of carbachol were studied under different experimental conditions. In experiments employing soft glass pipettes with resistances of 3-4 MOhm, carbachol (20 µM) did not inhibit L-type calcium current. However, when GTP was added to the internal solution, carbachol significantly reduced calcium current to 68 ± 10% (N=4) of control. Using hard glass pipettes with resistances of 10 - 11 MOhm, we obtained a pronounced reduction of calcium inward current (to 35 ± 2%, N=3) in the presence of carbachol. It is suggested that a) direct inhibition of calcium inward current by muscarinic agonists requires intracellular GTP and b) excessive dialysis of the cell by the use of low resistance pipettes impairs the effect, possibly due to the loss of cytoplasmic second messengers.

Supported by grants from the Fonds zur Förderung der Wissensch. Forschung (S45-3).

Tu-Pos78 **DIRECT RECORDING OF L-TYPE CA^{++} "WINDOW" CURRENT IN CARDIAC PURKINJE CELLS.**

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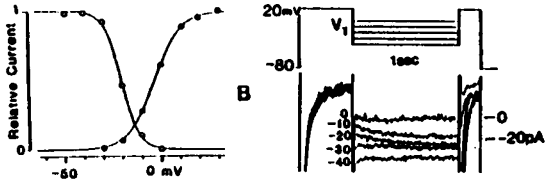
Activation and inactivation relationships for L-type Ca^{++} current overlap for voltages between about -20 and 0 mV (Panel A, 5 mM $[Ca]_o$), suggesting the presence of steady-state or "window" current. To confirm the presence of "window" current, and to separate it from slowly inactivating current elicited with depolarizing steps, we used an alternative approach. L-type Ca^{++} current was inactivated with an initial large depolarizing step, and the cell was then repolarized to test voltages (V_t) near those for "window" current. Panel B (same cell as A) shows this voltage clamp protocol and superimposed high-gain current traces. Leak current measured at the holding potential, the end of the initial step, and following clamp back to V_t was approximately linear. A slowly developing inward current was recorded at V_t within the "window", whereas at V_t of 0 mV (above the "window") or to -40 or -30 mV (below the "window") no inward current developed. The second depolarizing step elicited Ca^{++} current providing V_t was sufficiently negative to permit channel recovery to a closed state. The slowly developing inward current was abolished by nifedipine and increased by isoproterenol or Bay k 8644. Bay k 8644 shifted the "window" negatively by an amount similar to the shift in activation and inactivation curves. Our approach permits the direct measurement of "window" current and its separation from slowly inactivating current.



Tu-Pos79 EFFECTS OF INTRACELLULAR FREE MAGNESIUM ON CA^{2+} AND K^+ CURRENTS IN ISOLATED CARDIAC MYOCYTES. H.C. Hartzell, A.A. Lagrutta, I. Gourdon, and R.E. White. Department of Anatomy & Cell Biology, Emory University School of Medicine, Atlanta, GA 30322 (Intr. by S.R. Ikeda).

Recent studies from our laboratory indicated that increasing intracellular free $[Mg^{2+}]$ depresses I_{Ca} elevated by cAMP-dependent phosphorylation. We now report that in addition, internal Mg^{2+} increases voltage-dependent inactivation of I_{Ca} in ventricular cells, and inhibits basal and isoproterenol (ISO)-stimulated I_K in atrial cells. Myocytes were dispersed enzymatically from frog hearts, and ionic currents were measured using the whole-cell configuration of the patch-clamp technique. Intracellular $[Mg^{2+}]$ was varied by internal perfusion with solutions having different calculated free $[Mg^{2+}]$. Increasing $[Mg^{2+}]_i$ from 0.3 mM to 3.0 mM caused a 20% reduction in the peak amplitude of I_{Ca} or I_{Ba}, and shifted the current-voltage relationship and inactivation curves ~10 mV toward negative potentials. Relief from I_{Ca} inactivation, measured after a 200 msec prepulse to +90 mV, was depressed 56% by increasing $[Mg^{2+}]_i$ from 0.3 mM to 3.0 mM. With Ba^{2+} as charge carrier, the prepulse voltage which produced 50% of maximum inactivation was shifted 8 mV in the negative direction, and the plateau phase of the inactivation curve (prepulses between 0 mV and +100 mV) was diminished by > 50% when $[Mg^{2+}]_i$ is increased. The increased rate of I_{Ba} inactivation was due to an increase in both the steady-state level of inactivation as well as an increase in the rate, as measured by two-pulse inactivation protocols. Decreasing $[Mg^{2+}]_i$ from 3.0 mM to 0.3 mM increased the peak amplitude of I_K by ~400%. ISO (1 μM) produces a 2-fold increase in I_K amplitude. These results indicate that Mg^{2+} inhibits the activity of both cardiac Ca^{2+} and K^+ channels by acting upon a site on the cytoplasmic side of the sarcolemma. Since Mg^{2+} speeds the inactivation kinetics of I_{Ba}, but not I_{Ca}, it is likely that Mg^{2+} enhances voltage-dependent inactivation of currents. This effect could result from entry of Mg^{2+} entry into the pore or stimulation of processes associated with channel dephosphorylation. (Supported by the N.I.H.).

Tu-Pos80 NA-EXCHANGE IN ISOLATED VENTRICULAR MYOCYTES. M.A. Wallert and O. Fröhlich. Dept. Physiol., Emory University School of Medicine, Atlanta, GA 30322.

Isolated adult ventricular myocytes recover from an intracellular acid load by a Na-dependent, amiloride-sensitive proton extrusion mechanism, the Na-H exchanger. Using the pH-sensitive fluorescent dye BCECF, we characterized the kinetics of net proton extrusion after NH_4Cl-induced acidification, and we tested the response of steady-state pH_i to several different receptor agonists. The exchanger exhibited a K_m for Na_o of 58±16 mM and a V_{max} of 55±7 mmol H^+ (l cell $H_2O)^{-1}$ min^{-1}. Amiloride inhibited proton extrusion with a K_i of 3.5 μM and competed with Na_o. The rate of proton efflux was stimulated by intracellular protons and inhibited by extracellular protons. The stimulation was very steep and was nearly complete within 0.5 pH units, consistent with the generally observed involvement of an additional proton modifier site. In contrast, the pH_o dependence was less steep: proton net efflux decreased from a maximum near pH_o=8.0 to essentially zero near pH_o=6.0.

The resting intracellular pH in these cells was 7.16 ± 0.11. It increased upon addition of several different agonists (by 0.10-0.15 units): the phorbol ester PMA, ATP, the α_1-adrenergic agonist 6F-NE and the α_2-agonist UK14304. These responses were absent in the absence of Na_o and in the presence of amiloride. Isoproteronol and acetylcholine had no direct effect on Na-H exchange. Supported by AHA/Ga Affil.

Tu-Pos81 DIRECT PROTON BLOCK OF SODIUM CURRENT IN GUINEA PIG VENTRICULAR MYOCYTES. I. Hisatome, R. Sato, and D. H. Singer, Northwestern University Medical School, Chicago, Illinois.

It is well known that extracellular protons can decrease I_{Na}. However, it has not been determined whether this reflects direct proton block of the Na^+ channel or shifts in gating kinetics caused by screening effects on the negative membrane surface charge. The site of proton block also is not yet clarified. High concentration of $[Ca^{2+}]_o$ are known to screen surface charge effects. We, therefore, studied proton effects on I_{Na}, in association with variations in $[Ca^{2+}]_o$ to minimize surface charge effects, in ten guinea pig ventricular myocytes, using whole cell voltage clamp methods. Experiments were conducted at 17°C using 10mM $[Na^+]_o$ and 10mM $[Na^+]_i$. When $[Ca^{2+}]_o$ was increased above 30 mM, peak I_{Na} decreased in the absence of shifts in gating kinetics, a finding consistent with Ca^{2+} block of Na^+ channels. Reduction in extracellular pH from 7.4 to 6.0 in conjunction with increases in $[Ca^{2+}]$ to >30 mM decreased peak I_{Na} amplitude without changing gating kinetics. In addition, at a constant pH_o, increases in $[Ca^{2+}]_o$ resulted in a progressive, dose dependent, decrease in the proportion of I_{Na} blocked by H^+ [e.g., at pH_o 6.0 as compared to pH_o 7.4, peak I_{Na} was reduced by 57.5 ± 13.5% at 1.8 mM $[Ca^{2+}]_o$ (N=6), by 32.0 ± 11.4% at 30 mM $[Ca^{2+}]_o$ (N=6) and by 19.0 ± 6.3% at 40 mM $[Ca^{2+}]_o$ (N=4)]. These results suggest that both Ca^{2+} and protons act to block the Na^+ channel and that extracellular protons block the channel in the pore.

Tu-Pos82 INACTIVATING PULSE DURATION AND RAMP RATE INFLUENCE Na WINDOW CURRENT IN CAT CARDIOCYTES Ruth L. Martin, Peggy L. Barrington, and Robert E. Ten Eick (Intr. by Arthur Veis)
Department of Pharmacology, Northwestern University, Chicago, IL 60611

Tetrodotoxin can shorten the plateau duration of action potentials in cat cardiac papillary muscles suggesting Na window current (NaWC) is present in cat heart. In contrast, no steady state (SS) Na current (I_{Na}) could be detected during 0.3 - 1.0 s pulses to membrane voltages (V_m) in the range of overlap of SS activation (m_∞) and SS inactivation (h_∞) vs. V_m curves. Because of these divergent results, we determined whether enzymatically isolated cat cardiac myocytes (n = 24) can exhibit NaWC using a whole-cell-patch voltage clamp (VC) technique to apply VC ramps. Using pipet and bath solutions which permit only I_{Na} to flow, net inward current (I_{in}) was detected during ramps from -70 to -40 mV. Peak I_{in} was maximal at V_m = -44.5 mV with a ramp rate of 100 mV/s. Reducing ramp rate decreased peak I_{in} amplitude and negatively shifted the voltage at which peak (V_{peak}) occurred. With ramp rates $\leq$ 30mV/s, no I_{in} was detected. During repeated trials with 100 mV/s ramps, peak I_{in} decreased and V_{peak} became more negative with time and trial number. These results reflect the expected behavior of a Hodgkin-Huxley-like NaWC. The question then became why was NaWC not seen during long pulses to V_m in the range m_∞ and h_∞ overlap. It could be that by the end of long VC pulses to V_m at which NaWC is expected, m_∞ and h_∞ no longer overlap. The idea was tested by varying the inactivating conditioning pulse duration (CPD) of a 2-pulse protocol. When CPD was increased from 30 ms to 1 s, the V_m at which h_∞ = 0.5 shifted from -45 mV to -90 mV. Less than 10% of this shift can be explained by the well known time dependent shift of h_∞. This suggests that overlap of m_∞ and h_∞ is dependent on CPD and explains why long CPD seem to remove NaWC.

Tu-Pos83 AMIODARONE SHOWS STRONG RESTING BLOCK OF CARDIAC I_{NA} AFTER REMOVAL OF INACTIVATION GATE WITH CHLORAMINE-T. J. Miyamoto, R. Sato, J. A. Wasserstrom, and D. H. Singer, Northwestern University Medical School, Chicago, Illinois.

Amiodarone (AM, 5 μM) has been shown to produce resting and use-dependent block of I_{Na} in cardiac cells from a number of species. However, interactions between AM and the Na^+ channel have not yet been investigated. Chloramine-T (CT) is known to remove the inactivation process of Na^+ channels. We, therefore, studied AM effects on I_{Na} before and after exposure to CT in guinea pig ventricular myocytes, using whole cell voltage clamp techniques. Both pipette and bath solutions contained 10mM Na^+. After 5 minutes of exposure to AM, the I_{Na} availability curve was shifted to more negative potentials. AM-induced resting block reduced I_{Na} to 65% of control at peak potential (-60 mV). AM block was more marked at -100 mV than at -140 mV, suggesting a high affinity for inactivated Na^+ channels. Following superfusion with CT (3mM), I_{Na} amplitude was reduced at all voltages and I_{Na} decay was markedly slowed. Resting block was unchanged by CT. In addition, the slope factor for the Na^+ availability curve increased from 4.21 to 7.60 mV. After removal of inactivation, use-dependent block could no longer be induced by a train of pulses to -60mV at either 1, 2 or 5 H_z. Furthermore, the blocked ratio $(I_o-I)/I_o$ was greater at -140 mV than at -100 mV. In summary, our results showed that, following removal of inactivation: 1) AM-induced resting block was unchanged; 2) AM block was enhanced by hyperpolarizing holding potential; in contrast, in the presence of the h-gate, block was increased by depolarization; 3) AM no longer induced use-dependent block. Our results indicate that the inactivation process plays an important role in the development of AM-induced use-dependent block.

Tu-Pos84 VOLTAGE-DEPENDENT RECOVERY FROM FLECAINIDE BLOCK IS DUE TO ACTIVATION UNBLOCKING.
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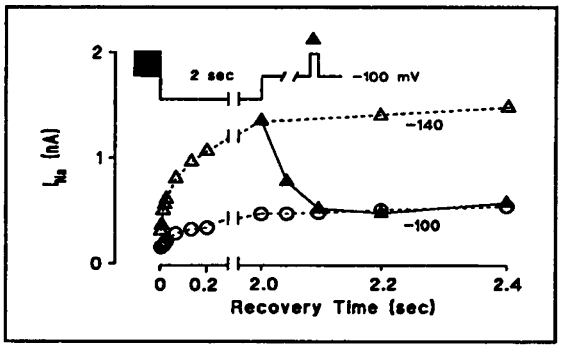
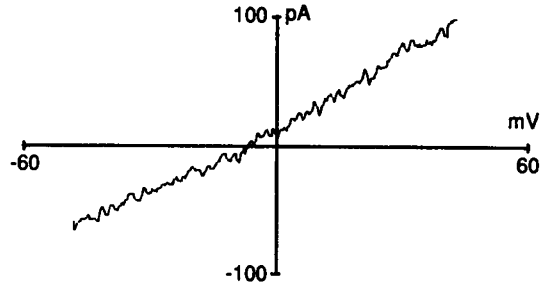
Diastolic recovery from use-dependent block of sodium channels is voltage-dependent. Recently this phenomenon has been recognized as the sum of a fast drug-dissociation from activated channels (activation unblocking) and a slower drug-dissociation from rested and inactivated channels. Cationic drug can only exit through a hydrophilic pathway, whereas neutral drug can unblock through a lipophilic pathway (Hille). The former is expected to be voltage-dependent, but the latter is not. To test this hypothesis, I_{Na} was recorded by whole cell voltage clamp in collagenase dispersed guinea-pig myocytes. Following induction of nearly 100% block (fast pulse train, flecainide (6μM), pKa 9.3), the recovery from block (open symbols) consists of two phases: a fast early phase that is strongly voltage-dependent and a slower late phase that is much less voltage-dependent. When the potential is clamped at -100 mV following a 2 sec recovery period at -140 mV, then I_{Na} (solid symbols) declines to the recovery level observed at -100 mV. Thus the fast unblocking cannot materialize unless activation is closely associated with hyperpolarization. We conclude that hyperpolarization induces a state (RD ⇐ ID) from which fast activation unblocking can occur (RD ⇒ AD ⇒ A). During subthreshold depolarization, availability for fast unblocking dissipates (RD ⇒ ID). Since the 2 sec hyperpolarization left no permanent recovery (RD ⇒ R), it must be activation unblocking that endows diastolic recovery with apparent voltage-dependence.



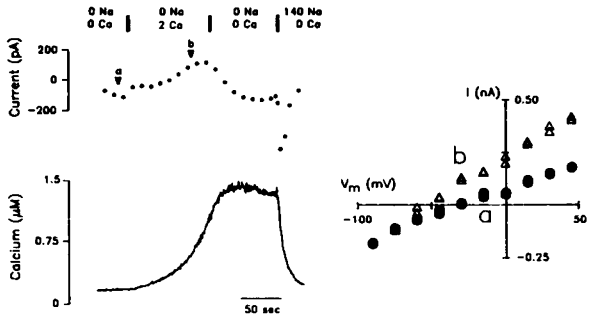
Tu-Pos85 STRONTIUM-INDUCED CREEP CURRENTS AND TONIC CONTRACTIONS IN GUINEA-PIG CARDIAC MYOCYTES.
Ernst Niggli, Department of Physiology, University of Maryland School of Medicine, Baltimore MD, 21201.

Strontium can replace calcium in a number of physiological and biochemical processes. Modifications of the mechanical response in cardiac myocytes after replacing Ca^{2+} with Sr^{2+} indicate a prolonged and augmented transient of activator cation, possibly exerting secondary effects on membrane currents. These effects of Sr^{2+} were investigated in ventricular myocytes of guinea-pigs. Membrane currents were measured with the patch-clamp technique in the whole-cell configuration. Mechanical activity was assessed using a laser diffraction system for sarcomere length measurements. Voltage-clamp pulses of 3.3 sec duration revealed large slow changes of membrane currents produced by Sr^{2+}. The identity of these currents was investigated after blocking I_{si}, Na^+ and K^+ currents. The remaining currents had many of the characteristics of "creep currents". The creep currents were paralleled by changes of $[Sr^{2+}]_i$, as determined by tracking the sarcomere length during the accompanying tonic contractions. The creep currents were suppressed by Ni^{2+} (5 mM), a finding that suggests that the Na^+-Ca^{2+} exchanger may be responsible for producing these currents. However, the same currents could also be measured despite the presence of Ni^{2+} if the cytosol was loaded with Sr^{2+} by means of a second patch-pipette sealed to the same cell. The figure shows the difference current before and after loading the cell with Sr^{2+}. The I/V relationship, obtained with voltage-clamp ramps of 2.5 sec duration, reversed at -7 mV and showed a slight outward rectification. These results suggest that the Sr^{2+}-induced creep current may be due to the activation of non-specific cation channels by intracellular Sr^{2+}.



Tu-Pos86 SODIUM-CALCIUM EXCHANGE CURRENT IN INDO-1 LOADED CARDIAC MYOCYTES. J.R. Berlin, J.R. Hume* and W.J. Lederer, Dept. of Physiology, Un. of Maryland Sch. of Medicine, Baltimore, MD 21201 and *Dept. of Physiology, Un. of Nevada Sch. of Medicine, Reno, NV 89557.

The sarcolemmal sodium-calcium exchange (Na-Ca) current has been described in cardiac cells (Hume and Uehara, J. Gen. Physiol. 87, 857-884, 1986; Kimura et al, Nature 319, 596-597, 1986) but the dependence of this current on intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) has not been well studied. In the present experiments, Na-Ca current was examined in guinea pig cardiac myocytes in which $[Ca^{2+}]_i$ was buffered with 1 mM of the Ca^{2+} indicator, Indo-1 (Na^+ salt). Cells were superfused in a Na^+-free (N-methyl glucamine), K^+-free salt solution which contained 25 uM D-600, 25 uM Ryanodine, 4 uM Strophanthidin and 0.1 mM Ba^{2+}. The patch electrode contained a tetramethylammonium ion salt solution with a total Na^+ of 11 mM. The figure shows that changing extracellular Ca^{2+} from 0 to 2 mM produced an outward current at -50 mV and a rise in $[Ca^{2+}]_i$. The elevated $[Ca^{2+}]_i$ was maintained in the absence of extracellular Na^+ and Ca^{2+}. Addition of extracellular Na^+, however, led to the activation of an inward current and a fall in Ca^{2+}_i. The voltage dependence of the current activated by increasing extracellular Ca^{2+} from 0 mM (a) to 2 mM (b) was similar to that predicted for an electrogenic Na-Ca mechanism (DiFrancesco and Noble, Phil. Trans. R. Soc. Ser. B 307, 353-398, 1985). This work has been supported by the NIH and American and Maryland Heart Associations.



Tu-Pos87 INHIBITORY EFFECTS OF CYCLIC GMP ON Ca CURRENT IN SINGLE CELLS FROM GUINEA PIG VENTRICLE. Renzo C. Levi, Giuseppe Alloatti & Rodolphe Fischmeister*. Lab. Fisiologia Generale, Univ. di Torino, I-10125 Torino, Italy, and *Lab. Physiologie Cellulaire Cardiaque, INSERM U-241, Univ. Paris-Sud, F-91405 Orsay, France (Intr. by G. Vassort).

Cyclic GMP (cGMP) levels in heart are increased by various hormones, including acetylcholine, adenosine and atrial natriuretic peptides. Since these hormones also, and independently, inhibit adenylate cyclase activity, it has been difficult to establish clearly the participation of cGMP in the negative inotropic effects produced by these agents. Using a combined whole-cell patch-clamp and intracellular perfusion technique, Hartzell and Fischmeister (Nature 323:273-275,1986) recently demonstrated that cGMP reduced Ca current in frog ventricular cells by antagonizing the stimulatory effect of cyclic AMP (cAMP). In the present study, we applied the same technique to investigate the effects of cGMP on Ca current in single myocytes isolated from guinea pig ventricle. Like in frog heart, cGMP (5 μM) strongly reduced I_{Ca} which had been elevated by intracellular perfusion with cAMP (50 μM). However, unlike in frog heart, in guinea pig cGMP also antagonized the stimulatory effects of the hydrolysis-resistant analog 8-Bromo-cAMP (50 μM). In addition, cGMP prevented the stimulation of I_{Ca} by IBMX (100μM), a phosphodiesterase inhibitor. The membrane permeant cGMP analog 8-Bromo-cGMP (100 μM), when applied outside the cell, also antagonized the stimulatory effect of IBMX on I_{Ca}. It is concluded that cGMP inhibits I_{Ca} in guinea pig ventricular cells by a mechanism different from the activation of a cGMP-stimulated phosphodiesterase.

(Partly supported by an exchange program INSERM/CNR)

Tu-Pos88 **EFFECTS OF PHORBOL ESTER ON CONTRACTILE RESPONSES OF RAT ISOLATED CARDIAC MYOCYTES.** **Kenneth T. MacLeod and Sian E. Harding. Dept. Cardiac Medicine, National Heart & Lung Institute, Dovehouse St., London, SW3 6LY, U.K.**

The action of phorbol 12-myristate 13-acetate (PMA), which stimulates protein kinase C, on the shortening of isolated cardiac myocytes has been investigated. Shortening was measured by a video edge-detection system connected to an inverted microscope. Isolated, Ca^{++}-tolerant rat ventricular myocytes were electrically stimulated at 0.5 Hz at 32°C. Under normal conditions ($[Ca^{++}]_o$ = 1.0mM) rat myocytes shortened upon stimulation by 3.1 ± 0.6% (mean ± SEM; n=5) of their resting length. The presence of PMA (10^{-7}M) produced a positive inotropy as twitch shortening increased to 5.5 ± 1.1% of resting length. PMA produced a small tonic shortening of resting cell length. The concentration of extracellular Ca^{++} which elicited maximal twitch shortening (8mM) produced a 13.1 ± 2.5% change in length. In the presence of PMA both normalised rate of shortening and relaxation were significantly ($p < 0.05$; Student's T-test) faster when compared with control. The contractile responses to changes in pH_i were recorded. pH_i was changed by addition and subsequent removal of 10mM NH_4Cl. Shortening decreased as pH_i decreased. The recovery of shortening to baseline levels was always (n=4) more rapid in the presence of PMA consistent with protein kinase C stimulating Na^+/H^+ exchange.

Supported by the British Heart Foundation.

Tu-Pos89 DUAL EFFECT OF PHOSPHORYLATION ON THE CARDIAC TRANSIENT OUTWARD CURRENT

T. NAKAYAMA*, H.C. PALFREY, D.J. NELSON, AND H.A. FOZZARD
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The transient outward K current (I_{to}) in canine cardiac Purkinje cells (which resembles I_A in neuronal cells) activates at potentials positive to -20 mV and inactivates with two time constants. β-adrenergic stimuli alter I_{to} decay by reducing the fast decaying component and increasing the slowly decaying component, thereby greatly slowing overall current decay. This kinetic change appears to be cAMP dependent, and may result from phosphorylation of the I_{to} channel itself or alternatively some associated regulatory protein (Circ. Res. 62: 162, 1988). Intracellular perfusion with cAMP protein kinase catalytic (C) subunit produces the same kinetic effects and overrides the effect of isoproterenol (J. Mol. Cell. Cardiol., In Press, 1989). The present studies lend further support to the hypothesis that protein phosphorylation is critical to I_{to} function. Inclusion of the C-subunit inhibitor PKI_{5-24} in the pipette solution (0.5 - 50 μM) initially reduced the slowly decaying components while enhancing the fast decaying component, the rate of which was dose-dependent. Further exposure to PKI_{5-24} abolishes I_{to} and subsequent exposure to isoproterenol (10^{-6} M) is without effect. Perfusion with the catalytic subunit of protein phosphatase I (0.2mg/ml, n=4) reduced both components of I_{to} and the effectiveness of isoproterenol. One phosphorylation event appears to be essential for basal channel activity and is near saturation at resting levels of cAMP. A second c-AMP dependent phosphorylation step appears to regulate the channel by slowing inactivation. Supported by NIH HL20592 and GM36823.

Tu-Pos90 FORMATION OF GIANT SARCOLEMMAL VESICLES FROM ISOLATED CARDIAC MYOCYTES AND ELECTROPHYSIOLOGICAL CHARACTERIZATION. Donald W. Hilgemann, Dept. of Physiology, Univ. of Texas Southwestern Medical Center, Dallas, TX 75235.

Membrane "blebbing" of cardiac myocytes can be optimized to prepare sarcolemmal vesicles of 10 to 30 micron diameter. Immediately upon isolation, guinea pig, rabbit or rat ventricular myocytes are placed in a solution of 150 mM KCl, 10 mM EGTA, 10 mM HEPES and 15 mM dextrose (pH 7.3). Within 1 to 8 h at 4°C, myocytes develop several large spherical blebs which are readily detached by agitation. Profuse blebbing can occur in fully extended myocytes, although greater numbers of blebs form on contracted myocytes. Incubation with 3 mM ATP significantly decreases blebbing, and suggests that rigor may be the cause of shortening in calcium-free medium. At an early stage, the substitution of sodium for potassium can reverse the blebbing process. Further addition of 0.1 mM calcium (no EGTA) largely hinders bleb formation; 2 mM magnesium or low pH (6.5) does not. High EGTA facilitates both formation and detatchment of membrane blebs. With inclusion of 2 mM magnesium, giga ohm membrane seals are obtained with standard patch clamp techniques (40 to 60 gigaohm using 150 mM cesium pipette solution and 2 micron inner diameter pipette tip). Inward rectifier and ATP-inhibited K-channels are found in inside-out excised patches. No channel activity has been found with cesium-substituted incubation solution. Membrane rupture for whole-vesicle current recording has proven difficult. Suction methods generally cause vesicle implosion and electrical methods disrupt seals. An alternative rupture method is being tested, whereby a glass fiber pulled to a point is inserted through the patch electrode tip by a remotely controlled manipulator. The dimensions of these vesicles, their presumed lack of myofilaments and sparsity of organelles should make them an advantageous preparation to study electrogenic sarcolemmal ion transport mechanisms (e.g. by combining patch clamp and fluorescent dye techniques).

Supported by AHA Grant-in-Aid 850996.

Tu-Pos91 MODIFICATION OF ELECTRICAL ACTIVITY IN CHICK EMBRYONIC HEART CELLS BY VARIOUS CARDIOACTIVE AGENTS AND BY BRIEF DURATION CURRENT STIMULI. John R. Clay*, Alvin Shrier+, and Richard M. Brochu+. *NINCDS, NIH, Bethesda, MD 20892, and +Depart. of Physiology, McGill Univ., Montreal, Que., Canada H3GIY6

We have investigated the effect of 1)TTX; 2) ACh; 3) bretylium; and 4) current stiumuli on the action potential and on spontaneous activity of chick embryonic heart cells. We have used heart cell aggregates in this study. These preparations are well space clamped, based upon the similarity of the kinetics of I_{Na} and I_K in aggregates to the kinetics of I_{Na} and I_K in single cells (Fujii, S. et al. 1988, J. Membr. Biol. 101: 209.; Clay, J.R. et al. 1988, J Physiol. 403: 525). Some of the other current components appear to be modified by suction pippette recording from single cells. We have found that: 1) relatively low concentrations of TTX (10^{-8}-10^{-7}M) significantly reduce $\dot{V}_{max}$ and cause a biphasic upstroke of the AP. Surprisingly, the beat rate is not significantly affected. 2): ACh (10^{-6} M) abolishes spontaneous activity in atrial cells and hyperpolarizes the membrane by 30-40 mV. However, the duration of the elicited AP is reduced by only 5-10%. 3): Bretylium (10^{-5}-10^{-4} M) increases the AP duration in ventricular cells during the latter phase of repolarization. 4): A brief duration depolarizing current pulse applied near the foot of the action potential delays the occurrence of the subsequent beat by ~ 30%. All of these results can be described by our model of electrical activity (Shrier A. and Clay, J.R. 1986, Biophys. J. 50: 861) with: 1) a tonic block of I_{Na} by TTX; 2) addition to the model of a time-independent $I_{K(ACh)}$, which significantly rectifies so that $I_{K(ACh)}$~0 at V>0; and 3) blockade of I_{x_1} (not I_K) by bretylium. 4): The phase resetting result can be attributed to an interplay between I_{Na} and I_{x_1}.

Tu-Pos92 EXTRACELLULAR CAESIUM ACCELERATES THE ONSET OF USE-DEPENDENT i_f PACEMAKER CURRENT BLOCKADE PP van Bogaert (Intr. by E. Carmeliet) (Laboratorium voor Fysiologie, Universiteit Antwerpen, RUCA, B-2020 Antwerpen, Belgium)

The i_f pacemaker current was studied in short cardiac Purkinje fibres of sheep with the two microelectrodes voltage-clamp technique (di Francesco, J. Physiol. 1984. 348:341-367). The "specific bradycardic agent" UL-FS 49 blocks the i_f current in an use-dependent manner during trains of voltage-clamp pulses from -30 to -120 mV, lasting 1 sec, at 0.4 Hz (van Bogaert et al., Europ. Heart J. 1987. 8,Suppl.L:35-42). In the presence of 2×10^{-6} M UL-FS 49, the outward i_f tail currents disappeared exponentially during a train with a rate constant λ: 0.049 +/- 0.001 N^{-1} (n=3). When 1×10^{-5} M CsCl was added, λ increased to 0.081 +/- 0.007 N^{-1} (n=3). The slope of the linear relation between rate of block during a train, λ, and drug concentration was increased by CsCl. After a block inducing train, outward i_f tail currents recovered with an exponential time course following prolonged hyperpolarizations. At -120 mV, the rate of recovery from block, 1, was 0.0058 +/- 0.0005 sec^{-1} (n=5) without and 0.0037 +/- 0.0003 sec^{-1} (n=7) with CsCl present. This reduced rate of relief from block can explain part of the faster accumulation of block during a train in the presence of CsCl. The process of i_f block removal appears to be partially dependent on the amount of inward current crossing the i_f channel. Caesium, a blocker of inward i_f current, reduced the unblocking rate at -120mV, while an increase of external Na+ from 35 to 105 mM increased 1 to 0.0116 +/- 0.001 sec^{-1} (n=5).

Supported by the NFWO and Born-Bunge Research Foundation.

Tu-Pos93 CELLULAR BASIS FOR THE FUNCTIONAL REFRACTORY PERIOD. B.M. Ramza, R.C. Tan, T. Osaka, R.W. Joyner. Depts. of Pediatrics and Physiology, Emory University, Atlanta, GA, 30322 and The University of Iowa, Iowa City, IA, 52242.
It is well established that a premature action potential elicited in ventricular muscle during the functional refractory period of a preceding action potential requires an increased stimulus intensity for successful propagation. Our goal was to understand the cellular basis for these relative decreases in tissue excitability during the recovery phase by performing parallel experiments on rabbit left papillary muscle and isolated rabbit ventricular cells. For each preparation, the pacing protocol consisted of a train of 10 stimuli (S1) at an S1-S1 interval of 500 mSec with a premature stimulus (S2) of variable S1-S2 intervals following the tenth S1 action potential (SD Time). In the tissue preparation, the I2 increased sharply for SD Times <0 mSec to a value that was 100% above the S1 stimulus threshold for SD Time=-5 $\pm$ 2.4 mSec (n=8). Similar experiments on the isolated ventricular cell showed no changes in I2 as a function of SD Time but rather significant decreases in both the Action Potential Amplitude (APA) and the Vmax of the S2 action potential. The APA and Vmax for the S2 action potential were decreased to 50% of the S1 action potential values for SD Time=-4.5$\pm$2.2 mSec and SD Time=0.6$\pm$1.5 mSec respectively (n=8). Both parameters reached 100% recovery by SD Time= 10 mSec. These results are consistent with the hypothesis that the decreases in tissue excitability occur as a result of a decrease in the cellular responsiveness (APA, Vmax) rather than an intrinsic decrease in cellular excitability (I2).

Tu-Pos94 METOPROLOL ALTERS I_{K1}, I_{to}, AND I_{Ca} BUT NOT I_{Na} IN ISOLATED CAT MYOCYTES. Peggy L. Barrington and Robert E. Ten Eick. (Intr. by AL Bassett). Department of Pharmacology Northwestern University, Chicago, IL 60611
Metoprolol (Lopressor) is a cardio-selective β_1-adrenergic antagonist. Most published evidence indicates that metoprolol is a pure class II agent. However in vivo, long-term metoprolol use prolongs Q-T intervals, suggesting that metoprolol has some class III antiarrhythmic properties. Whole-cell voltage-clamp experiments were conducted on ventricular myocytes isolated following perfusion of cat hearts with collagenase-containing solutions. I_{K1}, I_{to}, I_{Ca}(L-type) and I_{Na} were isolated using appropriate internal and external solutions, holding potentials and voltage clamp protocols. Metoprolol (10 μM) reduced peak I_{K1} at test potentials negative to E_K, while no effects were seen on the outward current positive to E_K. Hyperpolarizing test pulses negative to -130 mV altered inactivation with an inward "droop" that began within 20 ms of pulse onset and continued to develop during the next 100 ms. I_{to} and I_{Ca} were identified as the Cd^{2+} insensitive and sensitive currents respectively. Metoprolol (10 μM) decreased peak I_{to} at depolarizing pulse > 30 mV but had no effect on inactivation kinetics. Peak I_{Ca} amplitude decreased but the inactivation time course did not change. Peak and steady-state I_{Na} were not altered by 100 μM metoprolol. These results suggest metoprolol can exert direct effects on cardiac cells that can at least partially block I_{K1}, I_{to} and I_{Ca}. I_{K1} is more sensitive to metoprolol than I_{to} or I_{Ca}, while I_{Na} does not appear to be affected by drug. The nature of these direct effects suggests that metoprolol may have some activity as a class III agent. (Supported by a generous gift from the Ciba-Geigy Pharmaceutical Co.)

Tu-Pos95 **ANALYSIS OF AMILORIDE-SENSITIVE H^+ EFFLUX DURING α-ADRENORECEPTOR STIMULATION OF CARDIAC PURKINJE FIBERS.** Milton L. Pressler, D. Eugene Lovelace and Timothy E. Breen, Krannert Institute of Cardiology, Indiana University School of Medicine, Indianapolis, IN
Intracellular pH (pH_i) in cardiac Purkinje fibers may be modulated by stimulation of α-adrenoreceptors that are coupled to effects on the Na/H exchanger. We used ion-selective microelectrodes to study pH_i homeostasis in sheep Purkinje fibers after NH_4Cl prepulses. Phenylephrine (Phen; 100-300 μM) + timolol (300 nM) were used to stimulate α-adrenoreceptors and phorbol 12,13-dibutyrate (PDBu; 1.5-7.5 μM) to activate protein kinase C. Resting pH_i ranged from 7.18 to 7.35 at 25 °C. Phen increased pH_i by a mean of 0.03 units (n=4) after 2-5 min Greater alkalinization occurred after PDBu (mean increase 0.16; n=3) but was poorly reversible. An inactive phorbol ($4-\alpha$ phorbol 12,13-didecanoate) had no effects on pH_i. During acidosis after NH_4Cl withdrawal, continued exposure to Phen accelerated the rate of $[H^+]_i$ recovery [1.21 $\pm$ 0.03 (Phen) vs 0.85 $\pm$ 0.20 nM/min (CONTROL; n=3)] by a process inhibited by 1 mM amiloride (Amil) [0.49 $\pm$ 05 nM/min (Phen+Amil)]. The kinetics of $[H^+]_i$ recovery have been analyzed by a mathematical model. Partial differential equations have been solved for diffusion through a barrier into a space with diffusivity D to which a surface antiporter(s) adds or subtracts H^+. Residual errors were random and D was constant over up to 11 transitions. Surface fluxes of H^+ were derived after subtraction of passive diffusion. The results suggest that pH_i recovery after a prepulse of NH_4Cl involves a multiexponential ($\geq$2) process of surface H^+ efflux. Preliminary fits indicate that the H^+ efflux was: (i) maintained at a 2-3 fold greater rate during exposure to Phen; and (ii) inhibited strongly by Amil. The findings suggest that α-adrenoreceptor stimulation promotes pH_i recovery by enhancing H^+ efflux, probably through involvement of the Na/H exchanger.

Tu-Pos96 **NON-IDEAL OSMOTIC BEHAVIOR OF RABBIT VENTRICULAR MYOCYTES**

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Dept. of Physiology, Medical College of Virginia, Richmond, VA 23298-0551

Single myocytes are suitable for recording cell volume and ion activity. Volume alterations in response to osmotic stress were studied in myocytes isolated from ventricular septum of rabbit hearts by a collagenase digestion procedure. For volume measurements, isotonic solution (1T) contained (mM): 130 mannitol, 65 NaCl, 5 KCl, 2.5 $CaCl_2$, 1.75 Na_2HPO_4, 1.2 $MgSO_4$, 24 $NaHCO_3$, 10 glucose (pH 7.4, 37°C, 95% O_2 - 5% CO_2). Hypertonic solution (1.8T) was made by adding 260 mM mannitol to 1T, and hypotonic medium (0.6T) was created by omission of mannitol. The osmolarity of 0.6T, 1T and 1.8T was 199, 321 and 577 mOsm/l, respectively. Myocytes were placed in a perfusion chamber on the stage of an inverted microscope and viewed with video system (1750×). Images were recorded during exposure to 0.6T-1.8T, and cell length, width and area were measured with a computerized system. Cell volume was estimated from area × thickness, assuming that changes in cell width and thickness were proportional. All parameters were normalized to values in 1T (length = 121±6 μm, width = 30±4 μm, area = 3461±326 μm^2). In 0.6T, cell length and width increased to 1.03±0.01 and 1.21±0.04 (n=15, 5 cells), respectively. In 1.8T, the cells shortened to 0.93±0.01 and narrowed to 0.88±0.02 (n=9, 3 cells). Myocytes volume swelled to 1.45±0.09 and shrank to 0.75±0.02 when bathed in 0.6T and 1.8T, respectively. Observed volume changes were significantly different from expected values of 1.67 in 0.6T and 0.56 in 1.8T. When anisosmotic media were replaced with 1T, cells returned to their initial size and shape within 5-7 min. Changes in myocyte length and width in anisosmotic media were not proportional. Elongation and shortening of single cells may be resisted more than width changes because of greater difficulty in altering sarcomere length than in altering radial myofilament spacing. The non-ideal osmotic behavior of myocyte volume may suggest activation of compensatory ion transport in both hypotonic and hypertonic media.

Tu-Pos97 **COMPARISON OF THEORETICAL AND OBSERVED CONDUCTION CHANGES DURING SIMULATED ISCHEMIA IN VENTRICULAR MUSCLE**

Stephen Knisley, Yoshihiro Hiramatsu, Jack Buchanan, Leonard Gettes, Univ. of North Carolina, Chapel Hill, NC

To determine the relative importance of changes in active and passive membrane properties to ischemia-induced conduction slowing, we measured simultaneously $\dot{V}_{max}$ of the action potential (AP) upstroke, AP amplitude (APA), internal and external longitudinal resistance (r_i, r_o) using the voltage ratio method, time constant of the AP foot (τ_f) using a least squares technique, conduction velocity (θ), and from them, effective capacitance (C_{eff}) in guinea pig papillary muscles (n = 8) driven at 2.0 Hz during 30 minutes of simulated ischemia (SI: K^+ = 9 mM, pH = 6.5, pO_2 < 30 mm Hg, glucose = 0). To prevent changes in superfusate K^+ from influencing the results, we used a 9 mM K^+ control superfusate. We compared the observed changes in θ to those predicted by cable theory, i.e., $\theta = \sqrt{\dot{V}_{max}/[(r_i + r_o) \cdot APA \cdot C_{eff}]}$. During SI, $\dot{V}_{max}$ decreased by 23.1%, r_i increased by 66.6%, r_o was unchanged, APA decreased by 7.0%, τ_f increased by 53.5%, C_{eff} decreased by 39.3%, and θ decreased by 17.4%. The observed change in θ was 13.4% less than predicted by the combined changes in $\dot{V}_{max}$ and r_i alone. The discrepancy decreased to 10.8% when the change in APA was included. The observed change in θ became 9.6% greater than predicted after incorporating the change in C_{eff}. Our results suggest that C_{eff} decreases during ischemia and may lessen the conduction slowing. Moreover, when each of the changes in $\dot{V}_{max}$, r_i, APA, and C_{eff} are incorporated into the cable prediction, the predicted changes in θ become increasingly similar to the observed changes.

Tu-Pos98 **LASER SCANNER MAPPING OF ANISOTROPIC CONDUCTION IN CARDIAC VENTRICULAR MUSCLE: ARRHYTHMOGENIC SUBSTRATES PRODUCED BY PREMATURE STIMULATION AND HEPTANOL.** Bruce C. Hill, Research Institute, Palo Alto Medical Foundation, Palo Alto, CA and Mario Delmar, SUNY Health Science Center at Syracuse, NY.

Reentrant arrhythmias are readily induced in areas of two-dimensional conduction of oriented heart cells. In order to study pre-conditions for arrhythmia formation, we monitored activation wavefronts in small (<60 mm^2) thin (<1 mm thick) slices of rabbit left ventricular epicardium with a laser scanner. Alterations in the pathways of action potential propagation were induced by two interventions: premature stimulation and reduction in cell-to-cell coupling after exposure to heptanol. The tissue was stained with potentiometric dye WW781 and scanned with a pattern of 63 laser spots (50 micron diameter.) These slices were paced at 500-700 msec intervals for controls. For premature stimulation, extrastimuli having an S1-S2 coupling interval slightly longer than the effective refractory period were delivered after every third beat. In separate protocols, heptanol (1.75 mM) was added to increase intercellular resistance.

Heptanol produced lines of conduction block in 4 of the 5 preparations. Most lines of block were parallel to fiber orientation (as determined by the direction of slow conduction); i.e., they blocked conduction transverse to the fibers. In some instances, the activation wave pivoted around one end of the block, approached it from a retrograde direction, and blocked from that side also. Premature stimulation caused blocks that were parallel to fiber direction in 2 cases and perpendicular in one case. In some cases this block produced a similar pivoting of the wave and retrograde travel as was seen with heptanol exposure.

We found, thus, that premature stimulation and heptanol produce similar effects in breaking up the pathway of excitation into circuitous routes having delays and blocks. This alteration would be expected to increase the likelihood that a stable reentrant circuit around a functional obstacle would be formed.

Tu-Pos99 MODULATION OF K^+ AND Ca^{2+} CURRENTS BY ISOPROTERENOL IN BULLFROG ATRIAL MYOCYTES.
T. Nakajima, K. Ono, E.F. Shibata and W. Giles. University of Calgary, Calgary, Canada.

The effects of isoproterenol (ISO) on the action potential, (AP), the calcium current (I_{Ca}) and the delayed rectifier K^+ current (I_K) were examined using a tight-seal whole-cell voltage clamp technique in single cells from bullfrog atrium. ISO-induced changes in the AP were dose-dependent. Low doses ($5x10^{-9}$ M) prolonged the AP. Higher doses (10^{-6} M) increased the plateau height, but shortened AP duration by enhancing the early repolarization phase. ISO increased I_K and I_{Ca} in a dose-dependent fashion. Both of these effects were blocked by propranolol ($3x10^{-7}$ M). The inwardly rectifying K^+ current was not changed significantly by ISO. The ISO-induced increase in I_K was observed in the presence of $CdCl_2$ ($3x10^{-4}$ M), demonstrating that Ca^{2+} influx due to I_{Ca} is not essential for this effect. Since the reversal potential of I_K in normal Ringer solution (-83 ± 2 mV) was not significantly changed by ISO, stimulation of the Na^+/K^+ pump resulting in a hyperpolarization of E_K is not responsible for the increase in I_K. In the presence of ISO (10^{-6} M) the steady-state activation curve for I_K was shifted to more negative values (by approx. 10 mV) and kinetics of I_K were changed. Activation was accelerated, deactivation was slowed. ISO (10^{-6} M) increased I_{Ca} dramatically—approx. 6-fold at 0 mV, and decreased the time constant of I_{Ca} inactivation at 0 mV by approx. 30%. These ISO-induced dose-dependent changes in I_{Ca} and I_K can explain the observed changes in AP shape: (i) in approx. 10^{-8} M ISO only I_{Ca} is increased resulting in a higher plateau and a small lengthening of the AP (ii) in 10^{-6} M ISO the increase in I_{Ca} is much larger and it inactivates faster. In addition, I_K is increased significantly, tending to shorten the AP.

Tu-Pos100 ALPHA ADRENERGIC AGONISTS REDUCE A TRANSIENT OUTWARD CURRENT IN SINGLE CELLS FROM RABBIT ATRIUM.
D. Fedida, Y. Shimoni and W.R. Giles (Intr. by N. Anderson), University of Calgary, Calgary, Canada.

Single atrial cells were obtained from rabbit hearts by enzymatic dispersion and were voltage clamped using a whole-cell microelectrode technique. All experiments were done at 22 - 23°C. Transient outward currents were elicited from a holding potential of -80 mV, and at a constant rate (usually 0.1 Hz). The Tyrode solution included 0.3 mM Cd^{2+} to block I_{Ca} and propranolol (10^{-6} M) to prevent Beta-adrenergic effects. Alpha-adrenergic agonists were applied either by superfusion or by pressure ejection from a second microelectrode placed near the cell. Applying brief pulses of the adrenergic drugs allowed rapid recovery and avoided development of desensitization.

Methoxamine (10^{-4} M), phenylephrine (10^{-4} M) and noradrenaline (10^{-6} - 10^{-5} M) all produced marked reductions in the transient outward K^+ current which were readily reversible. This inhibition of the transient outward current was partially blocked by prazosin, (2×10^{-6} - 4 or 10^{-5} M) an alpha-1 antagonist. Atrial action potentials were recorded in the presence of propranolol (10^{-6} M). Alpha agonists produced a marked slowing of the initial repolarization of the action potential. This effect resembles the changes seen when the stimulus rate is increased or when quinidine (10^{-5} M) is applied. The reduction in the transient outward current by alpha-adrenergic agonists may be responsible for some of the species and frequency-dependent effects of these agents on atrial excitability and contractility.

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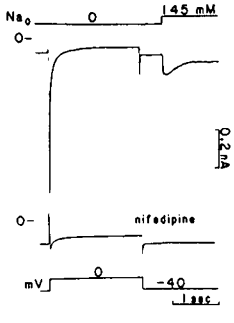
Tu-Pos101 SEPARATION OF INWARD Ca CURRENT (I_{Ca}) AND INWARD Na-Ca EXCHANGE CURRENT ($I_{Na\text{-}Ca}$) IN ISOLATED GUINEA PIG VENTRICULAR MYOCYTES.
John H.B. Bridge and Kenneth W. Spitzer, Nora Eccles Harrison CVRTI, University of Utah, Salt Lake City, Utah 84112



We used a rapid solution changer to separate I_{Ca} and $I_{Na\text{-}Ca}$. Isolated myocytes were voltage clamped at -40 mV (30°C) with suction micropipettes. The micropipettes contained 145 mM Cs, 0Na and 0K (pH 7.1). Initially, a cell was superfused in a stream of HEPES buffered solution (pH 7.4) containing 0Na, 145 mM Li and 10 mM caffeine (to prevent SR Ca sequestration). A 2 sec clamp pulse to 0 mV elicited I_{Ca} (see Fig.) and caused the cell to contract (not shown). Relaxation was induced 500 msecs after repolarization by rapidly moving an adjacent stream of solution containing 145 mM Na (+10 mM caffeine) over the cell,. ($t_{1/2} \cong$ 40 msecs, "A Simple Device for Rapidly Exchanging the Solution Surrounding a Single Cardiac Cell." Spitzer KW, Bridge JHB Am J Physiol: Cell, in press). This application of Na elicited a transient inward current (see Fig.) and caused the cell to relax. The current peaked prior to and decayed during relaxation. This current could not be activated by Na_o if Ca_i was buffered (14 mM EGTA in pipette) or if the preceding Ca current was blocked with 10 µM nifedipine(see Fig.). The integral of the nifedipine sensitive inward current (I_{Ca}) was approximately twice the integral of the Na_o dependent inward current. We suggest that the Na_o dependent current is $I_{Na\text{-}Ca}$ and is generated when Ca_i is transiently extruded in exchange for Na_o. The 2:1 integral relationship is expected when $3Na_o$ exchange with each Ca_i that entered as I_{Ca}.

Tu-Pos102 HCO_3/Cl EXCHANGE IN CAT VENTRICULAR MUSCLE. Kenneth W. Spitzer. Nora Eccles Harrison CVRTI, University of Utah, Salt Lake City, UT 84112.

The role of HCO_3/Cl exchange in mediating sarcolemmal Cl and HCO_3 transport in adult mammalian ventricular muscle was studied using resting cat papillary muscles and ion-selective microelectrodes to measure intracellular activities of $Cl^-(a^i_{Cl})$ and H^+ (pH_i). The mean (±SD) steady state values of Vm, a^i_{Cl}, pH_i and $[HCO_3]_i$, i.e.($0.032PCO_2 antilog_{10}(pH_i-6.1)$), in normal Tyrode's solution (136.8mM Cl, 18.0mM HCO_3, 5.0%CO_2, pH 7.40, 37°C) were -85.5±2.3mV(n=73muscles), 9.8±2.1mM (n=33), 7.14 ±0.06 (n=40) and 10.4 ±1.3mM (n=40), respectively. Non-CO_2 intracellular buffering power (mean pH_i interval=7.12-6.86) = 26.8 ±5.4mM/pH (n=10). Exposure to zero-Cl_o(pH7.4) elicited a monoexponential, DIDS sensitive, decline in a^i_{Cl} which was faster in 18mM HCO_3/5%CO_2 ($k=18.3\pm6.6\times10^{-4}$/sec, n=8) than in HCO_3/CO_2-free solution ($k=6.9\pm1.0\times10^{-4}$/sec, n=9). Exposure to zero-Cl_o (18mM HCO_3,5%CO_2,pH7.4) generated a DIDS-inhibitable, energetically uphill increase in pH_i. After equilibration in zero-Cl_o (HCO_3/CO_2-free,pH7.4), exposure to normal Cl_o (HCO_3/CO_2-free,pH7.4) elicited a monoexponential increase in a^i_{Cl} which was markedly slowed by rapid application of 18mM HCO_3,5%CO_2, (pH 7.4). Energetically uphill increases in steady state a^i_{Cl}, inhibited by DIDS, accompanied both the decline in pH_i elicited by lowering $[HCO_3]_o$ from 18mM(5%CO_2,pH7.4) to 4.0mM(5%CO_2,pH6.8) and the increase in pH_i produced by exposure to 10mM NH_4Cl (pH 7.4).Exposure to 20%CO_2(pH6.8) caused a sustained fall in pH_i but no change in a^i_{Cl}. pH_i in normal Tyrode's was decreased by DIDS (0.2mM) but largely unaffected by amiloride (0.5-2.0mM). The energetically uphill increase in pH_i that follows internal acid loading with either NH_4 or 4mM HCO_3(5%CO_2,pH6.8) was inhibited by DIDS. Cat ventricular muscle appears to have a reversible HCO_3/Cl exchange which may mediate acid extrusion.

Tu-Pos103 MODULATION OF CARDIAC Na, Ca AND K CURRENTS BY AMIODARONE AND DES-OXO-AMIODARONE. PB Bennett, C. Valenzuela, LM Hondeghem, Depts. of Pharmacology and Medicine, Vanderbilt University, Nashville, TN. (Intr. by J. A. Johns)

Ionic currents were measured in voltage clamped guinea pig ventricular cardiocytes using the whole-cell patch clamp method. Solutions and clamp potentials were selected to optimize measurements of the particular current under investigation. Amiodarone (A) is a highly effective antiarrhythmic agent with severe toxic side effects. Des-oxo-amiodarone (DA) was synthesized with the goal of producing a less toxic but still effective antiarrhythmic agent. DA, which is much less toxic than A in acute animal studies, caused use-dependent reduction of both I_{Na} and I_{Ca}, and caused a negative shift in the voltage dependence of channel availability. DA also reduced the delayed outward potassium current. These effects are similar to A. In contrast to A, DA (16 μM) enhanced I_{Ca} at negative (< -45 mV) potential while reducing current at more positive potentials. In addition the 1st peak inward I_{Ca} after a long rest period was increased in DA, but subsequently the currents were smaller than control. Amiodarone (16 μM) reduced Ca channel availability and caused use-dependent block. Thus, A appeared solely as a ion channel blocker, whereas the reduction of its carbonyl group produced an agent (DA) that could both enhance and inhibit Ca channel current depending on conditions.

Tu-Pos104 SODIUM CURRENT CAN BE AFFECTED BY INTRODUCTION OF ISOLATED CAT VENTRICULAR MYOCYTES INTO A PRIMARY CELL CULTURE ENVIRONMENT TE Schackow*, PL Barrington*, M Cook†, D Simpson†, RS Decker†, and RE Ten Eick* (Intr. by R Novak)
Departments of Pharmacology* and Cell Biology†, Northwestern University, Chicago, IL 60611

Adult cat cardiac ventricular myocytes (VM) obtained from collagenase-perfused hearts and maintained in primary culture represent an attractive experimental model to study aspects of cardiac function while reducing animal number and cost. To determine if cultured VM could be used as a stable experimental substrate for electrophysiological studies of the Na channel, we compared sodium currents (I_{Na}) in freshly isolated VM (FVM) with those in VM maintained in culture for 12 days (CVM). The results suggest that I_{Na} in CVM and FVM are different. Sodium currents were recorded from cells using the single suction-pipette voltage-clamp technique. CVM had developed prominent attachment plaques and were not beating. The current-voltage relationship in CVM [n=9] was shifted negative to that of FVM [n=5] at test pulses that elicited peak inward I_{Na}. The conductance-voltage relationship was not significantly changed in CVM. The steady-state inactivation curve (h_∞) in CVM [n=9], determined by a 100 ms test pulse to -10 mV preceded by a 1 s conditioning pulse, was shifted approximately 25 mV negative to that of FVM [n=6] (h_∞=1, 0.5, and 0 at conditioning potentials of -129±2 (mean±S.D.), -100±3, and -79±4 mV respectively for CVM; -103±4, -76±3, and -48±6 mV for FVM). I_{Na} inactivation during test pulses from -140 to -10 mV was significantly faster in CVM (τ_f=1.9±0.2 ms, τ_s=13.1±2.2 ms) [n=7] as compared to FVM (τ_f=4.7±1.6 ms, τ_s=19.9±2.6 ms) [n=5]. Faster I_{Na} inactivation in CVM suggests that at least some gating properties of Na channels have undergone change while in culture. Whether the changes result from being in culture or lack of beating remains to be determined.

Tu-Pos105 EFFECTS OF PHENYLEPHRINE ON INTRACELLULAR SODIUM ION ACTIVITY, TWITCH FORCE AND MEMBRANE POTENTIAL IN GUINEA PIG VENTRICULAR MUSCLE. Q.Y. Gong, D.Y. Wang, S.W. Chae and C.O. Lee, Dept. of Physiology, Cornell University Medical College, New York, N.Y. 10021

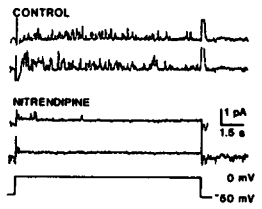
The effects of stimulation of alpha adrenergic receptor on intracellular sodium ion activity, twitch force and membrane potential were studied in guinea pig papillary muscle driven at a rate of 1 Hz. Intracellular sodium ion activity (a^i_{Na}), twitch force and action potential of the ventricular papillary muscles were measured simultaneously and continuously during exposure to phenylephrine (10^{-6} - 10^{-5} M) and subsequent washout in the absence and presence of prazosin, atenolol and strophanthidin. Phenylephrine decreased a^i_{Na} from 5.5±1.3 to 5.0±1.2 mM (mean±SD, n=6, $P < 0.001$), produced a biphasic change in twitch force; a transient decrease and then increase, and hyperpolarized diastolic membrane potential. The decrease in a^i_{Na}, transient decrease in twitch force and hyperpolarization in diastolic membrane potential produced by phenylephrine were abolished by prazosin ($3x10^{-7}$ M), but not by atenolol (10^{-5} M). Application of both prazosin and atenolol abolished the decrease in a^i_{Na}, biphasic change in twitch force and hyperpolarization in diastolic membrane potential produced by phenylephrine. Strophanthidin (10^{-5} M) had no effect on the decrease in a^i_{Na}, transient decrease in twitch force and hyperpolarization in diastolic membrane potential produced by phenylephrine. The results suggest that stimulation of alpha adrenergic receptor by phenylephrine causes a decrease in a^i_{Na}, a transient decrease in twitch force, and hyperpolarization in diastolic membrane potential, and that the decrease in a^i_{Na} may be related to the transient decrease in twitch force. Further, our study suggests that in ventricular papillary muscle the decrease in a^i_{Na} produced by phenylephrine may not be caused by a stimulation of the Na^+-K^+ pump. (Supported by USPHS HL 21136).

Tu-Pos106 LOW EXTERNAL Ca ON CALCIUM CHANNELS OF SQUID NEURONS (SQN). L. Tabares and C.M. Armstrong. Department of Physiology, School of Medicine, University of Pennsylvania, PA 19104-6085 - (Spon. L. Peachey).

The effect of lowering the external Ca concentration on the calcium channels of neurons dissociated from the squid giant fiber lobe and maintained in culture was studied with the whole cell variant of the patch clamp technique. Experiments were performed between 15-23 °C., with the holding potential set to -80 mV. The amplitude of the current through the calcium channels at the end of 10 ms depolarizing pulses was compared at 100, 50, 10, 1 and .5 mM external Ca, as well as at 10 mM Ba. At 1 mM Ca and below, the current recorded was typically bigger than at 10 mM with either Ca or Ba, and in some cases greater than that obtained at 50 mM Ca. The conductance in each of these solutions was calculated from the amplitude of the tail current after a 13 ms pulse to a range of activating potentials. The conductance curve in 1 mM Ca was shifted to the left between 15-30 mV relative to the 10 and 50 mM Ca curves, and about 6 mV to the left of the 10 mM Ba curve. In some cases the slope conductance from instantaneous I-V curves was found to be even bigger at 1 mM Ca than at 50 mM. These results suggest a change in the selectivity of SQN calcium channels when the external Ca concentration is 1 mM or lower. Although, this phenomenon may be similar to the one described in calcium channels both from vertebrates and invertebrates when the external Ca is in the micromolar range, it is interesting that in SQN the selectivity loss may occur even with 1 mM external Ca. Supported by NIH Grant No. NS12543.

Tu-Pos107 VOLTAGE INACTIVATION OF T-TUBULE CALCIUM CHANNELS INCORPORATED INTO LIPID BILAYERS. M. Fill, R. Mejia-Alvarez, S. Hamilton, and E. Stefani. Dept. Physiol. & Mol. Biophys. Baylor College of Medicine. Houston, TX 77030.

Vesicles from 3 membrane fractionations were incorporated into planar lipid bilayers (1:1, POPE:POPS, 50 mg/ml decane) using the Mueller-Rudin technique. Solutions contained 50 mM NaCl, 10 mM Hepes-Na (pH 7.2), 10 μM added $CaCl_2$, and 2.5 μM Bay K 8644. The current carrier (100 mM $BaCl_2$) and the vesicles were added to the "cis" chamber. Potential convention is "cis-trans". Two types of nitrendipine (20 μM) sensitive calcium channels were identified. One type, main conductace 10 pS, was observed at steady state potentials and showed no sign of voltage dependent inactivation. The second channel type, main conductance 12 pS, was strongly voltage inactivated. This channel was observed only if the bilayer was polarized (cis positive) and pulsed to depolarizing potentials (Po << 0.01 at steady HP=0 mV). During pulses, the channel inactivated. Channel inactivation was steeply voltage dependent (voltage range +20 to 0 mV). These observations may indicate that there are two distinct types of DHP sensitive calcium channels in the T-tubule. Alternatively, it is possible that some channels, in a single population of DHP-senitive channels, lose their inactivation "gate" during the fractionation process. Supported by NIH.



Tu-Pos108 SENSITIVITY OF Ca CHANNELS TO DIHYDROPYRIDINES IN RAT PITUITARY CELLS. J.L. Torres-Escalante, A. Marin, and G. Cota (Intr. by F. Ramón). Dept. of Physiology, Biophysics and Neurosciences, CINVESTAV, IPN, AP 14-740, México, DF 07000, México.

We have been studying the effects of nifedipine (NIF) and BAY K 8644 (BK) on the activity of SD and FD Ca channels in cultured pars intermedia (PI) cells. SD channels are low-threshold, transient, slowly-deactivating channels, whereas the opposite is true for FD channels (Cota, J. Gen. Physiol. 88:83, 1986). We used whole-cell recording with patch pipettes at 21°C. The permeant ion was external Ba (20 mM), and the HP was -80 mV. Tail currents were recorded at -80 mV following 10-ms pulses to +60 mV. NIF (1 μM) reduced the amplitude of the FD tail by ∿50% and that of the SD tail by ∿25%. In contrast, BK (0.5 μM) increased the amplitude of the FD and SD currents by ∿40% and ∿150%. Closing kinetics of the Ca channels was affected by BK, but not by NIF. The closing time constant was ∿100 μs for FD channels and ∿2.1 ms for SD channels in the control condition. With BK, the closing time constants were ∿150 μs and ∿1.6 ms, respectively. Neither NIF nor BK significantly altered the voltage dependence of FD and SD channel activation, as determined from tail current amplitudes following 10-ms steps. However, BK prolonged the rising phase of the SD current and removed current inactivation during small depolarizations. BK also affected the voltage dependence of steady-state inactivation for SD channels. The fraction of available SD channels at a HP of -40 mV was ∿20% in the control condition, and >80% with BK. A possible interpretation of these results is that in the presence of BK a fraction of FD channels activates at lower depolarizations than usual and closes at -80 mV so slowly that its macroscopic activity overlaps with that of normal SD channels. Alternatively, BK may remove the inactivation of SD channels, without affecting the voltage dependence of FD channel activation.

Tu-Pos109 CHANGES IN Ca and K CHANNEL ACTIVITY INDUCED BY ACUTE AND CHRONIC STIMULATION OF CATECHOLAMINERGIC RECEPTORS IN PITUITARY CELLS. G. Cota Dept. of Physiology, Biophysics and Neurosciences, CINVESTAV, IPN, AP 14-740, Mexico, DF 07000, Mexico.

Whole-cell membrane currents were recorded with patch pipettes at 21°C from rat pars intermedia (PI) cells kept in primary culture 1-20 days. Acute stimulation (for some minutes) of D-2 dopaminergic receptors with dopamine or bromocriptine (1 μM) reduced the amplitude of the current through voltage-gated Ca channels by a selective inhibition of the activity of high-threshold, fast-deactivating (FD) channels. Acute stimulation of beta-2 adrenergic receptors with isoproterenol (1 μM) had the opposite effect. Neither the dopaminergic agonists nor isoproterenol significantly affected the activity of low-threshold, slowly-deactivating (SD) Ca channels. This dual, "direct" modulation of FD channel function does not seem to be the only way to alter Ca influx by acute stimulation of the catecholaminergic receptors in PI cells. Acute exposure to dopamine also stimulated the voltage-dependent K outward current, an effect that is expected to change indirectly the activity of both types of Ca channels, SD and FD. In addition, chronic stimulation (for 1 day or longer) of the catecholaminergic receptors induced long-lasting changes in Ca channel activity, which appear to involve modifications of the surface density of FD and SD channels in the plasma membrane. Both effects, acute and chronic, of the stimulation of catecholaminergic receptors on Ca channel activity are probably mediated by changes in the intracellular levels of cyclic AMP. It is then reasonable to propose that modulation of Ca channel function and regulation of the total number of Ca channels in a cell contribute to the catecholaminergic control of hormone synthesis and release in PI cells.

Tu-Pos110 SD AND FD Ca CHANNELS IN NORMAL PROLACTIN-SECRETING CELLS. M. Hiriart*, A. Navarrete, and G. Cota. *Dept. of Neurosciences, IFC, UNAM, AP 70-600, México, DF 04510, and Dept. of Physiol., Biophysics and Neurosciences, CINVESTAV, IPN, AP 14-740, México, DF 07000.

Two components of voltage-dependent Ca current have been reported in normal lactotrophs (Lingle et al., J. Neuroscience 6:2995, 1986). We have confirmed this finding, and demonstrated that lactotrophs express slowly- and fast-deactivating (SD and FD) Ca channels whose kinetics properties are similar, but not identical, to those present in GH3 cells (Armstrong and Matteson, Science 227: 65, 1985) and pars intermedia (PI) cells (Cota, J. Gen. Physiol. 88:85, 1986). Lactotrophs were identified in primary cultures of the rat pars distalis using the reverse hemolytic plaque assay. Whole-cell Ca channel currents were recorded with patch pipettes at 21°C (HP -80 mV). With external Ba (20 mM) as the permeant ion, pulsing to -20 mV induces an inward current that inactivates quickly and completely during the maintained depolarization, while the inward current induced at +20 mV inactivates only partially. The current at -20 mV is carried by SD channels that close at -80 mV with a time constant of ∿2.5 ms, whereas the current at +20 mV flows through SD and FD channels. Closing time constant for FD channels is ∿110 us at -80 mV. Closing kinetics and inactivation properties of SD and FD channels in the lactotrophs are about the same than in GH3 and PI cells. There is, however, a notorious difference in the voltage range over which SD and FD channels activate in these three pituitary cell types, under identical recording conditions. The activation thresholds for SD and FD channels using 10-ms pulses are (mV): -40 and 0 in lactotrophs; -40 and -20 in GH3 cells; -30 and -10 in PI cells. Half-maximal activation levels are (in the same order; mV): -20 and +25; -20 and 0; 0 and +20. Then, different pituitary cells in culture may express distinct subtypes of SD and FD Ca channels.

Tu-Pos111 **D600 BLOCK OF THE L-TYPE Ca^{2+} CHANNEL REQUIRES MEMBRANE PENETRATION IN VASCULAR SMOOTH MUSCLE.** Normand Leblanc, and Joseph R. Hume, Dept. of Physiology, University of Nevada School of Medicine, Reno, Nevada, USA, 89557.

It has been recently suggested that D600 blocks the Ca^{2+} channel (I_{Ca}) from the outside in single visceral and vascular smooth muscle cells (*, Ohya et al., Pflugers Arch. 408: 80, 1987). We have reinvestigated this hypothesis in whole-cell voltage clamped smooth muscle cells of the rabbit portal vein by comparing the effects of externally applied D600 with its permanently charged non-permeant derivative D890. At low frequencies of stimulations (0.05 Hz), D600 inhibited I_{Ca} in a dose-dependent fashion with a complete block occuring at 10^{-4} M. Externally applied D890 was approximately 1000 times less potent than D600 for inhibition of I_{Ca} using this protocol. During a train of stimulations at 0.5 Hz, D600 (10^{-6} M) produced additional frequency-dependent block of I_{Ca} as shown in other preparations. During superfusion with D890 (10^{-4} M), a similar protocol produced little if any decline in the amplitude of I_{Ca}. Internal application of D890 (10^{-4} M) inhibited I_{Ca} as compared with control experiments conducted in absence of drug in the pipette in order to obtain an estimation of run-down. We conclude that in vascular smooth muscle cells, D600 does not block the L-type Ca^{2+} channel from the outside as originally proposed (*). Instead, the molecule must penetrate the surface membrane in an uncharged form to reach its inhibitory site intracellularly as demonstrated in cardiac myocytes (Hescheler et al., Pflugers Arch. 393: 287, 1982). This work was supported by HL 30143. N.L. is a fellow of MRC of Canada and J.R.H. is an established investigator of AHA.

Tu-Pos112 STABILIZATION OF CARDIAC CALCIUM CHANNEL ACTIVITY IN EXCISED PATCHES. **Chr. Romanin, P. Größwagen,** and **H. Schindler** (Intr. by L. Hymel) Inst. for Biophysics, Univ. Linz, A-4040 Linz, Austria

Studies of L-type calcium currents in cell-free patches have been impeded by a rapid and irreversible rundown of channel activity. We investigated calcium currents in inside-out patches under different conditions in an attempt to prevent rundown. Based on the finding of Yatani et al. (Science 238, 1288, 1988), we examined the possible influence of G proteins on calcium channel activity. Specifically, we compared the proportion of open time in the cell-attached mode during 40 sec before ($P_{c.a.}$) and immediately after patch excision ($P_{i.o.}$). The ratio Pi.o./Pc.a. was then used to estimate calcium channel survival. When GTP (0.2 mM) was present in the bath, calcium channel activity was prolonged ($P_{i.o.}/P_{c.a.}$ = 0.29 ± 0.2 (N = 7), compared to $P_{i.o.}/P_{c.a.}$ = 0.07 ± 0.04 (N = 4) in the absence of GTP). Although GTP stabilized calcium channels during the first 40 sec after patch excision, activity usually disappeared completely within 1-4 min. In contrast to the moderate effect of GTP, addition of myocyte cytosol (including 1 mM ATP and 0.2 mM GTP) before excision fully stabilized calcium channel activity ($P_{i.o.}/P_{c.a.}$ = 1.69 ± 1.36, N = 6) for up to 40 min. This effect was pH dependent, being most pronounced at pH 6.5 and clearly diminished at pH 7.2. Following complete rundown, however, perfusion with cytosol, ATP, and GTP led only to a minor recovery of channel activity. In addition, phosphorylation by the cAMP-dependent protein kinase and subsequent perfusion with cytosol was insufficient to reactivate calcium channels. These results indicate an important and complex role for cytosolic components in the regulation of cardiac calcium channel activity.
Supported by the Fonds zur Förderung der Wissenschaftlichen Forschung (S45-3).

Tu-Pos113 MULTIPLE CONDUCTANCE STATES OF THE DIHYDROPYRIDINE (DHP) SENSITIVE CALCIUM CHANNEL IN GH_3 CELLS. Diana L. Kunze and Aileen K. Ritchie*, Department of Physiology and Molecular Biophysics, Baylor College of Medicine, Houston, TX 77030 and *Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550.

Single channel recordings of the DHP sensitive calcium channels in the anterior pituitary cell line, GH_3, showed multiple conductance levels in cell attached (bathing solution in mM: 140 KAsp, 10 EGTA, 1 $MgCl_2$, 10 Hepes) and outside out (pipette solution in mM: 124 $CsCl_2$, 11 EGTA, 1 $CaCl_2$, 2 $MgCl_2$, 10 Hepes) patches when 100 mM Ba was the ion carrying species (n=9 patches). The amplitude histograms were fit by multiple Gaussians which distinguished at least four amplitude levels: 0.42, 0.60, 0.82 and 1.03 pA during voltage steps from -40 mV to 0 mV. Another channel of 1.49 pA was observed occasionally. When single channel open time was prolonged by the addition of the DHP calcium channel agonist Bay K 8644 (10^{-8} to 10^{-6} M) single channel conductances of 8-9, 12-13, 16-18, 24-25 and 28-29 pS were calculated from channel currents recorded at membrane voltages between -30 and 0 mV (n=18 patches). These corresponded to 0.39, 0.57, 0.77, 1.18, and 1.45 pA at 0 mV. Additional amplitudes which did not fit these conductance ranges were also observed, although infrequently. The frequency of occurrence of partial closures from a larger amplitude state or transitions from a smaller to a larger level followed by complete closure suggests the existence of multiple conductance states. This could be due to a single intraconvertible channel or an array of channels that can open or close in concerted fashion as has been suggested to occur in reconstituted skeletal muscle calcium channels (Hymel et al. PNAS 85:4290, 1988; Ma and Coronado, Biophys. J. 53:387, 1988).

Tu-Pos114 DO SIALIC ACID RESIDUES ACCOUNT FOR SURFACE CHARGE MODULATION OF T- AND L-TYPE CALCIUM CURRENTS IN CULTURED PACEMAKER CELLS FROM THE SINO-ATRIAL NODE? Bernard Fermini and Richard D. Nathan, Dept. Physiology, Texas Tech Univ. Health Sciences Center, Lubbock, TX 79430.

We tested the hypothesis that sialic acid residues (NANA) constitute much of the negative surface charge associated with calcium channels. Voltage-dependent currents, $i_{Ca,T}$ and $i_{Ca,L}$, were recorded in cells isolated from rabbit sino-atrial node and cultured for 3-7 days (R. D. Nathan, Am. J. Physiol. 250: H325-H329, 1986). A highly purified preparation of neuraminidase (Sigma, Type X) was used to remove the surface NANA. The whole-cell voltage-clamp technique was used with pipettes containing (in mM/l): 120 CsCl; 20 TEA; 10 EGTA; 5 Mg_2ATP; 5 Na_2CP; 5 HEPES; 1.0 $MgCl_2$; pH 7.4, KOH. The bathing medium consisted of: 126 NaCl; 5.4 KCl; 20 CsCl; 2.5 $CaCl_2$; 5.5 dextrose; 0.33 NaH_2PO_4; 5.0 HEPES; pH 7.4, NaOH. All experiments were performed at 36° C in the presence of 30 μM TTX and 4.0 mM 4-AP. Separation of $i_{Ca,L}$ and $i_{Ca,T}$ was performed by using different holding potentials (-50 and -90 mV, respectively) and subtracting the two I-V relationships. Currents could be recorded for 52 ± 7 min (n=11) without marked rundown. Following a 1-hr incubation of the cells with neuraminidase (1.0 U/ml, 37° C), we found a significant ($P < 0.05$) increase in the amplitude of $i_{Ca,T}$ between -30 and 0 mV, but no change in $i_{Ca,L}$. The enzyme failed to affect the half-inactivation potential of either current, but significantly reduced the slope factor for $i_{Ca,T}$ inactivation from 5.5 ± 0.9 mV (n=4) to 3.5 ± 0.1 mV (n=5). These preliminary results suggest that sialic acid residues might be important for the function of T-type (but not L-type) calcium channels, but in ways unrelated to the channel's surface charge.
Supported by grant HL 20708. Dr. Fermini holds a fellowship from the Canadian Heart Foundation.

Tu-Pos115 CALCIUM CURRENT ACTIVATION KINETICS IN DISSOCIATED BULLFROG SYMPATHETIC NEURONS.
Francisco Sala and Paul R. Adams, Howard Hughes Medical Institute, Dept. Neurobiology and Behavior, SUNY , Stony Brook, NY 11794.

Calcium currents were recorded in dissociated bullfrog sympathetic ganglion cells through low resistance patch pipettes using discontinuous voltage clamp (40-50 KHz). After 10 ms depolarizing pulses, turn-off tail currents were measured and fitted with the sum of two exponentials. One component decayed with a fast time constant (τ =230 us at -40 mV), and the other, much smaller (1/40th), decayed with a time constant of 1-2 ms in a voltage-independent manner. Only the fast component was undoubtedly associated with the closure of calcium channels. Since the existence of two types of calcium channels has been reported in this preparation (Lipscombe & Tsien, Neurosci.Abst. 13, 1239) we performed two kinds of test in order to dissect the two components. First, tail currents were measured after short and long depolarizing pulses from Vh=-90 mV, and were found to decay with the same time constant. Second, Ca currents evoked from depolarized holding potentials activated and deactivated with the same kinetics as when evoked from -90 mV. Thus, we studied the activation kinetics as if there was only a single population of Ca channels. Tail current amplitudes grew as the depolarizing pulse was made larger, and the activation curve was well fitted by the Boltzmann expression raised to the second power with a midpoint voltage of -3 mV and a voltage sensitivity factor of 11 mV. After minimizing gating currents, the time course of the onset of Ca currents follows m^2 kinetics. The activation time constants estimated from turn-on and tails (assuming a m^2 scheme) showed a bell-shaped dependence on voltage with a maximum of 1.1 ms near 0 mV. The values of the rate constants alpha and beta were derived from the activation curve and time constants. The instantaneous I/V relationship showed a strong rectification at positive potentials, but could not be matched by the constant-field equation..

F. Sala supported by Fundacion Juan March (Spain).

Tu-Pos116 DIHYDROPYRIDINE CALCIUM CHANNEL ANTAGONISTS IN RAT CEREBRAL CORTICAL SYNAPTONEUROSOME MEMBRANES
Jill Moring, William J. Shoemaker, and Leo G. Herbette, Biomolecular Structure Analysis Center and Alcohol Research Center, Depts. of Radiology, Medicine, Biochemistry, and Psychiatry, University of Connecticut Health Center, Farmington, CT 06032

The remarkable effects of dihydropyridine (DHP) antagonists of the voltage-dependent calcium channel on the ethanol withdrawal syndrome have aroused interest in the interaction of dihydropyridines with brain membranes. We are investigating the nonspecific binding of the dihydropyridines to rat cerebral cortical synaptoneurosome (SNM) membranes. Small angle x-ray scattering was used to investigate the structure of the SNM membranes. Electron micrographs of the SNM preparation showed synaptoneurosomes with classical synaptic appositions intact, other vesicles, occasional mitochondria, and some myelin. Multibilayer diffraction samples yielded two to five diffraction orders; calculated electron density profiles indicated a typical membrane bilayer structure with scattering contribution from protein, especially outside the phospholipid headgroup region. Unit cell repeat distances were 70 - 80Å. Partition coefficients of the DHPs such as the calcium channel antagonist nimodipine (1900 ± 200) into SNM membranes were found to be high. Small angle x-ray scattering indicated that the DHP calcium channel agonist BAY K 8644 partitions into SNM membranes from the upper acyl chain region through the phospholipid headgroup region. Ethanol also partitions into membranes at the phospholipid headgroup/hydrocarbon core interface, where the membrane is hydrated. Because ethanol and other drugs partition into SNM membranes at approximately the same location, they are likely to interact there with each other and with receptors in the membrane. Supported by NIAAA center grant #01350 and RJR Nabisco, Inc.

Tu-Pos117 **ANALYSIS OF THE PURIFIED DIHYDROPYRIDINE RECEPTOR COMPLEX FROM SKELETAL MUSCLE: SUBUNIT COMPOSITION AND INTERACTIONS.**
S.L. Hamilton†, M.J. Hawkes‡, K. Brush†, R.-J. Chang‡, and H. Smilowitz‡. †Dept. of Physiology and Molecular Biophysics, Baylor College of Medicine, Houston, TX 77030 and ‡Dept. of Pharmacology, University of Connecticut, Health Center, Farmington, CT 06032.

The dihydropyridine (DHP) receptor from rabbit skeletal muscle has been characterized by affinity labeling, purification and partial reduction. Two procedures were used for purification: one which was a procedure modified from that of Curtis and Catterall [Biochem. 23:2113 (1984)] and one which employed an anti α_1 monoclonal antibody (Mab) [Smilowitz et al., Neurosci. XIII:31.3 (1987)] affinity column. In addition, both digitonin and CHAPS were utilized with each purification procedure. The major findings were: 1) In contrast to its behavior in digitonin, the 140K α_2 polypeptide does not quantitatively co-purify with the 170K α_1 polypeptide when the purification is carried out in the detergent CHAPS. This has been shown using both wheat germ and Mab affinity columns. For example, digitonin extracted receptor complex bound to the Mab affinity column loses α_2 when the digitonin is replaced by CHAPS. 2) The β-subunit also dissociates from α_1 in the detergent CHAPS. A major contaminant of the purified preparation in CHAPS is calsequestrin. 3) More DHP binding is lost due to CHAPS extraction compared to digitonin extraction; the data raises the possibility that the α_1-α_2 association may have a functional role. 4) The 190K unreduced (α_2) polypeptide exhibits a disulfide reduction intermediate of 160K; in the course of disulfide reduction, 60K and 25K/30K polypeptides are released; the 160K intermediate and the 60K polypeptide have not been described previously. Supported by MDA and NIH grant HL37028 to S.L.H. and NIH Program Project grant HL33026 to H.S.

Tu-Pos118 ANALYSIS OF THE PUTATIVE SUBUNITS OF A VOLTAGE-DEPENDENT CALCIUM CHANNEL. E. McKenna, P.L. Vaghy, K. Naito, K. Itagaki, T.L. Kirley, and A. Schwartz, Dept. of Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267.

Purified skeletal muscle voltage-dependent calcium channel preparations consist of five putative subunits (kDa): α_1 (165), α_2 (150), β (50), γ (33), and δ (28). Photoaffinity labeling and immunological studies have identified the α_1 subunit as the receptor for calcium antagonists in skeletal muscle. Fragments of the α_1 subunit also bind calcium antagonists. Amino acid sequencing of these fragments is used for localizing drug binding sites. Sequencing of cDNA clones has revealed the complete amino acid sequence for the α_1 and α_2 subunits. The primary structure of the β, γ, and δ subunits has not yet been determined. Based upon partial amino acid sequence(s), oligonucleotide probes are constructed for screening cDNA libraries, isolation and sequencing of clones, and deducing the complete amino acid sequence for each subunit. Towards this goal, we have isolated individual subunits from purified skeletal muscle calcium channel preparations using sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by electroelution or electroblotting onto PVDF membranes. Amino acid composition and partial amino terminal sequences have been determined. (Supported by NIH grants T32 HL07382 and RO1 HL41088.)

Tu-Pos119 FURTHER CHARACTERIZATION OF THE Ca^{2+} CHANNELS FROM SEA URCHIN SPERM PLASMA MEMBRANES. A. Liévano and A. Darszon, Dept. of Biochemistry, CINVESTAV-IPN, Apartado Postal 14-740, 07000 México City.

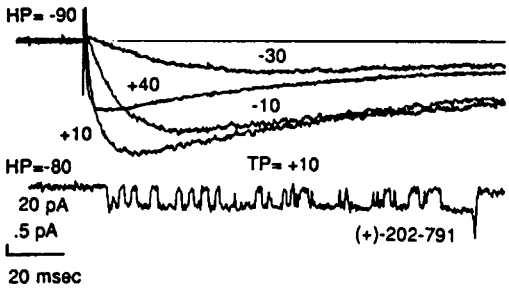
The influx of Ca^{2+} across the sea urchin sperm plasma membrane is a necessary step during the egg-jelly induced acrosome reaction. There is pharmacological evidence for the involvement of Ca^{2+} channels in this influx. We have approached the study of the sea urchin sperm plasma membrane Ca^{2+} channels by fusing them into planar lipid bilayers. With this strategy, we have detected a Ca^{2+} channel which displays a complex behaviour (Biophys.J.53:559a), with the following characteristics: a high main-state conductance (MS-γ) of 172 pS in 50 mM $CaCl_2$, which relaxes to levels of lesser conductance in a voltage-dependent form at negative Em; it is blocked by mM concentrations of Cd^{2+}, Co^{2+} and La^{3+}, which also inhibit the egg-jelly induced acrosome reaction; the MS-γ decreases for the following divalent cations $Ba^{2+}>Sr^{2+}>Ca^{2+}$; the channel discriminates poorly between divalent over monovalent cations. The sperm Ca^{2+} channel rectifies in 10 mM $CaCl_2$, having a maximal conductance value of 94 pS at +100 mV. Under these conditions a different channel of lesser conductance becomes apparent fluctuating above the MS-γ at positive potentials. This channel can not be observed in 50 mM divalent cation solutions, possibly because it is inactivated by high $[Ca^{2+}]$. Also in 10 mM Ca^{2+} solutions, Mg^{2+} permeates through the main channel when added to the *cis* side, with a $P_{Mg}/P_{Ca}=0.35$ while it blocks when added to the *trans* side. In 50 mM $CaCl_2$ solutions the MS-γ open probability has values of 1.0 at voltages more positive than -40 mV, and it decreases at more negative ones, following a Boltzmann function with an $E_{0.5}$ = -72 mV, and an apparent gating charge value of 3.9. These results suggest that the main channel works as a single macromolecular assembly and that it appears to be a new type of Ca^{2+} channel. Supported by WHO, CONACYT and OEA grants.

Tu-Pos120 CALCIUM CURRENTS IN THE DEVELOPING MOUSE OOCYTE. J.M. MURNANE & L.J. DeFELICE (spon. W. Sale). Emory Univ., Anatomy and Cell Biology, Atlanta, Georgia 30322

Peres (J Physiol 391:573;1987) identified a voltage-dependent Ca current in zona-free mouse eggs which did not differ significantly in current-voltage relationship with the Ca current found in the immature, germinal vesicle-containing (GV) oocytes collected from eight week old, post-pubescent mice (Pflugers Archi 407: 534;1986). Using whole-cell methods we compared the membrane properties of the pre-pubescent, immature, (GV) oocyte and the mature mouse egg. GV oocytes were collected from neonatal (1-10 days) mice and compared to eggs collected from post-pubescent, estrus and superovulated mice. The cumulus mass, follicular cells and the zona pelucida were removed to provide a clean surface for electrophysiological recording. A distinct inward current was observed in both the oocyte and the egg. The extrapolated reversal potential and kinetics suggests the inward current is carried by Ca. Furthermore, the increase in peak amplitude in 20 mM external Ca was accompanied by a positive shift in the reversal potential, current-voltage relationship, activation time, and time to peak. Two components of a Ca channel current could be distinguished by voltage-dependence. In neonatal oocytes, an inward, rapidly activating (10-20 msec), fully inactivating current was observed in response to 500 msec depolarizing pulses from -80 mV. Steps from -30 mV did not elicit the early peak current but produced an inward, slowly activating (50-200 msecs), slowly inactivating current. The rapidly activating Ca-sensitive current increased ten-fold (approximately 0.1 to 1.0 nA) in the mature eggs. Furthermore, GV oocytes exposed to endogenous gonadotrophins expressed mature Ca currents, suggesting desynchronization of nuclear and cytoplasmic (membrane) maturation of the mouse oocyte. In summary, the immature mouse oocyte membrane contains two types of Ca currents (I slow, I fast). I fast increases after gonadotrophin exposure. Supported by NIH grant 19770-01A1.

Tu-Pos121 DIHYDROPYRIDINE-SENSITIVE CALCIUM CHANNELS IN DISSOCIATED GRANULE CELLS CULTURED FROM MOUSE CEREBELLUM. Paul A. Slesinger and Jeffry B. Lansman, Graduate Program in Neuroscience and Dept. of Pharmacology, School of Medicine, University of California, San Francisco, CA 94143.

Whole-cell and cell-attached recordings were used to study Ca channels in mouse cerebellar granule cells grown *in vitro*. Cerebella from 7 day old mice were dissociated mechanically into single cells and cultured for up to 5 weeks. Ca current density was negligible after the first day of plating and increased during maturation in culture. With 20 mM $BaCl_2$ or $CaCl_2$ in the bathing solution, voltage steps from a holding potential of -90 mV produced Ca currents that activated near -30 mV and reached a peak near +10 to +20 mV. A slowly inactivating component of the Ca current was observed during the voltage pulse in most cells. The extent of inactivation varied from cell to cell and was independent of the size of current.



Whole-cell currents produced in response to a fixed step were reduced with more positive holding potentials showing a 1/2 maximal inactivation of -65 mV and were completely blocked in the presence of 20 μM Gd^{3+}. Recordings of single channel activity from cell-attached patches were dominated by an ~25 pS channel in 110 $BaCl_2$. The DHP agonist (+)-202-791 or prepulses to +50 mV increased mean channel open time. In the presence of DHP agonist, 20 μM Gd^{3+} produced discrete interruptions of the unitary current at 0 mV which was relieved at negative potentials, suggesting open channel block at an intrapore site. Mouse cerebellar granule cells express predominantly a single type of Ca channel which is sensitive to dihydropyridines and is blocked by multivalent cations.

Tu-Pos122 CALCIUM FLUXES DURING KCl INDUCED LIGHT EMISSION IN THE HYDROZOAN *PHIALIDIUM GREGARIUM*.

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The embryos of the hydrozoan *Phialidium gregarium* contain an endogenous calcium-sensitive photoprotein. When depolarized with KCl, these embryos give a flash of luminescence that depends, in part, on the entry of extracellular calcium through voltage-sensitive calcium channels. We have measured this entry using $^{45}Ca^{++}$ and find a calcium influx of approximately 2 x 10^{-12} moles per embryo which, considering the embryo volume, leads to an increase of roughly 0.5 mM in the intracellular calcium concentration. Simultaneous measurement of calcium influx and calcium-mediated light emission (made by injecting 20 embryos into a small, 200 μL, drop of high K^+ artificial seawater containing $^{45}Ca^{++}$ that was positioned above a photomultiplier tube) showed an approximately linear relationship between the integrated calcium entry and the square root of the integrated light emission. This result suggests that the endogenous photoprotein *in vivo* behaves similarly to other photoproteins, e.g. aequorin, *in vitro*. While it is possible that the calcium entry accounts for all of the KCl-mediated light emission, we have several pieces of evidence suggesting an amplification step which involves the release of calcium from internal stores. To test such a release, we measured the $^{45}Ca^{++}$ efflux from preloaded embryos. KCl treatment causes the rapid efflux of approximately 60% of preloaded (presumably compartmentalized) intracellular calcium. Thus, the increased intracellular calcium resulting from KCl treatment probably includes both influx from extracellular and release from intracellular calcium sources. This work was supported by NSF grant DCB-8602722, and NIH grants AM35597 and GM20024.

Tu-Pos123 SINGLE CALCIUM CHANNELS IN GRANULOSA CELLS. J.L. Schwartz*, G.A.R. Mealing*, J.F. Whitfield*, E.C. Rousseau+, and M.D. Payet+. *Division of Biological Sciences, National Research Council of Canada, Ottawa, Canada, and +Département de Physiologie et Biophysique, Université de Sherbrooke, Sherbrooke, Canada.

Extracellular calcium is essential for hormonal regulation of steroidogenesis in granulosa cells of the hen (1). In previous studies, we reported the existence in these cells of two types of potassium channels, one of them being modulated by intracellular calcium (2). We have also reported a transient inward calcium current (3) using the whole-cell patch-clamp technique. Here, we report single-channel calcium currents in granulosa cells in short-term primary cultures. Cells were patch-clamped at room temperature, in the cell-attached configuration, with 110 mM Ba-acetate in the pipette and 136 mM K-acetate and 20 Ba-acetate in the bath. Three types of channel were observed: (i) a T-type, low conductance channel (g_{Ca1} less than 10 pS) that displayed short openings and was only active for a short time at the beginning of the command pulse; (ii) an L-type higher conductance channel (g_{Ca2} = 30 pS), that was less frequently observed, displayed long-lasting openings and was activated by 5 μM BAY-K8644; and (iii) a channel with a conductance intermediate between the T and the L-types. This is the first report of single calcium channel activity in ovarian endocrine cells in primary culture. Their modulation by hormones and neurotransmitters may play an important role in steroid production.

(1) Asem E.K. and F. Hertelendy (1986) Gen Comp Endocr, 62: 120-128; (2) Asem E.K., J.L. Schwartz, G.A.R. Mealing, B.K. Tsang and J.F. Whitfield (1988) BBRC, 155(2): 761-766; (3) Schwartz J.L., G.A.R. Mealing, E.K. Asem, J.F. Whitfield and B.K. Tsang, FEBS (in press).

Tu-Pos124 CALCIUM CURRENTS IN HUMAN PANCREATIC B-CELLS. R.P. Kelly, R. Sutton* and Frances M. Ashcroft. University Laboratory of Physiology, Parks Road, Oxford OX1 3PT and *Nuffield Department of Surgery, John Radcliffe Hospital, Oxford OX3 9DU, U.K.

Single pancreatic B-cells were isolated from heart-beating cadaver organ donors, with permission, and maintained in short-term tissue culture (FEBS Letts 215:9). Calcium currents were recorded using the whole-cell patch-clamp method and a holding potential of -70mV. The pipette solution contained (mM): 145 NMG, 110 HCl, 2 $MgSO_4$, 5 HEPES (pH 7.2), 11 EGTA, 1 $CaCl_2$ (free Ca^{2+} 0.06 uM), 3 ATP. The bath solution contained (mM): 5 KCl, 135 NaCl, 5 $CaCl_2$, 2 $MgSO_4$, 5 HEPES (pH 7.4) plus 5 uM TTX. Inward currents were activated at -50mV and had a peak amplitude of -5.27 ± 1.14 pA/pF (n=5) at -10mV. Significant rundown of current amplitude occurred within 10 minutes. Inward currents were unaffected by TTX but reversibly inhibited by 1mM $CdCl_2$ applied from a puffer pipette. Ca currents showed time-dependent inactivation during the pulse. Evidence that inactivation is, at least partly, Ca-dependent includes: 1) Ba currents do not inactivate ; 2) in two-pulse experiments the voltage-dependence of inactivation is U-shaped ; 3) the rate and extent of inactivation is related to the amount of Ca^{2+} entry. These results resemble those found for rodent B-cells (J. Physiol. 374:531; J. Physiol. 404:731). We suggest these Ca currents underlie the action potential of the human B-cell and mediate the increase in Ca^{2+} influx required for insulin secretion.

Supported by the British Diabetic Association, the Wellcome Trust and Nordisk UK.

Tu-Pos125 TRANSMITTER REGULATION OF SINGLE CALCIUM CHANNELS AND CONTRACTION OF ARTERIES. Jennings F. Worley*, Nicholas B. Standen and Mark T. Nelson. Department of Pharmacology and Toxicology*, West Virginia University, Morgantown, WV, 26506 and Department of Pharmacology, University of Vermont, Burlington, VT, 05405.

The effects of serotonin (5HT) and norepinephrine (NE) were examined on single voltage-dependent calcium channels in on-cell patches and contraction of ring segments of rabbit basilar (cerebral) and mesenteric (peripheral) arteries. 5HT (300 nM) when added to the bath solution increased the open state probability (2-4 fold) of single calcium channels in isolated basilar artery smooth muscle cells without affecting unitary currents. Similarly, NE (10 uM) increases in the open state probability of single calcium channels in mesenteric arteries where observed with no change in unitary currents. 5HT and NE action may be mediated by an intracellular messenger since they were added to the isolated cell surface and did not directly contact the calcium channels. In support of 5HT and NE opening voltage-dependent calcium channels, agonist induced contractions of the basilar and mesenteric arteries were increased by agents that open calcium channels (membrane depolarization and Bay R 5417) and were reduced by agents that close or block calcium channels (membrane hyperpolarization with BRL 34915, cadmium, cobalt, verapamil, diltiazem, nisoldipine and nimodipine). These results suggest that maintained force development to 5HT and NE is a consequence of opening voltage-dependent calcium channels. Furthermore, in 5HT and NE, extracellular calcium entry through voltage-dependent calcium channels can regulate vascular smooth muscle tone at physiological membrane potentials.

Tu-Pos126 MODULATION OF SINGLE SLOW (L-TYPE) CALCIUM CHANNEL BY INTRACELLULAR ATP IN VASCULAR SMOOTH MUSCLE. Yusuke Ohya & Nicholas Sperelakis, Dept. of Physiology & Biophysics, Univ. of Cincinnati, Cincinnati, OH 45267-0576.

To investigate the role of ATP in the regulation of slow (L-type) Ca^{2+} channel of vascular smooth muscle cells, single Ca^{2+} channel currents (conductance of 18-20 pS) were recorded by patch clamp technique in cell-attached mode. Intracellular compositions were modified by permeabilizing the cell membrane at one end of the cell. Single cells were freshly isolated by collagenase treatment from guinea-pig portal vein. The pipette contained isotonic Ba^{2+} solution, and the bath contained high K^+ solution (with 5 mM EGTA) to depolarize the membrane to near 0 mV. Without Bay-K-8644 (Ca^{2+} channel agonist), the channel activity was low, and disappeared usually within 5 min after permeabilizing the membrane and exposure to the ATP-free solution. If ATP (1-5 mM) was applied to the bath (access to cell interior) before complete rundown of channel activity, the channel activity was partially recovered. ATP did not change single-channel current amplitude or mean open time, whereas it increased number of the channels available for opening and/or open probability. AMP-PNP did not have, but ATP-γ-S had effects similar as ATP; however it was weaker than ATP. With 1 μM Bay-K-8644, the activity of the Ca^{2+} channel was high; channel activity persisted for more than 10 min after permeabilizing the cell and exposing to the ATP-free solution containing metabolic poisons (1 mM CN^- and 10 mM 2-deoxy-glucose). These results indicate that activation of slow Ca^{2+} channel requires some phosphorylation mechanism. Bay-K-8644 may change the nature of the Ca^{2+} channel, making it resistant to rundown.

Tu-Pos127 INDUCTION OF CALCIUM CURRENTS AND ENHANCEMENT OF MECHANICAL ACTIVITY IN CRUSTACEAN MUSCLE BY SULFHYDRYL REAGENTS. L. Lizardi, M. C. García, J. A. Sanchez and C. Zuazaga, Inst. of Neurobiology, U. of Puerto Rico Med. Sci. Campus, San Juan, P.R. 00901.

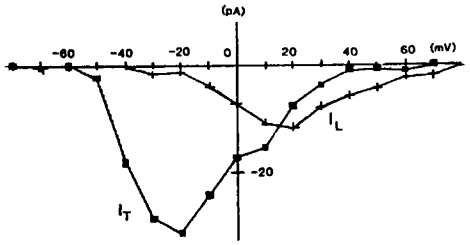
Completely inexcitable striated muscle fibers of the freshwater crustacean Atya lanipes, which normally show no graded responsiveness, generate all-or-none action potentials following exposure to the sulfhydryl reagents known as α,β-unsaturated carbonyl compounds (Zuazaga & del Castillo, Comp. Biochem. Physiol. 82C: 409, 1985). The ionic currents which underlie these responses were investigated using the three-microelectrode voltage clamp technique (T=13-15 °C). Normal solution was prepared according to van Harreveld; 300-400 mM sucrose was added in some experiments to block contraction. In Cl-free solutions, Cl^- was replaced by $CH_3SO_3^-$. Na^+ was isomotically replaced by TEA^+, and Ca^{2+} by Mg^{2+} in TEA-containing and nominally Ca-free solutions, respectively. In the normal preparation, K and Cl currents were present since outward currents were partially blocked by TEA^+ and decreased in Cl-free solutions; no inward current could be detected. However, following exposure to the sulfhydryl reagent 4-cyclopentene-1,3-dione (4-CPD, 4mM), net inward currents which were abolished in Ca-free solutions, were observed. I_{Ca} decayed during maintained depolarizations. Peak inward current (ca. 50uA/cm^2) was maximal near 0mV. Isometric tension was measured on bundles of ca. 20 fibers at 20-22 °C. Tetanic tension (20 Hz) increased 300% following exposure to 4-CPD in normal Ca^{2+}.Tension was strictly dependent on extracellular Ca^{2+} before and after 4-CPD exposure. The effect of this reagent on both electrical and mechanical activity was transient, lasting ca. 1 hour. It is concluded that Ca channels are made functional by 4-CPD in this crustacean muscle and that increased tension in the chemically-modified fibers is probably mediated by an increase in Ca^{2+} influx. (Supported by NIH grant #NS-07464).

Tu-Pos128 CALCIUM CHANNELS IN CHICK EMBRYO VENTRICULAR MYOCYTES. W. Bamrungphol-Watanapa and C.M. Armstrong. University of Pennsylvania, Philadelphia, PA 19104-6085.

Single ventricular myocytes were enzymatically dispersed (DeHaan, Dev. Biol. 16:216-49,1967, modified) from chick embryo hearts (6 to 8 days old). Calcium currents in these cells were studied with the whole-cell patch clamp method, within two days after dissociation. External solution (mM): 135 Tris(7.0), 20 $CaCl_2$, 5 glucose, 10 Hepes (acid), pH 7.5 (TrisOH), with or without 1 uM TTX. Internal solution: 120 Tris, 25 CsF, 10 EGTA, 10 Hepes, pH 7.2 (TEAOH); 22-24°c; HP = -80 mV. Maximal Ca currents recorded from approximately spherical myocytes of 20-25 um diameter are 0.2-1 nA. Tail current analysis suggested at least two populations of calcium channels, with deactivation time constants of 1.8±0.4 and 0.3±0.05 ms at -80 mV (SD and FD respectively). The voltages at half maximal conductance are about -20 (SD) and +10 to +20 mV (FD). The SD channel inactivates to 50% peak current in 20 ms at 0 mV. The FD channel inactivates more slowly, and 80-90% of the peak current remains after 100 ms at 0 mV. Thus, the two channels seem to be similar to the high-voltage and low-voltage activated Ca channels described in mammalian heart preparations. The total Ca current is increased by 100-200% with 400nM BayK 8644 externally. In preliminary studies, the tail current in BayK is best fitted by a single exponential, with a time constant of 1.2±.3 ms at -80 mV.

[Supported by NIH grant NS 12543 to CMA, and a grant from the Royal Thai Government to WBW.]

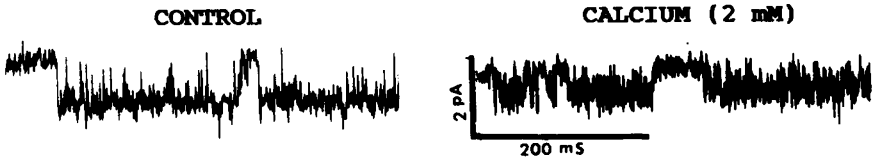
Tu-Pos129 **THE LOW THRESHOLD CALCIUM CURRENT (I_T) IS LARGER THAN THE HIGH-THRESHOLD CURRENT (I_L) IN EMBRYONIC CHICK VENTRICLE CELLS.** **Seiko Kawano and Robert L. DeHaan, Woodruff Health Sciences Center, Emory University, Atlanta GA 30322**



The calcium current in cardiac cells can be separated into I_L and I_T by differences in their voltage-activation range and drug sensitivities. We measured developmental changes in calcium currents in cells isolated from 7d and 14d embryonic chick ventricle with the whole-cell clamp technique. In 45% of 7d and 65% of 14d cells, I_L and I_T could be separated by their voltage dependence. In these cells, peak amplitude of I_T was about twice that of I_L. At both stages, I_L was blocked by dihydropyridines (2 μM nifedipine or D-600) but was unaffected by Ni^{2+}. I_T was insensitive to nifedipine or D-600, but was depressed in a dose-dependent manner by 40-160 μM Ni^{2+}. The half-inactivation potential (V_h) of I_L in cells from both 7d and 14d hearts was -18 mV; complete inactivation occurred at +10 mV. In I_T, in contrast, a marked change in the steady-state inactivation relation took place with development. Between 7 and 14 days, V_h shifted from -43 mV to -59 mV; complete inactivation occurred respectively at -10 mV and -30 mV. We conclude that I_T predominates in the chick ventricle, and that the voltage dependence of I_T channels changes between 7 and 14 days of incubation. (Supported by NIH HL-27385 to RLD).

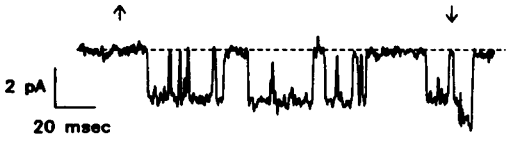
Tu-Pos130 **INTRACELLULAR CALCIUM BLOCK OF SINGLE CALCIUM CHANNELS FROM RABBIT MESENTERIC ARTERY.** Huang Yu, John Quayle, Nicholas Standen, and Mark Nelson. Department of Pharmacology, University of Vermont, Burlington VT 05405.

Barium (80 mM) currents through single voltage-dependent calcium channels were measured in excised patches of smooth muscle membrane. The solution bathing the intracellular surface of the channels was 120 mM NaCl, 20 mM CsCl, pH 7.4, and 0-10 mM calcium. The Ca channel agonist, Bay R 5417, was included in the solutions to lengthen channel open time. As shown in the Figure, intracellular calcium (2 mM) induced a flickery block of the single channels, with the mean unitary current reduced and open-level noise increased at 2 kHz. At 5 kHz, the mean unitary current was little affected and discrete blocking events could be observed. The power spectra from the channel currents have a component associated with the blocking events, with a corner frequency of about 1 kHz with 2 mM Ca present. The half blocking concentration of calcium for the channel was estimated to be about 3 mM, a value similar to the one obtained by Rosenberg et al. (JGP, 1988). Association and dissociation rate constants of calcium were estimated to be 0.9 mM^{-1} ms^{-1} and 4.5 ms^{-1}, respectively. These results are consistent with intracellular calcium modulating Ca channel currents via a low affinity binding site.



Tu-Pos131 UNITARY CALCIUM CURRENTS IN A SMOOTH MUSCLE DERIVED CELL LINE. T. N. Marks, G. R. Dubyak, and S. W. Jones. Dept. of Physiol. and Biophys., Case Western Reserve University, Cleveland, OH, 44106.

The A7r5 cell line has previously been shown to express functional dihydropyridine sensitive calcium channels by the whole cell voltage clamp technique. The present report examines the properties of individual ion channels using the cell attached configuration. With 90 mM Ba^{2+} in the pipette, single channel openings were poorly resolved in the absence of Bay-K-8644. Addition of 1 uM Bay-K-8644 to the bath consistently resulted in long lasting openings. Slope conductance was 25 pS between -20 and +10 mV. Bay-K-8644 sensitive currents were also carried by Na^+ in the absence of divalents. With Na^+ as the charge carrier (120 mM) activation voltages were shifted ~60 mV in the hyperpolarized direction. At pH 7.4 the slope conductance of the channel was 25 pS between -60 and -30 mV, and openings were quite noisy. At pH 9.0, the slope conductance increased to 50 pS between -80 and -40 mV and there was less noise during openings, consistent with a reported proton block of the channel.



HP −80 mV, Step −60 mV, 120 mM Na^+, pH 9.0

Tu-Pos132 A SINGLE CALCIUM CURRENT TYPE IN RABBIT VENTRICULAR MYOCYTES. R. Gonzalez-Rudo, J. B. Patlak, and W. R. Gibbons. Department of Physiology and Biophysics, University of Vermont, Burlington, VT 05405. (Intr. by Norman R. Alpert.)

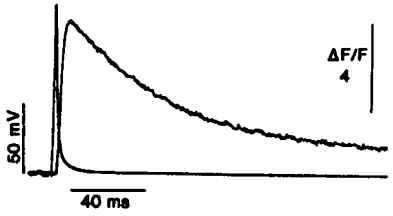
A fast transient ("T" type) Ca current has been reported in a variety of cardiac cells. T current activates and inactivates at more negative voltages than does the Ca current ("L" type) originally described in heart, has a faster transient time course, and is less sensitive to cadmium and dihydropyridines. We have examined Ca current of rabbit ventricular myocytes using the tight seal whole cell voltage clamp method. Cells were dialyzed with (in mM): 120 CsCl, 10 CsEGTA, 5 $MgCl_2$, 10 HEPES (pH 7.45). Cells were bathed in zero Na solutions containing 10 uM tetrodotoxin, with 3.6 mM Ba or Ca as charge carrier. In Ca, mean time to peak current at 0 mV was 16.3±1.2 ms; mean time to decay to half peak amplitude was 55.7±9.6 ms at 0 mV. Modal threshold was -20 mV; current usually peaked at +10 mV. In Ba, time to peak current was unchanged, but current decay was slower. Threshold and peak current voltages in Ba were 10-20 mV negative to the values in Ca. The current-voltage relations in Ca and in Ba were smooth functions of voltage with a single inward peak. Changing holding potential from -100 to -40 mV did not alter current during subsequent pulses in 10 of 14 cells bathed in Ca solution. Prepulses to -40 mV partially inactivated current in 4 cells in Ca and most cells in Ba, but the inactivated current had essentially the same time course as the parent current; depolarization did not selectively inactivate a fast transient (T) current. Cadmium (10 uM) and nisoldipine (50 nM) reduced current without the voltage-dependent sensitivity expected if T and L channels were both present. Results were consistent with a single type of Ca current resembling the classical "L" type Ca channel. No "T" type current was found. (Supported by NIH grants HL1416 and AR37606).

Tu-Pos133 A NOVEL CALCIUM CURRENT IN SKELETAL MUSCLE FROM MICE WITH MUSCULAR DYSGENESIS. Brett Adams and Kurt Beam, Department of Physiology, Colorado State University, Fort Collins, CO 80523.

The whole-cell patch-clamp technique was used to study voltage-dependent calcium currents in primary cultures of myotubes and in freshly dissociated skeletal muscle from mice with muscular dysgenesis. In addition to the transient calcium current (I_{fast}) previously described by Beam et al. (Nature 320: 168), a dihydropyridine (DHP) sensitive calcium current was found in dysgenic skeletal muscle. This current, here termed I_{dys}, is largest in dysgenic myotubes grown on a substrate of killed fibroblasts and in fetal or neonatal dysgenic muscle. In dysgenic myotubes grown on standard plastic culture dishes, I_{dys} is so small that it usually cannot be detected without potentiation by Bay K 8644. I_{dys} becomes apparent at about -20 mV and peaks at about +20 mV. The current activates rapidly (rise-time of peak current ~5 ms), and with 10 mM Ca as charge carrier inactivates little during a 200 ms test pulse. Substituting Ba for Ca as charge carrier doubles the size of I_{dys} without altering its kinetics. Thus, I_{dys} is kinetically distinct from I_{slow} of normal skeletal muscle and the L-type calcium current of cardiac muscle. I_{dys} is 75% blocked by 100 nM (+)PN 200-110 and is increased 3-fold by 500 nM racemic Bay K 8644. The very high sensitivity of I_{dys} to these DHP compounds distinguishes it from I_{slow} and from the rapidly activating calcium current of normal skeletal muscle first described by Cota and Stefani (J. Physiol. 370: 151). The significance of I_{dys} is not yet known; it may represent a mutant product of the gene for the skeletal muscle DHP receptor, or the product of a gene normally expressed in non-muscle tissue. Supported by grants from M.D.A. and N.I.H. (NS 24444) to K.G.B.

Tu-Pos134 **MYOPLASMIC CA TRANSIENTS MEASURED WITH RHOD-2 AND FLUO-3 IN SINGLE SKELETAL FIBERS FROM RAT AND FROG.** J. García[1], D. Compagnon[2], J. Vergara[2] & E. Stefani[1]. [1]Dept. Physiol. & Mol. Biophys., Baylor College of Medicine, Houston TX, 77030, and [2]Dept. Physiol., UCLA, Los Angeles CA, 90024.

Ca signals during action potentials (AP) were recorded from single cut fibers of rat e.d.l. and frog peroneus muscles. Fibers were mounted at a slack length in a double vaseline gap. The middle-pool contained normal solution. End-pools contained (mM): K-glutamate 125 for rat, or 98 for frog, K_2-EGTA 0.1, $CaCl_2$ 0.0082, Na_2-ATP 5, $MgCl_2$ 5.5, K-HEPES 5, Na_2-phospho-creatine 5, and glucose 5. Temperature 15°C. Rhod-2 (287 μM) and Fluo-3 (514 μM) were added to this solutions. Fluorescence was measured at 180°. For Rhod-2: excitation filter (EF) 550 nm (FWHM 520-570 nm) and barrier filter (BF) long pass (590 nm). For Fluo-3: EF 480 nm (FWHM 460-500 nm) and BF 550 nm (FWHM 520-570 nm). The delay between AP and the Ca signal was 2 ms for the rat. Signals with Fluo-3 were smaller with the same time course. Absorbance changes at the excitatory wave length due to Ca-dye binding and to the fiber movement were ≈100 times smaller than fluorescence. In rat with Rhod-2 F/F is ≈8 at the peak. Ca transient can be estimated from cuvette measurements in intracellular solution which gave a K_d of about 2 μM. Supported by NIH.



Tu-Pos135 **PROTEIN KINASE/PHOSPHATASE MODULATION OF PURIFIED SKELETAL MUSCLE CALCIUM RELEASE CHANNEL ACTIVITY IN PLANAR BILAYERS.** **Lin Hymel** and **Hansgeorg Schindler**, Inst. for Biophysics, Univ. of Linz, A-4040 Linz, Austria; **Shiaw-Der Yang**, National Tsing Hua Univ., Hsinchu, Taiwan; **Makoto Inui**, **Stephan Reif**, and **Sidney Fleischer**, Dept. of Molecular Biology, Vanderbilt Univ., Nashville, TN 37235 USA

The purified ryanodine receptor from skeletal muscle sarcoplasmic reticulum has been incorporated into vesicle-derived planar membranes and identified as the calcium release channel (PNAS USA 85, 441-445, 1988). The calcium release channel was found to be activated by Ca^{2+}, ATP, and ryanodine. We now find that the purified receptor preparations fall into three categories with respect to activatability: Type I preparations, such as previously described, are highly activated by 100 nM free Ca^{2+}, 1 mM ATP, and 200 nM ryanodine. Type II preparations are similarly activated, but only after pretreatment with membrane phosphatase. Type III preparations can be highly activated only after combined treatment with phosphatase and cAMP-dependent protein kinase (A-kinase). Preparations which require only Ca^{2+}, ATP, and ryanodine to be highly activated (type I) can nevertheless be further activated by A-kinase. Preparations which are only weakly activated by phosphatase treatment (type III) cannot be activated by A-kinase alone, but are activated by A-kinase when applied after phosphatase in the bilayer cell. We conclude that there are two classes of phosphorylation sites on the calcium release channel: inhibitory sites which are selectively hydrolyzed by membrane phosphatase and which, when phosphorylated, down-regulate the channel in a dominant fashion, and activating sites which are phosphorylated by A-kinase. Protein kinase C was without effect on highly activated channels.

Supported by the Fonds zur Förderung der Wissenschaftlichen Forschung (S45-3), NIH AM14632 and HL32711, and grants from the Muscular Dystrophy Association and American Heart Association.

Tu-Pos136 Regulation of Ca^{2+} channels in osteosarcoma cells. Sandra E. Guggino and Gerda E. Breitwieser. Department of Medicine and Neuroscience, and Physiology, Johns Hopkins University, Baltimore, MD 21205.

We measured whole cell inward currents carried by 20 mM $BaCl_2$ in freshly dissociated osteoblast-like osteosarcoma ROS 17:2.8 cells. Inward currents at holding potentials of -40 and -100 mV were measured. The initial peak inward current at 0 mV is about 200 pA; pretreatment with 10^{-4} M dibutyryl cAMP and 10^{-4} M isobutylmethylxanthine (IBMX) increased currents fivefold. Half maximal block of Ba^{2+} currents was achieved with 0.5 μM $CdCl_2$. Threshold increases in currents were observed with 1 μM BAY K8644 and a rapid block of current was achieved with 1 μM nitrendipine in the absence of BAY K8644. The whole cell Ba^{2+} currents exhibited significant rundown: in less than two minutes current was 50% of that observed immediately upon patch rupture. We tried several manuevers to control rundown: preincubation with dibutyryl cAMP with or without IBMX, vanadate or BAY K8644 or addition of forskolin or phorbol ester. Pipette addition of trypsin, leupeptin, antipain, aprotinin, catalytic subunit of protein kinase A, staurosporine, an inhibitor of protein kinase C, ATPγS, GTPγS, GppNHp, cAMP, ± EGTA, or up to 3 mM free Mg^{2+} did not alter rundown. We conclude that the channels in ROS cells are regulated in a manner unlike L channels in cardiac cells but may resemble the regulation of Ca^{2+} channels in GH_3 cells. Supported by AHA Grant-in-Aid, with funds contributed in part by AHA, Texas Affiliate, Inc.

Tu-Pos137 ANGIOTENSIN II INCREASES ONE TYPE OF Ca^{2+} CURRENT IN CULTURED CHICK HEART CELLS.

A. Sculptoreanu and G. Bkaily, Department of Physiology and Biophysics, Faculty of Medicine, University of Sherbrooke, Sherbrooke, Quebec, Canada, J1H 5N4.

It is known that Angiotensin II (ANG II) has a positive inotropic effect in the heart. More recent studies suggest that ANG II receptors may be coupled to phospholipase C by a pertussis-insensitive nucleotide regulatory protein. This results in production of IP_3 and stimulation of protein kinase C by DG. In this study we investigate the effect of ANG II on the slow inward Ca^{2+} currents of 10-day-old chick embryo heart cells in culture using the whole cell voltage clamp technique.

Three types of slow inward currents were reported recently by our group (Bkaily et al., 1988): a fast transient type, which is activated from a holding potential (HP) of -80 mV, a slow transient and a susteined which are equally activated from HP's of -80 mV or -50 mV. Superfusion with ANG II (10^{-8}M) increased the fast transient type of Ca^{2+} current within 10 minutes. With a similar time course, the slow and susteined types of Ca^{2+} currents were reduced. The ANG II antagonist, (leu^{8}) ANG II (10^{-9}M), reversed the effects of ANG II.

Therefore, the positive inotropic action of ANG II in heart could be due to an increase in a fast transient type of Ca current. Further studies are necessary in order to elucidate the mechanism by which ANG II modulate the Ca^{2+} current in heart muscle.

This work was supported by MRCC(MT-9816 and ME-9788) to G. Bkaily who is a scholar of CHF. A. Sculptoreanu is a Ph.D. fellow of CHF.

Tu-Pos138 EFFECTS OF GUANINE NUCLEOTIDES ON THE HIGH THRESHOLD Ca CHANNELS OF IDENTIFIED DORSAL HORN PROJECTION NEURONS. Li-Yen Mae Huang, Ph.D. Marine Biomedical Institute and The Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550.

Dorsal horn projection neurons which include spinothalamic and trigeminothalamic tract neurons are responsible for transmission of pain signals into the central nervous system. A procedure was developed to identify and isolate these projection neurons so that they are suitable for patch-clamp recordings (Huang, Neurosci. Lett., 82:267-272, 1987). Voltage-dependent Na, Ca, K and Ca-dependent K channels are shown to be present in these cells. We have identified different types of Ca channels. They are low threshold transient, high threshold inactivating and non-inactivating Ca channels (Huang, J. Physiol., in press). The effects of intracellularly applied guanosine 5'-O-3-thiotriphosphate (GTP-γS) and guanosine 5'-O-2-thiodiphosphate (GDP-βS) on these Ca channels were studied using whole cell patch-clamp technique. GTP-γS has little effect on the low threshold transient I_{Ca} but markedly slows the activation and inactivation of high threshold I_{Ca}. Cyclic AMP and forskolin do not mimic the effect of GTP-γS. When the cells were treated with pertussis toxin, the effect of GTP-γS was not observed. On the other hand, the rate of activation and inactivation of I_{Ca} does not change when GDP-βS is included in the pipet solution. These results are consistent with the suggestion that GTP-γs directly modulated the activities of high threshold Ca channels in the identified dorsal horn projection neurons. This work is supported by NS23061 and NS01050.

Tu-Pos139 DOPAMINE-DEPENDENT DECREASE IN CALCIUM CONDUCTANCE AND PROTEIN PHOSPHORYLATIONS IN HELIX ASPERSA NEURONS. Young-Kee Kim, Steven A. Dotson and Michael L. Woodruff, Department of Chemistry and Biochemistry, Southern Illinois University, Carbondale, Ill 62901-4409.

In the land snail, Helix aspersa, dopamine application leads to a sustained hyperpolarization and a decrease in the duration of the calcium-dependent action potential in cells of the right-parietal lobe of the subesophageal ganglion (F16-F19 and F28-F32). Voltage clamp experiments reveal that these phenomena are accompanied by a dopamine-dependent decrease in inward calcium current. Calcium current is reduced by over 90% by 5 μM dopamine. Half maximal response is obtained with 1 μM. With prolonged application the membrane shows an "adaptation": the action potential duration slowly increases again, approaching the original value after 10 to 20 mins. The calcium current does not seem to recover and we are exploring the possibility that time-dependent changes in potassium conductance may underlie this recovery. A dopamine-dependent decrease in outward current is observed.

The Helix neurons studied are large, 50 to 100 μm in diameter, and over 130 phosphoproteins can be assayed in microdissected neurons with two-dimensional electrophoresis/autoradiography. Dopamine increases the phosphorylation in 39 proteins and decreases phosphorylation in five others. The time-dependence some of these phosphorylations shows a recovery phenomenon similar to that seen in the physiology: both phosphorylation-dephosphorylation and dephosphorylation-rephosphorylation cycles are observed. Dopamine may not be acting through cyclic AMP-dependent protein kinase. The proteins that show dopamine-dependent phosphorylation in vivo are not substrates for in vitro phosphorylation by purified catalytic subunit of protein kinase A. Also, in our physiology experiments the effect of dopamine in decreasing the action potential duration is not mimicked by forskolin.

Tu-Pos140 B. Edmonds, M. Klein and E.R. Kandel. MODULATION OF DIFFERENT COMPONENTS OF Ca^{2+} CURRENT IN SENSORY NEURONS BY SEROTONIN AND FMRFamide. Center for Neurobiology & Behavior and Howard Hughes Medical Institute, Columbia University, 722 West 168th Street, New York 10032.

We examined the Ca^{2+} current (I_{Ca}) of Aplysia sensory neurons using whole-cell patch clamp techniques. We found that I_{Ca} has two components, a slowly-inactivating component sensitive to nifedipine and a rapidly-inactivating component inhibited by FMRFamide (FMRFa). In previous two-electrode voltage-clamp experiments, modulation of I_{Ca} by serotonin (5-HT) was not observed; however, under whole-cell voltage-clamp we now find that 5-HT increases a slowly-inactivating, nifedipine-sensitive component of I_{Ca}. This effect is simulated by cAMP and by activators of protein kinase C (Braha et al., 1988). Together with alterations in Ca^{2+} buffering (Blumenfeld et al., 1988), the increase in this slowly-inactivating current may contribute to the 5-HT-induced increase in the intracellular Ca^{2+} Arsenazo signal in response to a long depolarizing voltage-clamp step (Boyle et al., 1984).

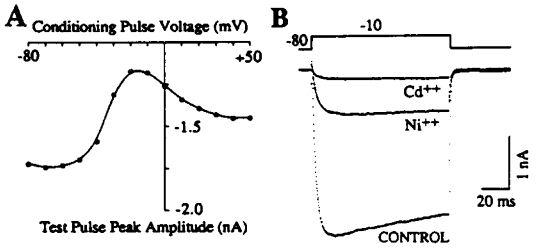
The effects of 5-HT on both synaptic potentials and on the S-potassium channel are overriden by FMFRa. Thus in the presence of 5-HT, FMRFa reopens S-channels previously closed by 5-HT (Belardetti et al., 1987). This override by FMRFa of 5-HT-induced responses can also be observed on other effector responses and even in the pattern of protein phosphorylation (Sweatt and Kandel, 1988). However, the 5-HT-induced increase in I_{Ca} is not reversed by FMRFa. This suggests that alternative molecular machinery may be involved in the modulatory effects of 5-HT and FMRFa on Ca^{2+} channels.

Tu-Pos141 **DIFFERENTIAL CALCIUM CURRENT BLOCK BY HYDROGEN PEROXIDE IN LOBSTER CARDIAC GANGLION NEURONS MEDIATED BY INTERNAL CALCIUM.**
D. Livengood and D. Miller, Department of Physiology, Armed Forces Radiobiology Research Institute, Bethesda Maryland, 20814.

H_2O_2 (0.35 mM to 3.5 mM) blocks I_{Ca} by 50% to 100% in a partially reversible manner with no change in I_K, I_A or in I_{leak}. The late outward calcium mediated K current in the presence of 30 mM TEA, 4 mM 4-AP and 10^{-7}M TTX and was greater after exposure to H_2O_2 even though the I_{Ca} was decreased. We postulated that the H_2O_2 induced increase in the late outward K current and the decrease in I_{Ca} were at least partially due to an increase in $[Ca^{++}]_i$ (Biop. J. 53:1988 p.555a). We report now that H_2O_2 blocks a calcium current that peaks at -30 mV following steps from a holding potential of -50 mV while leaving intact a somewhat smaller current that peaks at approximately -15 mV. In 25 mM Ca^{++} saline, the current that peaks at -30 mV inactivates in approximately 50 msec. In 30 mM Ba^{++} saline the current inactivates more slowly and peaks at -15 mV. A return to Ca^{++} saline at this time results in a recovery of the -30 mV peak. In Ba^{++} saline, 3.5 mM H_2O_2 has no effect on the -15 mV peak. However, with a subsequent return to 25 mM Ca^{++} saline following the peroxide treatment in Ba^{++} the - 30 mV Ca^{++} peak does not recover. We conclude that the two calcium peaks are different in nature and that the -30 mV peak current can be inactivated by H_2O_2 induced $[Ca^{++}]_i$ increase whereas the - 15 mV peak current is unaffected by H_2O_2 and the corresponding $[Ca^{++}]_i$ shift.

Tu-Pos142 **PHARMACOLOGY OF CALCIUM CURRENTS IN ENZYMATICALLY DISSOCIATED GUINEA-PIG CA1 HIPPOCAMPAL NEURONS** J.M.H. ffrench-Mullen, N.T. Slater and J.L. Barker. Lab of Neurophysiology, NINCDS, N.I.H. Bethesda, MD 20814 and Dept. of Physiology, Northwestern University Medical School, Chicago, IL 60611.

CA1 neurons were enzymatically dissociated from hippocampi of adult guinea-pigs (Kay and Wong, 1985) and patch-clamped in solutions containing 3 mM external Ba++ and internal Cs-BAPTA and Mg-ATP. The barium current (IBa) was studied using 100 ms or 1 s depolarizing command steps from holding potentials (Vh) of -80 or -40 mV. Steps from -80 mV evoked inward currents which displayed two exponential components of inactivation and a unimodal I-V relation of the peak IBa. The peak IBa evoked by 100 ms steps to -10 mV following a 1 s conditioning step to varying membrane potentials, showed a U-shaped dependence on the prepulse command voltage (Fig 1A). IBa was blocked by divalent cations with a potency order of La>Cd=Co>Ni. Ni blocked the rapidly inactivating component of IBa (Vh = -80; Fig 1B) and less potently blocked the slowly inactivating component of IBa at Vh = -40 mV. Other calciumchannel antagonists tested did not significantly discriminate between these two components (potency order: nifedipine > (-)-pentobarbital > verapamil > D-600 > (+)-pentobarbital). BAY K8644 (5-10 uM) potentiated both components of IBa, and amiloride (100-500 uM) was without effect. These data therefore provide no evidence for a transient (T-type) calcium channel in CA1 neurons, and suggest that two types of high voltage-activated calcium channel exist which may play a role in repetitive firing and epileptiform burst generation. Supported in part by NS 25682.



Tu-Pos143 EFFECT OF BENZODIAZEPINE LIGANDS ON Ca CHANNEL CURRENTS IN XENOPUS OOCYTES INJECTED WITH RNA FROM RAT HEART. Eli Gershon and Nathan Dascal. Department of Physiology and Pharmacology, Sackler School of Medicine, Tel Aviv University, Ramat Aviv 69978, Israel.

Voltage dependent Ca channel (VDCC) currents have been measured in Xenopus oocytes injected with heart RNA purified from 7-days old rats, using voltage clamp . The currents were evoked by a depolarization voltage step, using Ba^{2+} as charge carrier. Electrophysiological analysis of the current reveals two components: a slow L-type, DHP-sensitive current and a fast transient, DHP-insensitive, current. The benzodiazepine (BZ) ligands diazepam and Ro5-4864 decreased the current with micro molar affinity, with potency order of Ro5-4864 > diazepam > clonazepam. The central antagonist Ro15-1788 did not interfere with the effect of these drugs, suggesting their effect is not mediated through the "central type" receptor. The slow current that increases about 3 fold in the presence of 0.5 uM Bay K8644, is less potentiated when previously treated with Ro5-4864 or diazepam. Current-voltage relation of the peak inward current shows a steeper decline at positive test voltage in comparison with the control i-v curve. Steady state inactivation curve of the current reveals a small shift towards hyperpolarizing voltages. It is concluded that the benzodiazepine ligands block voltage dependent Ca^{2+} currents with a preferential affinity of these drugs for the resting state of the channel. The results agree with an involvment of a low affinity BZ peripheral receptor.

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

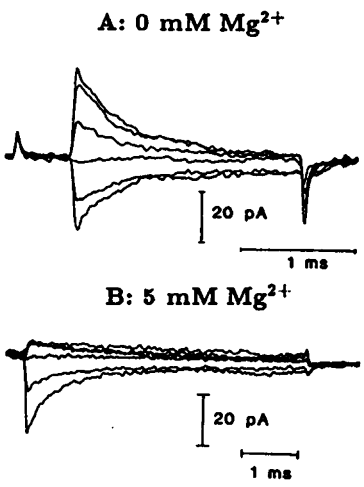
Tu-Pos144 THE ACTIONS OF LEAD AND ZINC ON THE CALCIUM CURRENT OF APLYSIA NEURONS. Dietrich Busselberg, Martyn L. Evans and David O. Carpenter. Wadsworth Cntr. for Labs & Research, NYS DOH & School of Public Health, Albany, NY 12201. (Intr. by M.V. King)

Lead and zinc block the voltage dependent calcium current of Aplysia neurons. Threshold concentrations are 0.5 μM for lead and 250 μM for zinc. Concentrations for 50% reduction are 0.1 mM for lead and 4.7 mM for zinc. Nearly complete (> 90%) inhibition of the calcium current occurs for lead at 400 μM and for zinc at 20 mM. Reduction of calcium current amplitude starts immediately after solution change and steady state is reached within 3 to 5 min of application. On washout 90 to 100% of the control level is restored within 5 min for both ions. The calcium current block is voltage dependent and the maximum of the current-voltage curve is shifted 5 to 10 mV in the hyperpolarizing direction by 250 μM lead and 10 to 15 mV in the depolarizing direction by 10 mM zinc. The inactivation curve of the calcium current is not significantly affected by lead or zinc. These data suggest that lead blocks the calcium channel directly whereas zinc may be acting by a combination of channel block and screening of surface charges. The low concentrations at which lead is effective make charge screening an unlikely mechanism. The blocking effect of lead is specific to the calcium channel, as the sodium channel and the delayed rectifier potassium channel are not significantly affected by doses of lead which inhibit the calcium current by 50%. The voltage dependence of the activation and inactivation of the Aplysia calcium current and its sensitivity to block by nickel and nifedipine suggest a strong similarity to the L type calcium current described in mammalian neurons. These data indicate that the toxic effects of lead in mammals may be due in part to the block of neuronal calcium channels. (NIH Grant #NS23807 to DOC).

Tu-Pos145 **Block of Na^+ outward currents by intracellular Mg^{2+}**

by Michael Pusch*, Franco Conti+ and Walter Stühmer*
(*Max-Planck-Institut für biophysikalische Chemie, Am Faßberg, D-3400 Göttingen and +Istituto di Cibernetica e Biofisica, CNR, I-16146 Genova)

We have investigated macroscopic Na^+ outward currents in inside-out patches from *Xenopus laevis* oocytes injected with cDNA derived mRNA coding for the type II rat brain sodium channel (Noda *et al.* (1986) *Nature* 320: 188-192) using standard patch clamp techniques (Stühmer *et al.* (1987) *Eur. Biophys. J.* 14 : 131-138). The pipette solution contained (in mM): 115 NaCl, 2.5 KCl, 1.8 $CaCl_2$, 10 HEPES-NaOH, pH 7.2. The bath solution contained 90 KCl, 30 NaCl, 10 NaOH-EGTA, 10 HEPES-KOH, pH 7.2 and different amounts of $MgCl_2$. The Figure shows 'tail' currents elicited by voltage steps to -30, 0, ..., 120 mV after a prepulse to 30 mV from two different patches at 0 (A) and 5 mM Mg^{2+} (B) respectively. The reduction of the outward currents as compared with the inward currents at the higher Mg^{2+} concentration is obvious. A quantitative analysis yielded a half blocking concentration between 3 and 4 mM at 0 mV and a voltage dependence of e-fold per 49 mV. We conclude that the reduction of the Na^+ conductance at large depolarizations which has been observed frequently in several preparations can be mainly attributed to a voltage and dose dependent block by intracellular Mg^{2+}.



Tu-Pos146 **SODIUM CHANNEL ACTIVATION IN CRAYFISH AXONS: 3. EFFECTS OF DEUTERIUM OXIDE.**

D.A. Alicata, J.G. Starkus and M.D. Rayner.
Békésy Laboratory of Neurobiology and Department of Physiology, U. of Hawaii, Honolulu, HI. 96822

Schauf and Bullock (1979, Biophys. J. 27:193) have reported for *Myxicola* giant axons that substitution of heavy water (D_2O), externally and internally slows the kinetics of sodium channel ionic current (INa) without significant change in gating current (Ig) kinetics. In our axons we confirm Schauf and Bullock's findings: (1) INa is delayed without change in gating current kinetics or total charge movement. (2) D_2O has no apparent affect on the "Cole-Moore-type" shifts of INa rising phase. (3) Sodium channel deactivation (monitored by ionic tail currents), seems to be insensitive to D_2O. (4) In the presence of D_2O activation of INa is delayed. However, the change in time to peak remains a constant fraction of control time to peak.

We further observe that D_2O both prolongs the "initial delay" before the start of the INa rising phase and slows the subsequent rate of sodium channel activation. Thus the shift in peak INa is greater than the prolongation of the "initial delay". We conclude that voltage sensitive "gating" charge movement is generated in non-conducting transitions which precede transition to the "open" or conducting state.

Our confirmation in crayfish axons of Schauf and Bullock's data strengthens their argument that no significant component of Ig arises in the final opening step and supports their suggestion that Ig is generated within a hydrophobic rather than a hydrophilic environment. We interpret these findings as indicating a physical and functional separation between the voltage sensitive gating particle and the activation gate.

Supported by PHS grant #NS21151-04, and partially from Hawaii Heart Association, UHBRSG and NIH RCMI grant RR-03061.

Tu-Pos147 **SODIUM CHANNEL ACTIVATION IN CRAYFISH AXONS: 4. EFFECTS OF CHLORAMINE-T**

J.G. Starkus, D.A. Alicata, P.C. Ruben and M.D. Rayner
Békésy Lab. of Neurobiology and Department of Physiology, University. of Hawaii, Honolulu, Hawaii 96822

Internally applied 10mM chloramine-t (CT) removes fast inactivation in crayfish axons but leaves slow inactivation intact (Yeh et al., Biophs. J. 1987). When fast inactivation is completely removed, two components of activation become visible, one with fast kinetics (fast activation) and a second with slower kinetics (slow activation).

Since CT does not affect the kinetics of sodium gating current, Ig, we have compared the temporal relationships of sodium gating and ionic current before and after CT treatment. We find that, in CT treated axons, slow activation continues through the time course of early decay in control ionic currents. This macroscopic observation from crayfish axons is similar to the conclusions reached from single channel measurements in neuroblastoma cells (Aldrich and Stevens, J. Neurosc. 1987). We also compared the kinetic components of gating current to sodium channel activation. The intermediate component of gating current appears to be the major "driver" for the fast activation of sodium current, while the slow gating component reflects an eigenvalue equivalent to that seen during slow activation of INa.

The effect of prepulse on the initial delay ("Cole Moore type" shift) in the sodium current was studied before and after CT treatment. The same shift was observed in the fast activation component but no apparent shift was seen in the slow activation component.

These data can be effectively simulated using a simple model in which a single voltage sensitive gating particle interacts electrostatically with a largely voltage insensitive M-gate (with two preopen positions).

Supported by PHS grant #NS21151-04, and partially from Hawaii Heart Association, UHBRSG, and NIH RCMI grant RR-03061.

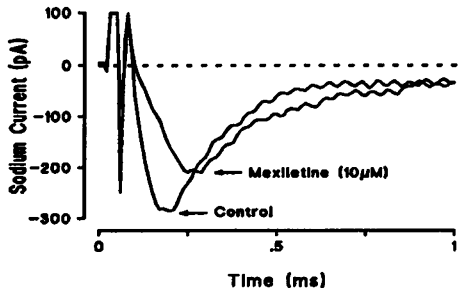
Tu-Pos148 MODIFICATION OF Na CHANNEL GATING BY AN ALPHA SCORPION TOXIN FROM TITYUS SERRULATUS. G.E. Kirsch, A. Skattebøl, L.D. Possani† and A.M. Brown. Baylor College of Medicine, Department of Physiology and Molecular Biophysics, Houston, Texas 77030 and †Universidad National Autonoma de Mexico, Department of Biochemistry, Mexico DF, 04510 Mexico.

The effects of TsIV-5, a toxin isolated from the Brazilian scorpion Tityus serrulatus, on whole-cell and single channel Na currents were determined in N18 neuroblastoma cells. In whole-cell records at a test potential of -10 mV, external application of 500 nM TsIV-5 slowed inactivation 20-fold and increased peak current by about one-third without changing time-to-peak. Both the steady-state activation and inactivation curves were shifted to more negative potentials. Other alpha scorpion toxins produce similar effects but the single channel mechanism is not known. TsIV-5 caused a voltage-dependent prolongation of mean single channel open time such that at a test potential of -60 mV no change was observed whereas at -20 mV mean open time increased about 3-fold and prolonged bursting was observed. Macroscopic current reconstructed from summed single channel records showed a characteristic toxin-induced potentiation of peak current and a 20-fold slowing of the decay phase. TsIV-5 does not discriminate between tissue-specific Na channel subtypes. Prolonged open times and bursting also were observed in toxin-treated Na channels from rat ventricular myocytes, rat cortical neurons and mouse skeletal muscle. The toxin effects are shown to be consistent with a kinetic model in which TsIV-5 selectively interferes with the ability of the channel to reach the inactivated state. Supported by grants from the NIH (HL-25143 and HL-33662) and the American Heart Association (876197).

Tu-Pos149 USE-DEPENDENT INHIBITION OF SODIUM CURRENTS BY FORSKOLIN IN SINGLE SCIATIC NERVE FIBERS FROM TOAD. Neil Castle, Anesthesia Research Laboratories, Harvard Medical School and Brigham and Women's Hospital, Boston, MA 02115

Forskolin (FSK), routinely used as a tool specifically to activate adenylate cyclase (AC), has recently been shown to alter the gating of potassium channels in a manner which appears to be independent of its AC activating ability. In the present study the effect of FSK on voltage dependent Na^+ and K^+ currents in single Bufo marinus sciatic nerve fibres has been examined under voltage-clamp conditions. Consistent with work by others, FSK and dideoxy FSK (no effect on AC activity) (50-150 μM) induced a reversible, concentration dependent acceleration of the delayed K^+ current inactivation. At higher concentrations (150-450 μM) FSK also reduced the peak Na^+ current (I_{Na}) (by up to 50%) and increased the rate of I_{Na} inactivation. However, there was no effect on the voltage-dependence of steady-state inactivation or the recovery from fast inactivation. Upon repetitive stimulation at frequencies of 1-10 Hz, I_{Na} was further reduced (by up to 60%). The magnitude of this use-dependent (or phasic) inhibition did not appear to be voltage dependent, since a similar degree of block was seen with repetitive pulses to -20 and +100 mV. Recovery from phasic inhibition was relatively slow, $\tau \sim 10$ sec. Phasic inhibition could be prevented by preceding each test pulse with a 50 ms prepulse to -120 mV. Near maximal phasic inhibition was observed at pulses of 4 ms duration, suggesting that phasic binding of FSK occurs to the open state of the channel. In summary, FSK appears to block both Na and K channels in nerve albeit at higher concentrations than required to activate AC. It is interesting to note, however, that unlike most other agents that produce phasic block of the Na^+ channel, forskolin is not charged.

Tu-Pos150 **VOLTAGE CLAMP OF CARDIAC SODIUM CURRENT AT 37°C: REDUCTION BY MEXILETINE.** **KT Murray and LM Hondeghem. Vanderbilt University, Nashville TN 37232.**

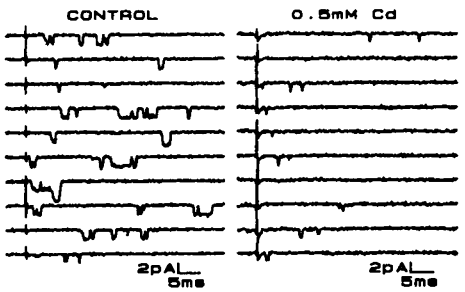


In order to voltage clamp I_{Na} it has been necessary to reduce the temperature and the sodium gradient. Guinea pig ventricular myocytes, dispersed using collagenase, were bathed in normal Tyrode's solution at 37°C. A GΩ seal was formed with a glass pipette (1-2 MΩ) that was carefully coated with sylgard to minimize pipette capacity. After optimal series resistance compensation, the capacity transient for steps of up to 150 mV lasted 50-70 μs. By the end of the capacity transient less than 10% of the maximal sodium current had developed. The I-V relationship increased smoothly over about 40 mV range. The maximum sodium current had an amplitude between 50 and 300 pA, and reached its peak 180 to 200 μs following the beginning of the clamp pulse, and was almost completely inactivated in less than 1 ms. Currents reduced by inactivation had a constant time to peak and could be scaled to superimpose. Furthermore, deactivation current tails obtained at various times during the sodium current decayed smoothly and monoexponentially with a constant time constant. These results suggest that the sodium current was adequately controlled. Contrary to broken-in cells, these on-cell patches exhibited little drift of inactivation and were easily maintained for long periods of time (up to 3 hrs). Since the action of sodium channel blockers is dependent upon both temperature and sodium gradient, the on-cell patch method could provide a more desirable preparation for studying the action of antiarrhythmic agents. Mexiletine (10 μM) reduced the amplitude of the sodium current as shown in the figure. This new technique provides an opportunity to study the action of antiarrhythmic agents under conditions more closely encountered *in vivo*.

Tu-Pos151 **Cd^{2+} AND Zn^{2+} BLOCK UNITARY Na^+ CURRENTS IN PURKINJE AND VENTRICULAR CELLS**

Clive Marc Baumgarten and Harry A. Fozzard
Medical College of Virginia, Richmond, VA and The University of Chicago, Chicago, IL

Ca^{2+} induces a voltage-dependent decrease of unitary Na^+ conductance without altering mean open time (τ_o). Cd^{2+} and Zn^{2+} are more potent inhibitors of whole-cell I_{Na} in heart than Ca^{2+}. Their mechanism of action was studied in cell-attached patches on enzymatically isolated canine Purkinje and ventricular cells. Pipettes contained (mM): 280 NaCl, 2 $CaCl_2$, 10 HEPES (pH 7.4) ± $CdCl_2$ or $ZnCl_2$ (0.1, 0.2, or 0.5 mM); bath solution was 150 K-aspartate, 0.1 or 1 EGTA, 5 HEPES (pH 7.4) at 10°C. Consecutive sweeps from two ventricular cell patches stepped from -130 to -50 mV are shown. Cd^{2+} (0.5 mM) reduced unitary conductance (γ_{Na}) from 16.1±0.8 to 12.8±0.9 pS ($p<0.01$, $n=9$) and shortened τ_o at all E_m tested, e.g., at -50 mV, from 1.1±0.1 to 0.4±0.1 ms ($p<0.01$, $n=6$). These effects were dose-dependent, and similar results were obtained in both ventricular and Purkinje cells. Block by Cd^{2+} and Zn^{2+} are different than block by Ca^{2+}. Cd^{2+} caused a voltage-*independent* reduction in γ_{Na} and a voltage-dependent reduction in τ_o. Block by Zn^{2+} involved voltage-dependent decreases in both γ_{Na} and τ_o. Voltage-dependent abbreviation of τ_o by divalent cations suggests a long-lived block partway through the pore. Voltage-independent effects on γ_{Na} may be caused by a diminution of the negative surface potential at the extracellular membrane face. *(NIH HL20692, HL24847, AHA EI 83-113).*



Tu-Pos152 **ENDPLATE NA CHANNELS IN MOUSE SKELETAL MUSCLE FIBERS ARE PREFERENTIALLY AFFECTED BY THE METHYLATING AGENT TRIMETHYLOXONIUM.**

R.L. Milton, Department of Physiology & Biophysics, University of Health Sciences/The Chicago Medical School, North Chicago, Illinois 60064.

Mouse flexor digitorum brevis (FDB) muscles were treated *in vivo* with the methylating agent trimethyloxonium (TMO). This agent has been shown to eliminate the sensitivity of Na channels to the blocking action of tetrodotoxin (TTX) (Sigworth and Spalding, Nature 283:293-295, 1980).

Using the loose patch voltage clamp technique, the percentage of Na channels insensitive to TTX after treatment with TMO was measured in the junctional and extrajunctional regions of dissociated FDB muscle fibers. It was found that Na channels at or near the endplate were affected by the TMO treatment to a much greater degree than were extrajunctional channels. Approximately 90% of Na channels at the endplate were insensitive to TTX as compared to only about 20% of the extrajunctional channels. Measurements made two to four days following treatment revealed that this insensitivity to TTX disappeared with a half life of about 35 hours, probably due to channel turnover, but slow hydrolysis of the methyl ester bond may also have occurred.

These results suggest that endplate Na channels of fast twitch skeletal muscle fibers may represent a distinct Na channel subtype.

Tu-Pos153 ISOLATION OF POLYPEPTIDE TOXINS FROM THE SCORPION *LEIURUS QUINQUESTRIATUS* THAT ALTER THE GATING OF BATRACHOTOXIN-ACTIVATED SODIUM CHANNELS IN PLANAR LIPID BILAYERS. M.E. O'Leary* and B.K. Krueger, Dept. of Physiology, Univ. of Maryland School of Medicine. Baltimore, MD 21201

We recently reported (O'Leary and Krueger, Soc. Neurosci. Abst. 14: 835, 1988) that external application of the venom of the north African scorpion, *Leiurus quinquestriatus quinquestriatus* suppresses the gating of batrachotoxin (BTX)-activated Na channels so that the single channel probability of being open (P_o) remains high (> .95) at very hyperpolarized membrane potentials (< -140 mV). We have now fractionated the venom by HPLC cation exchange chromatography and identified at least two Na channel toxins by their ability to stimulate the binding of ^{3}H-BTX to Na channels in rat brain synaptosomes. Of the more than 20 absorbence peaks (280 nm) eluted from the column by an ammonium acetate (NH_4Ac) gradient, two well separated active peaks eluted between 275 and 350 mM NH_4Ac. Samples containing these fractions caused a hyperpolarizing shift in the P_o vs V_m curve of BTX-activated Na channels. Preliminary data indicate that both these fractions are required to cause the shift in Na channel gating produced by crude venom. The requirement for both toxins to cause the suppression of Na channel closing while either one alone is sufficient to stimulate ^{3}H-BTX binding, suggests that effects of whole scorpion venom may reflect the interaction of more than one toxin at multiple binding sites on the Na channel. (Supported by NIH NS16285 and NS20106 and U. S. Army Medical Research and Development Command.)

Tu-Pos154 REGULATION OF SLOW CHANNELS BY CYCLIC NUCLEOTIDES AND METABOLISM. Jacques, D. and G. Bkaily, Department of Physiology and Biophysics, University of Sherbrooke, Sherbrooke Quebec, Canada, J1H 5N4.

In 3-day-old embryonic chick heart cells, 3 types of TTX- and Mn^{2+}- insensitive slow Na^+ currents were investigated using the whole-cell patch clamp technique. The first type, a fast transient slow inward current (ft) was activated from a holding potential of -80 mV. This current is highly sensitive to melittin (10^{-8}M). From a holding potential of -50 mV, there was activation of two other components i.e. a slow and a sustained current (sts). Melittin at a high concentration (10^{-4}M) completely blocked the slow transient component (st) but left unblocked the sustained component (s). The effect of 8-Bromo cGMP, 8-Bromo cAMP and cyanide (CN) on the TTX-Mn^{2+} insensitive ft Na^+ current was studied. 8-Bromo cGMP (10^{-3}M) decreased by 80% the relative amplitude of the ft slow Na^+ current within 25 min. However, 8-Bromo cAMP (10^{-3}M) decreased by 40% the relative amplitude of this current within 30 min. The effect of the metabolic inhibitor, cyanide (NC- 10^{-3}M) on the relative amplitude of the slow Na^+ current was biphasic. First, there was a 45% decrease of the relative amplitude of the ft slow Na^+ current that reached the steady state in 7 min and remained stable for approximately 13 min. Secondly, after 17 min, there was a sudden separation of two peaks of the slow Na^+ current ft and st. After 22 min, the st peak was completely blocked and there was a further decrease of the ft component. After 30 min, the ft component was completely blocked. These results suggest that in heart cells the regulation of the ft slow Na^+ channels by cAMP is different from that of the slow Ca^{2+} currents, however, it seems to be similar to that of Ca^{2+} channels in vascular smooth muscle. This work was supported by MRCC (MT 9816) and Dr. Bkaily is a scholar of CHF.

Tu-Pos155 PURIFICATION OF TWO PROTEIN TOXINS FROM LEIURUS QUINQUESTRIATUS THAT SPECIFICALLY MODIFY Na CHANNELS. R. Hahin and J. Borneman, Biological Sciences Department, Northern Illinois University, DeKalb, IL 60115

Toxins were purified from crude venom obtained from Middle Eastern scorpions (Leiurus quinquestriatus hebraeus). Active fractions were purified using cationic exchange chromatography, and tested for their ability to lengthen propagated compound action potentials (A.P.) recorded from frog sciatic nerves. A.P. recordings were made using the sucrose gap recording method.

Insoluble material was removed from dissolved crude venom by sedimentation at 12,000XG for 15 min.; the supernatant was applied to an Amberlite CG-50 (1 X 25 cm) column equilibrated with 10 mM NH_4 Acetate. Fractions were eluted with a step to 166 mM, a linear gradient from 166 to 400 mM, followed by a final step to 500 mM NH_4 Acetate. Four major peaks in absorbance (280 nM) were observed; toxins in the third peak lengthened compound A.P.'s. All active fractions were pooled and filtered (Amicon UM-2) and then were applied to a FPLC Mono-S cation exchange column equilibrated with 10 mM Phosphate Buffer. Fractions (0.35 ml) were eluted using a linear gradient (0-500 mM) of NaCl, followed by a final step to 1000 mM NaCl using a flow rate of 1 ml/min. 6 major peaks in absorbance were seen, but only 2 were active in prolonging the Compound A.P.

The dose response curve for toxin (LqtxI) obtained from one of the peaks matched the dose response curve obtained from Leiurus quinquestriatus quinquestriatus toxin V purified and kindly provided by Prof. H. Rochat (Université d'aix-Marseille). The second active fraction (LqtxII) exhibited a distinctly different dose-response curve with a potency which was approximately 1/1000 of that observed for LqtxI.

Tu-Pos156 **GUARDED RECEPTOR ANALYSIS OF COCAINE INTERACTION WITH CARDIAC SODIUM CHANNELS.** William J. Crumb Jr., Craig W. Clarkson, Department of Pharmacology, Tulane University Medical School, New Orleans, Louisiana 70112.

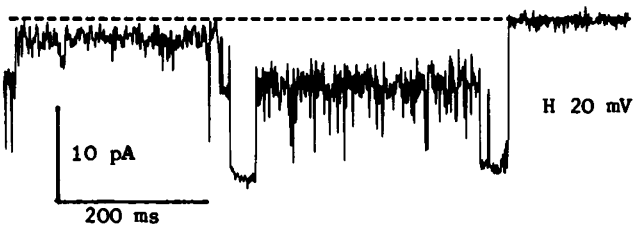
Recent reports suggest that cocaine can produce ventricular arrhythmias at plasma concentrations achieved during an overdose (e.g. 10-20 μM). A possible mechanism for this effect is slowing of impulse conduction due to Na channel block. To our knowledge, no one has investigated the effects of cocaine on cardiac Na channels since Weidmann (1955). We therefore investigated its effects on cardiac Na current in isolated guinea pig ventricular myocytes using the whole-cell variant of the patch clamp method (15-16 ^{o}C, $[Na]_i$ = 10 mM $[Na]_o$ = 25 mM). Cocaine (10-100 μM) reduced Na current in a use-dependent manner (V_h = -140 mV, V_{test} = -20 mV, 0.2-5 Hz). The mechanisms for use-dependent block development and recovery were characterized. At 30 μM cocaine, two phases of block development were defined: a rapid phase (τ = 1-7 ms) and a slower phase (τ = 1-3 s). Recovery from block at -140 mV was monoexponential (τ = 8.6 ± 1.3 s; n=10). In comparison, the time constant for recovery from block by lidocaine (100-200 μM) was 1.0 ± 0.4 s at -140 mV (n=4). The effects of both lidocaine and cocaine could be well described by the guarded receptor model. The estimated dissociation constants for cocaine binding to channels primarily in rested, activated and inactivated states were: 732 μM, 14 μM and 8 μM respectively (n=11-13). Estimates for lidocaine were similar: 976 μM, 17 μM and 4 μM for rested, activated and inactivated states (n=5). These data indicate that cocaine can block cardiac Na channels at concentrations achieved during an overdose, and that cocaine unbinds more slowly than lidocaine at hyperpolarized potentials.

Tu-Pos157 HYDROGEN IONS AFFECT THE COCAINE BINDING AFFINITY IN BTX-ACTIVATED Na CHANNELS IN PLANAR BILAYERS. Jilda Nettleton and G.K. Wang. Department of Anesthesia Research Labs, Harvard Medical School and Brigham and Women's Hospital, Boston, MA 02115.

The effects of pH on cocaine(-) binding interactions with skeletal muscle BTX-activated Na channels incorporated into planar bilayers, were studied both under symmetrical and asymmetrical NaCl conditions. An increase in internal pH reduces the apparent binding affinity of the internally applied cocaine molecules in the pH range 6.2-9.0, which is consistent with the idea that the charged cocaine form is more active than the neutral form. Most of the reduction in the cocaine affinity can be accounted for by a decrease in the apparent on-rate constant (k_{on}), ($k_{on}(6.2) = 3.5 \times 10^4\ M^{-1}s^{-1}$, $k_{on}(9.0) = 5.0 \times 10^2\ M^{-1}s^{-1}$). The off-rate constant, (k_{off}), is little changed ($k_{off}(6.2) = 3\ s^{-1}$, $k_{off}(9.0) = 5s^{-1}$). The magnitude of the change in binding affinity suggests that the apparent pK_a of cocaine at its binding site is about 7.0, which is 1.5 units lower than the pK_a in the bulk solution. In contrast, elevation of external pH increases the internal cocaine binding affinity exponentially in the pH range 6.2-8.4. A plot of log K_D vs pH exhibits a slope of about -0.4. External H^+ ions appear to inhibit the binding interactions primarily by lowering k_{on}, k_{off} again being relatively unaffected. Finally, when cocaine is applied externally, an increase in external pH also increases the cocaine binding affinity exponentially in the pH range 6.2-8.4, but with a slope of about -0.8 (log K_D/pH). The reason for this increased slope may be that at high pHs more cocaine is in the neutral form; thus, more can cross the membrane barrier, become charged and block the sodium channel. Supported by NIH-GM35401

Tu-Pos158 EFFECTS OF TWO SODIUM CHANNEL 'AGONISTS': VERATRIDINE AND DELTAMETHRIN ON SINGLE CHANNEL ACTIVITY IN INEXCITABLE EMBRYONIC COCKROACH NEURONES. M. Amar and Y. Pichon, Dpt. of Biophysics, CNRS Laboratory of Cellular and Molecular Neurobiology, F-91190 Gif sur Yvette.

It has been recently suggested that, in two preparations, the alkaloid veratridine acts on open sodium channels. This suggestion is in contrast with our earlier studies (Pichon and Boistel (1969), J.Physiol., Paris, 61, 373-374). In the present experiments, we have reinvestigated the effects of small concentrations of veratridine (1 µM) or of deltamethrin (0.01 to 1 µM). The cultured embryonic cockroach neurones which were used were devoid of functional sodium channels and were inexcitable. The two molecules were added to the normal saline contained in the patch pipettes. The cell attached configuration of the patch-clamp technique was used. As illustrated in the figure for veratridine, the two molecules induced single channel activity in the form of long openings with several conductance states and flickering. The existence of sodium channels in the open configuration is therefore apparently not prerequisite for sodium channel 'activation' by veratridine and deltamethrin in this preparation. (Supported by CNRS and Roussel UCLAF).



Tu-Pos159 SYNTHESIS AND CHARACTERIZATION OF CONOTOXIN IIIa. Becker S., Atherton E.*, Michel H., and Gordon R.D.. (Intr. by F. Pepe). Max-Planck-Institut für Biophysik, Frankfurt am Main, Federal Republic of Germany, and * Cambridge Research Biochemicals Ltd., Harston, Cambridge, England.

We have synthesized Conotoxin IIIa (Geographutoxin I) a voltage dependent sodium channel specific polypeptide neurotoxin, using solid phase peptide synthesis together with Fmoc chemistry. After cleavage from the resin, the peptide was isolated by reverse phase HPLC, and then the six cys-Acm groups were deprotected with mercuric acetate. The reduced product was again purified by reverse phase HPLC and it's chemical authenticity was confirmed by peptide sequencing, amino acid analysis and fast bombardment mass spectrometry. We have developed protocols to oxidise this reduced peptide in high yield although complete oxidation was never obtained and so we repurified the oxidised peptide by reverse phase HPLC. We next examined the blockade of specific (3H) saxitoxin binding to electric eel membranes by this cyclised toxin. We found that the oxidised toxin was able to completely block (3H) saxitoxin binding in a competitive manner. IC 50 plots and double reciprocal plots of the inhibition of binding gave Ki values of 2.0 and 2.5nM. Thermal stability studies revealed that the activity of conotoxin IIIa as assayed in these binding studies was stable at 4 C and 20 C for at least 24 h. We conclude that we have established a synthetic route to produce cyclised active toxin which can be used to probe sodium channels.

Tu-Pos160 HIGH RESOLUTION RECORDING OF Na GATING CURRENTS FROM SQUID REVEAL A FAST INITIAL COMPONENT. J.M. Bekkers, I.C. Forster* and N.G. Greeff*. Section of Molec. Neurobiol., Yale Univ. Medical School, CT06810 and Physiologisches Inst. Univ. Zurich, Switzerland.*

Non-inactivating gating currents (Ig,n) were recorded from the giant axon of squid (Loligo forbesii) following inactivation of the sodium gating system using various prepulse protocols. Axons were typically dialyzed at 5°C with TMA-F and externally bathed in slightly hyperosmotic Tris-Cl with TTX. Measurements of Ig with 100 kHz bandwidth were made using a fast, low noise voltage clamp (<0.5 μVrms noise referred to input) and a 16 bit ADC sampling at up to 3 μs per point. In addition to one or two slower components of Ig,n , a very fast component relaxing with a time constant from 5-25 μs was resolved which accounted for up to 50% of the initial amplitude of Ig,n and approximately 6% of the total charge transfer. This component was best observed with freshly mounted axons and when precautions were taken to ensure rapid charging of the membrane with a time constant of 3 μs or less without overshoot. We have also observed a corresponding component in the total (fully recovered) Ig which appears qualitatively similar to that observed in crayfish axons (Starkus et al., Biophys. J., 35,521-33) and is suggestive of an asymmetric charge group insensitive to the state of Na channel activation. Studies have shown that system non-linearities or inhomogeneous charging of the membrane cannot account for the reported observations. Supported by Swiss NFG 3.143.0.85.

Tu-Pos161 **MEASURING DWELL-TIME DISTRIBUTIONS OF SMALL SUBCONDUCTANCE LEVELS USING VARIANCE-MEAN ANALYSIS.** Joseph Patlak. Dept. of Physiology, U. of Vermont, Burlington, VT 05405.

Most channels display subconductance levels (sublevels) during their function. Although sublevel lifetimes can be determined directly in large channels with well spaced sublevels, background noise prevents their accurate measurement in the Na^+, Ca^{++}, and other small-conductance channels. I present a method of obtaining the time constants of the dwell time distribution for small sublevels that is based on Variance-Mean analysis (Patlak, 1988. JGP, Oct 88). Variance-Mean analysis consists of sliding a window , W time points wide, one point at a time over periodically sampled data. The mean and variance of the points in the window are determined, and the means are sorted by their associated variances. Levels are defined as periods of constant current without transition to another level. Windows that include one or more transitions have high variance, while those that have constant current throughout have variance as low as that of the background noise. The mean currents from the low-variance windows are entered into a "levels histogram" to determine the distribution of sublevel currents. Once the magnitude of possible current levels has been established, the total number of entries to each component of the levels histogram can be determined. Each sublevel event contributes, on the average, N entries, where N is a function of the dwell time of the sublevel event, T_S, and the window width as follows: For $T_S < W$, $N=0$; For $T_S >= W$, $N=T_S-W+1$. The sum of the entries for all events of a record, N_S, is thus proportional to the integral of the dwell time distribution from W to infinity. For currents from channels with exponentially distributed dwell times (or the sum of exponentials), N_S is an exponentially decreasing function of W, with time constant(s) that are identical to those in the original function. These time constants can be experimentally determined by fitting N_S as a function of W for the currents from an individual channel. Analysis of simulated channel data accurately measured the time constant at each possible level, even with substantial background noise. A similar analysis of Na^+ channel bursts from frog and mouse skeletal muscle showed that both the fully open state and the 35% sublevel state were well described by a single exponential in the N_S vs. W plots, with time constants of 1.5 and 0.5 ms respectively at approximately 14°C. Supported by NIH grant AR37606 and the American Heart Association.

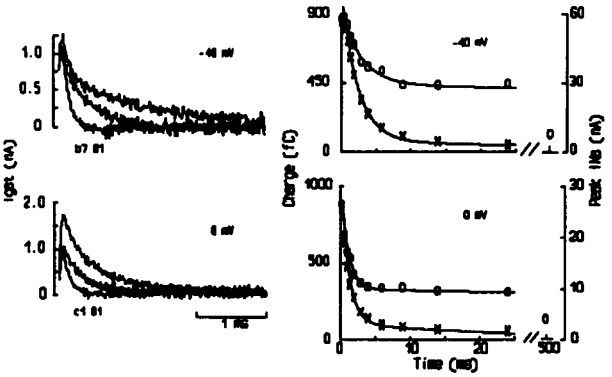
Tu-Pos162 **REMOVAL OF NA CHANNEL FAST INACTIVATION IN SINGLE CARDIAC PURKINJE CELLS.** *M.F. Sheets, D.A. Hanck and H.A. Fozzard, Cardiac Electrophysiology Labs, The University of Chicago and Northwestern University, Chicago, IL.*

Removal of Na channel fast inactivation has been used to help understand channel kinetics. For example, in squid axon internal perfusion with enzymes such as pronase or α-chymotrypsin removes inactivation without altering I_{Na} onset ("activation"), but in neuroblastoma cells internal enzymes remove inactivation as well as altering I_{Na} onset. We studied the internal application of 1.25 mg/ml α-chymotrypsin in single canine cardiac Purkinje cells that were voltage clamped with a large suction pipette (20-30 μm) and perfused with external (mM) 30 NaCl, 120 CsCl, 2 $CaCl_2$, 10 HEPES (pH 7.4) and internal (mM) 30 NaF, 120 CsF, 10 HEPES (pH 7.4) at 13°C. I_{Na} responses to step depolarizations from a V_{hp} of -150 mV and I_{Na} tail currents from +40 mV to -150 mV were recorded at 300 KHz with a 16-bit ad-converter (Masscomp 5520), and then leak and capacity corrected using a modified P-4 technique. Peak γ_{Na} vs. membrane potential (V_m) were fitted to a Boltzman equation using a modified Gauss-Newton nonlinear least squares algorithm (NAG). In a typical cell, 30 min after perfusion with internal α-chymotrypsin, I_{Na} response to a step depolarization to -45 mV for 10 ms caused a more rapid onset of I_{Na}, a delay in time to peak I_{Na} from 2.5 ms (control) to 3.7 ms, an increase in peak I_{Na} from -37 nA (control) to -67 nA and nearly complete removal of I_{Na} decay. In 3 cells V_m at one-half peak γ_{Na} shifted by -18 mV (± 2 mV, S.D.) while the slope remained unchanged (-8.7 ± 0.8 in control, -9.0 ± 2.2 after α-chymotrypsin), and maximum γ_{Na} increased by a factor of 1.2 ± 0.1. After removal of inactivation, time constants of I_{Na} tail currents to -150 mV increased from 109 μs (± 38, control) to 243 μs (± 112). The changes in the onset of I_{Na} to step depolarizations and in I_{Na} tail currents may have been caused by modification of the internal surface charge potential by α-chymotrypsin. In cardiac cells internally applied α-chymotrypsin has complex effects and alters both Na channel activation and inactivation.

Tu-Pos163 **IMMOBILIZATION OF NA CHANNEL GATING CHARGE IN SINGLE CANINE CARDIAC PURKINJE CELLS.**

D.A. Hanck, M.F. Sheets, and H.A. Fozzard. Cardiac Electrophysiology Labs, The University of Chicago and Northwestern University*, Chicago, IL*

Inactivation of Na channels has been reported to manifest primarily as charge immobilization, i.e. charge not recovered over the time of integration of the gating current (I_g). In cardiac cells decay of Na current (I_{Na}) is slower than in nerve and long conditioning steps are required to achieve "steady-state" availability. We assessed the time course of charge immobilization and compared it with the development of inactivation as assessed by peak I_{Na} in cardiac Purkinje cells at 13°C. I_{Na} and I_g were recorded in response to two-pulse protocols with a V_{hp} of -150 mV; cells were stepped to -40 or 0 mV for a variable time, clamped back to -120 mV for 2 ms, and stepped again to -40 mV or 0 mV to assess availability. Data were recorded in quadruplicate at 300 kHz with a 16-bit a/d-converter, averaged, and leak and capacity corrected using a modified P-4 technique. At 0 mV I_{Na} inactivation developed with two τ's (1 and 30 ms). Charge also immobilized with two τ's (1 and 230-365 ms). Unlike I_{Na} where the fast τ comprised 90% of the response, only 60% of the charge immobilized with the fast τ. At -40 mV I_{Na} inactivation also developed with two τ's (2 ms and 50 ms); the fast τ represented greater than 90% of the amplitude. Sixty percent of the charge immobilized with a similar fast τ; the remainder immobilized with a τ greater than 400 ms. After long conditioning steps I_g relaxed more quickly and may represent charge distribution between inactivated states. Supported by NIH HL20592 and HL01447.



LEFT: I_g recorded after inactivating for 0.3, 24, and 500 ms at potentials indicated. RIGHT: Peak I_{Na} (X) and charge (O). Lines are drawn using fitted values as described in text.

Tu-Pos164 SODIUM CHANNEL OPENING RATE PREDICTIONS IN FROG SKELETAL MUSCLE. R. Hahin, Biological Sciences Department, Northern Illinois University, DeKalb, IL 60115.

Linear systems convolution analysis of muscle Na currents was used to predict the opening rate of Na channels as a function of time during voltage clamp pulses. If open Na channel life-times are exponentially distributed, the channel opening rate can be predicted analytically, given knowledge of the single channel open-life-time and current amplitude. The opening rate, n(t), was computed using the equation (Cohen et al. 1981; Brain Res.): $n(t) = (dI/dt + I/\tau)/i$, where i and τ are the single channel current and open-life-time respectively.

Predictions of n(t) during voltage clamp pulses shows that Na channel inactivation arises from an exponential decline in opening rate. Contrasting with the previous observation, Na currents pharmacologically modified so they do not inactivate show a predicted sustained channel opening rate.

Large depolarizing voltage clamp pulses produce channel opening rate functions that resemble gating currents. Comparisons of channel opening rate functions with gating currents suggests that: 1. most charge movement occurs prior to channel opening, and 2. there may be subtypes of Na channels that contribute more charge movement per channel opening than others.

The opening rate predictions suggest that Na channels open on average only once during the transient period of channel opening; however Na channels can reopen during long voltage pulses and contribute to steady-state currents. The convolution predictions necessarily will overesti-mate the opening rate of channels contributing to the steady-state currents.

Tu-Pos165 **VOLTAGE-GATED NA^+ AND K^+ CHANNELS FROM THE AXOPLASM OF *LOLIGO PEALEI***

W.F. Wonderlin & R.J. French, Department of Medical Physiology, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

We have addressed the question of whether voltage-gated Na^+ and K^+ channels similar to channels observed in axolemma can be obtained from axoplasmic organelles. Giant axons were removed from *Loligo pealei*, cleaned of surrounding connective tissue and the axoplasm extruded using the roller method. Organelles were released from the cytoskeleton by gentle trituration of the axoplasm in 0.6 M K^+-iodide, which was then centrifuged at low speed (5' @ 1300xg). The supernatant was examined at 2000x using video-enhanced microscopy and found to consist predominately of small vesicles less than ~0.5 um in size. The supernatnant was pressure-applied to a painted lipid bilayer (80:20 phosphatidylethanolamine: phosphatidylcholine in decane) bathed by either 200 mM NaAc, KAc or CaAc. Single-channel recordings revealed several classes of ion channels. In the presence of batrachotoxin (BTX), a voltage-gated, TTX-sensitive Na^+ channel was observed. The i-E relationship of the Na^+ channels was linear, with an average slope conductance of ~18 pS. Under biionic conditions, the channels were highly selective for Na^+ over K^+. P_{open} was greater than 90% at positive potentials but decreased with hyperpolarization beyond ~-50 mV, with a $P_{0.5}$ between -75 and -90 mV. These Na^+ channels are very similar to BTX-modified, TTX-sensitive Na channels incorporated from axolemmal vesicles prepared from squid optic nerve (Latorre et al, *Biophys. J.*, 51:195a; our unpublished observations). Two classes of K^+ channel were also observed, with conductances of 15-23 and 50-60 pS, respectively. Both classes of K^+ channels exhibited rapid, voltage-dependent gating; P_{open} was generally highest between 0 and +-50 mV and decreased with further polarization. These K^+ channels are qualitatively similar to single K^+ channels in the axolemma of the giant axon (Llano et al, *J. Gen. Physiol.*, 92:179-196, 1988). Thus, voltage-gated Na^+ and K^+ channels exist in axoplasmic organelles of the squid giant axon and exhibit permeation and gating properties very similar to channels previously studied in squid axolemma.

This work was supported by the Grass Foundation, the Alberta Heritage Foundation for Medical Research and the Medical Research Council of Canada.

Tu-Pos166 SURFACE CHARGE EFFECTS ON GATING OF SKELETAL MUSCLE SODIUM CHANNELS INCORPORATED INTO PLANAR LIPID BILAYERS. Robert J. French, Bernard A. Pailthorpe* and Murray C. Lee. Department of Medical Physiology, University of Calgary, Alberta, Canada T2N 4N1, and *Department of Applied Physics, University of Sydney, N.S.W. 2206, Australia.

We have examined the influence of surface charge on the gating of batrachotoxin (BTX)-activated single sodium channels from rat skeletal muscle incorporated into neutral lipid bilayers formed from phosphatidylethanolamine and phosphatidylcholine (PE:PC, 80:20) in decane. Open probability, P_o, was determined as a function of voltage, E, from steady-state single channel records. When the channels were bathed in symmetric 200 mM NaCl the mid-point ($E_{0.5}$) of the activation curves (P_o vs. E) was at -90 to -100 mV. When channels were incorporated in the presence of a NaCl gradient (200/20 mM, extracellular/intracellular) and the gradient of ionic strength was subsequently removed by addition of either NaCl or N-methyl-glucamine-HCl the activation curve shifted in the negative direction. The observed shifts indicate an effective negative surface charge density of < 1e/1000 Angstrom^2 adjacent to the channel's voltage sensor, based on Gouy-Chapman theory. Addition of divalent cations (10-20 mM Ca or dimethonium - McLaughlin et. al., J. Membrane Biol. 76:83) to the 'extracellular' solution produced a positive shift in $E_{0.5}$. Subsequent addition of an equal concentration to the 'intracellular' solution caused smaller negative shifts. In contrast to Ca, dimethonium induced no detectable reduction of the unitary currents. Dimethonium may thus be a useful probe in further studies of the effects of surface charge on channel function.
Supported by the Alberta Heritage Foundation for Medical Research and the Medical Research Council of Canada.

Tu-Pos167 RAT BRAIN TYPE IIa SODIUM CHANNEL α-SUBUNITS EXPRESSED IN CHINESE HAMSTER OVARY (CHO) CELLS. *T. Scheuer, +S. Boyd, +V.J. Auld, +R.J. Dunn & *W.A. Catterall. *Dept. of Pharm, U. Wash, Seattle WA, +Dept. Medical Genetics, U. Toronto, Toronto, Ont.

A clone for the α subunit of the rat brain type IIa[1] Na channel has been expressed in CHO cells. This clone differs at 6 amino acid positions from the rat brain type II Na channel[2] and has been previously expressed in *Xenopus* oocytes[1]. CHO cells were stably co-transfected with pECE which contained a SV40 early promoter and the Na channel clone and pSV2-NEO. Cells were grown in RPMI containing G418. Na channel expression was assessed by whole cell voltage clamp. The solutions used were (in mM): External: 150 NaCl, 5 KCl, 1.5 $CaCl_2$, 1 $MgCl_2$, 5 glucose, 5 NaHEPES, pH 7.4. Internal: 90 CsAspartate, 60 CsCl, 5 NaCl, 20 MgATP, 5 NaEGTA, pH 7.4. These cells expressed a transient inward current in the voltage range from -20 to +60 mV in 38 out of 47 cells tested. This current was TTX-sensitive (200 nM). Cells transfected with the vector without the Na channel clone expressed no detectable inward current (5 of 5 cells). Mean inward current at the peak of the current-voltage (I-V) curve was 148 pA, n=27. I-V curves for I_{Na} peaked between +10 and +20 mV, ~20 mV more positive than peaks of I_{Na} I-V curves in rat brain cells under similar recording conditions or in rat brain polyA mRNA-injected *Xenopus* oocytes[1]. This confirms the finding of a similarly altered voltage-dependence after expression of this clone in oocytes[1]. In contrast to rat IIa expression in oocytes, inactivation was rapid in these cells (τ= 400 μs at +10 mV, 22°). Five distinct toxin binding sites have been defined on the Na channel molecule. Toxins acting at three of these sites, TTX, veratridine, and α-scorpion toxin from *Leiurus quinquestriatus*, each had effects on I_{Na} that were consistent with their effects on currents mediated by native Na channels. Thus, the primary receptor sites for these 3 toxin types must be on the α subunit. [1]Auld et.al. *Neuron* **1**:449-461, 1988. [2]Noda et. al.*Nature* **320**:188-192, 1986.

Tu-Pos168 **A MODEL OF THE SODIUM CHANNEL: MACROSCOPIC AND SINGLE CHANNEL BEHAVIOR.** **PB Bennett, JR Balser and LM Hondeghem. Vanderbilt University, Nashville TN 37232.**

The Hodgkin-Huxley model reasonably reproduces macroscopic sodium currents but does not faithfully reproduce single channel activity. Published models of single channel behavior accurately reproduce single channel activity but do not correctly reproduce macroscopic currents over a wide range of membrane potentials. We therefore formulated the simplest possible model that could reproduce both. The derived amino acid sequence of the sodium channel indicates that there exist four homologous domains, therefore we assumed four activation subunits. We further assumed a single inactivation subunit that was coupled to only one of the activation subunits: $D_i \Leftrightarrow A_i$ where i=1, 2 or 3, and $D_4 \Leftrightarrow A_4 \Leftrightarrow I$. The transitions between D and A are governed by two voltage-dependent rate constants: k_{AD} and k_{DA}, those between A_4 and I are controlled by k_{IA} and k_{AI}. When all four subunits are activated, the channel is open. This model could accurately fit (global fitting procedure) macroscopic sodium conductance from neonatal mouse cardiocytes obtained at 15°C (largest deviation < 1%). A Monte Carlo simulation of 50,000 channels using the best fitting rate constants **predicted** single channel behavior in concordance with that observed experimentally: time to first opening shortens with depolarization, null sweeps become less likely with depolarization but never approach a probability of zero. Reopenings were maximal (mean ≈ 3) near -25 mV, but reopenings were rare at potentials more negative than -50 mV and more positive than 0 mV. Mean open time peaked near -25 mV, was weakly voltage-dependent over a 25 mV range, but shortened quickly at more positive or more negative potentials. If the modulated receptor for sodium channel blockers is on the activation-inactivation gating pair (A,I), then activation from the standpoint of drug binding has one open and three closed states. Consequently, activation associated use-dependent block and unblock without opening of channels are predicted. Both these predictions were verified by experimental test. We conclude that this simple model which has only 4 rate constants and is compatible with known protein structure, can accurately reproduce macroscopic and single channel sodium currents, as well as provide testable predictions about drug action.

Tu-Pos169 **CLONING OF A cDNA FOR A RAT CARDIAC SODIUM CHANNEL ISOFORM.** R.B. Rogart, L. Cribbs, L. Muglia, D. Kephart, M. Kaiser. Dept. of Medicine/Cardiology & Cell Physiology Committee, University of Chicago, Chicago, IL 60637

Voltage-gated Na^+ channels are the transmembrane proteins essential for mediating the upstroke of the cardiac action potential, and therefore play a prominent role in arrhythmogenesis and anti-arrhythmic drug action. Na^+ channels exist as multiple subtypes, distinguishable by their developmental and tissue distributions, as well as by their sensitivity to a variety of toxins. Na^+ channels can be divided into two major subgroups, TTX-sensitive (TTX-S) and TTX-resistant (TTX-R). In rat brain, three TTX-S Na^+ channel isoforms are encoded by a closely related multigene family. However, the molecular identity of other mammalian Na^+ channel subtypes remains to be elucidated. Mammalian heart expresses a TTX-R Na^+ channel isoform with an affinity for TTX which is 1000-fold less than for the TTX-S brain isoforms. We characterized the hybridization of brain and heart mRNA with a cRNA probe (Goldin, et. al., PNAS, 1986) coding for the rat brain II Na^+ channel. Northern blots showed hybridization bands at 9-kb which were of 32-fold lower intensity for RNA from rat heart. We employed an RNA hybrid-melting technique to detect various mRNAs which are homologous with the rat brain cRNA probe. These studies showed two major Na^+ channel mRNA species in rat brain with about 84% homology, as expected from cloning studies. A further distinct Na^+ channel mRNA species with about 81% homology was found in rat heart. These studies demonstrated that the major Na^+ channel isoform in rat heart is encoded by a distinct gene species. We then screened a rat heart cDNA library with probes derived from the rat brain Na^+ channel sequences, and obtained several positive cDNA clones. Upon further characterization by Northern blots, we have identified clones which hybridized selectively to a 9-kb mRNA species in cardiac mRNA, as compared to brain and skeletal muscle mRNA. One clone, 4-23, is 4-kb in length, and encodes about 70% of the protein coding region of the Na^+ channel.

Tu-Pos170 CHARACTERIZATION OF cDNA CLONES ENCODING A SECOND SODIUM CHANNEL SUBTYPE IN RAT SKELETAL MUSCLE. R.G. Kallen, Z. Sheng, L.Q. Chen, and R.L. Barchi. Mahoney Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, PA 19104.

Electrophysiological, biochemical, and immunological data suggest the presence of multiple subtypes of voltage-dependent sodium channels in rat skeletal muscle. As part of a collaborative effort, we previously reported the full-length sequence of one rat skeletal muscle sodium channel (RSMSC-I) (Trimmer et al., Neurosci. Abs. 14, 1988). We now report the partial sequencing of a second channel subtype from rat muscle. Initially using a probe (RB-211) provided by G. Mandel (Tufts-NEMC) derived from the rat brain channel, multiple clones were isolated from a denervated rat muscle cDNA library. One was found to differ from others that were used to determine the RSMSC-I sequence. Probes derived from this clone were used to screen an innervated rat muscle cDNA library, generating additional RSMSC-I and non-RSMSC-I clones. From among the non-RSMSC-I clones, a cDNA clone containing 3.2 kb of contiguous sequence located within the coding region for a second sodium channel has been isolated (RSMSC-II) and the corresponding amino acid sequence deduced. In general, RSMSC-II is highly homologous to RSMSC-I in regions corresponding to the putative internal repeat domains but shows considerable divergence from RSMSC-I and from the brain and eel channels in the interdomain regions. The region between domains III and IV is the exception, with 98% homology among RSMSC-I, -II and rat brain I-III. Specific probes have been generated to each subtype and data from Northern blot analyses with these probes will be presented. (supported by NIH grant NS-08075 and a grant from the Muscular Dystrophy Association.)

Tu-Pos171 ESCHERICHIA COLI K-12 WILD TYPE OMPC PORIN DIMER ISOLATION AND PRELIMINARY CHARACTERIZATION. Warren J. Rocque and Estelle J. McGroarty, Dept. of Biochemistry, Michigan State University, E. Lansing, MI 48824.

Escherichia coli K-12 strain PLB3255 produces an OmpF protein with a 15 amino acid deletion in the primary sequence. The mutation appears to increase the porin channel size since the PLB3255 cells can grow on maltodextrins in the absence of a functional maltoporin. Porins were isolated from strain PLB3255 and separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Approximately 50-60% of the isolated porin contained trimeric aggregates and 35-40% was a 50 KDa "dimer." When the porin isolate was heated at 100°C and separated on SDS gels with 8 M urea, both the trimer and "dimer" migrated as a single band at 36 KDa corresponding in mobility to wild-type OmpC porin. The "dimer" was less heat stable and denatured at 66°C compared to 81°C for the trimer. The aggregates, suspended in 30% SDS, were separated on a Sephadex G-200 gel filtration column eluted with 0.5% sodium deoxycholate. Two peaks were recovered containing purified trimers and "dimers" as determined by SDS-PAGE. Circular dichroism spectra of the trimer and "dimer" were similar in shape and indicated similar β-structure content. Electrical conductance across planar bilayer lipid membranes and liposome swelling assays showed the two aggregates had similar channel-forming activity. OmpC porin "dimers" were also found in four other ompF deletion mutants of the same phenotype. However, an ompF point mutant and an ompC deletion mutant showed no dimer formation. We believe that specific changes in the OmpF porin may alter the assembly of the OmpC porin. In addition, the "dimer" is a stable porin configuration that may represent the smallest functional channel forming unit of the OmpC protein.

Tu-Pos172 ELECTROPHYSIOLOGY OF PURIFIED CHLORIDE CHANNELS IN PLANAR BILAYERS.

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Chloride channels are essential to the normal functioning of many epithelial transport processes. Defects in the regulation of these channels results in the disease cystic fibrosis. To purify these channels, a family of compounds, the indanyloxyacetic acids (IAA), were identified as high affinity inhibitors of electrogenic chloride fluxes in vesicles. An affinity column was synthesized by linking an IAA to an activated Sepharose matrix. Bovine kidney cortex membrane vesicles were solubilized with N-octylglucoside and bound to the affinity column. The column was washed to remove nonspecifically bound proteins. The specifically bound proteins are eluted with the potent IAA-94. Silver stained SDS gels reveal three bands at 97, 64, and 45 kD. The specifically eluted proteins have been reconstituted into phospholipid vesicles by a detergent dialysis procedure and then incorporated into planar lipid bilayers via vesicle fusion. Two types of chloride channels are observed. One is a small conductance (26 pS in 150 mM KCl), ideally Cl^- selective channel with slight voltage dependence to its open probability. The second channel is a large conductance (~400 pS in 150 mM KCl) channel. The channel has 5:1 Cl^-/K^+ selectivity and displays multiple subconductance states with horrendous transition kinetics between the substates. Neither of these channels is blocked by IAA or stilbene derivatives.

Tu-Pos173 RECONSTITUTION OF ADENOSINE DEAMINASE COMPLEXING PROTEIN IN LIPOSOMES: INTERACTION WITH SMALL SUBUNIT ADENOSINE DEAMINASE.

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Adenosine deaminase (ADA) has a polymorphic quarternary structure. It can be present as a small subunit ADA (SS-ADA), 45,000 D or as large membranal form when it is bound to a complexing protein (ADCP), 200,000 D, whose physiological function is not yet understood. The interconversion between membrane bound and soluble forms might be involved in regulation of ADA activity. We report the first attempt of reconstitution of the membranal ADCP in liposomes and the study of its interaction with the catalytic small subunit. ADCP was isolated from cow kidney. Liposomes were prepared from asolectin soybean lipids and different phosphatidyl cholines by bath or tip sonication. Dynamic light scattering techniques were applied to determine liposomes size distribution and stability. ADCP reconstitution was carried out by ultracentrifugation. Reconstitution yield ranged from 40-85%. Reconstituted ADCP retained its ability to bind SS-ADA. Binding depended only on the amount of ADCP in the liposomes. Specific activity of ADCP in asolectin liposomes seemed to fit with the formation of a 2 ti 1 form (2 SS-ADA to 1 ADCP) which fully retained the activity of ADA in aqueous neutral solution. SS-ADA non-specific binding to liposomes in the absence of ADCP occured to a different extent but always an abrupt decrease of the enzymatic specific activity was detected. Preliminary experiments have been carried out on liposomes enriched with cholesterol the presence of which seemed to slightly increase the specific activity of ADCP in DMPC vesicles. These observations were also supported by experiments with fluoresceine isothiocyanate labeled ADA following the fluorescence of the fluorophore at 520 nm.

Tu-Pos174 INSERTION OF BACTERIORHODOPSIN INTO POLYMERIZED DIACETYLENIC PHOSPHATIDYLCHOLINE BILAYERS. Patrick L. Ahl, Alok Singh, Ronald Price, John Smuda, and Bruce P. Gaber. Bio/Molecular Engineering Branch, Code 6190, Naval Research Laboratory, Washington, DC 20375.

We have developed a method to incorporate the membrane protein bacteriorhodopsin (bR) into polymerized bilayers composed of a diacetylenic lecithin (1,2-bis(10,12-tricosa-10,12-diynoyl)-sn-glycero-3-phosphocholine (DC8,9PC)), and a non-polymerizable short acyl chain length spacer lipid (dinonanoyl phosphatidylcholine (DNPC)). Polymerized bilayers were formed by UV irradiation at 4 C. The protein was inserted into the membranes after UV polymerization to avoid inactivation due to UV radiation. The extent of polymerization was estimated by the absorbance increase due to long chain length polydiacetylene. The visible absorbance of polymerized bilayers composed of DC8,9PC and DNPC (1:2 mole ratio) was several fold greater than that of pure DC8,9PC bilayers indicating the membranes with DNPC had more long chain length polymer. Octyl-glucopyranoside (OG) solubilized bR was inserted into the polymerized bilayers by overnight incubation at 4 C (lipid/protein 2.5:1 wt/wt, 30 mM OG) followed by dialysis. Insertion of bR into the polymerized DC8,9PC/DNPC membranes was confirmed by density gradient centrifugation, UV/visible spectroscopy, and freeze fracture microscopy. The polymerized membranes were 10 to 20% protein by weight. The insertion of bR was enhanced by DNPC. (Supported by an NRL ARI.)

Tu-Pos175 A VOLTAGE DEPENDENT ANIONIC CHANNEL FROM THE SARCOPLASMIC RETICULUM OF Bufo marinus RECONSTITUTED IN PLANAR LIPID BILAYERS. Laura Larralde and Enrico Nasi, Dept. of Physiology, Universidad Central de Venezuela, Caracas, Venezuela and Dept. of Physiology, Boston University School of Medicine, Boston, MA.

The properties of an anionic channel from native sarcoplasmic reticulum membrane vesicles fused with lipid bilayers were investigated. Microsomal fractions obtained from skeletal muscles were isolated by differential centrifugation, using a protocol that allows separation of t-tubules, intermediate SR membranes, and heavy SR membranes. Planar lipid bilayers with a capacitance of 200-300 pF were painted across a 250-300 μm orifice, using a 7:1 mixture of POPE and POPS dissolved in n-decane. The observed channel has a conductance of approximately 140 pS in 200 mM symmetrical Cl^-. Several other anions were shown to permeate, according to the sequence: $Cl > Acetate > PO_4 \gg$ Gluconate. A small permeability to cations was also detected. Millimolar levels of Mg^{2+} cis cause a voltage-dependent block of the channel. The gating behavior exhibits a pronounced dependence on voltage: near 0 mV the channel is open most of the time, while at more positive voltages (cis) it spends progressively more time in the closed state. Furthermore, a time- and voltage-dependent inactivation process was observed: after positive voltage steps gating activity is typically followed by a long quiescent period. The duration of the burst is inversely related to the magnitude of the voltage step. Dwell-time distributions indicate the presence of only one closed state and one fully open state during bursts. A subconductance state, however, was often seen. Ca^{2+} is apparently not required for channel activity.

Tu-Pos176 REGULATION OF PERMEABILITY OF RECONSTITUTED LENS FIBER CELL MIP26. Beth A. Scaglione, Robert A. Uphaus*, & David A. Rintoul. (Intr. by Ruth Welti). Division of Biology, Kansas State University, Manhattan KS 66506 and *Department of Chemistry, University of Nebraska, Lincoln NE 68588.

A novel fluorescence quenching assay has been developed to study permeability of liposomes containing reconstituted proteins from the bovine and human lens fiber cell membrane. This assay has allowed us to identify the Major Intrinsic Protein (MIP26) as the protein responsible for permeability of the reconstituted liposomes. Permeability of reconstituted liposomes containing lens fiber cell membrane proteins was shown to be altered by changes in the pH of the assay buffer. Similar regulation of permeability by pH was noted in liposomes containing electrophoretically purified, homogeneous MIP26, indicating that the MIP26 protein itself could be gated by pH. Permeability of liposomes containing purified bovine MIP26 was maximal at pH 7.0. MIP26 from bovine lenses was also extracted into chloroform/methanol, using the method of Broekhuyse *et al.*, *Experimental Eye Research* (1976) *23*, 365-371); this material was capable of forming a stable monolayer in a Langmuir-Blodgett apparatus. The apparent molecular area of the MIP26, as measured by surface/area isotherms, also varied with pH. The molecular area of the protein was maximal at pH 7.0. These observations indicate that permeability of lens fiber cell junctions can be regulated by pH, that this regulatory ability resides in the MIP26 molecule itself, and that Langmuir-Blodgett monolayer techniques will be very useful in future experiments aimed at elucidation of the molecular dynamics of this channel-forming protein.

Supported by grants from the NEI (R01-EY06493) and the NCI (T32-CA09418).

Tu-Pos177 ROLE OF CARBOHYDRATE IN THE BILAYER STABILIZATION ACTIVITY OF GLYCOPHORIN A IN DIOLEOYL PHOSPHATIDYLETHANOLAMINE LIPOSOMES. Purnima Pinnaduwage and Leaf Huang, Department of Biochemistry, University of Tennessee, Knoxville, TN 37996-0840.

The importance of carbohydrates on the stabilization of dioleoyl phosphatidylethanolamine (DOPE) bilayers has been investigated using glycophorin A, the major sialoglycoprotein of the human erythrocyte membrane, as a stabilizer. Two major types of glycophorin, differing in the sialic acid content, were used in the study. Type MM contains 19.2 ± 2.5 sialic residues per molecule of glycophorin and type NN contains 10.8 ± 1.2. Type MM could stabilize DOPE bilayer at 0.5 mole % whereas type NN was unable to do so even at 1 mole %. The importance of the sialic acid content in the stabilization activity of glycophorin was further confirmed by the observation that the neuraminidase-treated type MM shows a lower stabilization activity than the untreated parent. Since the type NN had no stabilizing effect, we have attempted to couple a trisaccharide, NeuNAc→Gal→Glc, to type NN by reductive amination. 2.5 ± 0.8 saccharide chains were added per molecule of type NN. The trisaccharide attached type NN showed a greater stabilization activity compared to that of the parent type NN molecule, indicating again that the sialic acid content of the stabilizer molecule determines the stabilization activity. Addition of wheat germ agglutinin (WGA), which binds to the sialic acid residues of a glycoprotein, to type MM stabilized DOPE liposomes caused a rapid aggregation of liposomes resulting in leakage of an entrapped marker, calcein. The aggregation increased with increasing amount of the lectin, however, the leakage rate was maximum at an optimum concentration of WGA. These results are discussed in terms of the contact-induced bilayer destabilization. Supported by a contract with LipoGen, Inc.

Tu-Pos178 CRYO-TRANSMISSION ELECTRON MICROSCOPIC ELUCIDATION OF THE EGG PHOSPHATIDYLCHOLINE-OCTYLGLUCOSIDE VESICLE TO MICELLE TRANSITION. P.K. Vinson[1], Y. Talmon[2], and A. Walter[3], [1]Dept. of Chem. Eng. & Mat. Sci., Univ. of MN, Minneapolis, MN, [2]Dept. of Chem. Eng., Technion, Haifa, Israel, [3]Dept. of Physiol. and Biophys., Wright State Univ., Dayton, OH.

Dynamic, stable nonequilibrium, and equilibrium structures formed during the vesicle to micelle transition in the egg phosphatidylcholine (PC) -octylglucoside (OG) system have been directly imaged with Cryo-Transmission Electron Microscopy (CTEM) at high resolution (~2 nm). PC vesicles were prepared by dialysis from excess OG; some preparations contained headgroup labelled fluorescent lipids [M. Ollivon et al., Biochem. 27:1695, 1988]. Fluorescence intensity and optical density (OD) were monitored as PC vesicles were diluted with OG and a series of PC-OG samples at discrete concentrations taken for CTEM analysis. Sample films (<0.5 micron thick) for CTEM were prepared, in a temperature and humidity controlled chamber (25°C, 95-99% RH), which were plunge-vitrified in liquid ethane. The vitrified-hydrated specimens were viewed at T < 108 K. Thermal fixation from a controlled environment maintains sample composition and temperature prior to vitrification, thereby preventing artifacts associated with fixation processes. Several structural changes were imaged as the [OG] increased. First, the average vesicle size decreased with no apparent change in the OD. Then, open vesicles, and long cylindrical micelles were observed with an associated rise in OD. At higher [OG], cylindrical and spherical micelles were observed with a decrease in OD and changes in the fluorescence. At high [OG], spherical micelles are the dominant structure. Thus, the transition is a structural progression from a) vesicles, to b) smaller vesicles, to c) open vesicles coexisting with cylindrical mixed micelles, to d) cylindrical and spherical mixed micelles, to e) spherical mixed micelles. (Supported by AHA, DOE and BSF)

Tu-Pos179 SOLUBILITY PROPERTIES OF THE ACYL METHYLGLUCAMIDE DETERGENTS. S.E. Suchy, P.K. Vinson and A. Walter (Intr. by K. Strange), Dept. Physiol. Biophys., Wright State Univ., Dayton, OH and Dept. Chem. Engin. & Mat. Sci., Univ. MN, Minneapolis, MN.

Detergent critical micelle concentrations (cmc) and their ability to solubilize and form vesicles from phosphatidylcholine (PC) are important criteria for reconstitution protocols. The cmc and its temperature dependence were determined for an homologous series of acyl methylglucamides (MEGA-8, -9, -10). Each detergent was added continuously from a concentrated solution to a saline buffer with the fluorescent probe ANS held in a thermojacketed cuvette; ANS fluorescence increases at the cmc. The cmc's at 25 C were 47.6, 15.6 and 4.8 mM for MEGA-8, -9, and -10; the ΔG per -CH2- was -677 cal/mol, similar to other acyl series. The cmc's decreased slightly with increasing temperature (T=5 to 40°C) for MEGA-9 and -10 while that of MEGA-8 was virtually insensitive to temperature in this range. MEGA-9 solubilization of egg PC in aqueous solutions was determined as a function of [PC] and temperature. The lamellar-micellar phase boundaries were determined by simultaneous 90° light scattering and the resonance energy transfer using the headgroup labeled lipid probes NBD-PE and Rho-PE (Ollivon et al 1988 Biochemistry, 27:1695). The solubilization was linear with [PC]; the MEGA-9 to egg PC ratio in the structures at optical clarity was 2.3 while the monomeric [MEGA-9] was 14.3 mM or slightly lower than the cmc at 25°C, analogous to the octyl glucoside and egg PC system. In contrast, vesicles formed from MEGA-9 tended to be multilamellar. Unlike the cmc of MEGA-9, solubilization of egg PC by this detergent was very temperature dependent. Thus, the cmc alone is not a good indicator of how detergent will behave during a reconstitution protocol. (Supported in part by the American Heart Association Ohio Affiliate)

Tu-Pos180 "TIGHT" INSERTION OF CYTOCHROME B5 INTO LARGE UNILAMELLAR LIPOSOMES. Susan F. Greenhut and Mark A. Roseman, Dept. Biochemistry, Uniformed Services Univ., Bethesda, MD 20814.

Cyt b5 spontaneously binds to liposomes in a "loose", or transferable form, whereas in vivo it binds posttranslationally to the ER in the "tight" or nontransferable form. The mechanism of tight insertion is unknown, except that it does not require SRP or energy input. We have found that prolonged incubation of b5 with LUVs of POPC, DOPC, or DOPC/DMPC results in slow conversion of the loose to tight form, with a half-time of days. However, the process is complex. When the b5-LUVs are incubated for 96hr at 30° then depleted of loose b5 (by transfer to an excess of SUVs, followed by sedimentation in a glycerol gradient), the tight b5 is found to be concentrated in about 10% of the LUVs. If the recovered LUVs devoid of tight b5 are reincubated with fresh b5, the same phenomenon occurs. All attempts to isolate or identify a pre-existing "active" subpopulation of LUVs in the original LUV preparation have failed. Prolonged preincubation of the vesicles in the absence of b5 does not produce a population of vesicles that rapidly incorporates the b5 in the tight configuration. Apparently, a new population of vesicles (containing tight b5) is generated only during the prolonged incubation with the protein. The rate of tight insertion is independent of initial b5/lipid ratio or absolute lipid concentration. The b5-enriched fraction contains about the same level of trapped sucrose as does the original vesicle preparation, indicating that the process does not involve total breakdown of vesicle integrity. When fresh b5 is added to these tight-b5/LUV vesicles, all the freshly added protein rapidly inserts (< 2hr) in the tight configuration. This does not happen with tight-b5/LUVs that are prepared de novo by detergent-dialysis. Apparently, the newly formed population is "insertion-active". (Supported by NIH grant DK30432).

Tu-Pos181 AGGREGATION OF HAPTEN-BEARING LIPOSOMES BY ANTIBODIES: AN ELECTRON-MICROSCOPY STUDY OF KINETICS AND AGGREGATE MORPHOLOGY. Lee, Kyung-Dall, and Owicki, John C., Dept. of Biophysics and Medical Physics, Univ. of California, and Cell and Molecular Biology Division, Lawrence Berkeley Laboratory, Berkeley, CA 94720.

The aggregation of hapten-bearing liposomes by monoclonal antibody (MAb) was studied by freeze-fracture electron microscopy of samples frozen throughout the course of the aggregation. Rapid freezing was achieved with a double propane-jet apparatus. By this method the time evolution of the morphology and size distribution of aggregates was obtained. Liposomes were made of dipalmitoyl-phosphatidylcholine: cholesterol (2:1) by multiple extrusion through polycarbonate filters and had a mean diameter of 58 nm (11 nm S.D.). The fluorescein hapten (<0.2 mole % of lipid) was attached to dipalmitoyl phosphatidylethanolamine by a triglycine spacer as described previously (Petrossian *et al.* (1985) J. Lipid Res. **26**:767-773). Anti-fluorescein MAb's were a gift from E. Voss, Jr. The binding of hapten by MAb, monitored by the quenching of hapten fluorescence, was rapid and substantially plateaued prior to appreciable aggregation. Aggregate size distributions for oligomers were obtained from the two-dimensional fracture views by a stereological correction. The aggregation data were fit well by a Smoluchowski model with vesicle association rates proportional to the products of numbers of available haptens and antibodies on reacting vesicles, and dissociation rates small for aggregates larger than dimers. Association and dissociation rates were small compared with the corresponding rates for antibodies and haptens in solution, but the equilibrium constants (ratios of rate constants) were more similar in the two cases. Most aggregates were compact rather than highly ramified. Specific (antibody-mediated) and non-specific (colloidal forces) aggregation could be distinguished morphologically. Auxiliary turbidimetric measurements showed that aggregation rates increased with the number of haptens and antibodies per vesicle, but no sharp threshold behavior was observed. Supported by NIH Grant AI-22860 to JCO.

Tu-Pos182 PHOTOGATING OF ION CURRENTS ACROSS THE BILAYER MEMBRANE (BLM)
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The photoinduced interfacial charge separation between Mg-octaethylporphyrin in a BLM and various aqueous electron acceptors on both sides of the membrane places positive charge in the BLM on the nanosecond time scale. This charging of the BLM increases the ionic current of negative, and decreases that of positive, hydrophobic ions. At low concentrations of the mobile, hydrophobic ion, a 30% change in conductivity is observed, which decreases with increasing concentration of positive tetraphenylphosphonium ($TPhP^+$), and increases drastically with increasing concentration of negative tetraphenylboride ($TPhB^-$). In the region of saturated $TPhB^-$ conductance, the increase in conductivity due to the photogenerated charge in the BLM is 360% (10^{-4}M $TPhB^-$). In this case, the net charge gated is 300 times greater than the photogenerated charge in the bilayer membrane, thus there is a net gain in the "bilayer photodiode". The photogating is readily explained as space charge effects in the BLM, and can be accounted for by a simple implicit equation for hydrophobic ion binding and electrostatics. We conclude that the hydrophobic ion conductance in BLM is diffusive and largely space charge limited. This research is supported by PHS grant GM25693.

Tu-Pos183 DIRECT OBSERVATION OF ETHANOL BINDING TO PHOSPHOLIPID BILAYERS: A ^2H NMR STUDY.
Joao Figueirinhas* and Philip W. Westerman*#, *Dept. of Biochemistry, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272, and #Dept. of Physics and Liquid Crystal Institute, Kent State University, Kent, Ohio 44242.

The interaction of ethanol with DMPC/H_2O bilayers in the L_α phase has been studied by ^2H NMR. Orientational order has been measured at many sites in all three components. The most sensitive sites in DMPC to solvent-induced changes in order are the choline and glycerol backbone segments. Water ordering increases with the addition of EtOH to DMPC bilayers. Both of these observations are supportive of interactions between the added solute and the polar headgroup of DMPC. The ordering of ^2H-labelled EtOH was also measured. At DMPC/H_2O = 1/12, one population of CD_3CHDOH is observed and its Saupe order tensor, as determined using quadrupole splittings ($\Delta\nu$) and H-D dipolar couplings was calculated to be, at 40°, S_{zz} = 0.126 or 0.109 and S_{xx}-S_{yy} = -0.005 or -0.021. At higher water contents, two spectral populations of EtOH are evident; one isotropic component in which the different chemical shifts of the EtOH are resolved, and a second component which is ordered. The isotropic signals are broader than in liquid EtOH supporting the view that this population is associated with the lipid polar headgroup yet in rapid exchange with aqueous EtOH. The ordered component arises from EtOH which is intercalated in the bilayer but in slow exchange with aqueous EtOH. The ordering of this component as a function of temperature and water content has been examined. (Supported in part by Project #87470 from JNICT (Portugal), and NSF Grant #85/03219, and an Research Challenge Grant from the Ohio Board of Regents.)

Tu-Pos184 MAXIMAL ORDERING AND CUT-OFF IN ANESTHETIC POTENCY OCCUR AT SAME CHAIN-LENGTH IN n-ALKANOLS.
Philip W. Westerman* and James M. Pope#, *Department of Biochemistry, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272 and #School of Physics, The University of New South Wales, Kensington, N.S.W., Australia.

The interaction of seven n-alkanols, with bilayers of egg yolk lecithin containing varying amounts of cholesterol, has been studied by deuterium nuclear magnetic resonance (^2H-NMR). At 37°C and comparable concentrations of solute in the bilayer (lipid/n-alkanol = 5/1) order parameters measured at the 1-methylene segment of the n-alkanols show a well-defined maximum for a chain length of 12, for bilayers containing equimolar amounts of EYL and cholesterol. For bilayers containing lesser amounts of cholesterol, the maximal ordering at this same site is poorly defined and tends to be associated with n-alkanols of longer chain length. The maximal ordering associated with n-dodecanol suggests that it is the n-alkanol, optimally accommodated in 1:1 EYL: cholesterol bilayers. The ordering maximum occurs at the same chain length where a "cut-off" in anesthetic potency in the n-alkanol homologous series occurs. The implications of our observations in the context of lipid-based unitary theories of general anesthesia are discussed. (Supported in part by an Academic Challenge Research Grant from the Ohio Board of Regents and the Australian Research Grants Scheme.)

Tu-Pos185 **MOTIONAL DYNAMICS OF STEARIC ACID SPIN LABELS AND LIPID-PROTEIN INTERACTIONS IN BOVINE ROD OUTER SEGMENT MEMBRANES AS STUDIED BY ELDOR AND SATURATION RECOVERY EPR.** Jimmy B. Feix,[1] Cherie Hubbell,[2] and Wayne L. Hubbell.[2] [1]National Biomedical ESR Center, Department of Radiology, Medical College of Wisconsin, Milwaukee WI 53226; [2]Jules Stein Eye Institute and Department of Chemistry & Biochemistry, University of California-Los Angeles, Los Angeles, CA 90024.

Electron-electron double resonance (ELDOR) and saturation recovery methods have been used to examine the interaction of ^{15}N stearic acid spin labels with ^{14}N stearic acid spin labels and with a ^{14}N spin probe covalently bound to rhodopsin in native bovine rod outher segment (ROS) membranes. Calculations based on the Heisenberg exchange rate (W_x) for bimolecular collisions between ^{14}N16doxyl stearate ($^{14}NC16$) and ^{15}N16doxylstearate ($^{15}NC16$) gave a lateral diffusion constant at 35°C of 1.8×10^{-8} cm^2/sec, in good agreement with previous determinations. Appreciable interaction of $^{14}NC16$ with ^{15}N-12doxylstearate and ^{15}N-5doxylstearate was also observed, indicating the occurrence of vertical fluctuation (i.e. motion of $^{14}NC16$ towards the membrane surface) in the ROS membrane.

In studies designed to examine the structure of rhodopsin in the bilayer, the protein was labeled with a mercurial spin label that bound selectively to a cysteine residue with a hydrophobic environment. Using ELDOR methodology, interaction could be demonstrated between the (^{14}N) protein-bound spin label and ^{15}N-16doxylstearate, indicating that the labeled cysteine residue is accessible to membrane phospholipid and suggesting a localization deep in the membrane bilayer. Utilization of these methods to obtain structural information on membrane proteins will be discussed.

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Tu-Pos186 SYNTHESIS AND CHARACTERIZATION OF N-PARINAROYL GANGLIOSIDE G_{M1}. Wenxia Song and David A. Rintoul, Biology Division and Biochemistry Department, Kansas State University, Manhattan KS 66506.

N-*cis* and N-*trans*-parinaroyl ganglioside G_{M1} were synthesized and characterized by HPLC, TLC, component analysis, absorbance spectroscopy, and proton NMR spectroscopy. Steady-state fluorescence anisotropy of the purified compounds, incorporated into phosphatidylcholine liposomes, was measured in the presence and absence of cholera toxin and choleragenoid. In gel-phase liposomes, anisotropy measurements indicated that the motion of the parinaroyl ganglioside was not affected by addition of cholera toxin or choleragenoid. In fluid-phase liposomes, however, addition of toxin resulted in increased anisotropy (decreased rotational motion) of the fluorescent gangliosides. This decreased motion was not observed with other parinaroyl lipid probes, such as phosphatidylcholine, glucosylceramide, or free fatty acids, indicating that the effect was due to specific ganglioside/toxin interactions. Varying the amount of ganglioside or the amount of toxin suggested that the effect of toxin on probe motion was saturable at approximately 1 toxin (or toxoid) molecule per 5 ganglioside molecules. These results are consistent with previous hypotheses regarding the ganglioside/cholera toxin interaction, and indicate that parinaroyl ganglioside probes will be useful in elucidation of the molecular details of this interaction.

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Tu-Pos187 **EFFECT OF PHOSPHATIDIC ACID ON MEMBRANE HETEROGENEITY**

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In biological membranes the structural organization and the physico-chemical properties of phospholipid matrix are involved in the control of membrane functions. The formation of compositional domains endowed with specific properties has been shown following transmembrane signalling and cellular activation. In this respect phosphatidic acid (PA) could be involved in calcium-mediated events. The effect of PA on membrane structural organization have been studied analyzing the fluorescence decay of 1,6-diphenyl-1,3,5 hexatriene (DPH) and 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH) by multifrequency phase fluorometry. In liposomes of dimyristoyl phosphatidylcholine the presence of PA, which forms intermolecular hydrogen bonds, decreases water penetration in the headgroup region and in the membrane hydrophobic core. Moreover PA induces an increase of structural heterogeneity. Both effects have different patterns in the gel and in the liquid-crystalline state. Similarly in intact cell, the formation of PA due to receptor mediated activation modifies the pattern of membrane heterogeneity.

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Tu-Pos188 MICROHETEROGENEITY IN ARTIFICIAL AND NATURAL MEMBRANES

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Fatty-acyl chain mobility and phase separation phoenomena can produce structural defects and domain segregation in phospholipid membranes. In biological membranes cholesterol and proteins could be further factors controlling the degree of membrane structural microheterogeneity.
To study the influence of cholesterol and proteins on microheterogeneity of the erythrocyte membranes we used steady-state fluorescence anisotropy and excited state decay of 1,6-diphenyl-1,3,5-hexatriene (DPH). DPH fluorescence decay was measured by multifrequency phase fluorometry and the data were analyzed using a continuous distribution of lifetime values. Cholesterol induces a decrease of the distribution width of lifetime values around the central value(10.27 ns). This homogenizing effect has been confirmed in liposomes containing a very low cholesterol concentration. In erythrocyte membranes the degree of protein aggregation influences DPH decay behaviour, indicating that the pattern of protein distribution in the membrane plane could be a factor involved in the formation of structural and compositional domains.
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Tu-Pos189 CALORIMETRIC STUDIES OF THE EFFECTS OF DEHYDROERGOSTEROL ON THE PHASE TRANSITION OF INTERDIGITATED PHOSPHOLIPIDS. Yvonne Kao and Parkson Lee-Gau Chong, Department of Biochemistry, Meharry Medical College, Nashville, TN 37208

The effect of dehydroergosterol (DHE) on the phase transition of multilamellar C(18):C(10) phosphatidylcholine (abbreviated as C(18):C(10)PC) vesicles was studied using differential scanning calorimetry. C(18):C(10)PC is an asymmetric lipid known to form highly ordered mixed interdigitated bilayers below the maximal transition temperature, T_m, and partially interdigitated bilayers above T_m. DHE is a fluorescent analog of cholesterol shown in previous studies to behave like cholesterol in non-interdigitated symmetric diacyl PC. In this study, DHE exhibited similar characteristics to cholesterol in another type of lipid membrane, i.e., interdigitated C(18):C(10)PC. DHE abolishes the phase transition of C(18):C(10)PC at 27 mol% compared to 25 mol% for cholesterol (Chong & Choate, 1989); and decreases T_m, the onset temperature, T_o, and the completion temperature, T_c, at a similar rate to cholesterol at about -0.24°/mol% DHE. DHE is believed to insert itself into the highly ordered interdigitated bilayers causing disruption and the eventual abolition of the phase transition in a mannar analogous to cholesterol. This study indicates that DHE can serve as a useful fluorescence probe to characterize the effect of cholesterol on interdigitated lipid bilayers. This study echoes previous observations that the phase transition between interdigitated lipid bilayers is much more sensitive to sterols. DHE abolishes the phase transition of non-interdigitated symmetric PC at around 42 mol% (Hale & Schroeder, 1982), a value much higher than 27 mol% for interdigitated C(18):C(10)PC observed in this study. Supported by NSF (R11-8714805), EI of AHA and CIBA-GEIGY.

Tu-Pos190 THE STRUCTURAL AND DYNAMIC PROPERTIES OF A PHOSPHOLIPID CONTAINING A DIUNSATURATED ACYL CHAIN. John E. Baenziger, Ian C.P. Smith, Robin J. Hill, and Harold C. Jarrell. Intr. by David J. Siminovitch. Department of Biochemistry, University of Ottawa; National Research Council of Canada, Ottawa, Canada.

Polyunsaturated lipids play a significant role in membrane function, yet little is known of their physico-chemical properties in membranes. To gain insight into their biological function, we have used the protozoan, Tetrahymena, to synthesize a series of isolinoleic acids ($18:2^{\Delta 6,9}$) deuteriated at ten positions along the chain. The specifically labeled fatty acids were esterified to form 1-palmitoyl-2-isolinoleoyl PC (PiLPC) and aqueuos dispersions were examined by ^2H NMR spectroscopy. Quadrupolar splittings and T_1-spin lattice relaxation times, measured as a function of temperature, indicate that in the plateau region, the average structural and dynamic properties of the diunsaturate differ significantly from saturated and monounsaturated chains. From proton-decoupled spectra of oriented bilayers of PiLPC labeled at the eight position, the dipolar coupling between the two C-8 geminal deuterons was also measured. The complete ordering matrix for this methylene sesgment and its average orientation were calculated and this had led us to propose a model describing the average conformation and types of motions of the diunsaturated chain. Comparison of this model to current models describing the motions of saturated and monounsaturated lipids should lead to a greater understanding of the role of polyunsaturated lipids in biological systems.

Tu-Pos191 DIPYRENYL PHOSPHOLIPID ANALOGS AS PROBES OF MEMBRANE DYNAMICS. M. Vauhkonen*, D.G. Perry, P. Somerharju**, M. Sassaroli and J. Eisinger. Dept. of Physiology and Biophysics, Mount Sinai School of Medicine, New York, NY 10029.
We have investigated the relationship between the lateral diffusivity of monopyrenyl phosphatidylcholine (pyPC) probes and the rate of intramolecular excimer production in dipyrenyl PC (dipyPC) in a series of host bilayer systems at temperatures below and above T_m. The lateral diffusion coefficient (D) of pyPC was determined from the probe concentration dependence of the monomeric fluorescence yield, analyzed according to the milling crowd model (Biophys.J. 49:987,1986), using computer simulations extended to probe ratios (x) of 0.001. To obtain the intramolecular excimer production rate (K), we derived the relationship $K = [r/r(x_0)]\tau_M^{-1}$, where τ_M is the monomer lifetime of the isolated pyPC probe and r and $r(x_0)$ are the excimer/monomer intensity ratios of dipyPC and pyPC, respectively, the latter measured at a probe ratio for which excimer production halves the τ_M, the pyrene moieties being attached to the same length acyl chains in pyPC and dipyPC. We found a quasi-linear relationship between D of pyPC (7-35 x 10^{-8} cm^2s^{-1}) and K for dipyPC (2-14 x 10^6 s^{-1}) measured at temperatures between 0 and 53 C, in several host vesicle systems in the fluid state, but not in their gel state. The short range (≈ nm) diffusivity of pyPC probes in fluid DMPC vesicles is comparable to the long range (≈μm) D measured in photo-bleaching recovery studies. These results suggest that dipyPC probes are valid indicators of local membrane fluidity and since the experimental r is concentration-independent at low probe ratios ($x \approx 10^{-3}$), they are particularly suitable for the fluorescence microscopic imaging of membrane fluidity.
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Tu-Pos192 CHOLESTEROL DYNAMICS IN MODEL AND BIOLOGICAL MEMBRANES. Philip L. Yeagle, James Frye*, Kathleen Battaglia and Arlene D. Albert. Department of Biochemistry, University of New York at Buffalo, Buffalo, N.Y. 14214 and *Department of Chemistry, Colorado State University, Fort Collins, CO 80523.

Time resolved flourescence anisotropy of the sterol analog cholestatrienol, and NMR spin lattice relaxation measurements of C-13 labelled cholesterol are exploited to determine the correlation times characterizing the major modes of motion of cholesterol in unsonicated phospholipid multilamellar liposomes. Two modes of motion are found to be important: rotational diffusion or wobble. The anisotropy decay of the cholestatrienol fluorescence is uniquely sensitive to wobble, and a correlation time of 1.8 ns is obtained, independent of temperature and cholesterol concentration. With the results from the fluorescence anisotropy decay, it is possible to deconvolute the contributions of these two modes to C spin lattice relaxation, employing CPMAS C-13 NMR to obtain the necessary high resolution spectra from this unsonicated system. A correlation time of 0.3 ns was obtained for rotational diffusion of the sterol about its long axis perpendicular to the membrane surface. In this manner, previous ambiguities in the assignment of correlation times to cholesterol motion in phospholipid bilayers are resolved. Data on cholestatrienol behavior in cell membranes will be presented, including the bovine rod outer segment disk membrane, the human erythrocyte membrane and the rabbit muscle sarcoplasmic reticulum.

Tu-Pos193 NMR AND OPTICAL PROPERTIES OF A FLUORINE-LABELED STYRYL MOLECULAR PROBE IN DIMYSTEROYLPHOSPHATIDYL CHOLINE VESICLES: A PROBE LOCATION MODEL, B. P. Bammel+, H. P. Hopkins+, R. Haugland×, J. Schuette#, W. Szwlecki×, and J. C. Smith+, Department of Chemistry, Georgia State University+ and Emory University#, Atlanta, GA and Molecular Probes, Inc.×, Euguene, OR - Using a combination of magnetic resonance and optical spectroscopic techniques, a model for the location of a fluorinated, potential-sensitive molecular probe of the styryl class in DMPC unilamellar vesicles has been developed. The distance from the unpaired electron site of lipophilic spin labels incorporated into the vesicles to the probe trifluoromethyl group has been determined from the effect of the paramagnetic agents on the probe ^{19}F T1 and T2 relaxation time values. The correlation time necessary for the preceding distance determination was estimated from vesicle-bound probe fluorescence lifetime and polarization analyses. From a knowledge of the nitroxide spin label N-O group site in the bilayer, determined from the effect of the unpaired electron on the lipid ^{13}C resonance linewidths, and the distance of the preceding site to the probe fluorine moiety, a model for the location of the probe in the DMPC bilayer has been developed and further refined using molecular mechanics energy minimization calculations. In this model, the long axis of the styryl probe is approximately parallel to the bilayer normal with the upper end of the probe optical chromophore located just below the lipid glycerol backbone; the remainder of the chromophore extends well into the hydrocarbon region of the bilayer. This location suggests that the optical properties of the probe should not be unduly sensitive to changes in the bilayer surface charge density.
Support: NIH award GM30552 and Department of Education award 2-0-01008.

Tu-Pos194 CHARACTERIZATION OF PHOSPHOLIPID TRANSFER BETWEEN PHOSPHOLIPID:BILE SALT MIXED MICELLES. Doug A. Fullington and J. Wylie Nichols, Department of Physiology, Emory University School of Medicine, Atlanta, GA 30322

The transfer of fluorescent-labeled N-(7-nitro-2,1,3-benzoxadiazol-4-yl) dimyristoyl phosphatidylethanolamine (N-NBD-DMPE) between phosphatidylcholine (PC):bile salt (BS) mixed micelles was measured by monitoring the increase in fluorescence as N-NBD-DMPE, initially contained in mixed micelles at self-quenching concentrations, was diluted into unlabeled mixed micelles. Kinetic analysis of transfer rate data obtained in previous experiments has shown that the transfer of phospholipid between taurocholate:phospholipid mixed micelles occurs by two different mechanisms. At low micelle concentrations, the predominant mode of transfer is by the diffusion of water-soluble monomers; whereas at high concentrations, transfer occurs during transient micelle collisions [Nichols, J. W. (1988), Biochemistry 27:3925-3931]. This investigation was continued by testing the effect of bile salt structure on the kinetics of N-NBD-DMPE transfer between mixed micelles. These results indicated that both the monomer diffusion and collision-dependent transfer mechanisms are a common property of PC:BS mixed micelles prepared from a wide range of bile salts. In general, we found that conjugation with taurine or glycine lowers the rate of collision-dependent transfer as does the addition of hydroxyl groups in the 7 and/or 12 positions. For acceptor mixed micelles at 1.5 mM PC concentration, the fastest rate of transfer was observed for mixed micelles prepared with chenodeoxycholate ($t_{1/2}$=26 msec); whereas the slowest rate was observed for glycocholate mixed micelles ($t_{1/2}$=34 sec), considerably faster than that measured for N-NBD-DMPE transfer between phospholipid vesicles ($t_{1/2}$=1.7 x 10^5 sec). Supported by NIH GM32342, DK40642, and a grant-in-aid from the AHA, Georgia Affiliate.

Tu-Pos195 MUTUAL DIFFUSION OF INTERACTING MEMBRANE PROTEINS. James R. Abney (1), Bethe A. Scalettar (2), and John C. Owicki (1,3), (1) Cell and Molecular Biology Division and (2) Chemical Biodynamics Division, Lawrence Berkeley Laboratory, 1 Cyclotron Road, Berkeley, CA, 94720, and (3) Department of Biophysics and Medical Physics, University of California, Berkeley, CA, 94720.

The generalized Stokes-Einstein equation is used, together with the two-dimensional pressure equation, to analyze *mutual* diffusion in concentrated membrane systems. These equations can be used to investigate the role that both direct and hydrodynamic interactions play in determining diffusive behavior; however, in this work, only direct interactions are explicitly incorporated into the theory. We look at diffusion in the presence of weak attractions, soft repulsions, and hard-core repulsions. It is found that, at low densities, attractions *retard* mutual diffusion while repulsions *enhance* it. Mechanistically, attractions tend to tether particles together and oppose the dissipation of gradients or fluctuations in concentration, while repulsions provide a driving force that pushes particles apart. At higher concentrations, changes in the structure of the fluid enhance mutual diffusion even in the presence of attractions. It is shown that the theoretical description of postelectrophoresis relaxation and fluorescence correlation spectroscopy experiments must be modified if interacting systems are studied. The effects of interactions on mutual-diffusion coefficients have probably already been seen in postelectrophoresis relaxation experiments.

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Tu-Pos196 FLUORESCENCE SPECTROSCOPY AND MICROSCOPY OF PHOSPHOLIPID CHOLINE MONOLAYERS LABELED WITH MEROCYANINE-540. Hao Yu and Sek Wen Hui, Membrane Biophysics Lab., Department of Biophysics, Roswell Park Memorial Institute, Buffalo, New York, 14263.

The characteristics of Merocyanine-540 (MC-540), as a reporter of the phyical state of phospholipid monolyers, was investigated by fluorescence microscopy and spectroscopy. MC-540 was mixed with dipalmitoylphosphatidylcholine (DPPC) or with dioleoylphosphatidylcholine (DOPC) before spreading on the air/water interface. The fluorescence emission spectra of the monolayer were recorded through a fiber optics light guide. The images were detected with a SIT camera attached to a fluorescence microscope equipped with a 40X objective. At low surface pressure (3 dyne/cm), a broad emission peak centered at 568nm was detected. The emission peaks of both types of lipid monolayers undergo a slight blue shift upon increasing surface pressure to 32 dyne/cm, while the intensity drops rapidly through this pressure range. Bright and dark domains were visible by low level fluorescence microscopy. For DPPC, small dark domains appear at 2.2 dyne/cm, and grow in size with increasing pressure up to 23 dyne/cm. The shape of domains are simular but not indentical to those seen in NBD-PC labeled DPPC monolayers. For DOPC,large dark patches gradually diminished with increasing pressure. The heterogeneity of monolayers should be taken into account in analyzing spectral data.

Tu-Pos197 LATERAL DIFFUSION AND AGGREGATION: A MONTE CARLO STUDY

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The aggregation of a membrane component is modeled by Monte Carlo calculations of cluster-cluster aggregation of particles on a square lattice. In these calculations, clusters are initially distributed randomly. They then execute a random walk, with a size-dependent diffusion coefficient, and adhere on contact.

Cluster growth is characterized by the time dependence of several quantities, including the cluster radius, the average coordination number of a cluster element, and the number-average, mass-average, and z-average cluster masses. Two types of aggregation measurement are simulated: scanning fluorescence correlation spectroscopy, and fluorescence measurements of the cluster fractal dimension. The cluster fractal dimension can be obtained from measurements of the fluorescence intensity as a function of the illuminating spot size, or from a two-dimensional Fourier transform of the fluorescence pattern. Other calculations yield the diffusion coefficient of the aggregating species, and the diffusion coefficient of an inert species obstructed by the aggregate. (Supported by NIH grant GM38133-01A1.)

Tu-Pos198 MEASUREMENT OF LIPID PACKING FLUCTUATIONS WITH LONG-LIVED FLUORESCENCE PROBES.

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Studies described here focus on the use of a long-lived fluorescence probe, coronene (τ~200ns), for examining phospholipid structural fluctuations in lipid bilayers. Previously we reported complex anisotropy decays for coronene imbedded in DPPC LUVs, at several temperatures below and up to the lipid phase transition temperature (T_c=41°C). Rotations of coronene in fluid-lipid surroundings are rapid (few nanoseconds), whereas gel-state phospholipids effectively 'immobilize' imbedded coronene molecules. In addition, we observe intermediate rotational correlation times (20-200ns), characteristic of (equilibrium) lipid 'melting' processes. We find these data are best analyzed using a 'chain' fluctuation model, derived from Landau phase transition theory, which employs distributions of lipid order and melting rates. A 'gating' factor is defined and reflects the number of lipid chains surrounding a coronene molecule which must disorder for probe rotation. Further, we have investigated the effects of the peptide melittin on these submicrosecond lipid fluctuations. Preliminary studies of coronene labelled DMPC LUVs containing melittin (1:50 peptide to phospholipid molar ratio) reveal a significant decrease (~50%) in the steady-state anisotropy values below the lipid phase transition. In contrast, DPH and TMA-DPH, both shorter-lived fluorescence probes, report little change. The application of our fluctuation 'gating' model to fluorescence decay data from other probes in lipid-peptide systems and the implications of peptide altered fluctuation rates will be discussed (*Supported in part by a PSC-CUNY award and a Petroleum Research Fund grant).

Tu-Pos199 EVALUATION OF ABILITY OF SPIN LABELS TO DETECT AND DIAGNOSE INTERDIGITATED LIPID BILAYERS.

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The behavior of a number of spin labels in several lipid bilayers, known to be interdigitated from X-ray diffraction studies, has been compared in order to evaluate the ability of the spin label technique to detect and diagnose the structure of lipid bilayers. The main difference between interdigitated and non-interdigitated gel phase bilayers which can be exploited for determination of their structure using spin labels, is that the former have a much less steep fluidity gradient. Thus long chain spin labels with the nitroxide group near the terminal methyl of the chain, such as 16-doxyl-stearic acid, its methyl ester, or a phosphatidylglycerol spin label containing 16-doxyl-stearic acid (PG-SL), are more motionally restricted and/or ordered in the interdigitated bilayer than in the non-interdigitated bilayer. This difference is large enough to be of diagnostic value for all three spin labels in the interdigitated bilayers of dipalmitoylphosphatidylcholine-glycerol, dipalmitoylphosphatidylglycerol-polymyxin B, and the asymmetric 1-stearoyl-2-caproyl-phosphatidylcholine. A difference can also be detected for all three spin labels in the interdigitated bilayers of dihexadecylphosphatidylcholine, dipalmitoylphosphatidylcholine-ethanol, and 1,3-dipalmitoylphosphatidylcholine. However, the difference is not large enough to be of diagnostic value at low temperatures. Use of probes with the nitroxide group closer to the apolar-polar interface reveals that these latter interdigitated bilayers are more disordered or less closely packed. As the temperature is increased, however, the motion of PG-SL does not increase as much in these interdigitated bilayers as in non-interdigitated bilayers. The difference in the motion and/or order of PG-SL between interdigitated and non-interdigitated bilayers is large enough at higher temperatures to be of value in diagnosing the structure of the bilayers. Thus by choice of a suitable spin label and a suitable temperature, this technique should prove useful for detection and diagnosis of lipid bilayer structure with a good degree of reliability.

Tu-Pos200 PROTONATION AND METAL-ION COMPLEXATION CONSTANTS OF IONOMYCIN IN 80% METHANOL. M.E. Craig[1], M.K. Stiles[2], D.R. Pfeiffer[2], and R.W. Taylor[1]. [1]Department of Chemistry, University of Oklahoma, Norman, OK 73019 and [2]The Hormel Institute, University of Minnesota, Austin, MN 55912.

Ionomycin, H_2I, is unique among the polyether ionophores in that it has two moieties with ionizable protons, a carboxylic acid and a β-diketone. Thus, ionomycin is able to form neutral 1:1 complexes with divalent cations. The protonation constants, $K_{Hi} = [H_iI]/[H_{i-1}I]a_H^*$, have been determined in 80% methanol-water at 25° C by spectrophotometric and potentiometric titration techniques. The logarithmic values of K_{H1} and K_{H2} are 11.8 and 6.84, respectively. Comparison with results for the model compounds, 2,6-dimethylheptanedione and 4-methylpentanoic acid, allows assignment of K_{H1} and K_{H2} to the b-diketone and carboxylic acid moieties, respectively. Changes in the UV-Vis spectra of ionomycin upon addition of metal ions were utilized to determine the complex formation constants with divalent cations. Conditional complexation constants, K_S', were determined by spectrophotometric titration of the ionophore with metal ion at fixed pH^* values. The values of the conditional constants were resolved from the titration data using a non-linear least-squares method. Data were consistent with 1:1 stoichiometry for the MI complex. Using the values of the protonation constants, the pH-independent complex formation constant, K_S, was determined from the relationship, $K_S = K_S'\{1 + K_{H1}a_H^* + K_{H1}K_{H2}(a_H^*)^2\}$. The selectivity pattern of the K_S values followed the sequence; Sr<Ca<Mg<Mn<Co<Zn≤Ni<<Cu. This pattern is similar to that found for the K_S values of the 1:1 complexes of a simple β-diketone, acetylacetone. Thus, ionomycin follows the so-called Irving-Williams series found for the complexation of a wide variety of ligands with divalent cations. (Supported by NIH grants GM 24701 and HL 08412).

Tu-Pos201 THE DISSOCIATION AND EXCHANGE KINETICS OF THE Mg(II), Zn(II), Co(II), AND Ni(II) COMPLEXES OF IONOPHORE A23197 IN 80% METHANOL-WATER. T.P. Thomas[1], D.R. Pfeiffer[2], and R.W. Taylor[1]. [1]Department of Chemistry, University of Oklahoma, Norman, OK 73019 and [2]The Hormel Institute, University of Minnesota, Austin, MN 55912.

Ionophore A23187 forms 1:1 and 1:2 metal-ion-ligand complexes with divalent cations in 80% methanol-water solutions. Using the stopped-flow technique, we have studied the kinetics of the dissociation of the 1:1 and 1:2 Mg-A23197 complexes in the presence of excess weak acid and the exchange reactions of the 1:2 Mg-, Zn-, Co-, and Ni-A23187 complexes with ethylenediaminetetraacetic acid (EDTA) at 25.0° C. The dissociation of the $Mg(A23187)_2$ complex was studied by mixing equal volumes of the complex with a buffer solution containing chloroacetic acid (HAc). Under the conditions employed ($pH^* = 4.2 - 5.2$, $[HAc] = 1.5 - 34.3$ mM, $I = 0.05$) the observed dissociation rate constant showed a dependence on both $[H^+]$ and [HAc] as well as an acid-independent pathway . The rate expression for the reaction was found to be; rate = $\{k_d + (k_H'[H^+] + k_{HAc}'[HAc])/(1 + k_{HAc}'[Ac^-])\}[Mg(A23187)]_{Tot}$. The observed rate constants for the exchange reactions exhibited a first-order dependence on $[EDTA]_{Tot}$ and, with the possible exception of the Mg-complex, a first-order dependence on $[H^+]$. The kinetic studies of the Mg-complexes were carried out at pH^* values above the protonation constant of A23187. The values of the exchange rate constants followed the sequence; Zn>Mg>Co>>Ni, which agrees with the trend expected based on the calculated values of the dissociation rate constants, $k_d = K_{os}k^{M-S}/K_1$, where K_{os}, k^{M-S}, and K_1 are the outer-sphere association constant, solvent-exchange rate constant, and 1:1 complex formation constant, respectively. (Supported by NIH grants GM 24701 and HL 08214).

Tu-Pos202 GENERAL FEATURES IN THE STOICHIOMETRY AND STABILITY OF IONOPHORE A23187-CATION COMPLEXES. C.J. Chapman, R.W. Taylor and D.R. Pfeiffer, Hormel Institute, Univ. of Minnesota, Austin, MN 55912 and Department of Chemistry, Univ. of Oklahoma, Norman, OK 73019.

A study on the stoichiometry and stability of complexes between A23187 and divalent cations in 80% methanol/water solutions [C.J. Chapman et al. (1987) Biochemistry 26, 5009-5018] has been extended to additional transition series cations, the heavy metal cations Cd^{2+} and Pb^{2+}, plus seven lanthanide series trivalent cations. Stability constants of 1:1 complexes between the ionophore and divalent cations vary by $10^{6.2}$ between Cu^{2+} and Ba^{2+}, the strongest and weakest bound examples, respectively. Considering alkaline earth and first series transition cations together, the pattern of stability constants obeys the extended Irving-William series as is seen with many non-ionophorous liganding agents. Cd^{2+} and Pb^{2+} are bound with an affinity similar to Mn^{2+}, whereas lanthanides are bound with little selectivity and slightly higher stability. Titration of the ionophore in the 10^{-5} M concentration range with di- and trivalent cations gives rise first to MA_2 complexes and subsequently to MA as the metal concentration is increased. The second stepwise stability constants for formation of the MA_2 species exceeds the first constant by ~10-fold. With lanthanides, heavy metals, and transition metal cation, OH^-, at near physiological concentrations, competes with free ionophore for binding to the 1:1 complexes. This competition is not apparent when Ca^{2+} or Mg^{2+} are the cations bound. Implications of the 1:1 complex selectivity pattern, the ionophore-hydroxide competitive binding, and potential ternary complexes involving 1:1 ionophore:cation complexes and other anions present in biological systems are considered with respect to the ionophore's biological actions. (Supp. by HL08214 and GM24701)

Tu-Pos203 LOCALIZATION AND HETEROGENEITY OF GLUCOSE TRANSPORTER PROTEINS IN SKELETAL MUSCLE. T. Plough*, E. Damiani, J. Vinten*, S. Salvatori, G. Salviati and A. Margreth, NRC Unit for Muscle Biology and Physiopathology, Institute of General Pathology, University of Padova, Italy and * Dept. of Medical Physiology B, The Panum Institute, University of Copenhagen, DK-2200, Denmark

The existence of more than one form of glucose transporter proteins in skeletal muscle, as well as its dual localization, in the plasma membrane (Wang, JBC 262, 15689, 1987) and in transverse tubular membranes (Burdett et al., Arch. Biochem. Biophys. 253, 279, 1987), are still controversial issues. Immunoblot techniques and monoclonal antibody against the human erytrocyte glucose transporter were used for reinvestigating this problem in purified membrane fractions from rabbit sleletal muscle. Antibody immunostianed glucose transporter in isolated plasma membrane (Seiler & Fleischer JBC 257, 13862, 1982), as well as in a TT-enriched membrane fraction (R1) prepared according to Saito et al. (J. Cell Biol. 99, 875, 1984). In contrast, transporter was not detected in either TC or LSR fractions obtained by the same procedure, as well as in isolated triads and in junctional TT, following disruption of triads by the method of Morgan & Kuypers (Arch. Biochem. Biophys. 253, 377, 1987). Our results indicate that glucose transporters are selectively expressed in free TT in addition to the plasma membrane, yet contain membrane-specific peptide components. Preliminary evidence likewise indicates differences in affinity for cyt B of plasma-membrane-bound and TT-bound glucose transporters of rabbit skeletal muscle.

Tu-Pos204 ANTIBODY MEDIATED LIPOSOME TARGETING TO MOUSE PULMONARY ARTERY ENDOTHELIAL CELLS. Eric G. Holmberg, Una S. Ryan, Stephen J. Kennel, and Leaf Huang, Department of Biochemistry, University of Tennessee, Knoxville, TN 37996-0840; Biology Division, Oak Ridge National Lab., Oak Ridge, TN 37831-8077; Department of Medicine, Univ. of Miami, P.O. Box 016960, Miami, FL 33101.

The rat monoclonal antibodies 273-34A and 411-201B have been shown to preferentially bind to mouse lung capillary endothelium in vivo. We have characterized the binding and endocytic properties of these monoclonal antibodies in a mouse pulmonary artery endothelial cell culture system and used them in an attempt to preferentially target liposomes to these cells. Both 273-34A and 411-201B exhibit saturable binding to the endothelial cells that can be blocked by the addition of excess non-labeled antibody. 34A and 201B bind to different epitopes on the cell surface as evidenced by lack of cross-blocking to each other. The dissociation constants, K_D, for 34A and 201B are 1.07 X 10^{-8} M and 1.40 X 10^{-8}M, respectively. Scatchard analysis reveals 3.8 X 10^6 and 2.4 X 10^6 binding sites per cell for 34A and 201B, respectively. The time course of endocytosis for both antibodies reveals that 85-90% of surface bound 201B and 75-80% of surface bound 34A is endocytosed within 15 minutes when incubated at 37°C. We have incorporated these monoclonal antibodies into liposomes via the attachment of palmitic acid residues to the monoclonal antibody and examined the binding properties of these immunoliposomes with the mouse artery endothelial cells. We have utilized the high binding affinity between avidin and biotin to specifically target biotin containing liposomes to mouse artery endothelial cells. The monoclonal antibodies were biotinylated and the subsequent addition of avidin to the cell bound antibodies allows the specific binding of biotin containing liposomes. Supported by NIH grants CA 24553 and AI 25834.

Tu-Pos205 PARTITIONING OF LIPOPHILIC DRUGS INTO MEMBRANES AND THE ROLE OF LIPID COMPOSITION. Stephen E. Wright and Leaf Huang, Department of Biochemistry, University of Tennessee, Knoxville, TN 37996-0840.

We have examined the partitioning behavior of the anticancer agent teniposide (VM-26) into multilamellar vesicles composed of various phospholipid species. Partitioning was found to be sensitive to the composition of the liposomal membrane since changes in the head group or acyl chain constituents could dramatically alter the affinity of the drug for the bilayer. [^{3}H]-VM-26 partitioned most readily into monounsaturated species of phosphatidylcholine (PC) with a partition coefficient (Kp) of 4290 ± 206 for dioleoyl-PC at 37°C. Inclusion of additional phospholipids having a different head group reduced partitioning in the order: cardiolipin > phosphatidylglycerol > phosphatidylserine > phosphatidylethanolamine with the Kp in the presence of 33 mol% cardiolipin reduced to 1370 ± 29. Partitioning into disaturated species of PC was much less than that for unsaturated species and was inversely proportional to the hydrocarbon chain length at temperatures either above or below the chain melting temperature. Partitioning into fluid-phase DMPC or DOPC was reduced in the presence of cholesterol in proportion to its bilayer concentration. Inclusion of the more hydrophilic derivative etoposide (VP-16) at concentrations up to 10 mol% did not compete with [^{3}H]-VM-26 for association with dioleoyl-PC. Addition of calf serum or serum albumin could significantly reduce the association of [^{3}H]-VM-26 with the liposomes. Supported by NIH grants CA 24553 and CA 08331.

Tu-Pos206 THE EFFECTS OF DOLICHOL ON MEMBRANE BILAYER PERMEABILITY

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The dolichols are a group of long-chain endogenous alcohols which are known to produce an L-alpha to hexagonal phase transformation within certain lipid bilayers. This study sought to correlate this structural finding with the effects of dolichol on the permeability of lipid multilayers to glucose under different physical conditions. Glucose was encapsulated within liposomes consisting of 1,2 dioleoyl phosphatidylethanolamine (DOPE) and phosphatidylcholine (DOPC) 1:1 prepared with 0-2 mol% dolichol C80-105. Release studies were carried out utilizing Sephadex gel-filtration and high-speed dialysis. When incorporated into lipid bilayers 0.5, 1, and 2 mol% concentrations of dolichol resulted in 2.5, 4, and 9 fold increases in the rate of glucose leakage from these vesicles at 35°C. Changes in liposome permeability were less evident in experiments conducted at 25°C. X-ray diffraction of multilamellar liposomes (50mg/ml) suspended in glucose/Hepes/NaCl buffer showed the existence of hexagonal phase along with a predominantly L-alpha bilayer liposomal structure. Hexagonal phase formation was most pronounced at temperatures of 35°C and higher, and became less apparent when the temperature was reduced to 25°C. This mechanism of membrane perturbation may be important for facilitating drug transport across membranes of biological origin. Supported by HL33026, RJR Nabisco, Inc. and by the State of Connecticut Department of Higher Education High Technology Program.

Tu-Pos207 ELECTROLYTIC SHIELDING OF MEMBRANE SURFACE CHARGES AND OF VESTIBULE CHARGE DISTRIBUTIONS. CONSEQUENCES FOR CHANNEL PERMEABILITY. Peter C. Jordan, Dept. of Chemistry, Brandeis University, Waltham, MA 02254-9110.

Vestibule charge distributions are believed to significantly influence the permeability of a number of physiologically significant channels, e.g. a K-channel from Sarcoplasmic Reticulum, a Ca activated K channel from skeletal muscle, etc. Evidence for this belief is the observation that these channels exhibit the apparently incompatible attributes of high selectivity and high conductivity.

Membrane surface charges can also significantly influence channel conductance. Comparison of the electrical properties of channels reconstituted in neutral and charged membranes has been used to provide an estimate of the distance between the membrane-water interface and a channel binding site or to demonstrate that the conductance pathway is effectively isolated from the membrane-water interface.

Using an efficient method for solving the nonlinear Poisson-Boltzmann equation applicable to general cylindrically symmetric geometries (Jordan, Bacquet and McCammon, 1988, Biophys. J., 53, 514a), we can assess the influence that altering ionic strength has on the electrical potential due to various surface and vestibule charge distributions and consider their influence on channel permeability.

Preliminary studies of vestibule charge distributions demonstrate that, even if the vestibule can accommodate an ion atmosphere, the channel potential created by charges localized on its outer surface falls off notably more gradually than would be expected on the basis of Gouy-Chapman theory. For narrow vestibules or channels, which can not accommodate counterions, the potential attenuates only slightly. The presence of the low dielectric surroundings clearly affects the potential within both the vestibule and the channel constriction. However, in the bulk solution, the presence of a vestibule has little influence; the potential decays essentially as predicted on the basis of the Gouy-Chapman model. Surprisingly, charges localized on the interior surface of a large vestibule (one that can accommodate counterions) produce only slightly greater axial potentials than if the charges were localized on the vestibule's outer surface.

Partial studies of a charged membrane shows that charges localized more than a Debye length from the entrance to a vestibule, while strongly influencing the solution potential, have little influence on the potential within the vestibule or the channel.

Tu-Pos208 Ca^{2+} UPTAKE BY CARDIOLIPIN (CL)-CONTAINING LIPOSOMES. EFFECT OF LIPID COMPOSITION. M.B. Kester and P.M. Sokolove, University of Maryland Toxicology Program and Department of Pharmacology & Experimental Therapeutics, University of Maryland Medical School, Baltimore, MD 21201.

Ca^{2+} uptake by phosphatidylcholine (PC) liposomes containing 20% CL has recently been reported [Smaal et al. (1987) Biochim. Biophys. Acta 897: 191]. In eukaryotic cells, CL is restricted to the inner mitochondrial membrane, a membrane composed of PC; phosphatidylethanolamine (PE), half of which may occur as the plasmalogen (PE_{pl}); and CL in a 4:4:1 mole ratio. Therefore, in order to investigate Ca^{2+} transport by more nearly physiological lipid mixtures and to define the role of individual lipids therein, we have compared Ca^{2+} uptake by PC/CL (4:1), PC/PE/CL (2:2:1), PC/PE/CL (4:4:1), and PC/PE/PE_{pl}/CL (4:2:2:1) liposomes prepared by extrusion. Ca^{2+}uptake from solutions containing 0.5-10 mM Ca^{2+} was monitored via encapsulated Arsenazo III with the following results: (1) Ca^{2+} uptake by PC/CL (4:1) liposomes was modest (ca. 0.3 mmoles/mole phospholipid), saturated at 2 mM Ca^{2+}, and occurred in the absence of Arsenazo efflux. (2) PE markedly increased Ca^{2+} uptake by CL-containing liposomes. (3) In liposomes containing 20% CL, PE-induced increases in Ca^{2+} uptake were associated with Arsenazo efflux; in PC/PE/CL (4:4:1) liposomes, increased Ca^{2+} uptake occurred in the absence of dye efflux. (4) In contrast to the results obtained with PC/CL liposomes, Ca^{2+} uptake by PE-containing liposomes increased with increasing Ca^{2+} concentration up to 10 mM or until Arsenazo release was elicited. (5) Ca^{2+} uptake in all preparations was biphasic, consisting of a rapid, temperature-independent phase, complete within one minute, and a slower uptake process, with an apparent activation energy of 30-45 kcal/mole. The slower phase accounted for a more significant proportion of the total uptake when PE was present. (6) PE_{pl} had no noteworthy effect on Ca^{2+} uptake. In sum, PE markedly altered all aspects of CL-mediated Ca^{2+} transport. These observations are consistent with participation of inverted lipid structures or their precursors in the transport process. [Supported by NIH (HL32615), the American Cancer Society (#JFRA-109), and the Graduate School, University of Maryland, Baltimore.]

Tu-Pos209 ALAMETHICIN TETRAMER CONDUCTANCE. Igor Vodyanoy *, **, Garland R. Marshall and Francis Chiu ***. *- Department of Physiology and Biophysics, University of California Irvine, Irvine, CA 92717; **- Office of Naval Research, Arlington, VA 22217; ***- Department of Pharmacology, Washington University, St. Louis, Missouri 63110.

Alamethicin is the best studied member of fungal antibiotics which produce voltage-gated channels in lipid bilayers and cell membranes. Details of its pore formation are still unclear. An alamethicin pore was designed with an amide backbone mouth at the C-terminus. The actual peptide is a tetramer of alamethicin 1-17 in which four monomers have been attached to a tetramer of lysine through the epsilon amino groups. Our preliminary data show that this peptide, which we refer to as Ac-(Lys(Alamethicin 1-17))$_4$-NH$_2$, after addition to the phosphatidylethanolamine bilayer, causes voltage-dependent conductance with a reduction of concentration dependence and a current-voltage curve steepness of about 5 mV per e-fold of the current. The conductance is expressed as channels of the basic unitary conductance (about 2.5 nS). These data are in accord with our channel model in which voltage-dependence is explained by interaction of the electric field with the dipole moment of the alpha helical part of alamethicin. Supported by the NIH grants GM30657 and GM33918.

Tu-Pos210 BETA SUBUNIT OF RICINUS COMMUNIS PERMEABILIZES LIPID BILAYERS.

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The lethal toxin, Ricinus communis is a dimer of M.W. 60,000 comprised of an alpha subunit responsible for intoxication and a beta subunit which is believed to permeabilize cells allowing the entry of the alpha subunit. Initial investigations aimed at elucidation of the mechanism(s) underlying the entry of the toxin into cells were performed using Mueller-Montal folded lipid bilayers. Bilayers (100 um in diameter) were formed by adding a mixture of 10 parts solvent (either decane or decane:hexane (1:1)) to one part lipid (azolectin, a gift from Dr. M. Colombini), to salt bathing solutions on either side on a saran interface in a divided chamber. Typically, 10 ul of the bilayer mixture resulted in bilayers with resistances of at least 10^{11} ohm, at room temperature (25 -27° C). Following formation of a bilayer 2 to 5 uL of a diluted (0.05 mg/ml)stock of beta subunit of Ricin toxin was stirred into one or both sides of the bilayer, for 30 seconds. Within seconds of stirring, current fluctuations appeared and conductance of the bilayer increased. This increase in conductance of the bilayer has been observed both in the presence and absence of pH gradients and at pHs ranging from 2 to 7.8. Voltage is not necessary for induction of fluctuations. Using reversal potentials and alterations in ion gradients, preference for H ≈ Na$\gg$K,Li,TMA, Cl, SO_4 has been observed. Fluctuations have not been observed under any conditions when the alpha subunit has been added to the bilayer alone. Thus these data indicate the beta subunit of Ricin toxin permeabilizes lipid bilayers yielding cation selective pores. The mechanism of permeabilization appears to differ from that of Diptheria toxin in that a pH gradient is not necessary.

Tu-Pos211 THEORETICAL STUDY OF BUNDLES OF α-HELICES. PARTIAL MODELLING OF THE ACETYLCHOLINE RECEPTOR.

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In view of the large amount of experimental data pointing to the possible role of bundles of hydrophobic α-helical polypeptide segments in the transfer of ions through membranes, theoretical studies have been performed using energy-optimization techniques, aiming at an understanding of the structure and properties of such aggregates relevant to the functionning of membrane channels.

Essential results concern the particular packing properties of hydrophobic segments, their ability to form packages of different shapes and similar energies enclosing a pore of modulable size, the aptitude of a bundle of purely hydrophobic α-helices to interact favorably with ions and/or water, and the respective roles of the peptide carbonyl groups and of polar side-chains in the transfer of cations.

An application of the same methodology to the modelisation of the inner wall of the acetylcholine receptor is considered, using the real sequence of the hydrophobic segments and the structural informations based on recent photolabelling experiments with inhibitors.

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Tu-Pos212 HEME INTERACTIONS WITH ARTIFICIAL LIPID BILAYERS.

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The biosynthetic centers of protoheme and apoprotein are isolated by the mitochondrial membrane *in vivo*. An *in vitro* system of CO-heme, apomyoglobin, and artificial liposomes was examined for evidence of heme partitioning and transmembrane movement. Heme association with, and dissociation from, the lipid bilayers could be followed spectrophotometrically. Partition constants were determined for thirty separate lipid vesicle compositions. A sharp decrease in the rate of heme partitioning into liposomes was observed as the lipid vesicles changed from liquid-crystalline to gel phase. A net negative charge generally decreased the overall rate and affinity of the vesicles for heme binding. The rate and extent of heme uptake in unsaturated lecithins was unaffected by cholesterol content at levels up to 40% per mole. Although there is some variation, most lecithins with low T_m values had an overall equilibrium constant of $\approx 5 \times 10^5$ with respective association and dissociation rate constants of $\approx 3 \times 10^6$ s^{-1} and 7 s^{-1} at 30° C.

An additional slow kinetic event was observed during association and dissociation measurements. Four lines of evidence indicate that this phase was heme transmembrane movement. 1) the spectral wavelength dependence was similar to that seen in heme partitioning. 2) the kinetics were essentially independent of lipid concentration but did decrease with increasing acyl-chain length. 3) the rates increased sharply at the T_m. 4) separate heme fractions, rapidly dissociating heme in the outer layer and heme in the inner portion of the bilayer, could be separated by column chromatography. These results indicate that *in vivo* free heme could be localized in a particular cell but be available for protein biosynthesis. This work was supported by the Robert A. Welch Foundation.

Tu-Pos213 PROTON AND SODIUM ION PERMEABILITIES OF DIOLEOYLPHOSPHATIDYLCHOLINE SINGLE LAMELLAR VESICLES CONTAINING CARDIOLIPIN AND POLYMORPHIC CARDIOLIPIN ANALOGUES.

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The proton permeability coefficients were measured at 25°for single lamellar vesicles (SLV) containing dioleoylphosphatidylcholine (DOPC) and varying amounts of cardiolipin and cardiolipin analogues prepared by extrusion (Mayer, Hope and Cullis, Biochim. Biophys. Acta 858, (1986) 161. The SLV were loaded with the pH sensitive, hydrophylic fluorescent probe, pyranine (8-hydroxy-1,3,6-pyrenetrisulfonic acid) (Kano and Fendler, Biochim. Biophys. Acta 509 (1978) 289). Using a the manual stop flow accessory (Hi-Tech Scientific Lts.) and computer digitization for 5 min, the following permeabilities (cm/sec x 10^{-3}) in DOPC were obtained: for pure DOPC 1.65 (± 0.163) and 1.81 (± 0.343) and 1.23 (± 0.110), 5% (w/w) bovine cardiolipin (CL) 1.66 (± 0.110), monolysoCL 3.54 (± 0.542) and dilysoCL 3.43 (± 0.503) at concentrations equimolar with 5% CL, 25% CL 2.44 (± 0.230), 5% synthetic cardiolipin with homogeneous acyl chains (TOCL) 1.46 (± 0.110), 6.3% TOCL 1.07 (± 0.047), 25% TOCL 2.56 (± 0.343) and finally dioleoylphosphatidylglycerol equimolar with the 25% TOCL 1.21 (± 0.0579). Sodium permeabilities were measured from the leakage rates of $^{22}Na^+$ from extruded vesicles. For DOPC SLV at 23° the permeability coefficient was 2.7 x 10^{-12}; egg PC, 8.3 x 10^{-12} and 7.6 x 10^{-11} for 20% TOCL. In conclusion, these polymorphic cardiolipins (Powell and Marsh, Biochemistry 24 (1985) 2902) while introducing slightly more permeability in DOPC in general maintain bilayer integrity. (Supported in part by the US Public Health Service (HL-38190).

Tu-Pos214 Na,K,Cl-COTRANSPORT IN CULTURED RETINAL PIGMENT EPITHELIUM. B.G. Kennedy. Indiana Univ. Sch. of Med. Northwest Center for Medical Education, Gary IN 46408.

The retinal pigment epithelium (RPE), a transporting epithelial cell layer separating the retina from its choroidal blood supply, controls the ionic and metabolic composition of the fluid bathing the photoreceptor cells. The present study examines ouabain-insensitive Rb transport by the RPE. Primary cultures, which grow as attached monolayers, were established from monkey RPE. Rb (used as a congener for K) uptake was assayed as unidirectional 86-Rb influx into confluent RPE monolayers. Approximately 50% of the Rb influx was inhibited by 0.1 mM ouabain. The ouabain-insensitive component of Rb influx was inhibited 90-95% by 1 mM furosemide. Uptake, 0.721 ± 0.095 umol Rb/mg prot/hr (mean ± SEM for n=17) in the presence of 0.1 mM ouabain, was reduced to 0.021 ± 0.004 (n=15) with addition of 1 mM furosemide. Maximal furosemide-sensitive Rb uptake was thus 0.700 umol Rb/mg protein/hr. Half-maximal inhibition of ouabain-insensitive Rb uptake was observed at 3.5 uM furosemide. Furthermore, Na-free (choline substituted) media reduced ouabain-insensitive Rb uptake by 90%. Furosemide sensitive uptake was only 0.017 umol Rb/mg protein/hr in this media. In low Cl media (5 mM Cl, with the balance replaced by gluconate), Rb influx was drastically inhibited, by about 95%. Furosemide-sensitive uptake was not detectable in the low-Cl media. In sum, the ouabain-insensitive fraction of Rb uptake is almost completely inhibited by the diuretic furosemide, and depends on both extracellular Na and Cl. This provides preliminary evidence indicating that RPE monolayers possess a prominent, furosemide-sensitive, Na,K,Cl-cotransport mechanism. (Supported in part by a grant from the American Diabetes Association, Indiana Affiliate, Inc.).

Tu-Pos215 THE BEHAVIOR OF THE SODIUM EFFLUX IN SINGLE BARNACLE MUSCLE FIBERS TOWARD ZINC. Huiwen Xie* and E. Edward Bittar, Department of Physiology, University of Wisconsin, Madison, WI 53706.

The ^{22}Na efflux in muscle fibers (from the barnacle B. nubilus) suspended in 10 mM-Ca^{2+}-10 mM-Mg^{2+}-10 mM-imidazole-artificial seawater (pH 7.3) is responsive to external application of Zn^{2+}. Zn^{2+} is always found to slowly reduce the resting level of the Na efflux in the absence or presence of ouabain. 2 mM-Zn^{2+} elicits a fall which averages 68±1% (SEM) (n=8). This is significantly greater than the 50±2% fall which 10^{-4}M-ouabain causes in companion controls, n=8 ($P < 0.001$). External application of 2 mM-Zn^{2+} after maximal inhibition by ouabain also results in a fall, the size of which averages 19±1% (n=8). By contrast, application of 10^{-4}M-ouabain to Zn^{2+}-treated fibers is without effect. To check whether the Zn^{2+} effect is maximal, 5 mM-Zn^{2+} (at pH 7.0) was tried. This causes a 55±4% fall in the Na efflux (n=7). A dose-response curve was also determined; this indicates an IC_{50} of ~200 µM and a minimal effective concentration of ~10 µM. Having next found that the injection of Zn^{2+} (e.g. 50 mM) fails to mimic its effect upon external application, the question asked was: Does Zn^{2+} act as a Ca^{2+} channel blocker, and if it does, is it also able to stop the entry of Na^+ via this channel? The results obtained with 100 mM-Ke show that 2 mM-Zn^{2+} is a potent blocker of the response of the ouabain-insensitive Na efflux to high Ke (41±11% stimulation, n=4 vs 334±29% stimulation in controls, n=4, $P < 0.001$). A dose-response curve also indicates that 10^{-5}M-Zn^{2+} halves the size of the response (n=5, $P < 0.01$). It is noteworthy that verapamil does not reduce the Na efflux, and that the addition of Zn^{2+} after 10^{-4}M-verapamil causes the efflux to fall. Whether Zn^{2+} fails to reduce the Na efflux when external Na^+ is replaced by choline remains to be seen.

Tu-Pos216 INTRACELLULAR SODIUM ACTIVITY FOLLOWING NA-K-ATPase INHIBITION IN ISOLATED RAT VENTRICULAR MYOCYTES. B.A. Biagi, B.T. Stokes, Q. Li, and R. Altschuld (Intr. by C.R. Hille). Dept. of Physiology and Division of Cardiology, The Ohio State University, Columbus, OH 43210.

We have previously demonstrated that the increase in intracellular calcium activity following ATP depletion with 3 mM amytal and 2 uM CCCP (A/CCCP) is dependent on the influx of extracellular calcium which may occur, in part, via the 3Na:Ca exchanger operating in the "reverse" mode. To evaluate the driving forces for 3Na:Ca exchange, intracellular sodium activity (A_{Na}) and membrane voltage (Vm) were measured during energy depletion with and without Na-K-ATPase inhibition with a zero K^+ (OK) perfusion solution. Ventricular myocytes were superfused with a Krebs-Henseleit solution at 37°C and A_{Na} and Vm were measured with double barreled Na^+ selective microelectrodes. Resting Vm was -57 ± 2.3 (26) mV and A_{Na} was 13 ± 2.0 (26) mM. OK perfusion for 4-5 min produced a transient hyperpolarization of Vm followed by a rapid depolarization to -27 ± 3.0 (15) mV. A_{Na} increased to 18 ± 3.0 (15) mM. During deenergization with A/CCCP for 10-15 min, Vm followed a complex pattern of rapid depolarization, hyperpolarization, and finally depolarization to -25 ± 6.4 (8) mV. A_{NA} increased steadily to 32 ± 9.3 (8) mM. The combined protocols (OK followed by A/CCCP) resulted in Vm and A_{Na} steady state values of -21 ± 9.7 (6) mV and 28 ± 5.2 (6) mM, respectively, and were not different from A/CCCP alone. These results demonstrate that in each case complex Vm changes are accompanied by a relatively slow and steady increase in A_{Na}. We conclude that the changes in electrical and Na^+ gradients under these conditions would result in a reversal of 3Na:Ca exchange which would contribute to the increase in intracellular calcium activity previously measured under these conditions.

Tu-Pos217 NA/K PUMP ACTIVITY IN CULTURED BOVINE VASCULAR ENDOTHELIAL CELLS (CBVEC), N.C. Adragna, Department of Pharmacology and Toxicology, Wright State University, Dayton, OH 45435.

All cells containing K as the predominant monovalent cation are expected to possess a Na/K pump that balances the inward Na and outward K "passive leaks" in order to achieve constancy of cell volume. However, recent studies on cultured cells from several tissues have reported "leak pathways" for Na and K larger than the pump in cells of supposedly normal volume. The present study investigated in CBVEC the causes for a smaller than expected fractional pump flux. The Na/K pump activity was measured as the net ouabain-sensitive (OS) Na gain and K loss in two clones, BFA-1c and BFAE-39. Na and K content were measured as a function of time in cells incubated in the absence and presence of ouabain, and in the original cultured medium (CM) or in a balanced salt solution (BSS) containing a similar electrolyte composition as the CM plus 1 mg/ml bovine albumin (Sigma C# A 7030). The OS Na gain and K loss in either flux medium was linear between 5 and 60 minutes. Under these conditions, the OS Na gain was 61 and 77% and the OS K loss, 44 and 66% lower in BSS than in CM for clone BFA-1c and BFAE-39, respectively. The K/Na ratios were: 1.7 ± 0.1 in CM vs 3.1 ± 0.7 in BSS ($p < 0.05$, n=4) for clone BFA-1c, and 1.2 ± 0.1 in CM vs 1.9 ± 0.3 in BSS ($p < 0.025$, n=4) for clone BFAE-39. Thus, a significant decrease in pump activity and an increased stoichiometry were observed in cells incubated in BSS. In addition, statistically higher fluxes were found in clone BFAE-39 than in clone BFA-1c, in CM but not in BSS due to a higher Na content in cells of clone BFAE-39 which had undergone about half the population doubling times of clone BFA-1c. Consistent with this finding, net OS Na gain and K loss decreased by increasing the passage number of the cells only in CM but not in BSS. It is concluded that with some balanced salt solutions the Na/K pump activity may be underestimated. (Supported by AHA/MV-87-01).

Tu-Pos218 COMBINED EFFECTS OF LIPID MODIFICATION AND DIVALENT CATIONS ON Na^+, K^+-ATPase ACTIVITY IN ISOLATED CARDIAC SARCOLEMMAL VESICLES. Kenneth S. Leonards, Dept. of Physiology, UCLA School of Medicine, Los Angeles, CA 90024-1760.

In order to investigate the relationships between sarcolemmal lipid composition, cation/sarcolemmal interactions, and the regulation of Na^+,K^+-ATPase activity, we examined changes in both Na^+,K^+-ATPase and 'basal' ATPase activities in the presence and absence of various divalent cations, before and after modifying the lipid composition of sarcolemmal membrane with PLA_2. Our results indicate that PLA_2 treatment significantly inhibits both 'basal' and Na^+,K^+-ATPase activities, and that this inhibition is completely reversible by removing the hydrolysis products with a BSA wash. Our results also show that divalent cations inhibit both 'basal' and Na^+,K^+-ATPase activities and that the cation sequences for the inhibition of these two ATPase activities are different. Most interesting, however, are the combined effects of divalent cations and PLA_2 treatment on the two ATPase activities. For the 'basal' ATPase, the cation inhibition sequence (-)PLA_2 treatment is: Ca=Sr>Ba>Co=Mn>Mg (most to least inhibitory). This sequence does not change when combined with (+)PLA_2 treatment, or (+)PLA_2(+)BSA wash treatment, although the activities themselves are significantly affected. In contrast to these findings the activity properties of the Na^+,K^+-ATPase are markedly changed by the combined effects of divalent cations and PLA_2 treatment. For untreated ((-)PLA_2) vesicles the cation inhibition sequence is: Mn=Co>Ca>Sr>Mg>Ba. After PLA_2 treatment this sequence is changed to: Ca>Sr>Mn>Ba>Co>Mg. Most interestingly, (+)PLA_2(+)BSA wash treatment not only restores Na^+,K^+-ATPase activity, but changes the cation inhibited sequence back to its original order. The results suggest that cations and membrane lipids can combine to regulate Na^+,K^+-ATPase activity in new ways. (Supported by NIH grant HL34517 and a AHA-GLAA Grant-in-Aid #843 G1.)

Tu-Pos219 MEMBRANE POTENTIAL AND PUMP ACTIVITY IN THE RECONSTITUTED Na,K-ATPase PROTEOLIPOSOMES. Atsunobu Yoda and Shizuko Yoda, Department of Pharmacology, University of Wisconsin Medical School, 1300 University Avenue, Madison, WI 53706.

In this study, Na,K-ATPase proteoliposomes (PL) containing 100 mM NaCl and 100 mM KCl were used; these concentrations were chosen to keep the PL in the Na-K exchange mode for a long time. In the presence of NaCl and KCl (total concentration, 200 mM) on the outside, MgATP activated both the Na-uptake and the ATP-hydrolysis on the inside-out PL, along with the generation of membrane potential (MP). The MP was estimated by the increase in the absorption of Oxonol VI by the PL. Initially the MP buildup rate depended on the amount of PL in the medium, but then the MP reached a steady level (about 100 mV) within 5 min at 15°C. The Na-uptake and ATP-hydrolysis, however, increased continuously with a constant coupling ratio between them (about 3.0) even after the MP reached steady state. Moreover, the steady state MP value did not vary with the quantity of PL in the medium, although the time required to reach steady state increased as the PL quantity decreased. These results cannot be explained by a simple relationship between the leakage and buildup of MP. It is possible that high MPs across the lipid bilayer (100 mV/5 nm = 200 kV/cm) break down the bilayer to discharge the MP until it reaches a steady level. (Supported by NIH Grant HL 16549).

Tu-Pos220 ELECTROGENIC REACTION STEPS OF NA,K-ATPASE FROM PIG KIDNEY AND EEL ELECTRIC ORGAN. Fendler K., Jaruschewski S., Hobbs A.S., and Froehlich J.P. (Intr. by Christensen B.) Max-Planck-Institut für Biophysik, Frankfurt am Main, Federal Republic of Germany

Charge translocations during the reaction cycle of NaK-ATPase from pig kidney and the electric organ of Electrophorus electricus were measured with a time resolution of 1 ms. Therefore ATPase containing membrane fragments were adsorbed to lipid bilayers and transient currents due to the pumping activity of the enzyme were recorded. The ATPase was activated by photorelease of ATP from a protected precursor, caged ATP.

The electrical experiments were complemented by rapid mixing ^{32}P incorporation studies. The results of both techniques are compared. This allows to correlate biochemically and electrically defined intermediates of the NaK-ATPase reaction cycle.

To investigate the inhibitory effect of caged ATP rapid mixing experiments were performed in the presence of caged ATP and (^{32}P)ATP. The time constants obtained under these conditions agree with the time constants of the electrical measurements.

From a comparison of the results obtained with both techniques and with both enzymes the two time constants observed in the transient currents can be assigned to caged ATP/ATP binding/exchange and the E1P->E2P transition.

Tu-Pos221 LYOTROPIC IONS ON ACTIVITY OF SODIUM, POTASSIUM-ATPASE. Kuniaki Suzuki and Robert L. Post, Department of Molecular Physiology and Biophysics, Vanderbilt University Medical School, Nashville, TN 37232.

The phosphoenzyme of (Na,K)-ATPase is composed of two principal reactive states designated E1P and E2P. E1P donates its phosphate group reversibly to ADP; E2P is stimulated by K^+ to donate its phosphate group reversibly to water but not to ADP; another state, E*P, responds either to ADP or to K^+. Lyotropic ions, 0.1 to 1 M, favor formation of E1P over E2P (Biophys J. 53:138a). From more lyotropic to less, the anion series is ClO_4^- > I^- > SCN^- > NO_3^- > Br^- > Cl^- > SO_4^{2-} > acetate$^-$ or other carboxylates. In order to estimate the effect of lyotropic ions on ATPase activity, we incubated the enzyme at pH 7 and 37 °C in 40 mM imidazole/MOPS, 4 mM ATP and measured the rate of release of P_i in the presence of various anions in place of the usual chloride. In 40 mM Na^+ and 3.5 mM Mg^{2+} the specific activity was 0.25 µmol/min/mg without K^+ and 13 with addition of 8 mM K^+ for all anions above (except I^- & Br^- not tested). In 400 mM Na^+ and 10 mM Mg^{2+}, with chloride, formate, acetate, propionate, and aspartate the specific activity without K^+ was 0.9 and with 80 mM K^+ it was 14. However, in these concentrations of Na^+ and Mg^{2+}, perchlorate and thiocyanate inhibited the activity to 0.1 without K^+ and to 3 with it. With nitrate the inhibition was about half a great with or without K^+. Thus lyotropic ions at concentrations that stabilized E1P in preference to E2P inhibited ATPase activity; this is consistent with a precursor-product relationship from E1P to E2P. Inhibition without K^+ was due to a concomitant reduction in the rate of spontaneous hydrolysis of the phosphoenzyme as shown by its rate of decay following a chase by unlabeled ATP. Supported by a grant, HL-01974, from National Heart, Lung, and Blood Institute of the N.I.H.

Tu-Pos222 SODIUM TRANSPORT BY THE GASTRIC H,K-ATPASE. Carlomaria Polvani, George Sachs* and Rhoda Blostein. Depts. of Biochemistry/Medicine, McGill University, Montreal, Canada and *Wadsworth Veterans Administration Hospital Center and UCLA, Los Angeles.

In view of the striking homology among various ion-translocating ATPases including Na,K-ATPase, Ca-ATPase and H,K-ATPase, and the recent evidence that protons can replace cytoplasmic Na^+ as well as extracellular K^+ in the reaction mechanism of the Na,K-ATPase (Polvani & Blostein, J. Biol. Chem., in press), we studied the role of Na^+ as a substitute for H^+ in the H,K-ATPase reaction. Using hog gastric H,K-ATPase-rich inside-out membrane vesicles we observed $^{22}Na^+$ influx which was stimulated by intravesicular K^+ (K_{in}) at pH 8.5 but not at pH 7.1. This Na^+ influx was observed in medium containing ATP and was inhibited by vanadate and a selective H,K-ATPase inhibitor, SCH28080. In order to determine the sidedness of H^+ effects, experiments were carried out for short periods (30 sec) under conditions of different initial pH gradients: pH_{in} /pH_o = 8.5/8.5, 8.5/7.1, 7.1/7.1, 7.1/8.5. Results from these experiments showed that K_{in}-activated $^{22}Na^+$ influx occurs only when pH_o = 8.5 and is unaffected by changes in pH_{in}. In separate experiments, the initial rate varied from 0.5 to 0.8 nmol/mg/hr (4 mM Na_o, 20 mM K_i; 0.2 mM ATP; 23°C). The coupling ratio for Na:ATP determined by measuring K_{in}-dependent ATP hydrolysis and Na^+ influx concurrently was close to 1.0. These results support a role for sodium ions as proton substitutes in the H,K-ATPase reaction mechanism and provide evidence for a similarity in ion selectivity and/or binding domains of the Na,K-ATPase and the H,K-ATPase enzymes. Supported by grants MT-3876 from the MRC (Canada) and SMI from the VA.

Tu-Pos223 BUMETANIDE INHIBITION OF H^+ AND Cl^- TRANSPORT, HIGH AFFINITY BINDING, AND BUMETANIDE LABELLING OF PROTEINS OF THE LYSOSOMAL MEMBRANE. John Cuppoletti and Michael Helmrath, Department of Physiology and Biophysics, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267-0576.

Rat liver lysosomes (tritosomes) possess an electrogenic H^+ ATPase which serves to acidify the lysosome interior. In lysosomal membrane preparations, N-ethylmaleimide-sensitive, vanadate-, Ca^{2+}-, ouabain-, and oligomycin- insensitive, Mg^{2+} ATP- dependent H^+ accumulation requires Cl^-. H^+ accumulation is inhibited by bumetanide, and the inhibition of H^+ pump activity can be overcome with K^+-valinomycin. In this sense, the H^+ pump is separable from the associated Cl^- channel. A similar channel serves an identical role in the chromaffin granule. In other studies, we have measured the Cl^- channel activity using 6-methoxy-N-(3-sulfopropyl)quinolinium (SPQ). Rates of Cl^- transport were measured in the presence of uM tetrachlorosalicylanilide (to maintain electroneutrality) in tetramethylammonium Cl^- solution. We found Cl^- transport to be slowed by uM levels of bumetanide. We demonstrated high affinity binding of ^3H-bumetanide to the lysosomal membrane preparation. Binding was largely insensitive to K^+, Na^+ or a combination of these cations. Irradiation of the membrane preparations in the presence of ^3H-bumetanide resulted in time-dependent incorporation of ^3H into membrane proteins of ca. 55 kD and ca. 70 kD. Label incorporation was slowed or prevented by the inclusion of excess unlabelled bumetanide. We are investigating the possibility that these proteins are associated with Cl^- channel activity required for H^+ transport in the lysosomal membrane. Supported by NIH DK 38808.

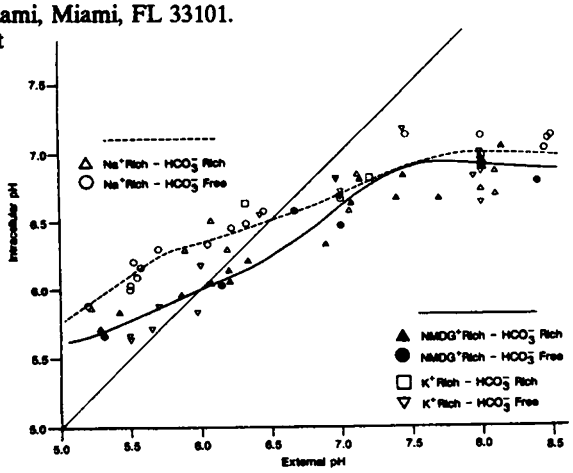
Tu-Pos224 FLUORESCENCE PROBING OF A MAJOR CONFORMATION CHANGE IN THE PLASMA MEMBRANE H^+-ATPase OF NEUROSPORA. Georg Nagel, Clifford Slayman, and Irena Klodos. Department of Cellular and Molecular Physiology, Yale University, New Haven, CT 06510.

Recently Klodos & Forbush (J. Gen. Physiol. 92:46a, 1988) discovered that the fluorescent dye RH-160 responds to rapid conformational changes of the Na^+,K^+-ATPase, specifically increasing its fluorescence during reactions leading to the E_2-P state. Among enzymes closely related to the Na^+,K^+-ATPase, that with the simplest reaction cycle is the H^+-ATPase of plant/fungal plasma membranes, and we have tested RH-160 on that enzyme in order to get information both about possible conformational changes in the H^+-ATPase and about the mode of action of the fluorescent probe. Standard conditions for the assays were: Plasma membrane fragments from Neurospora, containing protein: 0.1 mg/ml, and ATPase: 7 umols PO_4/min*mg protein; dye: 0.1 ug/ml; Mg^{++}: 1 mM; pH: 7.3; fluorescence assay: emission difference (>645 nm) for excitation at 550 nm and 450 nm.

With the H^+-ATPase, RH-160 fluorescence did not change on addition of ATP, but did increase slowly after addition of Na-orthovanadate (100 um). The fluorescence rise was accelerated by ATP (not ADP), and its rate was dependent upon concentrations of Mg^{++}, ATP, and vanadate. Boiling the enzyme, or pretreatment with trypsin (60 ug trypsin/mg protein, 20 min, 20°C), completely abolished the ATP effect, but trypsin left some residual fluorescence change in vanadate alone. The results are readily interpreted by supposing that H^+-ATPase increases RH-160 fluorescence in the E_2 state, but under normal conditions E_2-P is scarce because of fast dephosphorylation. The trypsin-sensitive fluorescence increase would indicate E_2 formation via ATP-phosphorylation. [Supported by Grant GM-15858 from N.I.G.M.S. and M12-7961 from the Danish Medical Research Council.]

Tu-Pos225 REGULATION OF INTRACELLULAR pH IN HUMAN PLATELETS. Peter A. Valant and Duncan H. Haynes, Dept. Pharmacology, Univ. Miami, Miami, FL 33101.

The mechanisms of homeostasis of internal pH (pHi) against variation of external pH (pHo) were investigated in BCECF-loaded human platelets. The platelets were introduced into media of variable pH and ionic composition, the approach steady-state was monitored, and pHi was calculated from the final fluorescence, using Kd = 1.07×10^{-7} and Fmin and Fmax values determined after digitonin permeabilization. The results, given in the figure, show the following: (1) At physiological pHo, pHi = 6.75 and is not strongly dependent on Na^+ or HCO_3^-. (2) In a Na^+ + HCO_3^--containing buffer, pHi is held within 0.65 units of this homeostatic value over a 3.0 unit variation of pHo (5.5-8.5). (3) In the acid range, extracellular Na^+ is essential in maintaining homeostasis of pHi. In its absence, pHi approaches pHo. This is consistent with Na^+/H^+ exchange. (4) In the alkaline range extracellular HCO_3^- makes a contribution to homeostasis. However it accounts for ca. 1/3rd of the alkaline delta pH. The rest may be accounted for by internal H^+ production. Supported by FL/AHA and NIH T32HI07188.



Tu-Pos226 INTERACTIONS OF DIVALENT CATIONS WITH TONOPLAST H^+-ATPASE. Shu-I Tu, Edwin Nungesser, and D.Brauer, USDA, ARS, Eastern Regional Research Center, Philadelphia, Pennsylvania 19118

The coupled activities, ATP hydrolysis and proton pumping, associated with the H^+-ATPase of tonoplast vesicles from corn roots require either Mg^{2+} or Mn^{2+}. The presence of Cd^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}, or their ATP complexes induced a differential inhibition to the coupled activities. In all cases, the proton pumping process was preferentially inhibited. Since (1) the potency of ATP-complexes was considerably higher than that of free cations and (2) the inhibition could be competitively relieved by Mg-ATP, it was concluded that the effective binding of the cations occurred at the active site of the enzyme. The results also suggest that the coupling between ATP hydrolysis and proton pumping may be indirect and the binding of Mg^{2+} at the active site may trigger the subsequent molecular events leading to a transmembranous proton movement.