T-Pos78 β-SHEET CONFORMATION IN THE GAP JUNCTION CONNEXON PROTEIN. W.C. Phillips, D.L.D. Caspar, R. Saper and D.A. Goodenough[+], Rosenstiel Center, Brandeis Univ., Waltham, MA 02254 and [+]Dept. of Anatomy, Harvard Medical School, Boston, MA 02215.

In X-ray diffraction patterns from oriented gap junction specimens, intensity on the equator at ~10Å spacing and on the meridian at ~4.7Å spacing indicates a substantial proportion of the trans-membrane protein is in a β-sheet conformation, with the strands of the sheet running parallel to the membrane surface. The location and relative amount of β-sheet in the connexon protein spanning the bilayer has been determined from an analysis of the meridional intensity observed from several specimens. A phase assignment was made using the minimum wavelength principle, assuming the connexon pair profile is centrosymmetric. The β-sheet is concentrated in two domains; a major domain running from ~20-50Å from the center of the gap, and a minor domain running from the center of the gap to ~15Å from the center. The major domain has from 4 to 7 β-strands, the minor domain from 2 to 3 strands. This assignment of β-sheet protein correlates well with the 3-D gap-junction model deduced by Makowski *et al.* from low-resolution X-ray data. The magnitude of the electron density fluxuation in the β-strands has been scaled by comparing the 4.7Å intensity with the low-resolution intensities, which were put on an absolute scale by solvent density contrast. Comparison with a 1-D profile calculated from a model β-sheet protein suggests that almost all the connexon protein in the region of the dominant domain may be in the β-sheet conformation. The amount of β-sheet estimated with this comparison does not depend on the choice of phases, although the positions of the β-sheet strands does.

T-Pos79 THE IONIZATION BEHAVIOR OF FATTY ACIDS BOUND TO BOVINE SERUM ALBUMIN: A ^{13}C NMR STUDY. David P. Cistola, Biophysics Institute, Boston University School of Medicine, Boston, MA 02118.

The metabolism of fatty acids (FA) in the bloodstream is largely influenced by the interaction of FA with serum albumin. This study demonstrates the usefulness of ^{13}C NMR for determining the ionization behavior of saturated FA bound to bovine serum albumin (BSA). Five carboxyl ^{13}C-enriched FA were used: octanoic (8:0), lauric (12:0), myristic (14:0), palmitic (16:0) and stearic (18:0) acids. All samples contained 10% BSA with a 5/1 FA/BSA mole ratio.

^{13}C NMR spectra for 8:0/BSA complexes exhibit one narrow FA carboxyl resonance which shifts from 181.9 ppm at pH 9.4 to 178.0 ppm at pH 3.1, with an apparent pKa of 4.2. Although 12:0/BSA and 14:0/BSA spectra each contain a FA carboxyl resonance which exhibits a complete titration shift, they also contain two and four, respectively, additional resonances which show no titration shift but which decrease in peak intensity with decreasing pH. For 16:0/BSA and 18:0/BSA complexes, a similar behavior is seen except all FA carboxyl resonances show a decrease in intensity with decreasing pH and disappear below pH 4.

The following conclusions are drawn: (1) at pH 7.4, FA bound to BSA is in the ionized form; (2) with respect to ionization behavior, BSA contains at least two types of FA binding sites: one type permits exposure of the FA carboxyl group to the aqueous solvent, and a second type requires interactions between the negatively charged FA carboxyl group and BSA; (3) for the latter type, the number of sites, based on the number of observed resonances, increases with increasing FA chain length.

T-Pos80 THERMODYNAMIC STABILIZATION OF CYTOCHROME C OXIDASE BY MEMBRANE PHOSPHOLIPID. C. Rigell, C. de Saussure and E. Freire, Dept. of Biochemistry, University of Tennessee, Knoxville, TN 37996.

The thermal stability of cytochrome C oxidase has been examined by high sensitivity differential scanning calorimetry and thermal gel electrophoresis. The studies have been performed on detergent solubilized and membrane reconstituted cytochrome C oxidase. The detergent solubilized enzyme undergoes a broad thermal unfolding transition centered at 56°C and characterized by a ΔH of 550 kcal/mole. When reconstituted into dimyristoylphosphatidylcholine (DMPC) vesicles the midpoint of the transition is shifted upwards to 63°C indicating that the phospholipid moiety stabilizes the native structure of the enzyme. The lipid bilayer environment contributes ~10 kcal/mole to the free energy of stabilization of the enzyme complex. The thermal unfolding of cytochrome C oxidase is not a two-state process. Deconvolution analysis of the heat capacity function indicates that the overall curve is composed of four well defined sequential melting steps. The calorimetric experiments have been complemented with thermal gel electrophoresis experiments directed to identify the enzyme subunits involved in each melting step. According to these experiments, the first melting step, at 52°C, involves subunits III and VIa (using the subunit nomenclature of Kadenbach et al., Anal. Biochem. (1983) 129, 517). This step is followed by two very closely spaced melting steps at 61 and 64°C involving the bulk of the enzyme complex (subunits I, II, VIc, VII and remaining low molecular weight subunits). The other melting step probably involves subunit IV. The melting of subunits V and VIb could not be detected by gel electrophoresis and therefore their melting temperatures could not be assigned. (Supported by NIH Grant GM-30819.)

T-Pos81 INTERACTIONS OF DIPHTHERIA TOXIN WITH DIPALMITOYLPHOSPHATIDYLCHOLINE VESICLES CONTAINING GANGLIOSIDES. G. Ramsay and E. Freire, Dept. of Biochemistry, University of Tennessee, Knoxville, TN 37996-0840.

The interactions of Diphtheria Toxin with DPPC large unilamellar vesicles containing different amounts of gangliosides have been studied by high sensitivity differential scanning calorimetry and fluorescence spectroscopy as a function of pH. In aqueous solution, the pH titration profile of Diphtheria Toxin shows two inflection points centered at pH 4.1 and pH 6.2, most likely corresponding to the protonation of aspartic and glutamic acid, and histidine residues, respectively. In the presence of vesicles containing ganglioside Gd_{1a}, only the inflection point at pH 6.2 is observed suggesting that penetration of the protein into the bilayer precludes the complete titration of the protein. High sensitivity differential scanning calorimetry experiments indicate that Diphtheria Toxin associates with the ganglioside containing membrane at all pHs; however, below pH 5, the association results in a dramatic phase separation process characterized by the appearance of a second peak in the heat capacity function centered 8°C above the main phospholipid transition peak. These findings are in agreement with an insertion model in which the toxin molecule undergoes a conformational change at low pH that exposes hydrophobic residues and allow penetration of the toxin into the hydrocarbon core of the bilayer. (Supported by NIH Grant GM-30819.)

T-Pos82 THERMODYNAMIC CHARACTERIZATION OF THE ASSOCIATION OF MYELIN BASIC PROTEIN WITH PHOSPHOLIPID BILAYERS. R. Prabhu and E. Freire, Department of Biochemistry, University of Tennessee, Knoxville, TN 37996.

The association of myelin basic protein (MBP) with phosphatidylserine and phosphatidylglycerol vesicles has been investigated using high sensitivity isothermal reaction calorimetry. These studies were performed using a newly designed multichannel isothermal microcalorimeter (LKB, Bioactivity Monitor) having a sensitivity better than 0.15 μW. Experiments were done by rapidly mixing predetermined amounts of MBP and phospholipid vesicles at temperatures below and above the phospholipid phase transition temperature. In the case of phosphatidylserine the reaction was endothermic (40 kcal/mole of protein) below the transition temperature and exothermic (-120 kcal/mole of protein) above the lipid phase transition temperature. These values are consistent with an interaction model in which the PS molecules perturbed by MBP are in an intermediate state between that of the gel and liquid crystalline molecules. In fact, according to the calorimetric data this state is energetically closer to the gel than to the crystalline state. In the case of dimyristoylphosphatidylglycerol (DMPG) and dipalmitoylphosphatidylglycerol (DPPG) the reaction was exothermic even below the phase transition temperature. The enthalpies of association were -145 kcal/mole for DMPG and -300 kcal/mole for DPPG. This behavior of PG vesicles is consistent with previous studies (Boggs et al (1982) Biochemistry 21, 1208) reporting the existence of metastable states below the lipid phase transition and that the magnitude of the exothermic processes induced by MBP is larger for DPPG than DMPG. (NIH Grant GM 30819).

T-Pos83 EFFECTS OF CYCLOSPORINE A ON BIOMEMBRANES

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The effects of cyclosporine A (CSA) - dipalmitoylphosphatidylcholine (DPPC) interactions were investigated using scanning calorimetry, infrared spectroscopy, and Raman spectroscopy. CSA reduced both the temperature and the maximum heat capacity of the lipid bilayer gel to liquid crystalline phase transition; the relationship between the shift in transition temperature and CSA concentration indicates that the peptide does not partition ideally between DPPC gel and liquid crystalline phases. This nonideality can be accounted for by excluded volume interactions. CSA exhibited a similar but much more dramatic effect on the pretransition; at concentrations of one mole percent CSA the amplitude of the pretransition was less than twenty percent of its value in the pure lipid. Raman spectroscopy confirmed that the effects of CSA on the phase transitions are not accompanied by major structural alterations in either the lipid headgroup or acyl chain regions at temperatures away from the phase transitions. Both infrared and Raman spectroscopic results demonstrate that CSA in the lipid bilayer exists largely in a β-turn configuration, as expected from single crystal X-ray data; the lipid phase transition does not induce structural alterations in CSA. In spite of the dramatic effects of the polypeptide on DPPC model membrane bilayers, CSA neither inhibits hypotonic hemolysis nor causes erythrocyte hemolysis, in contrast to many chemical agents which are believed to act through membrane-mediated pathways.

T-Pos84 ALAMETHICIN INDUCES A VOLTAGE-DEPENDENT CAPACITANCE CHANGE IN BLM's. I. Vodyanoy and J.E. Hall. Dept. of Physiol. and Biophys. Univ. of California Irvine, Irvine CA 92717 and T.M. Balasubramanian and G.R. Marshall Dept. of Physiol. and Biophys. Washington Univ. St. Louis MO 63110. Lipid bilayers formed from two monolayers have a capacitance which depends on the square of the voltage (Alvarez and Latorre Biophys.J. 21:1, 1978). We have measured the voltage-dependent capacitance (VDC) of phosphatidylethanolamine-squalene membranes (area about 7 10^{-4} cm^2) in two ways: first by using voltage pulses, and second by exciting the membrane with a sine-wave voltage and detecting the current with a lockin amplifier. Both methods showed a VDC change of about 1% of the total geometric capacitance when the voltage is increased from zero to 200 mV.

If alamethicin (a negatively charged molecule) is added to one side of the membrane, the VDC decreases when the voltage is positive (trans side ground). If BG, a positively charged alamethicin analogue which turns on when the voltage is negative (Hall et al, Biophys.J. 45: 233, 1984), is added to one side of the membrane, the VDC increases when the applied voltage is negative. BOC 2-20, a neutral alamethicin analogue which always produces a symmetrical current-voltage (I-V) curve, does not alter the VDC. These results imply that voltage changes the thickness of the membrane by moving alamethicin into or out of the membrane depending on the signs of the voltage and the charge on the alamethicin derivative.

We have also measured alamethicin "gating" charge movement using a membrane with alamethicin-induced negative resistance. Voltage was pulsed from a part of the I-V curve where channels are "off" to zero volts where current is zero but a large number of channels is open. Our preliminary results imply that all of the alamethicin molecules in the membrane are affected by the applied voltage but only a small fraction forms channels.

T-Pos85 INTERACTION OF FERRICYTOCHROME C WITH CARDIOLIPIN AND DIPALMITOYLPHOSPHATIDYLCHOLINE BILAYER SYSTEMS: RAMAN AND RESONANCE RAMAN SPECTROSCOPIC STUDY.
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The complex formed by the interaction of cardiolipin and ferricytochrome c exhibits the accepted resonance Raman spectroscopic markers implying that the iron atom is reduced by the association. No obvious reductant exists, however, in the system to induce this change. The visible absorption spectrum of the cardiolipin-ferricytochrome c complex shows shifts of the α,β bands, but does not exhibit the characteristic features of the reduced ferrocytochrome c species. The CH stretching mode region of the Raman spectrum of the lipid component of the system indicates that the number of gauche conformers in the cardiolipin acyl chains has increased upon interaction and that the double bond regions (cis double bond positions 9-10 and 12-13) of the acyl chains are involved in the formation of the complex. Interaction of the protein with cardiolipin appears to change the conformation of the heme coordination to mimic that of reduced cytochrome c without a transfer of an electron such that the iron atom remains in the ferric state. Extrinsic and intrinsic bilayer interactions of cytochrome c with multilamellar DPPC dispersions were studied as a function of pH; temperature profiles derived from specific Raman spectral intensity ratios were constructed. At pH 4, depressions in T_m, the gel to liquid crystalline phase transition temperature, were dramatic (>20°C), while at pH 7, the lowering of T_m was dependent upon protein concentration.

T-Pos86 STRUCTURAL STUDIES OF M13 COAT PROTEIN IN EITHER DEOXYCHOLATE MICELLES OR PHOSPHOLIPID VESICLES USING ^{19}F NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY. H.D. Dettman, J.H. Weiner and B.D. Sykes, Department of Biochemistry and M.R.C. Group on Protein Structure and Function, University of Alberta, Edmonton, Alberta, Canada T6G 2H7.

The coat (gene 8) protein of the filamentous bacteriophage, M13, is an intrinsic membrane protein during phage infection of *E. coli*. Its 50 amino acids may be divided into three regions: an acidic N-terminus; a basic C-terminus; and a hydrophobic core. The hydrophilic and hydrophobic domains have been biosynthetically labelled with the 3-fluoro-analogs of phenylalanine (Fphe) and tyrosine (Ftyr), respectively. Structural information has been obtained by monitoring the motion and exposure of the Fphe and Ftyr residues of the protein bound by either deoxycholate (DOC) or phospholipid vesicles using ^{19}F NMR.

The exposure of the fluoro-residues of the labelled coat proteins in DOC micelles was determined from the results of proteolytic digestions, solvent effects and ^{9}F photo-chemically induced dynamic nuclear polarization experiments; the motions of the Fphe and Ftyr rings were analyzed using a model dependent approach. Fphe11 was found to be outside the micelle, but, at least, partially buried in a hydrophobic pocket, Ftyr21 was at the solvent-micelle interface and Ftyr24 was inside the micelle. The exposure of the fluoro-residues of the labelled coat proteins reconstituted into phospholipid vesicles was determined by chymotryptic digestion and temperature studies; fluoro-residue ring motion analyses were also done. The Fphe residues were outside the bilayer while the Ftyr residues were inside the bilayer. The Fphe residues were not much more mobile than the Ftyr residues, suggesting that the hydrophilic ends were structured.

T-Pos87 PHASE BEHAVIOR OF PHOSPHATIDYLGLYCEROL/PHOSPHATIDYLCHOLINE VESICLES IN THE PRESENCE OF PROTHROMBIN FRAGMENT 1. Barry R. Lentz, Dennis R. Alford, Marcie E. Jones, and Frederick A. Dombrose. Department of Biochemistry, Univ. of North Carolina, Chapel Hill, NC 27514.

We have measured the phase behavior of mixed dipentadecanoyl phosphatidylglycerol ($DC_{15}PG$)/dimyristoyl phosphatidylcholine (DMPC) small, unilamellar vesicles (SUV) in the presence of saturating (> 95% occupancy of binding sites) concentrations of bovine prothrombin fragment 1 and 5 mM Ca^{2+}. Binding of the fragment 1 peptide in the presence of Ca^{2+} was verified by 90° light scattering. Only in the case of pure DMPC SUV were such light scattering measurements not reversible upon addition of EDTA to complex Ca^{2+}. The major effects of peptide binding on SUV phase behavior were to raise the phase transition temperature by between 2 and 15° C, depending on vesicle composition and, in general, to make the phase diagram closely resemble that of large, multilamellar vesicles (Lentz *et al.*, Biochemistry 17, 4475). No evidence was obtained for the existence of lateral membrane domains with distinct compositions induced by the binding of prothrombin fragment 1 plus Ca^{2+}. Surprisingly, fragment 1 without Ca^{2+} also altered the phase behavior of $DC_{15}PG$/DMPC SUV. Most striking was the effect of fragment 1 (with or without Ca^{2+}) on DMPC SUV phase behavior. Freeze-fracture electron microscopy demonstrated that pure DMPC vesicles were induced to fuse following treatment with fragment 1, while vesicles containing $DC_{15}PG$ remained intact. We conclude that Ca^{2+}-dependent binding of prothrombin fragment 1 to $DC_{15}PG$/DMPC does not induce formation of $DC_{15}PG$-rich domains, at least on a thermodynamic time scale (i.e., $\geq 10^{-3}$ to 10^{-5} sec), and involves some interaction in addition to the electrostatic interaction inherent in the commonly accepted Ca^{2+}-bridging model. Supported in part by USPHS grant HL-22771 and by an American Heart Association Established Investigator Award to BRL.

T-Pos88 SATURATION TRANSFER ^{1}H-NMR STUDIES AT 500 MHz OF RHODOPSIN RECONSTITUTED WITH PARTIALLY DEUTERATED LIPIDS: OBSERVATION OF DISTINCT PROTEIN RESONANCES: Alan Deese AND Edward Dratz, Chemistry Dept., University of California, Santa Cruz, California 95064

Saturation transfer studies have been carried out at 500 MHz on sonicated vesicles containing rhodopsin and 1(per-^{2}H-palmitoyl)-2-docosahexaenoyl-sn-glycero-3-phosphatidylcholine [16:Od_{31})(22:6)PC]. In addition to the expected lipid resonances, distinct aromatic and aliphatic protein resonances were observed. These resonances are not observed in sonicated, native ROS membranes. Selective presaturation of any of the protein or lipid resonances results in the transfer of spin polarization into the remaining protein and lipid resonances, but not into the residual HDO resonance.

The pure lipid sample behaves similarly, except that presaturation of single lipid resonances results in the transfer of spin polarization into the HDO resonance, as well as into the other lipid resonances. It appears that the rhodopsin forces water out of the membrane.

Upon bleaching of the rhodopsin sample, a significant broadening of the aliphatic protein resonance is observed, apparently indicating a protein conformational change resulting in motional restriction of that portion of the rhodopsin molecule contributing to the observed aliphatic protein resonance.

T-Pos89 ^{1}H-NMR STUDIES AT 500 MHz OF THE INTERACTION OF D-β-HYDROXYBUTYRATE DEHYDROGENASE (BDH) WITH PHOSPHATIDYLCHOLINE (PC) VESICLES. Alan Deese+, Oliver McIntyre*, Edward Dratz+, Perry Churchill*, Sidney Fleischer*, +Chemistry Dept. U.C. Santa Cruz, Santa Cruz, CA 95064 *Molecular Biology Dept. Vanderbilt University, Nashville, TN 37235.

BDH is a lipid requiring enzyme that specifically requires PC for enzymatic activity. The high resolution and sensitivity obtainable with NMR at 500 MHz have been utilized to study the interaction of BDH with the choline headgroup of PC. The ternary mixture of PC:N,N-dimethylphosphatidylethanolamine (DMPE): phosphatidylpropan-1,3-diol (PP) (1:4:1 wt/wt) was used in this study and mimics the optimal reactivation of BDH obtained by mitochondrial phospholipids. 500 MHz ^{1}H-NMR spectra of pure PC:DMPE:PP unilamelar vesicles show that the choline N-methyl resonance is resolved into two components; a larger downfield component assigned to PC's located in the outer monolayer and a smaller upfield component generated by PC's residing in the inner monolayer.

When BDH is added to PC:DMPE:PP vesicles at a ratio of one BDH per 4-6 PC, the outside choline N-methyl resonance is greatly broadened, whereas the inner PC resonance remains essentially unaffected. The interaction appears to be selective for PC. These observations are interpreted as showing that BDH inserts unidirectionally into the outer monolayer and does not gain access to the PC headgroups on the inner surface. BDH interacts strongly with the choline N-methyl region and greatly inhibits the motion of this segment of the PC headgroup, reflecting the specificity of the enzyme. (Supported in part by NIH Grants: AM 14632, ROI EY00175 and F32 EY05607-03)

T-Pos90 Dissolution and reconstitution of VSV G protein into egg PC vesicles: Studies using energy transfer between lipid probes. R. Blumenthal, M. Ollivon, D. Margolis and O. Eidelman. Section on Membrane Structure and Function, LTB, NCI, NIH, Bethesda, Md.

Reconstitution of membrane proteins requires fine tuning of the conditions in terms of a) the initial concentrations of protein, lipid and detergent and b) the kinetics of detergent removal. With excess detergent above the cmc, protein and lipid molecules are in separate detergent micelles. If the detergent concentration is dropped rapidly below the cmc, protein and lipid will tend to aggregate separately into protein oligomers and liposomes, respectively, before mixing of the two components can take place. There is presumably a critical phase of the detergent:lipid: protein mixture through which the system must go slowly in order to get proper mixing of components needed for functional reconstitution (Eidelman et al, J. Biol. Chem. 259,4622, 1984). In order to characterize this phase in detail we dissolved Egg PC vesicles containing NBD-PE and N-Rh-PE in octylglucoside (OG, cmc about 20 mM). Measurement of energy transfer efficiency (ETE) by donor quenching indicated that the area of the lipid bilayer expands linearly with increasing OG up to 10 mM. Above this concentration ETE decreased faster, followed by a sharp transition in ETE before the system went through the cmc. Leakage of vesicle contents and exchange of lipid probe between vesicles occured at OG concentrations as low as 1 mM. In an attempt to determine what was the optimal point in the phase diagram for insertion of VSV G protein, we incubated Egg PC and G protein at different OG concentrations and dialysed. The resulting virosomes were analysed by gel chromatography and their ability to fuse with negatively-charged acceptor vesicles.

T-Pos91 KINETIC AND STEADY-STATE PROPERTIES OF ACETYLCHOLINE RECEPTOR CHANNELS IN VOLTAGE-CLAMPED RAT MYOBALLS. Lee D. Chabala & Henry A. Lester, Div. of Biology, Caltech, Pasadena, California 91125.

Whole-cell or single-channel currents through acetylcholine (ACh) receptor channels were studied in voltage-clamped rat myoballs or in outside-out patches. ACh channels were activated by the photoisomerizable azobenzene derivative, Bis-Q. Myoballs and patches were bathed in solutions containing 500 nM cis-Bis-Q, the inactive isomer, and a flash was used to produce a concentration jump in agonist, trans-Bis-Q. Kinetic and steady-state properties of agonist-induced currents were studied between −160 mV and +110 mV. The experiments were carried out using symmetrical solutions (Na^+ or Cs^+ on both sides) or asymmetrical solutions (Na^+ in the bath and Cs^+ in the pipette). After the steady-state conductance, $g(V_m, \infty)$, is normalized for the open-channel conductance, it shows an exponential decrease with membrane depolarization. The apparent closing rates (α' ~0.07 ms^{-1} at -100 mV, 15°C) decreases with hyperpolarization and saturates at positive potentials; it cannot, however, account for the voltage dependence of $g(V_m, \infty)$. The effective opening rate, β', was derived from $g(V_m, \infty)$ and α' shows the opposite voltage dependence; it decreases with depolarization and levels off at hyperpolarized potentials. Thus, each rate constant shows both orientation and distortion polarization and thus depends on both the first and second power of membrane potential. In asymmetrical solutions, the dipole moment components are ~30 Debye for α' and ~47 Debye for b', while the polarizability components are ~$(38\ Å)^3$ for β'. When Cs^+ is the charge carrier, the open channel conductance is larger, the kinetics are faster, and β' appears to be smaller. [Supported by an MDA fellowship (L.D.C.) and USPHS grant #NS-11756].

T-Pos92 KINETICS OF OCTYLGUANIDINE BLOCK OF CLOSED ACETYLCHOLINE-ACTIVATED CHANNELS. STEPHEN M. VOGEL, JAY Z. YEH, and TOSHIO NARAHASHI. Northwestern University Medical School, Chicago, IL 60611

N-octylguanidine blocks the end-plate current (EPC) by interacting both with the closed and open states of acetylcholine-activated channels (Farley et al., J. Gen. Physiol. 77, 273, 1981). In the present study, the kinetics of the closed channel interaction were characterized. Frog cutaneous pectoris muscles were voltage clamped by the two-microelectrode method at 22°C. The membrane was held at -40 mV and a clamp step was made to -80 mV; the neurally evoked EPC was elicited at -80 mV following a variable time, Δt, at this potential (0.01-2 sec). Under control conditions, the EPC amplitude was not a function of the time spent at -80 mV. In the presence of octylguanidine (1-24 μM), the EPC amplitude declined with increasing Δt, reflecting a time-dependent block of the channels. Block development had an exponential time course. The time constant, τ, was shortened with increasing drug concentration. The relationship between $1/\tau$ and the drug concentration was linear, suggesting a first-order reaction between the drug molecule and a binding site. A preliminary estimate of the rate constants for the block yielded a forward rate constant of 1×10^2 $M^{-1}s^{-1}$ and a backward rate constant of 1×10^{-4} s^{-1}. The forward rate constant is some five orders of magnitude smaller than that describing block of open channels. When a second guanidino-group was added to octylguanidine to form 1,8-(bis)guanidino-N-octane (3-30 μM), open channel block occurred with a similar forward rate constant as with octylguanidine, but closed channel block disappeared. This suggests a crucial role for the free hydrocarbon tail of octylguanidine in interacting with the closed channel.

T-Pos93 ORGANOPHOSPHATE BLOCKADE OF ACh CHANNELS. B. Cohen and C. Edwards, Department of Biological Sciences, SUNY Albany, Albany, NY 12222 (Intr. by R. Rikmenspoel).

Previous work has shown that millimolar concentrations of organophosphates block acetylcholine receptors (AChR) at the neuromuscular junction. The purpose of this research was to use voltage clamp techniques to investigate the kinetics of organophosphate blockade of AChR. The effect of methanesulfonyl fluoride (MSF) on EPC's and noise served as a control for the anticholinesterase properties of organophosphate used. MSF is an anticholinesterase which is thought not to bind to the AChR. Ten millimolar MSF prolonged EPC decay, but decay remained monoexponential with no significant change in voltage sensitivity, and there was no effect on ACh-induced noise. In contrast, the organophosphate dichlorovos (DDVP) in mM concentrations reversibly produced biphasic EPC decay at membrane voltages from -120 to +40 mV and reduced the voltage sensitivity of the EPC decay. DDVP did not affect the EPC reversal potential. The lowest concentration of DDVP which produced biphasic EPC decay at 10°C was 0.5 mM. Four to eight mM DDVP completely blocked EPC's. The time constant of the fast component of EPC decay increased nearly linearly with DDVP concentration in the range 1-3 mM, while the time constant of the slow component remained nearly constant. DDVP produced double Lorentzian spectra and the cutoff frequency of the fast component increased linearly with DDVP concentration from 0.5 to 1 mM. The data on DDVP are consistent with the open channel blocking model (supported by a contract from the U.S. Army Research Office, No. DAAG 2982K 0065).

T-Pos94 THE BINDING OF A SPIN-LABELED LOCAL ANESTHETIC TO THE ACETYLCHOLINE RECEPTOR AT AN ALLOSTERIC SITE. A. Palma[1], J. Herz[2], H. H. Wang[1], and P. Taylor[2] 1 - Dept. of Biology, Univ. of Calif., Santa Cruz, CA 95064. 2 - Div. Pharmacology, Dept. Medicine, Univ. of Calif., San Diego, CA 92093.

The binding of a spin-labeled local anesthetic, C6SLMeI (a quaternary analog of intracaine), to enriched acetylcholine receptor membranes was studied by electron spin resonance (ESR) and competition with radio-ligands. In the absence of agonists, C6SLMeI bound to acetylcholine receptor membranes (at stoichiometric ratios, 1 - 10:1) shows moderately immobilized spectra. Addition of carbamylcholine results in the appearance of a highly immobilized signal superimposed on the spectrum observed in the absence of agonist. Prior exposure to α-bungarotoxin blocks the carbamylcholine-induced ESR component. In the presence of carbamylcholine, back titration of bound C6SLMeI with phencylidine (PCP) decreases the highly immobilized component suggesting a competitive interaction between C6SLMeI and PCP. This is confirmed by inhibition of ^{3}H-PCP binding by C6SLMeI in the presence of carbamylcholine which yielded a K_D of 6.8×10^{-7}M for the spin label. Scatchard analysis of binding indicated a competitive interaction. In the presence of α-toxin or absence of agonist, C6SLMeI binds with lower affinity ($K_D = 2 \times 10^{-5}$M. At concentrations less than 1×10^{-5}M, C6SLMeI does not bind to the agonist site, but causes an increase in ^{3}H-acetylcholine binding. These studies show that C6SLMeI binds to an allosteric site coupled to the high affinity binding of agonist. This site is characteristic of local anesthetic binding to the receptor.

T-Pos95 PROPERTIES OF SINGLE ACETYLCHOLINE (ACH) RECEPTOR CHANNELS ON DISSOCIATED CNS NEURONS OF LOCUST AND DROSOPHILA. S. Sombati & C.J. Lingle. FSU, Dept. Biol. Sci., Tallahassee, FL

ACh is a major transmitter in the insect CNS. Yet, properties of insect ACh receptor channels have been little studied. We are interested in whether ACh channels in locust neurons have properties similar to those of Drosophila (Wu et al., 1983, J. Neurosci.) and to what extent insect ACh channels correspond to vertebrate classes of nicotinic channels.

Neurons from the metathoracic ganglion of juvenile locusts, Shistocerca americana, were dissociated using a method similar to that described for Drosophila (Wu, et al., 1983). Patch clamp recordings were made from neuron somata after 20-24 hrs in culture. When the electrode contained 1-4 μM ACh, a single class of inward channels was activated in 12 patches. This class of channel was never observed in the absence of ACh. At the resting potential the inward current was about 1.5 pA and the single channel conductance was 21 pS. The extrapolated reversal potential was about 60 mV positive to rest. At the resting potential open time distributions contain two components, one of .26 mS and one of 5.1 mS. The fast component predominates.

In cultured CNS neurons of Drosophila larvae, two classes of ACh-activated channels were observed. One class exhibited a conductance and kinetic structure similar to that of the locust. The extrapolated reversal potential for this 21 pS channel was about 60 mV positive to rest. The second class of ACh-activated channel was observed in 4 of 16 patches where ACh channels were encountered. This class has about twice the single channel current of the first class and occurs with briefer openings. (Supported by NS-19139 and an FSU COFERS award to C. Lingle)

T-Pos96 STOPPED-FLOW TECHNIQUE WITH RECONSTITUTED ACETYLCHOLINE RECEPTOR VESICLES AND ITS APPLICATION

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A stopped-flow technique which is based upon Cs-induced fluorescence intensity decay and allows one to measure kinetic parameters of acetylcholine receptor (AchR)-mediated ion translocation has been optimized for reconstituted Torpedo californica AchR vesicles. A fluorescent dye, 1,5-anthraquinone disulphonate, was loaded into AchR vesicles by using a freeze-thaw method, and the dye outside the vesicles was removed by gel-chromatography. The optimum concentration of dye was found to be 10 mM. The total fluorescence decay increased with decreasing lipid-to-protein ratio, but with low lipid-to-protein ratios the rate of ion translocation by the AchR became too fast to be measured with the stopped-flow technique. A lipid-to-protein ratio of 1:20 was found to be optimum in terms of a good signal-to-noise ratio and a moderate reaction rate. Purification of the crude asolectin, which was used as lipid, was necessary in order to get a flat baseline. Crude asolectin gives a fluorescence decay without adding AchR agonists and this makes kinetic studies difficult. Using the stopped-flow technique, two rate coefficients have been measured over a range of acetylcholine concentration: J_A, the rate coefficient for ion translocation by the active state of AchR in the absence of inactivation (desensitization), and α, the rate coefficient for the fast activation of the receptor by acetylcholine. The method is being used to investigate the effect of inhibitors and chemical modification of the receptor on receptor function. Supported by NIH grant NS 08527.

T-Pos97 ANTICHOLINESTERASE (Anti-ChE) AGENTS INTERACT WITH PRE- AND POST-SYNAPTIC REGIONS OF THE GLUTAMATERGIC SYNAPSE. M. Idriss* and E. X. Albuquerque. (Intr. by Dr. H. Scofano) Dept. Pharmacol. & Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201.

The effects of the organophosphate (OP) and anti-ChE agnt, O-ethyl-S-2-diisopropylaminoethyl-methyl phosphonothiolate, were studied on the metathoracic flexor and extensor tibia muscles of Locusta migratoria. Exposure of the muscles to this OP (10 μM) for 15 min at normal external calcium concentration (2mM) caused repeated episodes of spontaneous firing of endplate potentials (EPPs) and muscle action potentials followed by periods of decreased activity in which miniature endplate potentials could be recorded. At low calcium concentration (0.8 mM) the EPPs were reduced to subthreshold level. This spontaneous activity was blocked by tetrodotoxin (0.3 μM) or a low concentration of external calcium (< 0.2 mM). Exposure to α-bungarotoxin or Naja toxin (10 μg/ml), or atropine (10 μM) for 1 hr did not affect the spontaneous release of the transmitter induced by OP. Coupled with this presynaptic effect, when endplate currents (EPCs) were evoked by nerve stimulation, other alterations were observed in the presence of the OP (10 μM): a decrease in the peak amplitude of the EPC and miniature endplate current, a marked nonlinearity in the current-voltage relationship and shortening of the decay time constant of these currents. Noise analysis disclosed a significant shortening of channel lifetime. Both the presynaptic and the postsynaptic effects of this agent were reversible upon washing the preparation. The present findings demonstrate that this OP anti-ChE agent interacts with the glutamate receptor directly and increases glutamate release. Preliminary experiments have also shown that other anti-ChE agents have similar effects. (Supported by USPHS Grant NS-12063 and U.S. Army Med. Res. & Develop. Command Contract DAMD-17-84-C-4219.)

T-Pos98 PHENCYCLIDINE ANALOGS AS PROBES FOR THE NICOTINIC RECEPTOR-IONIC CHANNEL COMPLEX OF THE NEUROMUSCULAR SYNAPSE. L.G. Aguayo* and E. X. Albuquerque, Dept. Pharmacol. and Exp. Ther., Univ. Maryland, School of Medicine, Baltimore, MD 21201.

The psychoactive drug (1-phenylcyclohexyl) piperidine (PCP) is a noncompetitive antagonist of the nicotinic receptor of the neuromuscular junction and Torpedo electroplax. Similarly to histrionicotoxin (HTX) it caused an increase in affinity of ACh for its binding site. However, electrophysiological studies revealed that, in contrast to HTX, PCP shortened channel lifetime in a concentration-dependent manner. One of its analogs 1-(1-phenylcyclohexyl) morpholine (PCM) also appeared to produce shortening of the decay time constant of the endplate current (τ_{EPC}). PCP produced two effects on the current-voltage (I-V) relationship: 1) a large curvature at potentials from -50 to -150 mV, and 2) a dependence of the reduction of the EPC peak amplitude on the duration of the conditioning pulse. In contrast, PCM caused a depression of peak EPC amplitude, but the I-V relationship (+60 to -150 mV) remained linear. In patch clamp studies on rat myoballs, PCP (4 μM + 100 μM ACh) reduced the frequency of opening and shortened the mean open time of ACh-activated channel currents to about 50% of control values. PCM (10 μM) reduced channel lifetime, but the frequency of channel opening was much less affected. Neither drug changed the channel conductance. The decrease in channel lifetime occurred without any bursting activity. These results suggest a good correlation between the potency of these agents in reducing τ_{EPC} and the mean open channel time of single channel currents. In addition, PCP had the ability to block the channel prior to activation. In conclusion, PCP and PCM block the ionic channel in open conformation, but even at low concentrations PCP also interacts with the closed or resting state of the ionic channel. (Supported by USPHS Grant NS-12063.)

T-Pos99 PERHYDROHISTRIONICOTOXIN (H_{12}-HTX) ENHANCES RECEPTOR DESENSITIZATION WHILE THE ANALOGS DEPENTYL-H_{12}-HTX AND BENZYLAZASPIRO-HTX INTERACT WITH THE ACETYLCHOLINE RECEPTOR-IONIC CHANNEL (AChR) COMPLEX PRIMARILY AS OPEN CHANNEL BLOCKERS. Y. Aracava* and E. X. Albuquerque (Intr. by Dr. P.M. Sokolove). Dept. Pharmacol. Exp. Ther., Univ. Maryland, Sch. Med., Baltimore, MD 21201.

Histrionicotoxins (HTXs), alkaloids obtained from the frog Dendrobates histrionicus, act as noncompetitive antagonists of the nicotinic acetylcholine (ACh) receptor. Biochemical and electrophysiological studies have shown that HTX and H_{12}-HTX increase the affinity of ACh for its binding site and produce a use-dependent depression of both nerve-elicited and microiontophoretically-induced endplate currents, suggesting generation of a desensitized species and block of the closed conformation of the AChR. Since these toxins also appeared to block the open channel of the AChR complex, we attempted to determine the effects of H_{12}-HTX and its analogs depentyl-H_{12}-HTX and benzylazaspiro-HTX on the properties of the single channel currents. H_{12}-HTX (up to 2μM) markedly decreased the channel opening frequency without affecting either the conductance or the open time of the ACh-activated channels. In agreement with the marked acceleration of the decay phase of the endplate currents, both depentyl-H_{12}-HTX (1-20μM) and benzylazaspiro-HTX (2-50μM) significantly shortened the channel open times while the conductance was unaltered and the channel opening frequency was less affected. The presence of the two side chains on the HTX and H_{12}-HTX molecules must play an important role in the effect of this compound on receptor desensitization and interactions with the closed conformation of the AChR. The removal of one side chain from H_{12}-HTX or the addition of a benzyl group to the core of the HTX molecule may account for the reduction of these effects and may satisfy structural requirements for open channel blockade. (Supported by USPHS Grant NS-12063 and U.S. Army Med. Res. & Develop. Command Contract DAMD 17-84-C-4219.)

T-Pos100 DENERVATION OF FROG SKELETAL MUSCLE INDUCES ACETYLCHOLINE-ACTIVATED CHANNELS WITH UNIQUE VOLTAGE AND CONDUCTANCE PROPERTIES. C.N. Allen* and E.X. Albuquerque, (Intr. by L. Mullins) Dept. Pharmacol. & Exp. Therap., Univ. Maryland Sch. Med., Baltimore MD 21201.

Chronic denervation of either mammalian or amphibian skeletal muscle results in the appearance of extrajunctional acetylcholine (ACh) receptor-ionic channel complexes which are grouped in patches of high and low density. The purpose of these studies is to evaluate the characteristics of the newly formed ACh receptors using the patch clamp technique. The interosseal muscles of Rana pipiens were denervated, under chloral hydrate anesthesia, by removing a 1.5 cm section of the right sciatic nerve. The left side remained innervated and served as a control. The muscles were enzymatically dispersed and mounted in a recording chamber using a parafilm-paraffin oil adhesive. Innervated muscles contained a single population of ACh channels characterized by a conductance of 30-32 pS at membrane potentials between -20 to -140 mV. Following denervation (42-44 days), ACh (100-300 nM) at membrane potentials more hyperpolarized than -110 mV activated single channel currents with a conductance of 30-32 pS. In contrast, at membrane potentials between -90 and -110 mV, 80% of the channels had a conductance of 20-22 pS; while the remainder had a conductance of 30-32 pS. At membrane potentials between -30 and -90 mV the majority (> 95%) of the channels had a conductance of 20-22 pS. These data indicate that nerve transection causes a new class of ACh-activated channels to appear which have different voltage and conductance properties. (Supported by USPHS Grant NS-12063 and U.S. Army Med. Res. and Develop. Command Contract DAMD-17-84-C-4219.)

T-Pos101 EFFECTS OF NEOSTIGMINE AND LOW Na ON THE ACETYLCHOLINE RECEPTOR-CHANNEL COMPLEX:VOLTAGE CLAMP AND SINGLE CHANNEL STUDIES IN ADULT FROG SKELETAL MUSCLE. L.Rojas,C.Zuazaga,M. Morales & J.del Castillo,Lab.of Neurobiol.,U.of Puerto Rico Sch. of Med.,San Juan, P.R. 00901.

In low (25%) Na Ringer's solution,neostigmine (NEO)induces an increase in the amplitude and a striking prolongation of the epps which develop a plateau lasting tens of ms (Fatt & Katz,J.Physiol. 115:320,1951).This is associated with similar changes in the epcs (Kordas,J.Physiol.198:81,1968). These effects cannot be accounted for by either AChE inhibition or by a change in the mean open time of the ACh channels,as derived from spectral analysis of voltage or current noise (Katz & Miledi, J. Physiol.231:549,1973;Proc.R.Soc.Lond.B192:27,1975).We have re-examined the combined effects of low Na and NEO.In voltage-clamped end-plates of frog sartorius, replacement of 70% of Na with sucrose decreases the amplitude of the mepcs and increases their half-decay time from 1.8 to 5ms.Addition of NEO (3μm) to the low Na solution increases the amplitude of the mepcs by a factor of about 40 and their half-decay time to 10ms.In low Na solutions,single ACh channels with a 10pS conductance and -40mV reversal potential were observed in 3- day denervated frog sartorius, using gigaohm patch clamp recording.Activity occurred in bursts interrupted by gaps less than 0.5ms, whose exponential distribution could be fitted by a 3.3ms time constant.When NEO (1μM) was added to the pipette,conductance increased to 22pS, the reversal potential remained unaltered and the time constant of burst duration increased to 12ms.These observations suggest a direct action of NEO on the ACh receptor-channel.In solutions of low ionic strength,NEO increases single channel conductance and hinders the dissociation of ACh,leading to repeated channel openings.These direct actions of NEO should be considered in the explanation of its effects on epps.(Supported by NIH grants#NS-07464,NS-14938&RR-08102;L.R.supported by C.D.C.H,U.C.V.,Venezuela.Contribution No.147 of the Lab. of Neurobiology)

T-Pos102 TWO PLASMA MEMBRANE POPULATIONS WITH DIFFERENT CONTENT OF MUSCARINE AND NITRENDIPINE RECEPTORS IN VENTRICULAR MUSCLE MICROSOMES. Neil Brandt, Dept. of Pharmacology, University of Miami School of Medicine, Miami, FL 33101.

Two plasma membrane populations have been identified in rabbit ventricular muscle microsomes fractionated on continuous sucrose gradients. PM-1, banding between 22 and 28% sucrose (w/w) had a high content of muscarine (MUS, 60% of total) receptors and a low content of nitrendipine (NIT, 10% of total) receptors (MUS/NIT=10). This band also contained 70% of all vesicles which entrapped [^{3}H] ouabain during Langendorff perfusion of the heart. PM-2 contained the bulk of the NIT receptors with 40% of total sites in a sharp peak at 35-37% sucrose. The relative content of MUS receptors (10% of total) was similar to that for sarcolemma (isopycnic point 18-20% sucrose) prepared by vesiculation of the cell debris pellet from the microsome preparation (MUS/NIT = 1). The PM-1 and PM-2 bands as determined by the distribution of MUS and NIT receptors were clearly distinguished from SR (31-35% sucrose) whose distribution was assayed by protein and by the specific marker, Ca^{2+} stimulated $Mg^{2+}K^{+}$ methylumbelliferyl phosphatase (Ca MUMBPase). NIT receptors were not found in mitochondrial preparations. PM-1 (MUS/NIT = 5) and PM-2 (MUS/NIT = 1) were also found in microsome preparations from feline ventricular muscle.

Digitonin (0.2 mg/mg protein) was employed to perturb the buoyant density of all plasma membrane vesicles. When PM-1 or the vesicles in the overlap region (28-32% sucrose) were detergent treated, MUS and NIT receptors shifted to densities greater than the SR in those bands. For PM-2, MUS, NIT and CaMUMBPase all shifted to a sharpened band at 40% sucrose, suggesting that some SR in PM-2 was linked to plasma membrane. (Supported by NIH NIRA HL 28804).

T-Pos103 THE EFFECTS OF SPIN LABELED LOCAL ANESTHETICS ON ACETYLCHOLINE RECEPTOR-MEDIATED ION FLUX. Gary D. Blickenstaff and Howard H. Wang, Department of Biology, University of California, Santa Cruz, CA. 95064

The effects of local anesthetics on cation flux mediated by the acetylcholine receptor have studied. Reconstituted vesicles were prepared using purified acetylcholine receptor from Torpedo californica and asolectin (a crude soybean lipid mixture). The local anesthetics used were: (1) tetracaine, (2) dibucaine, (3) a tertiary amine spin label which is also a potent local anesthetic 2-[N-methyl-N-(2,2,6,6-tetramethylpiperidinooxyl)]ethyl-4-hexyloxybenzoate (abbreviated C6SL), (4) the quaternary amine analog of (3)(C6SLMeI), (5) the butyloxybenzoate analog of (3)(C4SL), and (6) the ethyloxybenzoate analog of (3)(C2SL).

The carbamylcholine stimulated influx of radioactive rubidium into the vesicles showed a dose dependent inhibition by the local anesthetics tested. The effect of pH on the C6SL inhibition curve of ion flux was also examined. As the pH is decreased, the dose response curve is shifted, so that a lower concentration of drug is required to achieve an equivalent level of inhibition. Since a decrease in pH represents an increase in the ratio of cationic-to-neutral species of the tertiary amine anesthetic, this work supports the hypothesis that the charged cationic form of the drug is more effective in blocking receptor-mediated ion flux than is the neutral from of the drug.

T-Pos104 SINGLE CHANNEL RECORDING FROM THE AMILORIDE-SENSITIVE EPITHELIAL NA CHANNEL. K. L. Hamilton and D. C. Eaton. Department of Physiology and Biophysics, University of Texas, Medical Branch, Galveston, Texas 77550

We have examined unitary conductance events from the apical Na channel in cultured kidney cells (A6). We have used cell-attached patches with normal A6 media in the patch pipette and inside-out patches with K-Glutamate solution bathing what would normally be the inner surface of the cell membrane. These patches contain an amiloride-blockable Na conductance which has a unit conductance of 7-10 pS and a reversal potential at approximately +40mV (cell interior positive). The reversal potential implies a Na:K permeability ratio of approximately 3-5:1. The current voltage relationship was nearly linear over the range of +80 to -80mV. In cell-attached patches without amiloride, the mean open time when no potential was applied to the patch was 42.7 ± 6.5 msec (5 patches) and mean closed time was 72.3 ± 8.5 msec (5 patches). The ratio of mean open time to mean closed time varied with potential being small at cell negative potentials and approaching 0.5 for cell positive potentials. Amiloride reduced the mean open time in a dose-dependent manner which suggested that one amiloride molecule was blocking one open channel. The amiloride block was also voltage dependent suggesting an amiloride binding site approximately 40% of the way through the membrane field. There also appears to be a second class of Na channels with lower conductance and much higher selectivity. They are differentiated from the poorly selective channels by shorter mean open times, a very positive reversal potential, and a markedly non-linear I-V relationship.

T-Pos105 AMILORIDE RATE CONSTANTS ESTIMATED FROM CURRENT TRANSIENTS IN THE TOAD URINARY BLADDER. L.G. Palmer Dept. Physiol. Cornell U. Med. Coll. N.Y., NY 10021

The voltage dependence of amiloride block of epithelial Na channels in the toad urinary bladder was used to estimate unidirectional amiloride rate constants (k_{on}, k_{off}). Toad bladders were bathed on their serosal sides with high K to reduce basal-lateral voltage and resistance. Mucosal solutions contained submaximal doses of amiloride (0.1 to 0.4 μM). When the transepithelial voltage (V_T) was changed from zero to 50-200 mV, mucosa positive to serosa, the Na current increased instantaneously, due to the increased driving force for Na, and then relaxed exponentially (time constant τ) reflecting the voltage dependence of amiloride block. Plots of $r_A = 1/\tau$ vs. A, the amiloride concentration, were fitted according to the relationship $r_A = k_{on}A + k_{off}$. For V_T = 200 mV and mucosal Na (Na_m) 115 mM, values obtained were k_{on} = 9.6/sec·μM and k_{off} = 2.3/sec. Values are close to those found with noise analysis (Li et al, J. Membrane Biol. 64:77, 1982). When Na_m was reduced to 11.5 mM, k_{on} increased to 17.3/sec·μM, reflecting Na-amiloride competition. k_{off} decreased slightly to 1.7/sec. As V_T was increased from 50 to 200 mV, k_{on} increased by 30%, while k_{off} decreased by 15%. The amplitude of the current transient implied that 10-20% of the membrane voltage is sensed at the amiloride binding site, in agreement with results obtained with I-V analysis.

T-Pos106 EPITHELIAL CHANNEL BLOCKING: WHEN IS $K_A^{micro} = K_A^{macro}$?

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If the corner frequency of current fluctuations due to blocker concentration A has the form $f_c = (1/2\pi)(k_{off} + k_{on}A)$, then the concentration $K_A^{micro} = k_{off}/k_{on}$ can be determined from noise measurements. Is this equal to K_A^{macro}, the 50%-inhibition constant of the DC current? According to Li and Lindemann [J.Membrane Biol.76, 235 (1983)], the equality fails when the inhibition is competitive. But a 4-state model in which blocking competes with sodium clogging can satisfy the equality. We prove the equality for multi-state models in which the blocking ("on") step is the slowest step, i.e., in which the non-blocked states come to equilibrium after each unblocking ("off") step. The proof fails, for example, if a sodium-clogged state is longlived.

[Supported by NIH grant AM 05865].

T-Pos107 GLUTARALDEHYDE FIXATION PRESERVES THE PERMEABILITY PROPERTIES OF THE ADH INDUCED WATER CHANNELS. M.Parisi and J. Merot. CEN Saclay, Dept. Biologie, Lab Biomembranes, 91191 Gif sur Yvette, France

Glutaraldehyde fixation "freezes" ADH target epithelial barriers in a "high permeability state" (after ADH stimulation) or in a "low permeability state" (at rest). Nevertheless, while glutaraldehyde fixed epithelial tissues have been frequently employed, it was unknown if the fixative modified the intrinsec permeability of the water pathway. We have now studied the ADH-induced water channels in frog urinary bladders, before and after glutaraldehyde fixation, by a carefull estimation of the osmotic (Pf) and diffusional (Pd) permeability coefficients. The experimental approach was similar to the one previously employed with artificial lipid bilayers and non-fixed ADH-target tissues. Unstirred layers and non-osmotic pathways were evaluated from ^{14}C-butanol and ^{14}C-sucrose permeability studies. Amphotericin-B incorporation was used to test the validity of the employed methods. As in the case of artificial systems and non fixed toad urinary bladders, it was observed that $\Delta Pf/\Delta Pd$ = 3.08 (slope of correlation study; r=0.83; n=6) after amphotericin-B incorporation in glutaraldehyde fixed frog urinary bladders. In non stimulated preparations the Pf/Pd ratio was not significantly different from 1 but when fixation was performed after ADH treatment (oxytocin, 2.2 10^{-8}M), $\Delta Pf/\Delta Pd$ = 10.67; r=0.86; n=39. This value was not statistically different from the one observed, in similar experimental conditions, in non fixed bladders: $\Delta Pf/\Delta Pd$= 11.19; r=0.86; n=18. It was also compatible with previous reports on measures performed, in different experimental conditions, in non-fixed frog and toad urinary bladders. It can be concluded that the permeability properties of the ADH-induced channels are not modified by glutaraldehyde action, indicating that fixed preparations are a good model for the biophysical characterization of these water pathways.

T-Pos108 ENDOGENOUS CALCIUM INDICATOR REVEALS DEVELOPMENT OF CALCIUM CHANNELS IN HYDROMEDUSAN EMBRYOS. E. B. Ridgway and G. Freeman. Department of Physiology and Biophysics, Medical College of Virginia, Richmond; and Department of Zoology, University of Texas, Austin.

The eggs from a number of species of hydromedusans, including Phialidium, Mitrocomella and Eutonina, contain calcium-activated photoproteins similar to aequorin. In Phialidium the absolute amount of photoprotein per oocyte increases during oogenesis in proportion to oocyte volume. At spawning the eggs of a given female have roughly the same amounts of photoprotein, but there are large (presumably genetic) differences between the amounts in the eggs from different females. The photoprotein is apparently free in the cytoplasm, yet may be excluded from the yolk as judged by image-intensified micrographs of calcium induced light emission from centrifuged (stratified) eggs. KCl depolarization of oocytes or unfertilized eggs does not cause light emission. A KCl response does develop, however, during early cleavage in fertilized eggs and gradually increases in magnitude until approximately 50% of the endogenous photoprotein is flashed. The KCl response is probably related to the appearance of voltage sensitive calcium channels because it is blocked by Co^{++} and nifedipine and is increased with increases in bath calcium. Preliminary microelectrode recordings during cleavage (made with help from Richard Satterlie) show an active membrane response developing. It is not blocked by Ba^{++} or sodium-free saline but is blocked by Co^{++}; again suggesting the appearance of calcium channels in the membrane. Support by NIH grants NS10919 and GM20024.

T-Pos109 HIGH AND LOW AFFINITY NITRENDIPINE BINDING IN MEMBRANE PREPARATIONS FROM GUINEA PIG VENTRICLE: CORRELATION WITH INHIBITION OF CALCIUM CURRENT. W.P. Schilling, S.L. Hamilton, A. Yatani, K. Brush and A.M. Brown. Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550.

Dose-dependent inhibitory action of nitrendipine on I_{Ca} was determined in isolated adult guinea pig ventricular myocytes using patch clamp. Evaluation of the percent decrease in I_{Ca} after a 5 to 8 min. drug exposure at concentrations ranging from 10^{-10} to 10^{-5} M indicated an IC_{50} for effect on resting channels of 5 x 10^{-8} M and an IC_{50} for effect on inactivated channels of 10^{-9} M. In addition, a transient "agonist" effect of nitrendipine was observed at low concentrations (10^{-9} M). In comparison, specific nitrendipine binding in membrane preparations isolated from guinea pig ventricle by a modification of the procedure of Velema and Zaagsma (Arch. Biochem. Biophys. 212:678, 1981) was saturable and of high affinity (K_d = 0.12 ± 0.04 nM and B_{max} = 231 ± 46 fmol/mg protein) when measured in the presence of either 50 mM Tris-Cl, pH 7.4 or 40 mM KCl, 10 mM Tris-Cl, pH 7.4 (low salt). Elevation of KCl in the binding buffer to 150 mM (high salt) resulted in the appearance of low affinity binding sites. Inhibition of [^{3}H]nitrendipine binding by addition of unlabelled nitrendipine over a concentration range of 10 to 1000 nM indicated a K_d = 281 ± 93 nM and B_{max} = 94.8 ± 61 pmol/mg protein for this site. Although the affinity of this site for nitrendipine approximates that required for inhibition of rested Ca channels the B_{max} exceeds that expected for Ca channel density. The high affinity site may represent nitrendipine binding to inactivated channels and/or to the agonist site. Supported by grants HL25145 and HL 32935.

T-Pos110 BINDING AND PHARMACOLOGICAL STUDIES OF NITRENDIPINE ON PC12 CELLS. D.L. Kunze, M.J. Hawkes, S.L. Hamilton and A.M. Brown. Department of Physiology and Biphysics, University of Texas Medical Branch, Galveston, Texas.

^{3}H nitrendipine (NIT) binds in a saturable manner to high and low affinity sites of membranes prepared from PC12 cells. The respective K_D's are 0.7 ± .2 nM and 560 ± 300 nM and the corresponding B_{max}'s give values of about 5 x 10^3 and 5 x 10^5 sites per cell. The K_D and B_{max} for the high affinity sites are similar to values obtained for ^{3}H NIT binding to whole cells. Whole cell and single Ca channel currents were recorded with the patch clamp method. Whole cell Ca currents in 10 or 20 mM Ca_o produced by voltage steps to +10 mV from a holding potential of -100 mV were incompletely blocked, even with doses of NIT as great as 10 uM. The IC_{50} for the block that occurred was 10^{-6} M. However, when the holding potential was reduced to -20 mV where steady state inactivation (h_∞) = 0.5, 50% block occurred using a dose as small as 130 nM. NIT produced a large shift in the h_∞ (V) curve towards hyperpolarizing potentials and using a simple model of voltage-dependent block (Bean et al, J.G.P. 1983), a K_I of 8 nM was calculated for binding to the inactivated state. This is close to the K_D for the high affinity site, and the number of channels calculated from the electrophysiological experiments agrees with the values calculated for the number of high affinity binding sites. At low concentrations (10^{-8} M) a transient increase in Ca current was observed similar to the result in cardiac myocytes (Brown et al, Phys. Soc. Abs., 1984). A transient increase in opening probability was observed in the single channel records at these low doses. This was followed by an increased occurrence of failures or nulls. At higher doses the activity ceased completely. Supported by NIH HL32935, HL25145 and Miles Laboratory Grants.

T-Pos111 CHARACTERISTICS OF BINDING OF BAY k 8644 TO HIGH AND LOW AFFINITY SITES ON CARDIAC MEMBRANES. A.V. Shrikhande, J.G. Sarmiento, R.A. Janis, E. Rutledge and D.J. Triggle. Miles Inst. Preclin. Pharmacol., New Haven, CT, UCONN Med. Ctr., Farmington, CT and SUNY, Buffalo, NY.

The Ca^{2+} channel activator, BAY k 8644, binds with high and low affinity to ventricular membranes (1). Further characterization has shown that the apparent affinity (K, nM) and site density (B, pmole/mg) for the high (h) and low (l) affinity sites are:

TEMP. (°C)	K_h	B_h	K_l	B_l	ΔG° (kcal/mole)	ΔH° (kcal/mole)	ΔS° (e.u.)
15	0.66	0.30	138	14.36			
25	2.40	0.55	232	3.64	-11.8	-21.2	-31.6 (high affinity site)
37	9.12	0.41	72	1.41			

Agonist binding was enthalpy driven while antagonist binding was entropy driven (ΔS°=18.2 e.u.). Specific binding of BAY k 8644 to both sites was modified by diltiazem and verapamil. Ca^{2+} and Mg^{2+} greatly increased the amount of BAY k 8644 bound to the low affinity site under conditions where there was no apparent change in the amount of binding to the high affinity site. The regulation of the low affinity site by the above agents suggests that binding is to a specific site. The association rate of BAY k 8644 to cardiac membranes was biphasic and k_{obs} was independent of concentration. A model with a preexisting equilibrium between a high affinity site and a second low affinity site is consistent with the kinetic data, and is in agreement with the data of Weiland and Oswald (submitted) obtained for nitrendipine binding.

(1) Janis et al., Biochem. Biophys. Res. Comm. 121:317, 1984.

T-Pos112 CALCIUM CHANNELS IN SMOOTH MUSCLE: DIHYDROPYRIDINE ACTION. J.M. Caffrey, I.R. Josephson and A.M. Brown, Dept. of Physiology & Biophysics, Univ. of Texas Medical Branch, Galveston, Texas 77550.

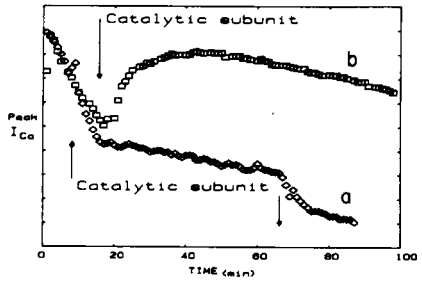
Smooth muscle contraction may be altered by nM concentrations of dihydropyridines but their mechanism of action on calcium channels is unknown. This question was investigated in single smooth muscle cells from Amphiuma stomach isolated by collagenase digestion. Calcium currents (I_{Ca}) were measured in 10 mM Ca_o using the whole-cell suction pipette voltage clamp method. I_K was suppressed with external K^+ channel blockers and by dialysis replacement of K_i^+ with Cs_i^+. At V_H -60mV, I_{Ca} had a threshold of -40 mV and a maximum inward current of ∿ 600 pA at +10 mV. Nitrendipine reduced I_{Ca} in a dose-dependent manner above 0.5 nM; 50 nM resulted in complete block. Agonist effects of nitrendipine (Brown et al, J.Physiol., in press, 1984) were not observed. The dihydropyridine agonist Bay K 8644 increased I_{Ca} in a dose-dependent manner with an ED_{50} of 11 nM. The increase in I_{Ca} was accompanied by a negative shift in activation. Thus there is a good correlation between binding (Bolger at al, JPET, 225:291, 1982) and present electrophysiological results in smooth muscle, a correlation that is weaker in other tissues. Unitary currents through single Ca^{++} channels were measured in the cell-attached mode with Ba^{++} (100 mM) as the permeant ion. At V_R + 60mV the mean current amplitude was 0.7 ± 0.1 pA. The open time distribution was fit by a single exponential with a mean value of 1.3 ± .05 msec. After Bay K 8644 (10^{-5}M) markedly prolonged openings appeared. The open time distribution became bi-exponential and the mean value for the long openings was about 10 times the control value. Similar results have been reported in cardiac muscle (Hess et al, Nat., 311:538 1984; Kokubun & Reuter, PNAS, 81:4824, 1984). Supported by NIH Grant HL25145.

T-Pos113 NITRENDIPINE BINDING TO TRANSVERSE TUBULE AND PLASMA MEMBRANE VESICLES PURIFIED FROM RAT SKELETAL MUSCLE Hemin Chin, NHLBI, NIH and Troy Beeler, USUHS, Bethesda, Maryland (Intr. by Marshall Nirenberg)

Transverse tubules (T-tubules) are invaginations of the surface membrane of muscle fibers that play an important role in excitation-contraction coupling. We have obtained T-tubule membranes of high purity and in high yield by a new method and have compared the properties of T-tubule membranes with plasma membranes. Muscle was homogenized with a Polytron at setting of 2 for 2 min, and extracted with a solution containing 500 mM LiBr, 50 mM Tris·HCl(pH 8.5), and protease inhibitors. T-tubule membranes and sarcolemma membranes were prepared from the supernatant and pellet fractions, respectively, by differential centrifugation and sucrose gradient centrifugation (20-40% step gradient for T-tubule membranes). The yield of T-tubule membranes was 0.4 mg of protein/g of muscle. Both the T-tubule and plasma membrane fractions contained much higher levels of cholesterol, and Na^+,K^+-ATPase and acetylcholinesterase specific activities than the sarcoplasmic reticulum fractions. The plasma membrane fraction consisted of spherical vesicles 2-10 uM in diameter, while the T-tubule fraction contained elongated vesicles approximately 2 uM in diameter (long axis). The protein compositions of T-tubule and plasma membrane fractions, analyzed by SDS-PAGE, were quite different. Voltage-sensitive calcium channels in T-tubule membranes were abundant, as indicated by specific [^{3}H]nitrendipine binding (45 pmol/mg protein), whereas little or no specific binding of [^{3}H]nitrendipine was detected to plasma membranes or sarcoplasmic reticulum membranes. These results suggest that the nitrendipine receptor is a specific marker for rat T-tubule membranes. (Supported by ONR, USUHS, and the NIH).

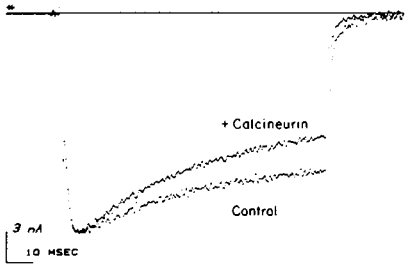
T-Pos114 LEUPEPTIN, AN INHIBITOR OF Ca-DEPENDENT PROTEASES, RETARDS THE KINASE-IRREVERSIBLE, Ca-DEPENDENT LOSS OF CALCIUM CURRENT IN PERFUSED SNAIL NEURONS. J. Chad and R. Eckert, Department of Biology, UCLA, Los Angeles, CA 90024.

Perfusion of Helix neurons is accompanied by a progressive 'wash out' of calcium current, I_{Ca}. This loss is slowed or partially reversed by means that enhance cAMP-dependent phosphorylation (a in fig.; Chad and Eckert, 1984, Neurosci. Abstr. 10:866). However, as Ca-mediated inactivation and wash out proceed, the effectiveness of phosphorylating agents in promoting recovery of I_{Ca} diminishes, suggesting irreversible loss of channels. Wash out is slowed by maneuvers that reduce Ca_i and Ca-dependent inactivation. This suggested a Ca-dependent degradation of the Ca channel that may occur preferentially from its inactivated state, and this was tested under voltage clamp (100ms pulses to +10 mV from -40 mV) with the tripeptidyl aldehyde leupeptin (Aoyagi et al., 1969, J. Antibiotics 22:558), an inhibitor of Ca-dependent proteases (Yoshimura et al., 1983, J. Biol. Chem. 258: 8883). Addition of 100 μM leupeptin to the perfusate had little effect on I_{Ca} amplitude, inactivation, or rate of washout in the absence of phosphorylating additives. However, irreversible loss of I_{Ca} was greatly reduced by perfusion with leupeptin (b in fig.), for introduction of catalytic subunit of cAMP-dependent PK plus ATP-Mg effectively restored I_{Ca} to nearly its original amplitude. The effect of leupeptin suggests that an endogenous Ca-dependent protease may account for the irreversible loss of Ca channel activity in perfused neurons. USPHS NS 8364.



T-Pos115 CALCINEURIN, A CALCIUM-DEPENDENT PHOSPHATASE, ENHANCES Ca-MEDIATED INACTIVATION OF Ca CURRENT IN PERFUSED SNAIL NEURONS. J. Chad and R. Eckert, Department of Biology, UCLA, Los Angeles, CA 90024.

Isolated neurons of Helix aspersa were perfused and voltage clamped under conditions that isolate the calcium current, I_{Ca}. Perfusate containing HEPES (100 mM, pH 7.8), aspartate (20 mM), CsOH (~70 mM) TEA-Cl (10 mM), catalytic subunit of cAMP-dependent protein kinase (~25 μg protein ml^{-1}), ATP-Mg (4 mM) and leupeptin (100 μM) maintained I_{Ca} with little loss for periods greater than 100 minutes. External Ca was 10 mM and internal was nominally 0.01 μM, but is predicted to undergo large excursions near Ca channels during Ca entry. Addition of calcineurin (~80 μg protein ml^{-1}), a Ca-dependent phosphatase (Steward et al., 1982, FEBS Lett. 137:80) to the perfusate in the presence of leupeptin had little effect on peak I_{Ca}, but produced a marked increase in rate of Ca-dependent inactivation during 100ms pulses to +10 mV from -40 mV. This is contrary to the behavior predicted from possible increased Ca-buffering capacity due to addition of a Ca-binding protein, and implies that the increased rate of Ca-dependent inactivation is due to the increased enzymatic phosphatase activity. The enhancement of Ca-mediated inactivation by a Ca-dependent phosphatase supports the hypothesis (Eckert and Chad, 1984, Prog. Biophys. Mol. Biol.) that a Ca-dependent dephosphorylation is the pivotal step effecting the Ca-mediated inactivation of the Ca channel, with phosphorylation required for returning the channel to an activatable state.



T-Pos116 ROLE OF CYCLIC NUCLEOTIDES IN REGULATION OF SLOW CHANNEL FUNCTION IN VASCULAR SMOOTH MUSCLE. Julia M. Ousterhout and Nick Sperelakis. Dept. of Physiology & Biophysics, Univ. of Cincinnati, Cincinnati, OH 45267.

Cyclic AMP (cAMP) is involved in the regulation of myocardial slow channels, presumably via activation of a cAMP-dependent protein kinase and phosphorylation of the slow channels or an associated regulatory protein. Our laboratory has recently shown that cyclic GMP (cGMP) also modulates the slow inward current in myocardial cells, but in a direction opposite to that of cAMP (Wahler and Sperelakis, 1984). The purpose of this study was to determine whether or not cyclic nucleotides regulate slow channel function in vascular smooth muscle (VSM). Cultured VSM cells were prepared from adult rat aortas by enzyme dispersion (collagenase or trypsin) and reaggregation. Ca^{2+}-dependent action potentials (APs) were elicited by electrical stimulation in the presence of tetraethylammonium (TEA, 5-15 mM). Superfusion of the reaggregates with dibutyryl cAMP was found to depress (0.1 mM) and abolish (0.5-1 mM) the TEA-induced APs. In contrast, in an intact strip preparation of the rabbit pulmonary artery, dibutyryl cAMP (0.1-2 mM) had little or no effect on TEA-induced APs. Superfusion of aortic reaggregates with dibutyryl cGMP (1 mM) or 8-bromo-cGMP (0.1-1 mM) also had little or no effect on TEA-induced APs; i.e., they were not inhibited or potentiated. These results suggest that cAMP, but not cGMP, may regulate the function of the voltage-dependent Ca^{2+} slow channels in rat aortic smooth muscle cells.
(This study was supported by NIH Grant HL-31942, Training Grant HL-07382, and Postdoctoral Fellowship HL-06835.)

T-Pos117 ELECTROPHYSIOLOGICAL PROPERTIES OF THE DEPOLARIZING AFTERPOTENTIAL OF HIPPOCAMPAL PYRAMIDAL NEURONS "IN VITRO". A. Konnerth* and H.D. Lux (Intr. by A.L. Obaid) Max-Planck-Institute for Psychiatry, 8033 Planegg-Martinsried, GRF. *Present address: Dept. of Physiology & Pharmacology, Univ. of Pennsylvania School of Dental Medicine, Philadelphia.

The ionic requirements of the depolarizing afterpotential (DAP), which underlies bursting of CA3 hippocampal pyramidal neurons, were studied using intracellular recordings in the brain slice preparation of the rat. The DAPs were associated with a decrease in membrane conductance. With certain assumptions it was possible to elaborate characteristics of the current responsible for the generation of DAPs. This "calculated" DAP-current is voltage dependent and reverses in the presence of K-currents around -50 mV. The DAPs persisted in the presence of anorganic Ca-antagonists (CO, Ni La) and EGTA, which blocked synaptic transmission and afterhyperpolarizations, presumed to be due to a Ca-activated K current. Reducing the Na concentration of the perfusion solution reduced the "calculated-DAP". Our data suggest that a part of the hippocampal pyramidal neurons possess a slow-inactivating Na conductance. We propose that this hypothesized somatic Na conductance plays a central role for the bursting behavior of a part of the hippocampal pyramidal neurons and is under certain circumstances important for epileptogenesis (1,2).

1. Konnerth, A., Heinemann, U., and Yaari, Y. Nature, 307, 69-71 (1984).
2. Taylor, C.P. and Dudek, F.F. Science 218, 810-812 (1982).

Supported by the DFG-grant He 1128/2-3 and a Feodor-Lynen-Fellowship of the Alexander von-Humboldt Foundation to A.K.

T-Pos118 COMPARATIVE STUDIES ON MEMBRANE DEPOLARIZATION IN VARIOUS EXCITABLE CELLS - V.Vasilescu, Eva Katona, Mioara Tripşa, Eugenia Kovács, D.Eremia Medical Faculty, Department of Biophysics, Bucharest, ROMANIA

The present work is designed to be a comparative investigation on the depolarization mechanisms in several types of excitable cells namely in nerve, retinal, muscle cells and in platelets as well.

In the attempt to define the excitation process, the impact between excitant and the excitable structure, the structure and function of excitable channels, the latency and dynamics of triggering and evolution of events in excitable cells, the stage refractory to excitant and the correlations with energetic couplings are taken into account. A co-operative process is considered to exist between the skeleton and channels.

Experimental data obtained in our Laboratory from a long series of investigations by means of the deuteration technique allowing knowledge of the physical state of water, from kinetical studies of transport processes and from certain pharmacodynamical investigations made possible to know the kinetics and the molecular and protonic bases of excitability.

According to all these studies, protons and the protonic configuration have an essential role in the evolvement of excitation and also in the blockage of this process, in this latter case they inducing inhibition of the energetic processes or a change in the conformation of ionic channels or of the membrane skeleton. Moreover when the energetic processes are inhibited always the conformation of ionic channels or of the membrane skeleton is modified wherever the impact between excitant and membrane may be.

T-Pos119 EFFECTS OF Na_i, Ca_i AND ATP ON THE VOLTAGE SENSITIVITY OF THE FORWARD Na/Ca EXCHANGE IN SQUID AXONS. ACTIVATION BY VOLTAGE CLAMP PULSES. Bezanilla, F., DiPolo, R., Caputo, C. and Rojas, H., Dept. of Physiology, UCLA, Los Angeles, CA, CBB, IVIC, Caracas, Venezuela.

Na_o-dependent Ca efflux has been measured in squid axons under voltage clamp and internal dialysis conditions. The standard internal medium contained (mM): 310, TRIS; 40 Na; 4 Mg; 1 EGTA; 350 Aspartate; 280 Glycine. The external medium was artificial sea water with Cl substituted by methanesulfonate and it contained TTX and Cyanide. In axons dialyzed without Na_i, and clamped at 0 mV, Na_o-dependent Ca efflux is about 3 $p.mole.cm^{-2}.s^{-1}$. Hyperpolarization to -40 mV causes an increase in the Ca efflux of 11%. Increasing Na_i (40 mM) decreases total Ca efflux but markedly increases its sensitivity to membrane potential (59%/40 mV). The $K_{1/2}$ for this effect is about 5 mM. Changing the Ca_i from 0.06 to 200 μM does not modify the voltage sensitivity of the Na_o-dependent Ca efflux. ATP increases the magnitude of the Na_o-dependent Ca efflux without apparently changing its voltage sensitivity. Stimulation of Na_o-dependent Ca efflux can be observed with hyperpolarizing pulses as short as 1 msec. When the pulse amplitude and duration were maintained constant, the increase in Ca efflux was found to be proportional to the duty cycle value. Steady hyperpolarizations produce a rise in the Ca efflux which is not sustained but decays with time, suggesting an inactivation-like mechanism. (Supported by CONICIT-NSF joint program, Int-8312953 and S1-1556. and the MDA of America.)

T-Pos120 THE PARADOX OF NEAR ZERO RESTING POTENTIAL OF THE HIGH CONDUCTION VELOCITY SHRIMP GIANT NERVE FIBER. S.F. Fan, P. Brink and M.M. Dewey. Dept. Anatomical Sciences, SUNY at Stony Brook.

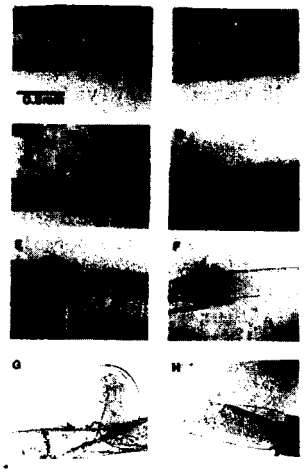
The conduction velocity of the giant nerve fiber of the ventral nerve cord of the shrimp (Penaeus) is high, ranging from 80 to 200 m/sec at 20°C (Fan et al., Kexue Tongbao (Sci. Bull., Peking) (4) 51, 1961; Kusano et al., J. Gen. Physiol. 68:361, 1966; J. Comp. Neurol 142:481, 1971). The diameter of the fiber may reach 250 microns including its thick myelin sheath; the diameter of the axon proper is only 10 microns except where it makes synaptic contact with the motor fiber, here it broadens. Between the myelin and axon there is a large fluid-filled space. The amplitude of the AP (monophasic) reaches more than 80 mV recorded from the fluid-filled space though only occasionally could a resting potential of several tens of mV be recorded. The resting potential of the cell body is -60 to -70 mV. Increasing the $[K^+]_o$ depolarized the cell body while the potential recorded from the giant fiber went to +20 mV at $[K^+]_o$ = 500 mM. After treating the giant fiber with 50% glycerol-50% 500 mM KCl for 5-6 days, the fluid-filled space had a positive potential which increased with decreasing ionic concentration and reached -60mV at pH 7 and $[K^+]_o$ = 5 mM. It reversed polarity at pH>8. The potential vanished when the electrode tip touched the axon. During the increase of ionic strength the cell body depolarized to zero at 500 mM. After heating to 80°C the nerve fiber gave a positive potential at neutral pH. Thus it seems that the shrimp giant fiber has the usual resting membrane potential but it is balanced by a Donnan type potential caused by the charge of the intracellular proteins. The negative resting potential occasionally recorded may be due to the tip of the electrode hitting the axon, thus the potential recorded then would reflect the true resting potential of the axon membrane. NIH GM 26392.

T-Pos121 WHOLE CELL AND SINGLE CHANNEL RECORDING FROM GUINEA-PIG CULTURED MYENTERIC NEURONS. D.J. Adams, K.M. Sanders* and G. Burnstock. Dept. of Anatomy & Embryology, University College London, UK; *Dept. of Physiology, University of Nevada, Reno, NV 89557.

Myenteric neurons from the guinea-pig caecum grown in tissue culture for 8-15 days (Jessen et al. 1978, Brain Res. 152, 573) were studied under current and voltage clamp conditions at 22°C. Whole-cell patch clamp recording with 2-5 M Ω electrodes filled with 140 mM KCl and 0.5 mM K_2EGTA was used to investigate passive and active membrane properties. The resting potential of the neurons was -52±10 mV (±SD, n = 16), and input resistances were 300-500 M Ω. Most myenteric neurons exhibited spontaneous activity; acetylcholine-mediated excitatory potentials sometimes reaching threshold (10-20 mV above the resting potential) to evoke an action potential. Neurons were characterized by action potentials that exhibit a brief after-hyperpolarization (20-50 ms) similar to S-type myenteric neurons described in situ (Hirst et al., 1974, J. Physiol. 236, 303). In response to depolarizing voltage-clamp steps the same myenteric neurons exhibited a transient inward sodium current and a maintained slow inward calcium current which underlie the rising phase of the action potential. Single channel recordings from cell-attached and inside out membrane patches were made using electrodes containing either 5 mM or 140 mM KCl. Ca^{++}-activated K^+ channels were identified by sensitivity to the cytoplasmic Ca^{++} concentration; seen kinetically as an increase in the probability of opening with increasing Ca^{++}. The Ca^{++}-activated K^+ channel had a conductance of ca. 180 pS and the current-voltage relationship was linear with symmetrical K^+ solutions. The open time distribution of Ca^{++}-activated K^+ channels was described by a single exponential function with a time constant of ca. 5 ms at -60 mV. This channel may contribute to the brief after-hyperpolarization observed in this type of myenteric neuron.

T-Pos122 "DESCHWANNING" THE SQUID AXON. H.M. Fishman, UTMB, Galveston, TX, 77550.

The Schwann cell sheath prevents external patch clamping of squid axons without insulating media (1). Desheathing (2) of a small region of axon was carried out by the following means: (a) A finely dissected axon was placed in filtered seawater (SW), which contained 4 mg/ml hyaluronidase (H), 2 mg/ml collagenase (C) and 1 mg/ml trypsin (T), for 40 min to 1 hr at 22°C (Fig. A). (b) The axon was then placed in a 0.5% glutaraldehyde-SW solution for 2 to 3 min. (c) Next, the axon was osmotically shocked in a 10% glycerol-SW solution. The axon shrank and pulled away from the sheath (Fig. B). After 3 min there was sufficient space to cut the sheath with a sharp needle, without damaging the axolemma (Fig. C). Upon reinflation of the axon by placement in normal osmotic strength SW, an area of about .001 cm^2 of axolemma, free of sheath, was obtained (Fig. D). Figs. E, F, G, and H show other desheathed axons. Voltage clamp measurements in axons exposed to HCT solutions for 1 hr showed no change in I_{Na} or I_K. However, glutaraldehyde (0.5% in SW) irreversibly reduced I_{Na} to 90% and I_K to 70% of control values in 3 min. Recovery of ionic currents from hyperosmotic shock yielded currents within 80% of controls. Thus the above solution treatments, when considered separately on intact axons, do not severely impair ion conduction processes. Supported by NIH NS11764. (1) Fishman, H.M. 1973 PNAS 70:876. (2) Metuzals, J. et al. 1981. Cell Tiss. Res. 221:1.



T-Pos123 THE PRESENCE AND ABSENCE OF ION CHANNEL ACTIVITY IN GROWING AND NON-GROWING GROWTH CONES.

C. S. Cohan, P. G. Haydon and S. B. Kater (Intr. by C.-F. Wu) Dept. of Biology, Univ. of Iowa, Iowa City, IA 52242.

Growth cones play an important role in neurite outgrowth and may have important consequences on the final patterns of neuronal morphology and connectivity. We are studying the ionic properties of the growth cone membrane with the aim of determining whether ionic currents are involved in the regulation of neurite outgrowth. Isolated, cultured, buccal neurons of the snail Helisoma produced neurite outgrowth over several days, after which all outgrowth ceased. This enabled us to study single channel ionic currents in both growing and non-growing growth cones. Patch clamp recordings from cell attached patches of growing growth cones revealed a voltage-sensitive, 70 pS ion channel which was associated with long channel lifetimes. In contrast, cell attached patches from non-growing growth cones were silent. This absence of channel activity was not due to the loss of channels since excision of these patches caused channel activity to appear. The presence of channel activity in these excised patches persisted when Ca was removed (EGTA added) from the intracellular bathing solution. In excised patches from both growing and non-growing growth cones, single channel currents were inward at negative membrane potentials and outward at positive membrane potentials when equimolar NaCl solutions bathed each side of the membrane. These Na currents were not blocked by the addition of 100 μM TTX. These data demonstrate that changes in ion channel activity are correlated with changes in the growth properties of neuronal growth cones.

T-Pos124 CA^{+2} AFFINITIES OF HUMAN PLATELETS. Terry Dowd and Bennie R. Ware, Department of Chemistry, Syracuse University, Syracuse, New York 13210

The binding and transport of Ca^{+2} by blood platelets plays a key role in thrombosis and hemostasis. We have studied the affinities of human platelets for Ca^{+2} over the concentration range 10 μM to 2.5 mM. Platelets were prepared by gel filtration and suspended in a physiological ionic strength medium that was buffered for pH (7.4) and for Ca^{+2} concentration. Electrophoretic mobilities at each Ca^{+2} concentration were measured using the laser Doppler technique of electrophoretic light scattering. The titration curves for reduction of electrophoretic mobilities by Ca^{+2} were fit to binding isotherms to determine affinity constants. In a sample of 60 donors, it was found that platelets from female donors had a significantly higher Ca^{+2} affinity than platelets from male donors. However, this sex-related difference was not found when comparing platelets from male and female cigarette smokers. Female smokers and non-smokers showed no significant difference in platelet Ca^{+2} affinities, but platelets from male smokers had a significantly higher Ca^{+2} affinity than platelets from male nonsmokers. Comparisons claimed to be significant had a p value less than 0.02. A number of sex-related differences in the physiology of human platelets have been established, and the correlation of smoking with clinical manifestations of thrombosis is well known. Our results indicate that differences in Ca^{+2} affinities at the external plasma membrane of circulating platelets could be a contributing factor to the mechanisms of these distinctions. (Supported by NIH Grant No. GM-27633)

T-Pos125 A CALCIUM REQUIREMENT FOR ELECTRIC FIELD-INDUCED CELL SHAPE CHANGES AND PREFERENTIAL ORIENTATION E.K. ONUMA and S.W. HUI, Dept. of Biophysics, Roswell Park Memorial Institute, Buffalo, NY 14263

C3H/10T½ mouse embryo fibroblasts stimulated by a steady electric field (10 V/cm) for 30 min exhibited lamellar retraction on the sides facing the electrodes. Some cells elongated and preferentially oriented with their long axis perpendicular to the field direction. Depletion of external calcium or blockage of calcium influx with lanthanum or D-600 resulted in a reduction of the field-induced response. When external calcium was elevated stepwise from 0 to 10 mM, the field-induced response increased correspondily. Electric stimulation in the presence of ionophore A23187 resulted in spindle-shaped cells with no preferential orientation. This response was blocked by calcium depletion and lanthanum, but not by D-600. The effects of the drugs W-12 and W-13 indicated a possible regulatory role for calmodulin. Calcium depletion also reduced the disruption of actin stress fibers induced by the electric field, as observed by fluorescence microscopy using rhodamine-phalloidin labeling. Some cell death resulted from prolonged electric field exposure, and the fatality was reduced by calcium depletion, lanthanum or D-600, but was not affected by the anticalmodulin drugs. We postulate that local calcium influx through channels opened by the electric field produces areas of high intracellular calcium which stimulate the cytoskeletal network to induce lamellar retraction. Prolonged field-induced calcium influx may eventually overcome the cell's mitochondrial calcium-buffer system, leading to necrotic calcification.

T-Pos126 SPATIALLY RESOLVED OPTICAL MEASUREMENT OF MEMBRANE POTENTIAL DISTRIBUTION IN SINGLE CELLS. David Gross,* Leslie M. Loew† and Watt W. Webb*. *School of Applied and Engineering Physics, Cornell University, Ithaca, NY 14853; †Department of Chemistry, SUNY Binghamton, NY 13901 (present address: Dept. of Physiology, Univ. of Connecticut Health Center, Farmington, CT.)

We have monitored cellular membrane potential changes quantitatively and mapped them at the resolution limit of the optical microscope by the use of the new fluorescent indicator of membrane potential, di-4-ANEPPS (#27a in Hassner et al., J. Org. Chem. 49, 2546, 1984). Spatial variation of membrane potential in isolated single cells and in clusters of cells in medium on the culture plate was induced by the application of a uniform external electric field. The epi-illuminated fluorescence image of the cell was detected, stored, analyzed, and displayed by a digital video microscopy system described previously (Gross and Webb, Biophys. J. 41, 215a, 1983; Biophys. J. 45, 269a, 1984). Single and clumped A-431 human carcinoma cells, rat basophilic leukemia (RBL) cells and Uromyces phaseoli fungus spores were studied. We found linear reversible response to induced membrane potential changes up to 9.5% membrane fluorescence increase per 100 mV hyperpolarization with λ_{ex}=546nm, λ_f=590nm. The spatial distribution of membrane potential change ΔVm induced by an external field E correspond to the behavior of the cell as an ideal insulating shell surrounding conducting cytoplasm; for spherical cells of radius r, $\Delta Vm = -1.5\ E\ r \cos\theta$. The membrane potential distribution in clumps of A-431 cells indicated that they are not directly coupled but do severely distort the applied field at the location of the cells. Supported by NSF 8303404, ONR N00014-84-K-0390, the Cornell Biotechnology Center (D.G. and W.W.W.) and NIH GM25190 and a RCDA, CA-677 (L.M.L.)

T-Pos127 REDOX MODIFICATION OF THE CARDIAC SODIUM-CALCIUM EXCHANGE SYSTEM. John P. Reeves and Calvin C. Hale. Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

When cardiac sarcolemmal vesicles were incubated with 1 mM dithiothreitol (DTT) for 60-90 min at 37° C, the initial rate of Na_i-dependent Ca uptake increased 5- to 8-fold. This increase in Na-Ca exchange activity was completely blocked by the inclusion of 0.1 mM EDTA in the medium, suggesting that it resulted from reactive oxygen intermediates generated by the metal ion-catalyzed oxidation of DTT. This was confirmed by the finding that the DTT-induced stimulation of exchange activity was inhibited by 48% in the presence of catalase, by 78% in the presence of superoxide dismutase and by 91% in the presence of both enzymes. Stimulation of exchange activity was not reversed by the subsequent addition of EDTA or by the removal of DTT. The effect of DTT was substrate-dependent, i.e. it was markedly enhanced by the presence of either Na or Ca. This was determined by incubating vesicles with 1 mM DTT in a KCl-based medium for 60 min at 37° C and then adding 0.1 mM EDTA to terminate DTT oxidation; the vesicles were centrifuged, resuspended in 160 mM NaCl, loaded with Na by incubation at 37° C for 30 min., and then assayed for Na_i-dependent Ca uptake. Little or no stimulation of exchange activity was observed unless Na or Ca ions were present during the period of exposure to DTT. Half-maximal stimulation by DTT required 25 mM Na; Ca was less effective than Na in promoting stimulation of exchange activity. The results suggest that oxygen radicals react directly with the Na-Ca exchange carrier to increase its activity, presumably by either oxidizing or reducing a critical group whose reactivity depends upon the conformation of the carrier. The response of the exchange system to oxygen radicals may be important in increasing the rate of Ca extrusion from myocardial cells during cardiac ischemia.

T-Pos128 THE CARDIAC SODIUM-CALCIUM EXCHANGER IS A GLYCOPROTEIN. Pilar de la Peña, Calvin C. Hale, and John P. Reeves. Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

The Na-Ca exchanger of cardiac sarcolemma (SL) has been tentatively identified as a broad band centered at approximately 82 kDa in SDS gel electropherograms (Hale *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, in press). The broadness of the band and its resistance to proteolytic degradation suggest that it might be a glycoprotein. SL vesicles were solubilized with 1% NP40 and 0.2% SDS and treated with endoglycosidase F, which cleaves asparagine-linked oligosaccharide moieties of both the "high mannose" and "complex" varieties. An increase in the mobility of the protein was observed along a broad front below 82 kDa with a sharp band appearing at approximately 65 kDa; these changes were detected with an antibody against the 82 kDa band following electrophoretic transfer from SDS gels onto nitrocellulose paper. A smaller increase in mobility was observed upon treatment with endoglycosidase H, which specifically cleaves the complex type. In order to correlate these changes with Na-Ca exchange activity, SL vesicles were reconstituted into proteoliposomes using soybean phospholipids and treated with endoglycosidases F and H. The initial rates of Na-Ca exchange increased approximately 2-fold as a result of the endoglycosidase treatments. Similar results were obtained when solubilized vesicles were treated with the endoglycosidases and subsequently reconstituted into proteoliposomes, but treatment of native vesicles gave variable results. The apparent activation of Na-Ca exchange activity was accompanied by a partial deglycosylation of the 82 kDa glycoprotein. These results indicate that the cardiac Na-Ca exchanger is a glycoprotein containing high mannose oligosaccharides and provide additional evidence that the 82 kDa band is the exchange carrier.

T-Pos129 CYCLOSPORIN A INFLUENCES MEMBRANE POTENTIAL OF HUMAN AND MOUSE LYMPHOCYTES. A CRITICAL COMPARSION OF STEADY STATE FLUORIMETRIC AND FLOW CYTOMETRIC MEASUREMENTS. Damjanovich S., A. Aszalos*, S. Mulhern*, G. Marti+, M. Balazs, and L. Matyus (Intr. by B. L. Eaton). Med. Univ. School Debrecen, Debrecen, Hungary, *FDA, Washington, D.C. and +NIH, Bethesda, Md.

Fluorescent cationic dyes signal changes in transmembrane potential through changing their location and fluoresence properties in cells as a function of membrane potential. Dihexyloxacarbocyanine iodide ($DiOC_6(3)$) was used in this study to monitor changes in membrane potential of human and mouse lymphocytes. Results from flow cytometric and steady state fluorimetric methods were compared. The direction of the changes in fluorescence signals was highly dependent on the dye concentration, the extracellular ion concentration, the method of measurement, as well as the presence and absence of compounds known to alter the membrane potential. The effect of cyclosporin A (CsA), a fungal metabolite, on the membrane potential of BALB/c and A/J mouse spleen, thymus and bone marrow lymphocytes as well as human peripheral blood lymphocytes was studied both in the flow cytometer and steady state fluorimeter. CsA decreased the membrane potential of both mouse and human lymphocytes. The possibile mechanism of this action was further studied by adding the CsA in combination with A23187, valinomycin, ionomycin, gramicidin and nystatin. Further correlations of membrane potential changes by CsA in combination with insulin, anti Tac antibody, OKT3, OKT4, or OKT8 antibodies were also monitored by flow cytometry. A three compartment model is proposed to explain data obtained from steady state fluorescence measurements, while a two compartment model describes the flow cytometric data.

T-Pos130 STOICHIOMETRY OF PROTON-AMINE ANTIPORT IN ISOLATED CHROMAFFIN GHOSTS. Robert G. Johnson, Frederick Racke, Sally E. Carty, and A. Scarpa. Dept. Medicine, Mass. General Hospital, Boston MA 02114, and Dept. Biochem/Biophysics, Univ. of Pennsylvania, Phila., PA 19104.

The stoichiometry of amine influx and proton efflux was measured in isolated chromaffin ghosts under varying conditions of internal and external pH using a sensitive on-line amperometric glassy carbon electrode for measurement of catecholamine fluxes and an on-line pH electrode for H^+ movement. The determination of the stoichiometry of the proton-amine antiporter contributes to an understanding of the charge of the amine species transported, the charge of the carrier, and the relative contributions of the ΔpH and $\Delta\Psi$ to the driving force. Chromaffin ghosts were formed in isotonic media from bovine chromaffin granules, purified in Percoll gradients by hypo-osmotic lysis of the granules and extensive washing followed by overnight dialysis. Ghosts formed in 185 mM KCl and 0.25-4 mM HEPES, adjusted from pH 6 to 8, and suspended in 0.27 M sucrose medium at the same pH had no pH across their membranes as measured by ^{14}C-methylamine distribution. The addition of nigericin, which catalyzes the electroneutral exchange of K^+ and H^+, resulted in generation of a stable ΔpH, inside acidic, approaching 1 pH unit, which could be maintained for over 10 min. The time course of dopamine accumulation under these conditions was followed with the glassy carbon electrode. Simultaneous recording with the pH-sensitive electrode revealed a time-dependent H^+ efflux. Both amine influx and proton efflux were inhibited by ammonia and harmaline. Preliminary results suggest that the ratio of proton-amine antiport ranges from 1 at pH 6 to 2 at pH 8. Supported by NIH grant HL-18708.

T-Pos131 MICROMOLAR CONCENTRATIONS OF ATP INDUCE RAPID INCREASES IN INTRACELLULAR FREE CALCIUM IN EHRLICH ASCITES TUMOR CELLS. George Dubyak and Mary Beth De Young, Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA 19104.

Ehrlich Ascites tumor cells were loaded with the calcium indicator Quin 2. Addition of 0.4-20 μM extracellular ATP resulted in an increase in cytosolic free calcium ($[Ca^{2+}]_i$) from the resting level of 80 nM to a peak value of 250-750 nM. When extracellular Ca^{2+} ($[Ca^{2+}]_o$) was low (<10 nM) this ATP induced Ca^{2+} transient was characterized by a rapid initial increase in $[Ca^{2+}]_i$ ($t_{\frac{1}{2}}$ = 2 sec.) followed by a decline ($t_{\frac{1}{2}}$ = 1.5 min.) to resting levels. Cells exposed to 1 mM $[Ca^{2+}]_o$ showed a triphasic response: an initial rapid phase identical to that found in the low Ca^{2+} medium, a second phase manifested as a slower increase or plateau in $[Ca^{2+}]_i$, and a third phase characterized by a gradual return to resting $[Ca^{2+}]_i$. The first component may be due to release from intracellular stores. Experiments with the Ca^{2+} ionophore ionomycin in low $[Ca^{2+}]_o$ support this conclusion. The second component of the transient (observed when $[Ca^{2+}]_o$ > 100 μM) may be related to increased permeability of the plasma membrane to Ca^{2+} since its magnitude and duration can be modified by manipulation of $[Ca^{2+}]_o$ or the presence of Ca^{2+} channel antagonists (La^{3+}, Mn^{2+}, diltiazem). ITP and GTP cause qualitatively similar effects but show differing dose dependencies. There was no response to adenosine or AMP and only a slow, reduced response to ADP. The nonhydrolyzable ATP analogue ß-γ(CH_2)ATP was ineffective even at concentrations exceeding 200 μM, suggesting that purine triphosphate hydrolysis may be a necessary step in the observed sequence of reactions. Supported by NIH grants HL18708 and GM-07229.

T-Pos132 CHARACTERISTICS OF INOSITOL TRISPHOSPHATE-INDUCED Ca^{2+} RELEASE FROM TUMOR MICROSOMES Suresh K. Joseph, Kathleen E. Coll, and John R. Williamson, Dept. of Biochemistry and Biophysics, Univ. of Pennsylvania, Philadelphia, Pennsylvania 19104 USA

Inositol trisphosphate (IP_3) mediates the rapid release of Ca^{2+} from the non-mitochondrial Ca^{2+} stores of a number of different insulin secretory tumor cells. In contrast to the hepatocyte, a subcellular fractionation of these tumors yields a microsomal preparation that retains its response to IP_3. We have studied some of the properties of the release mechanism using this preparation. Ca^{2+} release from these vesicles was found to be promoted by glycerolphosphatidylinositol (4,5)-trisphosphate ($GPIP_2$), an analogue of IP_3. The maximal amounts of Ca^{2+} released by this compound was the same as that obtained with IP_3 but the affinity of the release system for $GPIP_2$ was 10-fold lower than observed with IP_3. However, the release occurring with $GPIP_2$ was not followed by the gradual re-uptake of Ca^{2+} normally observed with IP_3. This is compatible with a slower rate of hydrolysis of $GPIP_2$ by the phosphatases contaminating the microsomal preparation. Sequential additions of maximal concentrations of IP_3 to microsomes indicates that the magnitude of Ca^{2+} release produced by the first addition is always greater than obtained with subsequent additions. This progressive decrease in the IP_3 response can be prevented either by using sub-optimal concentrations of IP_3 or by increasing the time interval between the additions. Similar phenomena can also be observed in the permeabilized hepatocyte. This apparent 'desensitization' could arise if Ca^{2+} released from an IP_3-sensitive vesicle were to redistribute into an IP_3-insensitive vesicle. Evidence compatible with this hypothesis will be presented. Supported by NIH grants AM-15120 and AA-05662.

T-Pos133 H^+ PERMEABILITY OF RENAL BRUSH BORDER VESICLES IS INCREASED BY MEMBRANE FLUIDIZING AGENTS. Harlan E. Ives and A. S. Verkman. Cardiovascular Research Institute, University of California, San Francisco, CA 94143.

H^+ permeability (P_H) of brush border membrane vesicles, isolated from rabbit renal cortex, was measured from the rate of collapse of preformed pH gradients using acridine orange fluorescence quenching. N-alkanols increased P_H from 0.005 to 0.1 cm/s in a dose dependent manner. At 25° C, P_H = 0.01 cm/s at [n-alkanol]= 90 mM (butanol), 30 mM (pentanol), 7 mM (hexanol) and 1.8 mM (heptanol). N-alkanols had no significant effect on electroneutral Na^+/H^+ countertransport. Activation enthalpy (ΔH) of P_H was 21.6 kcal/mole (5-50° C); in the presence of 24 mM hexanol, ΔH = 18.5 kcal/mole for T < 33° C and 7.8 kcal/mole for T > 33° C. Membrane fluidity was estimated from diphenylhexatriene fluorescence anisotropy (r). N-alkanols decreased r from 0.25 to 0.18 in a dose dependent manner. At 25° C, r = 0.22 at [n-alkanol] = 200 mM (butanol), 27 mM (pentanol), 9.5 mM (hexanol) and 2 mM (heptanol). Both the increase in P_H and the decrease in r correlated well with known n-alkanol water/lipid partition coefficients. Furthermore, there was a single functional relationship between P_H and r for all n-alkanols. Whereas Na^+/H^+ countertransport is amiloride inhibitable, independent of membrane fluidity and protein mediated - conductive H^+ transport is not amiloride inhibitable, is dependent on membrane fluidity and has a high ΔH suggesting transport by a lipid pathway.

T-Pos134 IN SQUID AXONS, "REVERSE" Na/Ca EXCHANGE IS MODULATED BY INTERNAL Ca AND ATP. R.DiPolo and L. Beauge. IVIC Caracas Venezuela. M.Y.M. Ferreyra, Córdova, Argentina.

We have previously reported (J. Gen. Physiol. 73:91,1979. Cell Calcium 3:194, 1982) that Na_i-dependent Ca influx in squid axons is modulated by the levels of both internal Ca and ATP. We now report that the Ca_o-dependent Na efflux is likewise modulated by the level of Ca_i and ATP. In this work, Ca_o and Na_o-dependent Na efflux has been examined in squid axons under internal dialysis conditions. The standard internal dialysis medium contained (mM): 310 K; 100 Na; 30 TRIS; 4 Mg; 3 EGTA; 310 Aspartate; 138 Cl; 100 glycine. The standard seawater contained ouabain, TTX and cyanide. The results show: 1) Axons dialyzed without both Ca_i and ATP do not show a Ca_o-dependent Na efflux even in 0Na_o-10 Ca_o, nor a Na_o-dependent Na efflux. The level of the flux [less than 1 pmole.cm^{-2}.s^{-1} (P/CS)] is close to that predicted by an electrical "leak". 2) In axons dialyzed with Ca_i (100 μM) and without ATP, Na efflux measured in the presence of 440 mM Na_o is about 4-5 P/CS and barely affected by varying Ca_o between 0 and 20 mM. However in the absence of Na_o, a Ca_o-dependent Na efflux similar in magnitude to that observed in the presence of external Na_o occurs. 3) In the presence of ATP and Ca_i, Na efflux into artificial seawater is close to 20 P/CS. Removal of external Ca drops the efflux to 9 P/CS; subsequent removal of Na_o brings the efflux to "leak" values. In the absence of Na_o, the $K_{½}$ for Ca_o activated Na efflux decreases from 9 to 5 mM. These results indicates an asymetry in the modus operandi of the Na/Ca exchange system with respect to Ca_i and ATP. These two substrates are require from the CIS side to promote Ca_o-dependent Na efflux under nearly physiological conditions. (Supported by CONICIT S1-1144. and NSF-BNS 8025570)

T-Pos135 EVIDENCE FOR AN ESSENTIAL CARBOXYL GROUP ASSOCIATED WITH THE ACTIVE SITE OF THE RENAL OUTER CORTICAL BRUSH BORDER MEMBRANE NA/GLUCOSE COTRANSPORTER. R. James Turner, Membrane Biology Group, Dept. of Medicine, University of Toronto, Toronto, Canada.

The carboxyl-specific reagent N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) irreversibly inactivates the renal outer cortical brush border membrane D-glucose transporter in a time and concentration dependent manner ($T_{1/2}$ ≈ 10 min at 37°C and 1 mM EEDQ). Both glucose transport via the transporter and phlorizin binding to the transporter are equally affected. The effect of EEDQ is to reduce the number of transporters (i.e., the number of phlorizin binding sites) without changing their affinity. The kinetics of inactivation is first order with respect to EEDQ indicating that this effect is due to the action of the reagent at a single essential carboxyl group. The transporter can be protected against EEDQ by glucose and phlorizin, but not by sodium. At high concentrations (> 10 μM) phlorizin provides almost complete protection against EEDQ while the maximal protection provided by glucose is approximately 50 per cent. The transporter can also be protected by nucleophiles such as hydroxylamine (2 mM) and glycine methyl ester (20 mM). This latter result is consistent with the proposed mode of action of EEDQ (formation of a transient intermediate with a carboxyl which then reacts with a neighboring nucleophile -- Belleau et al. Biochem. Pharmacol. 18: 1039, 1969). These results indicate the existence of an essential carboxyl group at or closely related to the active site of the transporter.

T-Pos136 DETERMINATION OF THE PROTON-HYDROXYL ION PERMEABILITY THROUGH PHOSPHOLIPID BILAYERS. Stephan Grzesiek and Norbert A. Dencher. Biophysics Group, Dept. Physics, Freie Universität Berlin, Arnimallee 14, D-1000 Berlin 33, FRG.

In order to compare the passive proton/hydroxyl ion permeabilities of phospholipid bilayers and of the membrane protein bacteriorhodopsin, H^+/OH^- fluxes across soybean phosphatidylcholine (SBPC) and diphytanoyl PC bilayers were measured. The recently published values of the H^+/OH^- permeability coefficient, Pnet, are quite diverse, ranging from 10^{-3} to 10^{-9} cm/s. In the present study, small unilamellar vesicles formed by sonication from SBPC (average diameter of ≈300 Å) or saturated non-oxidizable diphytanoyl PC (diameter ≈650 Å) were subjected to fast (~5ms) pH-jumps of 0.2-2.0 units and the response of different pH-sensitive optical probes was monitored. The buffer capacity of the system, i.e. of the buffer, of the dye molecules and of the lipids, was carefully determined. By creating only small pH-gradients and/or by using valinomycin (in the presence of K^+) or lipophilic ions, any influence of a transmembrane diffusion potential was minimized. From experiments with the pH-indicator pyranine entrapped in vesicles values of Pnet = 1.5×10^{-4} cm/s for SBPC and Pnet = 1.1×10^{-4} cm/s for diphytanoyl PC at 25°C and pH ~7 in the presence of 50 mM K_2SO_4 were calculated; the activation energies are 16.6 kcal/mol and 12.6 kcal/mol, respectively. The presence of Cl^- (100mM) induced a considerable increase in the apparent H^+/OH^- permeability: 1.1×10^{-3} cm/s for SBPC and 2.4×10^{-3} cm/s for diphytanoyl PC. This result indicates that even at neutral pH electroneutral H^+ fluxes can occur by the transmembrane diffusion of molecular HCl. The apparent Pnet determined with the fluorescent amine 9-aminoacridine was about ten times larger than that measured with other pH-probes applied.

T-Pos137 PROTON/HYDROXIDE CONDUCTANCE THROUGH LIPID BILAYER MEMBRANES. John Gutknecht and Anne Walter, Physiology Dept., Duke University, and Duke Marine Lab., Beaufort, NC 28516.

Proton/hydroxide (H/OH) permeability through phospholipid bilayer membranes at pH 7 is about a million-fold higher than alkali/halide ion permeability, but the mechanism of H/OH permeability is unknown. Previous studies of H/OH permeability have been generally limited to vesicle suspensions which provide sufficiently large surface areas for measuring small H/OH fluxes at physiological pH. Our report describes a simple and sensitive way of measuring H/OH conductance ($G_{H/OH}$) through planar bilayer membranes, using pairs of buffered solutions in which the only ionic activity differences are in H^+ and OH^-. $G_{H/OH}$ of bacterial phosphatidylethanolamine-decane bilayers ranges from about 1 nS/cm^2 at pH 1.6 to about 10 nS/cm^2 at pH 10.5. $G_{H/OH}$ of diphytanoyl phosphatidylcholine-decane bilayers ranges from 0.6 nS/cm^2 at pH 1.6 to about 2 nS/cm^2 at pH 12. Transmembrane pH gradients of up to 5 units (pH 4 vs. pH 9) have little effect on $G_{H/OH}$. Because $G_{H/OH}$ is nearly independent of pH, the calculated H^+ permeability coefficient is extremely pH dependent, ranging from 10^{-11} cm/sec at pH 1 to 10 cm/sec at pH 12. Addition of long-chain fatty acids, e.g. phytanic acid, to the membrane increases $G_{H/OH}$, and the pH dependence of $G_{H/OH}$ suggests that fatty acids are acting as H^+ carriers. When water activity is reduced 40% by substituting glycerol for water, $G_{H/OH}$ is reduced about 10-fold in both control and fatty acid containing bilayers. Current-voltage curves are non-linear in both control and fatty acid containing bilayers, and the voltage dependence of $G_{H/OH}$ suggests that the charge carrier crosses a trapezoidal energy barrier. Although the mechanism of H/OH transport is not known, the data are consistent with a proton carrier(s) mechanism, which could be due to traces of fatty acids, hydrolysis or oxidation products in the phospholipids. (Supported by NIH grant GM 28844.)

T-Pos138 INTRACELLULAR Mg INHIBITS INTRACELLULAR pH REGULATION AND CHLORIDE TRANSPORT BY BARNACLE MUSCLE. John M. Russell, Malcolm S. Brodwick and Eddie A. Fort, Department of Physiology and Biophysics, The University of Texas Medical Branch, Galveston, Texas 77550.

The intracellular pH (pH_i) of the giant barnacle muscle fiber is defended against acid insult by an ion transport mechanism believed to exchange intracellular Cl and perhaps H for extracellular Na and HCO_3, thereby neutralizing two acid equivalents for every Cl extruded (e.g., Russell, Boron and Brodwick, J. Gen. Physiol. 82: 47, 1983). Thus, a fall of pH_i stimulates Cl efflux and acid extrusion and both of these fluxes are blocked by SITS or DIDS. By means of the internally dialyzed barnacle muscle fiber preparation, we have shown that both the extra Cl efflux and the acid extrusion stimulated by an acidic pH_i can also be inhibited by raising the Mg content of the internal dialysis fluid. Under normal conditions, pH_i = 7.35, $[Mg]_t$ = 7 mM and $[ATP]_t$ = 4 mM, Cl efflux is about 50 pmol/cm^2·sec (p/c·s) of which about 30 p/c·s can be blocked by SITS or by raising $[Mg]_t$ to 50 mM. However, when pH_i is reduced to ∿ 6.9, Cl efflux increases to 1024 ± 93 p/c·s; application of SITS or DIDS will reduce Cl efflux to ∿ 80 p/c·s. Under these ionic conditions, Cl efflux can also be reduced by raising $[Mg]_t$. Increasing $[Mg]_t$ from 7 to 50 mM decreases Cl efflux to 220 ± 20 p/c·s and also reduces net acid extrusion in the presence of 6 mM HCO_3 from 316 ± 54 p/c·s to 82 ± 21 p/c·s. No effect of Mg could be observed in fibers treated with SITS. These results indicate that increased levels of Mg either directly or indirectly reversibly inhibit the ion transport mechanism responsible for acid extrusion/pH_i regulation in the barnacle muscle fiber. Supported by NIH NS 11946.

T-Pos139 DOMINANT SELECTABLE GENES ALTER TWO MECHANISMS OF MAMMALIAN K^+ TRANSPORT. John Jay Gargus. Department of Physiology and Section of Medical Genetics, Emory University School of Medicine, Atlanta, Georgia 30322.

Somatic cell mutants with alterations in net K^+ transport have been isolated from mutagenized cultures of the mouse LMTK$^-$ cell line by a single-step selection for their ability to grow at K^+ concentrations unable to support the growth of the wild-type cell line (0.2 mM K^+). These mutants fall into two classes: one class (LTK-5) possesses a functionally altered furosemide-sensitive Na-K-Cl cotransport system and the other (LTK-1) an altered K^+ channel (Gargus et al., 1978, PNAS 75:5589-5593). Somatic cell hybrids have been formed between the parent or mutant cell lines (all possessing the TK$^-$ genotype) and a wild-type L cell line possessing the HGPRT$^-$ genotype (501-C) by selection in HAT medium. Both HAT-resistant clonal isolates and plate-selected HAT-resistant clones were subjected to growth at the subthreshold selective K^+ concentration (0.2 mM) as well as to growth in HAT and 5 mM K^+ media. Hybrids formed between LMTK$^-$ and 501-C never gave rise to a cell line able to grow at 0.2 mM K^+; hybrids formed between LTK-5 and 501-C or LTK-1 and 501-C were uniformly able to grow at 0.2 mM K^+. All hybrids grew equally well in HAT or 5 mM K^+ media. In separate experiments, hybrids formed between LTK-5 or LTK-1 and 501-C arose at an equal frequency when selected in HAT medium containing 5 mM K^+ or in HAT medium containing 0.2 mM K^+. Hybrids formed between LMTK$^-$ and 501-C, on the other hand, never arose in HAT medium containing 0.2 mM K^+. These data demonstrate the LTK-1 and LTK-5 mutations to be dominant. Since these two mutations are also clearly selectable, a situation pertains which allows the use of DNA-mediated gene transfer in the isolation of the mutant genes encoding these two different transport proteins. Supported by the Emory University Research Fund.

T-Pos140 REVERSE FLUXES OF Cl THROUGH THE Na:K:Cl COUPLED TRANSPORTER IN SQUID AXON. John M. Russell (Intr. by Carlton R. Caflisch). Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550.

The axolemma of the squid giant axon possesses a tightly coupled 2Na:1K:3Cl uptake process (Russell, J.G.P., 81: 909, 1983). The absolute requirement for Na_o suggests that the overall transport process may be a [Na] gradient-driven process. However, the transport process also has an absolute requirement for ATP. Thus, the source of the driving energy to fuel net Cl uptake remains uncertain. Gradient-driven transport processes are expected to be rather easily reversible (compared with ATP-fueled transport processes). Even under net "forward" conditions, there ought to be a finite, measurable "reverse" transport. Cl efflux (the "reverse" flux) was monitored in internally dialyzed giant axons. It had been previously shown that the "forward" flux (influx) requires cis-side Na and K, can be inhibited by bumetanide and requires intracellular ATP. For the present experiments, fibers were dialyzed with a fluid which always contained 150 mM Cl. The control concentrations of ATP, K and Na in mM were respectively: 4, 350 and 50. Cl efflux from 17 fibers dialyzed with the control fluid was 11.3 ± 2.9 pmoles/cm^2•s (p/c•s). Removal of ATP reduced Cl efflux to 5.8 ± 1.3 (n=7) p/c•s. Replacement of intracellular K with either N-methyl-D-glucamine or TRIS resulted in a Cl efflux of 4.8 ± 2.2 (n=8) p/c•s Removal of intracellular Na in the presence of 350 mM K reduced Cl efflux to 7.8 ± 1.1 (n=8) p/c•s but when internal K was also reduced to 15 mM, Cl efflux declined to 2.4 ± 0.5 (n=4) p/c•s. These data show that a portion of Cl efflux (∿ 5-7 p/c•s) has the properties of Na, K, ATP-dependent Cl influx. These results support the hypothesis that a coupled, reversible Na:K:Cl transporter exists in the squid axolemma which may be gradient-driven. Supported by NIH NS 11946.

T-Pos141 CHARACTERISTICS OF THE Na-Ca EXCHANGER OF CULTURED KIDNEY CELLS OBTAINED BY MEASURING CYTOSOLIC FREE CALCIUM WITH AEQUORIN. Snowdowne, K.W., P.R. Cardillo and A.B. Borle, Department of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, PA, 15261.

Predictions about the characteristics of the sodium-calcium exchanger have been made from studying the effects of sodium on ^{45}Ca transport in surface membrane vesicles. Our objective was to test the validity of some of these predictions using cultured monkey kidney cells in which it is possible to monitor the effects of the removal of extracellular sodium (Na_o) on the cytosolic concentration of free calcium (Ca_i). Ca_i was measured with aequorin (as described in Science 217:252, 1982). The substitution of Na_o with tetramethyl ammonium, choline or lithium invariably caused a transient rise in Ca_i. Ca_i rose from a basal concentration of 60 nM to peak at 500-1000 nM within 2-3 minutes with maximal rates between 300-800 nM/min. Ca_i then fell during the next 15-30 minutes to a plateau at 50-100 nM above basal level. The rise in Ca_i was abolished by the removal of extracellular calcium suggesting that it was due to an enhancement of calcium influx. The size of the transient was enhanced by a brief period of anoxia, by 1 mM sodium cyanide, or by 2 ug/ml FCCP, which suggests that at least some of the enhanced Ca influx was taken up by the mitochondria. The concentration of Na_o yielding 1/2 maximal rise of Ca_i in FCCP poisoned cells was 16 mM. Alkalosis enhanced the effect of the removal of Na_o whereas acidosis reduced it. We conclude that the predictions obtained from investigations using isolated vesicles are valid but, in intact cells, one should consider the ongoing activities of other calcium transport processes.

T-Pos142 PHORBOL ESTER-INDUCED CHANGES OF CYTOPLASMIC pH IN NEUTROPHILS. ROLE OF EXOCYTOSIS IN THE ACTIVATION OF Na^+/H^+ EXCHANGE. S.Grinstein, B.Elder and W. Furuya. The Hospital for Sick Children, Toronto, Ontario, Canada.

Cytoplasmic pH homeostasis was studied in intact and granule-free porcine neutrophils following activation with 12-O-tetradecanoyl-phorbol-13-acetate (TPA). In intact cells TPA activated at least two separate processes: a Na^+-independent and amiloride-insensitive acidification, and a compensatory acid extrusion. The latter is Na^+-dependent and blocked by amiloride and is likely to represent Na^+/H^+ exchange. The acidification is associated with increased H^+ secretion and is parallel to the stimulation of superoxide production and of the hexose monophosphate shunt. Enucleated and degranulated neutrophils (cytoplasts) were prepared by sedimentation of cytochalasin B-treated neutrophils through a discontinuous density gradient. Cytoplasts responded to an artificially imposed acid load with activation of an amiloride-sensitive Na^+/H^+ exchange. TPA also activated both acid production and Na^+-dependent acid extrusion in cytoplasts. The magnitude of the responses was comparable in intact neutrophils and in cytoplasts. These data suggest that: a) The nucleus and the secretory granules are not involved in the acidifying response to TPA, and b) Exocytosis of secretory vesicles is not required in neutrophils for activation of Na^+/H^+ exchange during acid loading or following treatment with the phorbol ester TPA.

T-Pos143 Na^+/H^+ EXCHANGE ACTIVITY IN CANINE CARDIAC SARCOLEMMAL VESICLES. Seiler, S.M., Cragoe, E.J.,Jr.*, and Jones, L.R. The Krannert Inst. of Cardiol. and Dept. of Pharm., Indiana Univ. School of Med., Indianapolis, IN and *Merck, Sharp and Dohme Research Labs, West Point, PA (Intro. by H. Edenberg). Na^+/H^+ exchange was demonstrated in canine cardiac sarcolemmal (SL) membrane vesicles both by measuring rapid ^{22}Na uptake into SL vesicles in response to a transmembrane H^+ gradient, and by following H^+ transport in response to a transmembrane Na^+ gradient with acridine orange. The extravesicular K_m of the Na^+/H^+ exchange activity for Na^+ was determined to be between 2 and 4 mM (pH_i = 5.9, pH_o = 7.9), as assessed by measuring the concentration dependence of the ^{22}Na uptake rate, and the ability of extravesicular Na^+ to collapse an imposed H^+ gradient. Na^+/H^+ exchange was freely reversible. The Na^+/H^+ exchange activity was assayed in membrane subfractions and found most concentrated in SL vesicles, and absent from free and junctional SR vesicles. Na^+ uptake into SL vesicles, driven by a transmembrane H^+ gradient, was not stimulated (or significantly inhibited) by an imposed transmembrane electrical potential (inside negative), suggesting that the Na^+/H^+ exchange mechanism is not electrogenic. ^{22}Na uptake into SL vesicles mediated by Na^+/H^+ exchange was dependent on extravesicular pH, having an optimum around pH 9 (initial internal pH = 6). Although the Na^+/H^+ exchange activity was not inhibited by tetrodotoxin or digitoxin, it was inhibited by quinidine, quinacrine, and amiloride derivatives. The relative potencies of the various inhibitors tested was found to be: quinacrine > quinidine = ethylisopropylamiloride > methylisopropylamiloride > dimethylamiloride > amiloride. The Na^+/H^+ exchange activity identified in cardiac SL vesicles appears to be similar to Na^+/H^+ exchange recently described in intact cell systems. Supported by NIH, MDA, Herman C. Krannert Fund and AHA (Marion county affil.)

T-Pos144 INFLUENCE OF MEMBRANE HYDROLYSIS BY EXOGENOUS PHOSPHOLIPASE A2 ON ENZYME ACTIVITIES OF CARDIAC SARCOLEMMAL MEMBRANES. Robert A. Colvin Cardiology Division Univ. Ct. Hlth. Ctr. Farmington CT 06032

Exogenous phospholipase A2 (PLA2 bee venom) treatment of purified canine cardiac sarcolemmal vesicles resulted in a rapid and time dependent decrease in membrane phospholipid accompanied by accumulation of lysophospholipids and non-esterified fatty acids. After 2 hours of PLA2 exposure, membrane phospholipid decreased from 3.22 ± .31 to 1.06 ± .13 umole/mg. All classes of phospholipid, except sphingomyelin, were hydrolysed whereas total cholesterol content was unaffected. Increases in non-esterified fatty acids were reflected primarily in the unsaturated species oleic (18:1), linoleic (18:2), and arachidonic (20:4). Washing of membranes in 0.1% BSA removed the products of PLA2 hydrolysis while leaving the membrane lipid composition otherwise intact. Na/K ATPase activity was inhibited to 29% of control by 2 hours of PLA2 treatment; and this inhibition was reversed by removal of the hydrolysis products by BSA. Unmasking of latent 3-H ouabain binding by alamethicin suggested that PLA2 treatment resulted in a time dependent loss of sealed vesicles not reversed by BSA washing, that paralleled the time course of phospholipid hydrolysis. When 33% of membrane phospholipids were hydrolysed, Na/Ca exchange activity was inhibited by < 20%. Both peak uptake and initial uptake were inhibited to the same extent. After washing with BSA, initial and peak uptake were inhibited to 30% of control. This inhibition could not be explained by vesicle disruption. These data suggest a lipid requirement for Na/Ca exchange in canine cardiac sarcolemmal vesicles and suggest that the products of PLA2 hyrolysis may enhance exchange activity.

T-Pos145 EFFECTS OF ALCOHOLS AND OTHER HYDROCARBON DERIVATIVES ON INTERNAL Ca AND pH IN SQUID AXONS. G. Vassort, J. Whittembury and L. J. Mullins (Intr. by A. Hybl). Laboratoire de Physiologie Cellulaire Cardiaque U-241 INSERM, Universite Paris XI 91405 Orsay, France; Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, Philadelphia and Department of Biophysics, University of Maryland School of Medicine, Baltimore, U.S.A.

Squid axons were dye-injected to estimate rapid variations of Ca_i (Arsenazo III) or pH_i (Phenol Red); simultaneously membrane potential, Na_i and pH_i were monitored with voltage and ion-sensitive electrodes (Mullins et al., J. Physiol.(Lond.) 338, 1983). The effects of alcohols (pentanol, hexanol, octanol and decanol) and of some other hydrocarbon derivatives (chloroform, hexane, Br hexane, octane, octylamine) were investigated at the resting membrane potential and during alterations of transmembrane Ca movements by Na-free (Li) or K-rich solutions. At concentrations only slightly larger than the one necessary to block conduction and excitation, these substances increased Ca_i independent of the external [Ca] (0 to 112,3 mM); they simultaneously increased pH_i although the Na-H-Cl-HCO_3 exchange was inhibited by the absence of HCO_3 (and Na) in the external solutions, or the presence of SITS. During exposure to Na-free and K-rich solutions, Ca_i increase was larger in the presence of hydrocarbon derivatives although both rates of increase and decrease (on return) were significantly reduced. These effects were still observed in the presence of ryanodine, vanadate or CN. It is, thus, clear that the above substances alter both surface membrane properties and Ca buffering. This could be the result of the release of Ca^{++} and the binding of H^+ by the internal binding sites.

In an attempt to use caffeine, it was observed that it interferes with arsenazo III (and antipyrylazo III) and alters the absorption spectrum.

T-Pos146 CALCIUM REGULATION IN MYXICOLA GIANT AXONS STUDIED WITH OPTICAL SIGNALS AND COMPARED WITH RESULTS ON SQUID GIANT AXONS. R. A. Sjodin and A. M. Ahmed. Department of Biophysics, University of Maryland School of Medicine, Baltimore, MD 21201.

The value of $[Ca^{2+}]_i$ was monitored in intact Myxicola and squid giant axons microinjected with aequorin or arsenazo III. Myxicola giant axons injected with aequorin showed two phases of response to changes in $[Ca^{2+}]_o$. The first phase lasted about 1 hour after injection of aequorin and is characterized by large stepwise increases in light emission as the value of $[Ca^{2+}]_o$ is increased above 3 mM. When test pulses of $[Ca^{2+}]_o$ = 10 mM for 10 minutes duration are introduced against a baseline at 3 mM $[Ca^{2+}]_o$, each succeeding test pulse results in less of a light increase, grading into the second phase in which the basal light emission in 3 mM $[Ca^{2+}]_o$ is not increased even when $[Ca^{2+}]_o$ is increased to 112 mM. Squid giant axons did not show this behavior and were not as tight to changes in $[Ca^{2+}]_o$ as were Myxicola giant axons exhibiting phase II behavior. Injection of apyrase into Myxicola giant axons in phase II resulted in an increase in resting aequorin glow of about 3 fold while similar treatment in squid giant axons resulted in no significant change in resting glow. Apyrase injection made the light signal in Myxicola giant axons very sensitive to increases in $[Ca^{2+}]_o$. Apyrase injection in squid giant axons gave rise to decreases in calcium buffering but only at elevated values of $[Ca^{2+}]_i$. Responses to elevation of $[Ca^{2+}]_o$ near the resting glow in squid axons were relatively unaffected by apyrase, unlike Myxicola axons. Internally dialyzed Myxicola giant axons injected with arsenazo III showed decreased Ca buffering capacity when injected with vanadate, an inhibitor of Ca ATPase, while squid giant axons were relatively unaffected. It is concluded that significant differences between calcium regulation in Myxicola and squid axons occur which should be further investigated.

T-Pos147 FLUCTUATION OF THE Ca^{2+}-SEQUESTERING ACTIVITY OF PERMEABILIZED SEA URCHIN EMBRYOS DURING THE CELL CYCLE. F. A. Suprynowicz, G. Sluder and J. Zimmerberg. Dept. of Cell Biology & Anatomy, Johns Hopkins Medical School, Baltimore, MD; Worcester Foundation for Experimental Biology, Shrewsbury, MA; PSL, DCRT National Institutes of Health, Bethesda, MD

The intracellular Ca^{2+}-sequestering activity can be measured, using ^{45}Ca tracer, in Lytechinus pictus embryos permeabilized by 100-microsecond applications of a 1500-volt/cm electric field. We previously have shown that embryos that are permeabilized at different stages of the cell cycle accumulate tracer at different rates (Suprynowicz and Mazia, J. Cell Biol. 99, 429a), presumably into intracellular organelles since this calcium is released by ionophore A23187 in vitro.

The present work indicates that rates of ^{45}Ca accumulation progressively increase to five times the initial level at successive stages of the first cell cycle (one-cell stage), variably maximal between mitotic prophase and anaphase. Sequestration subsequently decreases by a factor of two and, during the second cell cycle (two-cell stage), undergoes a second oscillation (twofold amplitude) with the same temporal relation to mitosis as the previous fluctation. These same oscillations are present in 10 μM CCCP and 20μg/ml oligomycin.

We have measured rates of ^{45}Ca release at various stages of the cell cycle by diluting the ^{45}Ca specific activity of preloaded, permeabilized suspensions by a factor of 20. Following such dilution, isotope is released at 21-38% of the rate at which it accumulated. The variation in the rate of ^{45}Ca release, however, does not explain the fluctuation in rates of accumulation. Rather, this fluctuation largely represents changes in the rate at which Ca^{2+} is simultaneously sequestered.

Supported in part by NIH grant GM30758 to G.S.

T-Pos148 BAPTA, UNLIKE EGTA EFFICIENTLY SUPPRESSES CA-TRANSIENTS IN CHROMAFFIN CELLS.
E. Neher, Max-Planck-Institut f. biophys. Chemie, D-3400 Göttingen, F.R.G., and
A. Marty, Ecole Normale Supérieure, F-7500 Paris/France (Intr. by W. Stühmer).

We measured outward currents in bovine chromaffin cells using the whole-cell patch clamp configuration. Although we included 11 mM EGTA/1 mM Ca in the pipette solution we routinely observed large Ca-dependent outward currents, resulting in a typical N-shaped current-voltage characteristic. These currents are carried by the calcium-activated K-channel of large unit amplitude (BK-channel) which requires approximately 1 µM [Ca] for activation in the relevant voltage range[1]. Ca-activation occurs through a voltage-dependent Ca inward current[2] of typically $2 \cdot 10^{-5}$ A/cm^2. Since there is every reason to assume that EGTA is diffusing rapidly into the cell interior we must conclude that EGTA is not very efficient in suppressing a Ca-transient.

BAPTA, on the other hand, a Ca-chelator specially designed for fast Ca-uptake and release[3] suppresses the outward current at a concentration of 5.5 mM. Comparison of the two chelators at different concentrations showed that they are equipotent at a molar ratio of approximately 10.

[1]A. Marty, Nature Vol. 291, No. 5815, 497-500 (1981)
[2]E.M. Fenwick, A. Marty, and E. Neher, J. Physiol. 331, 599-635 (1982)
[3]R.Y. Tsien, Biochemistry 19, 2396-2404 (1980)

T-Pos149 SODIUM BINDING SITES ON THE INTESTINAL NA/GLUCOSE COTRANSPORTER. Brian E. Peerce* and Ernest M. Wright. Physiology Department, UCLA Medical School, Los Angeles, CA 90024.

A tyrosine group has been identified at, or near to, the Na^+ binding site of the Na/glucose and Na/proline cotransporters of rabbit intestinal brush borders. Three tyrosine group specific reagents, n-acetylimidazole (NAI), tetranitromethane (TNM), and p-nitrobenzene sulfonyl fluoride (NBSF), were used to evaluate the role of tyrosyl groups on Na^+-dependent glucose transport, Na^+-dependent phlorizin binding, and the Na^+-induced fluorescence quenching of fluorescein isothiocyanate (FITC) bound to the glucose site of the carrier. All three reagents inhibited glucose transport, phlorizin binding, and FITC quenching by 50-85% with K_i's in the range 7-50 uM. The presence of Na^+ during the exposure of membranes to the reagents completely protected against inhibition: the Na^+ concentration required to produce 50% protection was 14-36 mM. A fluorescent derivative of N-acetylimidazole was synthesized to identify the tyrosyl residues on SDS-PAGE. A total of five polypeptide bands were labeled with eosin or fluorescein n-acetylimidazole in a Na^+-sensitive manner. Two of these bands, previously identified as the proline (100,000 daltons) and glucose (75,000 daltons) binding sites of the proline and glucose carriers, account for 50% of the Na^+-sensitive tyrosyl-residues. On the basis of these studies we believe that the Na/glucose cotransporter contains both the Na and glucose active sites on the same polypeptide or that the cotransporter consists of two similar polypeptides, each containing one substrate binding site.

Supported by AM 19567.

T-Pos150 REGULATION OF Na/K/Cl COTRANSPORT IN VASCULAR SMOOTH MUSCLE CELLS. Nancy E. Owen, Dept of Biol. Chem. and Struc., Chicago Medical School, N. Chicago, IL 60064.

The regulation of Na/K/Cl cotransport was investigated in vascular smooth muscle cells. The system was studied using A7r5 cells originally isolated from embryonic rat thoracic aorta. Na/K/Cl cotransport was assayed by measuring K influx using ^{86}Rb as a tracer (Rb was found to substitute quantitatively for K). That a Na/K/Cl cotransport system exists was established by the finding that the ouabain insensitive K influx was sensitive to the "loop" diuretic bumetanide. Furthermore bumetanide sensitive K influx was dependent upon the presence of both Na and Cl in the extracellular milieu. It was found that bumetanide sensitive K influx was not inhibited by the Na/H exchange inhibitor amiloride or by the Cl/HCO_3 inhibitor SITS. However, bumetanide sensitive K influx was inhibited by agents which elevated cellular cyclic AMP (cAMP) levels such as isoproterenol, norepinephrine, prostaglandin E_1 and 8-bromo-cAMP. These agents caused 45%, 58%, 42% and 49% inhibition respectively. That they elevated cAMP levels was confirmed by radioimmunoassay. An agent which elevated cellular cyclic GMP levels, 8-bromo-cyclic GMP, was also found to inhibit bumetanide sensitive K influx (by 32%). In contrast to the cyclic nucleotides, when vascular smooth muscle cells were treated with mitogenic factors such as 10% fetal bovine serum or epidermal growth factor, bumetanide sensitive K influx was increased by 2-fold and 1.3-fold respectively. Thus, the present study provides evidence to suggest that Na/K/Cl cotransport in vascular smooth muscle cells is a regulated system. It can be inhibited by cyclic nucleotides and stimulated by mitogenic agents. The mechanism of regulation is at present not known, but it may involve protein phosphorylation. Supported by US PHS 31959, the Chicago Heart Assoc. and the Schweppe Foundation.

T-Pos151 Ca^{2+} BINDING TO CALSEQUESTRIN. Robert Williams and Troy Beeler, Department of Biochemistry, Uniformed Services University of the Health Sciences, Bethesda, MD 20814-4799

Calsequestrin is a Ca^{2+}-binding protein (Mw ≅ 40,000) located in the lumen of the sarcoplasmic reticulum at the terminal cisternae. It has the capacity to bind 40 moles Ca^{2+} per mole protein. Addition of 1-10 mM Ca^{2+} to isolated terminal cisternae vesicles causes calsequestrin to aggregate. Purified calsequestrin also aggregates in the presence of Ca^{2+}. The time course of aggregation is complex involving at least 4 steps. Following Ca^{2+} binding, calsequestrin undergoes a structural change which results in a large increase in tryptophane fluorescence. Aggregation as measured by turbidity follows a lag time which may be due to a nucleation step. The lag time and rate of calsequestrin aggregation is dependent on the ionic strength, pH, Ca^{2+} concentration, temperature, and dielectric constant of the medium. Aggregation is inhibited by Hg^{2+} and Zwittergent detergents. In the presence of Ca^{2+}, calsequestrin is resistent to trypsin digestion; however, in the absence of Ca^{2+}, trypsin rapidly digests calsequestrin. To further investigate the effect of Ca^{2+} on the structure of calsequetrin, the Raman spectrum of calsequestrin was measured. The Raman spectrum of physiological concentrations of calsequestrin (about 2 mM) does not change significantly as the concentration of potassium is varied from 0 to 1. M. Side chain bands in the Raman spectrum change as the concentration of calcium is varied from 0 to 30 mM, however the intensity distributions of bands sensitive to secondary structure, amide I and III, remain the same, indicating that calsequestrin does not undergo a change in secondary structure greater than 5% under these conditions. (Supported by ONR, NSF PCM-8302893 and USUHS)

T-Pos152 FLUORESCENCE PROBING OF THE CA^{2+} RELEASE CHANNEL OF SARCOPLASMIC RETICULUM Magotoshi Morii, Stefania Danko, and Noriaki Ikemoto Dept. Muscle Res., Boston Biomed. Res. Inst.; and Dept. Neurol., Harvard Med. Sch., Boston, Mass. 02114

Upon reaction of the isolated sarcoplasmic reticulum (SR) with a limited amount of a fluorescent derivative of maleimide, N-(7-dimethylamino-methylcoumarinyl) maleimide (DACM), e.g. 1.25 nmol per mg SR protein, a large fraction of the added DACM was incorporated into low molecular weight proteins (M_r = 32k and 40k). When DACM-labeled SR was used in Ca^{2+} release experiments, the amount of Ca^{2+} released nearly doubled for all types of Ca^{2+} release; viz. that induced by 1) Ca^{2+} jump, 2) drugs such as caffeine and quercetin, and 3) membrane depolarization. Stopped-flow fluorometry of the fluorescence intensity of the protein-attached DACM was carried out in parallel with rapid flow chemical quench studies of Ca^{2+} release. The fluorescence intensity of the protein-attached DACM decreased in parallel with Ca^{2+} release in a variety of conditions. One uM ruthenium red or 1 mM tetracaine, which produces complete inhibition of Ca^{2+} release, has no effect on the release-linked fluorescence change. These results suggest that the changes of DACM fluorescence represent conformational changes of the SR Ca^{2+} channel related to its opening, and that ruthenium red and tetracaine block Ca^{2+} movement occurring after the channel has opened. The results also suggest that the 32k and 40k dalton proteins are the prime candidates for the constituents of the Ca^{2+} channel. Supported by grants from NIH (AM 16922) and MDA.

T-Pos153 AUTOPHOSPHORYLATION OF GLYCERALDEHYDE PHOSPHATE DEHYDROGENASE AND THE INTERACTION WITH PROTEINS FROM SKELETAL MUSCLE MICROSOMES. R. M. Kawamoto and A. H. Caswell. Department of Pharmacology, University of Miami School of Medicine, P.O. Box 016189, Miami, FL 33101.

Glyceraldehyde phosphate dehydrogenase (GAPD) has been identified as a protein which promotes the formation of the triad junction (accompanying abstract). The glycolytic activity of the enzyme is not associated with a phosphorylated enzyme intermediate. However, we have found that in the presence of MgATP, this enzyme can be autophosphorylated. The extent of the phosphorylation has been found to be greater than 0.4mole phosphate per mole tetramer of GAPD. The phosphorylation proceeds in 0.1 mM EGTA with a $t_{\frac{1}{2}}$ of approximately 2 minutes. The phosphoprotein is acid stable but labile in neutral or alkaline conditions suggesting that the complex is an acylphosphate.

The phosphorylation is inhibited by NADH, G3P, calsequestrin and to a lesser extent by NAD^+. The phosphoprotein can be dephosphorylated by NADH and G3P. NAD^+ stimulates a moderate loss of phosphate whereas calsequestrin fails to dephosphorylate the enzyme. Unphosphorylated and phosphorylated GAPD has been incubated with a preparation of terminal cisternae and triads. Phosphorylated GAPD influences the extent of phosphorylation of some triadic proteins. The physiological significance of GAPD phosphorylation is being evaluated. Supported by Muscular Dystrophy Fellowship (to RMK), NIH training grant HL-07188 and NIH grant AM-21601.

T-Pos154 Cl^- STIMULATION OF Ca^{2+} RELEASE IN DISRUPTED CARDIAC CELLS VIA IONIC DEPOLARIZATION OF PLASMA MEMBRANE FRAGMENTS. Sue K. Donaldson, Robert A. Dunn, Jr., Christine E. Kasper and Daniel A. Huetteman. Dept. Physiology, School of Medicine; School of Nursing; University of Minnesota, Minneapolis, MN 55455.

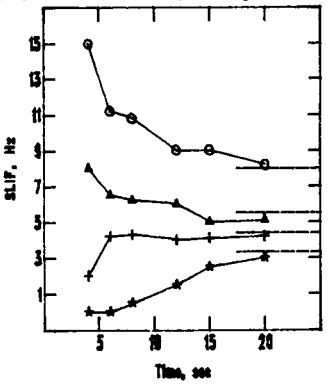
Small bundles of rabbit left ventricular cells were prepared by homogenization to disrupt plasma membranes (PM) (verified by electron micrographs). Disrupted bundles were mounted in a force transducer and immersed in aqueous solutions that mimicked intracellular ATP, CP, and ionic composition (with EGTA as Ca^{2+} buffer and oligomycin as mitochrondrial blocker pH=7.0, 23±1°C). Ca^{2+} releases were monitored as isometric tension transients. To determine if PM depolarization could trigger sarcoplasmic reticulum (SR) release of Ca^{2+} in this preparation, we abruptly substituted Cl^- for impermeant anion (propionate$^-$ or methanesulfonate$^-$) and choline$^+$ for K^+ (at constant $[K^+]x[Cl^-]$) in the bathing solutions. This Cl^- stimulus should depolarize SR and inside-out PM but not intact cells. Cl^- stimulation triggered Ca^{2+} release in the cardiac bundles as did caffeine. Digitoxin (0.01-1 mM) reversibly blocked the Cl^--induced release while the caffeine contracture was unaltered. Since digitoxin should affect only Na-K ATPase of plasma membrane and the caffeine contracture gave evidence of unaltered SR function, these data suggest that Cl^--stimulation triggered Ca^{2+} release by depolarizing PM. This preparation may be useful for studying plasma membrane activation of SR release of Ca^{2+}in cardiac muscle. Supported by NIH grant AM 35132.

T-Pos155 **Ca RELEASE BY NOREPINEPHRINE (NE) FROM INTERNAL SARCOPLASMIC RETICULUM (ISR) IN VASCULAR SMOOTH MUSCLE.** **D. Kowarski, H. Shuman, A.V. Somlyo, A.P. Somlyo. Penn. Muscle Inst., U. of Penn. Sch. of Med., Phil. PA. 19104.**

The subcellular distribution of Ca in cryosections of rabbit main pulmonary artery smooth muscle (MPA) was measured by electron probe microanalysis (EPMA). MPA was quick frozen in a relaxed state or during a 40 sec. maximal NE stimulated contraction (1.2mM Ca Krebs). To eliminate the K peak that normally overlaps the Ca peak in the X-ray spectra, Rubidium (Rb) was substituted for cell K. Maximal contractility of Rb substituted tissues was equal to that of normal K containing tissues. The cell [Rb]:[Na]:[Cl] ratios of 1.0:0.09:0.29 obtained are comparable to the [K]:[Na]:[Cl] ratios of 1.0:0.08:0.3 in normal MPA (1). We found that A) Non-mitochondrial micro-regions containing high [Ca] (up to 30 mmols/kg dry wt) occurred at sites distant (200 nm or more) from the plasma membrane. B) These regions contained the same [Na] and higher [P] compared to the adjoining cytoplasm. C) The frequency of high [Ca] regions ([Ca]>12.0 mmols/kg dry wt) was greater in relaxed than in contracted MPA (P<0.0005). We calculate, using a mathematical model for the inclusion by probes (50nm diameter) of cytoplasm as well as ISR (35nm diameter) and a 5% SR volume (2), that the true [Ca] of the ISR is from 43 to 50 in relaxed and 19 to 33 mmols/kg wt in contracted MPA (P<0.000005). This calculation assumes random sampling, since we could not visualize the ISR in the cryosections. E) Mitochondrial [Ca] was 2.5±0.24 SEM (N=155) mmols/kg dry wt. We conclude that A) NE causes Ca release from the internal SR (not attached to the surface membrane) as well as the junctional SR (3). B) NE can release Ca from the ISR in the presence of normal extracellular Ca. C) the relative sizes of the ISR and mitochondrial Ca pools in relaxed MPA are about 20:1. (1) Casteels et al. J. Physiol. 271,41,1977. (2) Devine et al. J. Cell Biol. 52,690,1972. (3) Bond et al. J. Physiol. 355,677,1984. Supported by HL15835 to PMI, and HL07499 to DK.

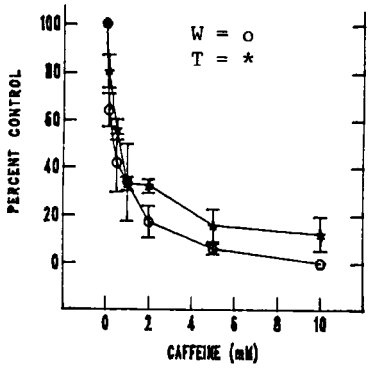
T-Pos156 Ca^{2+}-DEPENDENT OSCILLATIONS IN RAT CARDIAC MUSCLE: TRANSIENT STATE MEASUREMENTS FOLLOWING REGULAR ELECTRICAL DEPOLARIZATION. Arthur A. Kort, Edward G. Lakatta (Intro. by Bruce Simon). Gerontology Research Center, National Institute on Aging, Baltimore, Maryland

Spontaneous sarcoplasmic reticulum (SR) Ca^{2+} release which is asynchronous within and among cells interacts with the myofilaments to produce microscopic motion that causes scattered light intensity fluctuations (SLIF) when a laser beam is passed through unstimulated muscle; in the steady state, SLIF frequency varies with cell Ca^{2+} loading (Circ Res 54: 396, 1984). We now measure SLIF in the transient state following stimulation (S) at 60 min^{-1} at 29°C in $[Ca^{2+}]$ of 2 (*); 3 (+); 6 (▲); or 12 (o) mM. SLIF, quantitated as previously described (J Gen Physiol 82: 119, 1983), were measured during discrete time windows (see Fig. 1) utilizing time-gated averaging. In a single preparation, depending on the Ca^{2+} loading, S transiently (up to 20 sec) suppressed (*) or accelerated (▲, o) SLIF from the steady state level (horizontal lines). We interpret the transient: (1) suppression to indicate a decline in average myoplasmic $[Ca^{2+}]$ due to the absence of spontaneous SR oscillatory Ca^{2+} release; (2) augmentation as an augmentation of total cell Ca^{2+} loading which increase the frequency of Ca^{2+} oscillations and thus increases average myoplasmic $[Ca^{2+}]$. Thus, SLIF monitors transients in cell and average myoplasmic $[Ca^{2+}]$ that follow S without the perturbation of test depolarizations.



T-Pos157 SPONTANEOUS Ca^{2+}-DEPENDENT VS DEPOLARIZATION TRIGGERED DISPLACEMENT IN SINGLE ADULT Ca^{2+} TOLERANT RAT VENTRICULAR MYOCYTES. Maurizio C. Capogrossi, Harold A. Spurgeon, Edward G. Lakatta (Intro. by J. Froehlich). Gerontology Research Center, NIA, Baltimore, Maryland

Mechanical oscillations driven by spontaneous Ca^{2+} oscillations occur in some types of intact unstimulated mammalian cardiac muscle and isolated cardiac myocytes even in the absence of Ca^{2+} overload (Circ Res 54: 396, 1984; Biophys J 45: 94a, 1984). These originate at one or more foci within a cell and propagate as a contractile wave (W) with a velocity of 50-150 $\mu.sec^{-1}$ via diffusion of Ca^{2+} which induces Ca^{2+} release from the sarcoplasmic reticulum (SR) at the wave front. Does this mechanism of Ca^{2+} diffusion induced Ca^{2+} release share common properties with depolarization induced Ca^{2+} release? In single rat myocytes (Hepes buffer, 37°C, 1.0 mM Ca^{2+}) the displacement amplitude of spontaneous W and of twitch in response to depolarization by field stimulation at 72 min^{-1} (T) were measured with a video analyzer over a range of [caffeine]. In control, amplitude of T and W were 11.4±1.7 and 4.2±0.77 um respectively (n = 4). The fig. shows that Ca^{2+} diffusion in W retains the same relative potency as a trigger for SR Ca^{2+} release as depolarization (T) as SR Ca^{2+} loading is progressively reduced by incremental [caffeine] since the amplitude of T and W are reduced proportionately. This suggests that T and W are due to Ca^{2+} release from a common SR "Ca^{2+} pool" and possibly through a common release mechanism.



T-Pos158 NATURE OF SODIUM EFFECT ON HIGH AFFINITY SITES FOR CALCIUM IN CARDIAC SARCOLEMMA PREPARATIONS. M.B. Frankis and G.E. Lindenmayer (Intro. by H.G. Hempling) Depts. of Pharmacology and Medicine, Medical University of South Carolina, Charleston, SC 29425.

A previous study (Circul. Res. 55: in press, 1984) showed that cardiac sarcolemma preparations from canine ventricle possess a species of high-affinity Ca^{2+} binding sites (K_d = 0.205 μm; Bmax = 9.03 nmol/mg; assays at 5°C, pH 7.1 in 157 mM K^+, 3 mM Mg^{2+}, 1 mM EGTA, 0.01 - 10 μM free Ca^{2+}). Nearly complete inhibition of Ca^{2+} binding was observed as Na^+ was raised from 1 to 10 mM, and Scatchard plots showed that Na^+ increased the apparent K_d and decreased Bmax. Thus, Na^+ does not act as a simple competitive inhibitor. Experiments were carried out to test the reversibility of the Na^+ effect. Aliquots of the sarcolemma preparation were exposed to media identical to above except that free Ca^{2+} = 0.2 μM and (K^+ + Na^+) = 157 mM. After 17 hr, the suspension was diluted (" → "; see below) to allow a change in Na^+ in 2 of the 4 assays. The reactions were terminated at 41 hrs and Na^+-sensitive Ca^{2+} binding was determined. Case A: 10 mM → 10 mM Na^+ = 0.523 nmol/mg. Case B: 1 mM → 1 mM Na^+ = 2.453 nmol/mg. Case C: 10 mM → 1 mM Na^+ = 0.996 nmol/mg. Case D: 1 mM → 10 mM Na^+ = 0.725 nmol/mg. Since Case C did not equal Case B, the results suggest that inhibition of Ca^{2+} binding by Na^+ is irreversible for the assay conditions employed. In the absence of Na^+, the Ca^{2+} binding sites are labile in vitro. This is most apparent at 37°C, but is observable at 5°C. The loss of the binding sites with time also appears to be irreversible under the assay conditions used. This suggests that factors, present in vivo and necessary for the maintenance of the sites and reversibility of the sodium effect, are absent in the in vitro assay. (Supported by NIH grant HL29566).

T-Pos159 A FREEZE-FRACTURE STUDY OF THE ATRIAL AND VENTRICULAR MYOCARDIAL CELLS OF NORMAL ADULT CHICKENS D.M. Ryan and S.A. Shafiq - Downstate Medical Center, Brooklyn, NY

The fractured sarcolemmas of atrial (AMC) and ventricular (VMC) myocardial cells revealed profiles of surface caveolae, randomly distributed intramembranous particles (IMP's) ranging in size from 4-10nm in diameter, tall 10nm IMP's at sites of peripheral couplings, gap junctions and desmosomes, and differences with respect to all five structures were present. The surface caveolae were randomly distributed in both types of myocardial cells, but with a higher density of caveolae in AMC as compared to VMC. The density of randomly distributed IMP's was about two-fold higher in VMC as compared to AMC in both fracture faces. Freeze-fracture replicas revealed well-developed SR, and differences with respect to the geometry and quantity of individual components, and the overall archietecture of the SR were noted. The SR in VMC is composed of anastomosing tubular networks (FSR) which are linked by one or more longitudinal tubules (FSR) to broad, flattened expanses of extrajunctional SR (EJSR) overlying the I-Z-I band region. The FSR in AMC forms elaborate retes with fenestrations around the middle of A- and Z-bands. These retes are connected to distended pouches of EJSR which overlie the I-bands by several widely patent tubules (FSR) which run parallel to the long axis of the sarcomere. The architecture of the FSR in atrial and ventricular myocardial cells is strikingly similar to that of the posterior (PLD; fast) and anterior (ALD; slow) latissimus dorsi muscles (Ryan, D.M. and S.A. Shafiq. 1980. Anat. Rec. 198:147-161.), respectively, and the EJSR in cardiac muscles replaces the JSR in skeletal muscles. The distribution and density of IMP's in the SR (Ca^{2+} - ATPase) are similar in AMC and VMC and fall in a range in between that of the PLD and ALD muscles. These findings establish fiber type heterogeneity in the chicken heart based on freeze-fracture characteristics and further substantiate the immunocytochemical and biochemical findings from other labs.

T-Pos160 THE HIGH RESTING FREE CALCIUM CONCENTRATION IN HYPERTHERMIC MUSCLES MIGHT BE ASSOCIATED TO A DIMINISHED CAPACITY OF CALCIUM UPTAKE BY THE SARCOPLASMIC RETICULUM. M. Condrescu, J.R. López and L. Alamo, Centro de Biofísica y Bioquímica, Instituto Venezolano de Investigaciones Científicas, Apartado 1827, Caracas 1010-A, Venezuela.

In a recent study, Lopez et al. provided evidence for the existence of an alteration of the free calcium homeostasis in muscle fibers proceeding from Malignant Hyperthermic patients (Muscle and Nerve, 1984 in the press), and suggested that a deficient function of the sarcoplasmic reticulum membrane might be important in the pathophysiology of the hyperthermic syndrome. In this investigation we compared the capacity of calcium uptake and the (Ca^{2+} + Mg^{2+})-ATPase activity of crude sarcoplasmic reticulum vesicles prepared from Malignant Hyperthermia susceptible and control human muscles. The Calcium uptake was 0.29 ± 0.04 μmoles/mg protein.min (M ± SEM, n=8) in sarcoplasmic reticulum vesicles obtained from Malignant Hyperthermia susceptible patients while in control subjects it was 1.01 ± 0.05 μ moles/mg protein.min (M ± SEM, n=8). The (Ca^{2+} + Mg^{2+})-ATPase activity was also considerably lower in malignant hyperthermic sarcoplasmic reticulum (0.12 ± 0.01 μmoles Pi/mg protein.min, n=8) than in normal (0.36 ± 0.01 μ moles Pi/mg protein.min, n=8). These results were in good agreement with the intracellular free calcium determinations, suggesting that the increase of the free resting calcium concentration in malignant hyperthermic muscles might be due to a deficiency in the capacity of calcium uptake by the sarcoplasmic reticulum.

(Supported by CONICIT grant S1-1277 and MDA).

T-Pos161 PURIFICATION AND CHARACTERIZATION OF PHOSPHOLAMBAN : A REGULATOR OF Ca PUMP ATPase OF CARDIAC SARCOPLASMIC RETICULUM. M. Tada, M. Inui, M. Kadoma, J. Fujii, and K. Ohtsu. The First Department of Medicine, Osaka University School of Medicine, Osaka 553, Japan.

Phospholamban (PLN), a putative regulator of Ca pump ATPase of cardiac sarcoplasmic reticulum (SR), was purified from canine cardiac SR vesicles and its molecular characteristics were examined. After solubilization of SR proteins by deoxycholate (DOC) and fractionation by 25% ammonium sulfate, the resulting pellet was dissolved in non-ionic detergent $C_{12}E_8$ (dodecyloctaoxyethylene glycol monoether), and was further fractionated by high performance liquid chromatography on TSK-G3000SW column in 1 mg/ml $C_{12}E_8$ and 1 M KI. The fraction enriched in PLN was applied to CM-Sepharose CL-6B column and was eluted with a linear KCl gradient. Purified PLN showed a single band of 22,000 daltons on neutral sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (Weber and Osborn), while 27,000 daltons on alkaline SDS gel (Laemmli). Boiling of PLN in 2% SDS produced total conversion into the lower molecular mass component on SDS gels (10,500-dalton on Weber and Osborn gel and 11,000-dalton on Laemmli gel). While purified PLN retained its inherent properties to be catalyzed by cAMP-dependent protein kinase, phosphorylated PLN exhibited lower electrophoretic mobility (24,000-dalton on Weber and Osborn gel and 29,000-dalton on Laemmli gel). The reactivity of anti-PLN antisera was examined by indirect immunofluorescence labeling method on Western blot. The fluorograph showed that the antibodies to PLN specifically bound to cardiac SR PLN with 27,000- and 11,000-dalton form (Laemmli system). PLN may have a multimeric structure, although its submolecular structure remains to be examined. The antibody against PLN may allow us to gain further insights into the structural characteristics.

T-Pos162 EFFECTS OF DIMETHYLSULFOXIDE AND PROPRANOLOL ON CA TRANSPORT BY SARCOPLASMIC RETICULUM VESICLES. Hitoshi Takenaka, Catherine A. Childs, and Arnold M. Katz, Cardiology Div., University of Connecticut, Farmington, CT 06032.

To define the relationship between the Ca ATPase reaction and Ca fluxes by skeletal sarcoplasmic reticulum vesicles (SR), effects of propranolol (PROP) and dimethylsulfoxide (DMSO) were compared. Ca release from SR passively preloaded with Ca was inhibited by 10% DMSO, but markedly accelerated by 1 mM PROP. Ca uptake and ATPase activity in the absence of Pi were markedly inhibited by 1 mM PROP but DMSO had no effect. Prolonged incubation with PROP further inhibited Ca uptake while ATPase activity increased; DMSO did not show these time-dependent effects. When added during the Ca uptake reaction, DMSO slightly increased Ca content whereas PROP induced a Ca release that was much slower than that seen in the absence of ATP. DMSO had little effect on the Ca release induced by the addition of EGTA after most of the ATP has been hydrolyzed; PROP however caused an immediate Ca release. These findings suggest that PROP has immediate actions on the Ca pump ATPase molecule and/or its environment, and slower effects in which PROP modifies the Ca pump by a detergent-like action. The former can inhibit ATPase activity and, in the presence of EGTA and absence of ATP, induce rapid Ca release.

The amount of ADP-sensitive phosphoenzyme (EP) was increased by 1 mM PROP but decreased by 10% DMSO, whereas ADP-insensitive EP was decreased by PROP and increased by DMSO; neither affected significantly the total amount of EP nor the formation of ATP from Pi. These results may be explained if Ca efflux is accelerated by the formation of one or more of the intermediates that appear in the forward reaction mechanism before the formation of ADP-insensitive EP.

Supported by grants from NIH HL-21812 and HL-22135.

T-Pos163 LYSOPHOSPHOLIPID-MEDIATED EFFECTS ON SARCOPLASMIC RETICULUM CALCIUM TRANSPORT SYSTEM. Indu S. Ambudkar*, El-Sayed Abdallah**and Adil E. Shamoo. Department of Biological Chemistry, University of Maryland School of Medicine,660 W.Redwood Street, Baltimore, Maryland 21201.

Lysophospholipids are capable of inducing deleterious effects on membranes due to their ampiphilic properties. We studied the effects of various lysophospholipids on the calcium transport activity of sarcoplasmic reticulum from rabbit skeletal and canine cardiac muscles. In both cases the lipids decreased calcium transport, the effectiveness being in the order lysoPC>lysoPS, lysoPG> lysoPE. LysoPC, lysoPG and lysoPS all inhibited the activity maximally by more than 85%. LysoPE had a maximal inhibition of about 50%. Half maximal inhibition of calcium transport by lysoPC was achieved at 110 nmoles lysoPC/mg SR. At this concentration the Ca^{2+}+ Mg^{2+}-ATPase activity was inhibited to the same extent in rabbit skeletal membranes. In cardiac sarcoplasmic reticulum there was less than 20% inhibition of the ATPase activity at the same lipid/protein concentration. Studies with EGTA-induced passive calcium efflux showed that upto 200 nmoles lysoPC/mg SR did not alter calcium permeability significantly in cardiac sarcoplasmic reticulum. In skeletal muscle membranes the decrease in calcium uptake correlated well with the increase in passive calcium efflux due to the lysophospholipids. Phosphatidylcholine and phosphatidylethanolamine did not induce effects similar to those produced by the lysophospholipids. The differences in the lysophospholipid-induced effects on the sarcoplasmic reticulum from the two muscle types probably reflect variations in protein and other membrane components related to their calcium transport systems.

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T-Pos164 STUDY OF Eu^{3+} BINDING TO Ca^{2+} + Mg^{2+} ATPase OF SARCOPLASMIC RETICULUM. P. Gangola and A.E. Shamoo, Membrane Biochemistry Research Laboratory, Department of Biological Chemistry, University of Maryland, School of Medicine, 660 West Redwood Street, Baltimore, MD 21201.

We have utilized the laser excited Eu^{3+} luminescence to characterize the Ca^{2+} binding sites of the sarcoplasmic reticulum Ca^{2+} + Mg^{2+}-ATPase. The changes in the molecular environment of these sites are studied under the conditions of hydrolysis. In order to study the Eu^{3+} binding specifically to Ca^{2+}-translocating sites, the lipids bound to purified Ca^{2+} + Mg^{2+}-ATPase are exchanged with Dioleoyl-phosphatidyl choline (DEPC). The lipid exchange was achieved by adding excess DEPC and centrifugation. Eu^{3+} does not bind to DEPC. No significant change is observed in the ATPase activity after lipid exchange.

The excitation spectrum of Eu^{3+} bound to Ca^{2+} + Mg^{2+}-ATPase shows a peak at 579.3 nm and a shoulder at 579.6 nm. In the presence of Mg^{2+} and ATP a broadening of the excitation spectrum occurs and the fluorescence lifetime increases by about two-folds, which indicates a conformational change in the molecular environment of Ca^{2+} binding sites, following ATP hydrolysis.

A comparative study of Eu^{3+} binding to normal SR vesicles, purified Ca^{2+} + Mg^{2+}-ATPase and lipid exchanged Ca^{2+} + Mg^{2+}-ATPase has been done. The purification and lipid exchange results in the sharpening of excitation bands, which shows the minimization of the effects of negatively charged phospholipids or oxidized phospholipids on Eu^{3+} binding characteristics. Lipid exchange also causes a decrease in the fluorescence lifetime of Eu^{3+}, the reason for this is not clear to us at present.

T-Pos165 **MECHANISM OF MILRINONE-INDUCED POSITIVE INOTROPIC ACTION.** Judy Y. Su, Department of Anesthesiology, RN-10, University of Washington, Seattle, WA 98195

Milrinone, a potent cardioactive bipyridine, increases cyclic AMP (cAMP) level correlated with the increase in myocardial contractility in isolated papillary muscle and inhibits phosphodiesterase (PDE) activity in the crude extract (J. Cardiovasc. Pharmacol., 5:805, 1983). However, the cause-effect relation is not known. Cyclic AMP increases Ca^{2+} uptake by the sarcoplasmic reticulum (SR) in functionally skinned myocardial fibers (Pflügers Arch. 394:48, 1982). We hypothesized that milrinone-induced increases in myocardial contraction are due to increases in cAMP resulting from inhibition of phosphodiesterase activity. We studied the intracellular mechanisms of milrinone action using functionally skinned fibers from the right ventricular papillary muscle of rabbit. The muscle was isolated, pieces were homogenized (sarcolemma disrupted), and a fiber bundle was dissected from the homogenate. The fiber bundle was immersed in control solution (no drug), then in the test (containing drug) solution, and finally, in the control solution. The bathing solution contained (in mM) Mg^{2+}, 1 or 0.1; $MgATP^{2-}$, 2; K^{+}+Na^{+}, 70; creatine phosphate, 15; EGTA total, 0.05 or 7; and pCa = $[Ca^{2+}](M)$, > 8, 5.6-3.8. Ionic strength = 0.15 and pH = 7.00 ± 0.02 at 23°C with imidazole. Propionate or methansulfonate was the major anion. Caffeine-induced tension transient was used to measure the amount of Ca^{2+} release from the SR. We found that milrinone (0.01-1 mM) decreased the maximal, but not the submaximal (except at 1 mM milrinone on pCa 5.6) Ca^{2+}-activated tension development of the contractile proteins. Milrinone (0.1 µM-1 mM) caused biphasic effects, increasing at lower concentrations, and decreasing at > 0.5 mM both Ca^{2+} uptake and release from the SR. We conclude that milrinone induces positive inotropic action by increasing Ca^{2+} uptake and release from the SR. (Supported by grants #HL 20754 and #HL 01100 (RCDA) from the National Institutes of Health.)

T-Pos166 SPECTRAL AND ION-BINDING PROPERTIES OF FRAGMENTS OF SKELETAL MUSCLE CALSEQUESTRIN.
Mamoru Ohnishi and Reinhart A.F. Reithmeier. (Introduced by Robert S. Hodges) Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7

Calsequestrin (MW 44,000) binds 40 Ca^{2+} ions per molecule and is involved in calcium sequestration. We have determined the sequence of a 59 amino acid carboxyl-terminal tryptic peptide:

Thr Phe Asp Ile Asp Leu Ser Ala Pro Gln Ile Gly Val Val X Val Thr Asp Ala Asp^{20}
Ser Val Trp Met Glu Met Asp Asp Glu Glu Asp Leu Pro Ser Ala Glu Glu Leu Glu Asp^{40}
Trp Leu Glu Asp Val Leu Glu Gly Glu Ile Asn Thr Glu Asp Asp Asp Asp Asp Glu-COOH

This peptide bound only 2 Ca^{2+} ions per molecule in 100 mM KCl/2 mM $CaCl_2$, although it was highly negatively charged (Asp+Glu=44%). The fluorescence of Tb^{3+} was enhanced upon binding to calsequestrin and the carboxyl-terminal fragment suggesting that this calcium analogue is binding close to tryptophan residues in the protein. Circular dichroism spectra showed that the carboxyl terminal peptide was in the random coil form regardless of the calcium concentration. In contrast, intact calsequestrin has an 11% α-helical content that increases 2-fold upon binding calcium. A negatively charged peptide obtained from chymotrypsin digest also bound on 2 Ca^{2+} ions per peptide (Asp+Glu= 55%). Circular dichroism spectra of proteolytic digests of calsequestrin indicated that the digests were in the random coil form with or without calcium. These results suggest that fragmentation of calsequestrin results in a decrease in calcium binding capacity.
(Supported by the Alberta Heritage Foundation for Medical Research and the University of Alberta)

T-Pos167 PHYSICAL, ULTRASTRUCTURAL AND FUNCTIONAL ASPECTS OF GLYCEROL EFFECT ON SKELETAL SARCOPLASMIC RETICULUM.
W.B. Van Winkle, R.J. Bick, C.A. Tate, and M.L. Entman. Section of Cardiovascular Sciences, Departments of Medicine and Biochemistry, Baylor College of Medicine, Houston, Texas 77030.

Glycerol is the agent of choice to attenuate ice crystal damage in specimens prepared for freeze fracture electron microscopy. It has been noted however, that in many tissues, cells or isolated organelles, incubated (cryoprotection) in glycerol without prior glutaraldehyde fixation may produce aggregates or clusters of intramembrane particles and/or bare lipid patches devoid of particles. Glycerol at levels of 20-30% produces bare patches in skeletal SR with incubations as short as 10 min. At these concentrations, Ca^{2+} dependent, EGTA-sensitive ATPase activity is stimulated four-fold. However, whereas control Ca^{2+}ATPase is stimulated by the calcium ionophore A23187, there is no such stimulation in glycerol-treated preparations. Higher concentrations (40-50%) inhibit Ca^{2+}ATPase activity. Fluorescence polarization of DPH in SR shows little change in the presence of glycerol. However its cationic, surface-localized derivative TMA-DPH exhibits considerable decrease in polarization in glycerol treated SR. These data suggest that glycerol: (1) Exerts a dehydrating effect on phospholipid headgroups leading to close packing and "crowding" of Ca^{2+}-ATPase. In this respect TMA-DPH appears to be a more "sensitive" probe as it "sees" only the outer SR leaflet. (2) Stimulates Ca ATPase activity by rendering the lipid bilayer temporarily "leaky" to Ca^{2+}. Supported by the National Institutes of Health (HL 22856, HL 13870).

T-Pos168 ISOLATION OF JUNCTIONAL TERMINAL CISTERNAE, NONJUNCTIONAL TERMINAL CISTERNAE AND LONGITUDINAL TUBULES OF SARCOPLASMIC RETICULUM FROM MAMMALIAN FAST-TWITCH SKELETAL MUSCLE.
A. Chu, M.C. Dixon, R.J. Naukam, A. Saito, S. Fleischer. Dept. Molec. Biol., Vanderbilt University, Nashville, TN 37235.

The preparation of isolated junctional terminal cisternae from rabbit leg muscles has been described (Saito et al., J. Cell Biol. 99, 875-885, 1984). These vesicles are composed of two types of membranes, the calcium pump-containing membrane (~80%) and the junctional face membrane (~20%). The junctional face membrane contains characteristic and morphologically well-preserved "feet" structures. We have now achieved a large scale preparation using isopycnic zonal centrifugation. From the same microsomal preparation on sucrose gradient, we can obtain three distinct fractions: light sarcoplasmic reticulum (SR) vesicles (at 28-32% sucrose), heavy SR vesicles (at 33-37% sucrose), and a junctional terminal cisternae (at 38-45% sucrose). By a number of criteria including lipid/protein ratio, SDS gel electrophoretic protein profile, phospholipid composition, electron microscopic morphology, Ca^{2+},Mg^{2+}-dependent ATPase activity, and ruthenium red stimulation of Ca^{2+} transport (with and without phosphate), the light and heavy SR fractions were found to correspond to longitudinal tubules and nonjunctional terminal cisternae, respectively. The subpopulations of the two types of heavier SR vesicles therefore appear to be derived from different regions of terminal cisternae, the junctional area apposed to transverse tubules and the nonjunctional area. [supported by NIH grant AM 14632 and fellowship AM 07016 to A.C.].

T-Pos169 CALCIUM-PROTON TITRATIONS OF THE HIGH AFFINITY CALCIUM BINDING SITE OF THE SARCOPLASMIC RETICULUM ATPase. MARWAN K. AL-SHAWI AND GIUSEPPE INESI, INTRODUCED BY MARK KURZMACK, DEPARTMENT OF BIOLOGICAL CHEMISTRY, UNIVERSITY OF MARYLAND SCHOOL OF MEDICINE, BALTIMORE, MD. 21201

In order to detect and characterize the participation of ionizable groups and involvement of protons in the modulation of the calcium affinity sites of SR-ATPase, potentiometric titrations of the pH profile of H^+ release upon stoichiometric Ca^{2+} binding to the high affinity Ca^{2+} site were undertaken as follows. SR vesicles were preincubated overnight at 4^oC with tracer amounts of ^{45}Ca after the total Ca^{2+} contamination had been determined by atomic absorbtion spectroscopy. Endogenous Ca^{2+} was then removed to 0.3 nmol/mg of protein by passage through a Chelex X-100 column. The final suspending medium contained 80 mM KCl, 5mM $MgCl_2$ and 1 mM of a "Good" type zwitterionic buffer. Ca^{2+} titrations were performed at 25^oC under a N_2 atmosphere in a pH stat delivering 1mM NaOH to neutralize any H^+ release. The ratio of free to the bound ^{45}Ca was checked by a rapid filtration method and thence by radioactive counting. The initial plateau region of H^+ release of each titration at various pH values was used to generate the pH profile of the H^+/Ca^{2+} stoichiometry.

Such titrations indicate the release of $2H^+/Ca^{2+}$ at pH 5.9 which tends to OH^+/Ca^{2+} as pH 8.1 was reached. It is over this pH range that the change in Ca^{2+} binding affinity to the high affinty site as a function of pH is most pronounced. These results are consistent with the presence of two ionizable groups associated with the high affinity Ca^{2+} binding site having pK_{app} values of approximately 6.3 and 7.8. The mechanistic involvement of protons in calcium transport is currently under investigation. Supported by USPHS (HL27867) and MDA.

T-Pos170 ELECTRON MICROSCOPY OF QUICK-FROZEN SARCOPLASMIC RETICULUM MEMBRANES. D.J. Scales Dept. of Biological Chemistry, University of Maryland School of Medicine, Baltimore MD 21201 and Dept. of Physiology, University of the Pacific, San Francisco, CA 94115. Subfractions of muscle membranes, enriched with sarcoplasmic reticulum (SR), were fixed for electron microscopy by liquid-nitrogen-based quick freezing. These were the first subfractions prepared with the "Gentleman Jim" freezing device (Quick Freezing Devices, Baltimore, MD). Frozen membranes were then either freeze substituted or freeze fractured/etched. Freeze substitution in tetrahydrofuran (THF) was carried out according to Phillips & Boyne, J.Elect. Microsc. Tech. 1:9-29 (1984). The quality of freezing was excellent, and thin sections of freeze-substituted membrane pellets showed ice-free regions up to 25 microns from the freezing surface.

Some frozen samples were exposed to 1% tannic acid in THF before osmium. Tannic acid (TA) appeared to give the best ultrastructural preservation of SR membranes. Only those membranes exposed to TA demonstrated the hydrophilic moiety of the calcium-ATPase. When the step including TA was omitted, vesicle profiles showed only "naked" membranes. Other samples of SR pellets were applied to special holders designed to fit a Balzers freeze fracture device. Etched and rotary shadowed specimens revealed the cytoplasmic surfaces of SR vesicles in splendid detail. Some membranes were chemically modified before freezing. 5mM sodium vanadate is known to induce well-defined aggregates of the calcium ATPase. Fractured specimens showed arrays of 9nm particles on P faces and corresponding pits on E faces. Etched specimens of such membrane crystals made identification of the hydrophilic moiety of the ATPase particularly evident in metal replicas of frozen membranes. Supported by N.I.H. grant HL27867 and a grant from the Muscular Dystrophy Association

T-Pos171 KINETICS OF RAPID Ca^{2+} RELEASE BY SAROPLASMIC RETICULUM. Gerhard Meissner and Edward Darling, Department of Biochemistry, University of North Carolina, Chapel Hill, NC 27514.

A radioisotope flux-rapid quench-Millipore filtration method has been developed to determine the effects of Ca^{2+}, adenine nucleotides, calmodulin and drugs on the Ca^{2+} release behavior of "heavy" sarcoplasmic reticulum (SR) vesicles. Rapid $^{45}Ca^{2+}$ efflux was blocked by the addition of the two "quenching" agents Mg^{2+} and ruthenium red. Release rates of 0.1-0.3 μmol Ca^{2+}/mg protein/s were obtained for vesicles passively loaded with 5 mM $^{45}Ca^{2+}$ and diluted into media containing 1-5 μM free Ca^{2+}. Addition of the nonhydrolyzable ATP analog AMP-PCP to the release medium increased the initial release rate to 3-10 μmol Ca^{2+}/mg protein/s (k_1 = 30-100 s^{-1}). Ca^{2+} release was half-maximally activated at $5 \cdot 10^{-7}$ M Ca^{2+} with a Hill coefficient of 1 and half-maximally inhibited at $1.5 \cdot 10^{-4}$ M Ca^{2+} with a Hill coefficient of 1.65. The apparent n values of activation of Ca^{2+}-induced Ca^{2+} release by AMP-PCP and adenine were 1.65 and 0.85, respectively. Calmodulin reduced Ca^{2+}-induced and nucleotide-stimulated Ca^{2+} release rates by a factor of about 2. Calmodulin appeared to directly interact with the channel rather than via a calmodulin-dependent protein kinase. Ca^{2+} release from heavy SR vesicles was not inhibited by the Ca^{2+}-antagonists nitrendipine and D-600. Our studies suggest that heavy SR vesicles contain a Ca^{2+} release channel that is capable of mediating Ca^{2+} fluxes with a physiological rate. The channel is inhibited by calmodulin and contains interacting regulatory binding sites for Ca^{2+} and AMP-PCP (ATP). Supported by NIH grant AM18687.

T-Pos172 ISOMETRIC TENSION AND STIFFNESS AT VARYING IONIC STRENGTH IN MAMMALIAN SKINNED MUSCLE FIBERS. Peter J. Reiser and Richard L. Moss, Department of Physiology, Univ. of Wisconsin, Madison, WI 53706.

Experiments were performed to test whether isometric tension and stiffness change proportionately when single skinned muscle fibers were maximally activated in solutions of different ionic strengths. Segments of glycerinated fibers from adult rabbit soleus muscle were maximally activated at 10°C in solutions ranging in ionic strength from 125 mM to 300 mM. Sarcomere length ranged from 2.29 μm to 2.40 μm. Stiffness was measured from the force oscillations which resulted from small length sinusoids (0.04-0.06 % L_0) at 1.4 kHz which were imposed at one end of the fiber during activation. Control experiments indicated that the relatively compliant ends of the fiber segments at the points of attachment might have contributed to, but could not account for, the stiffness observations. Fiber to fiber variations in the phase shift between the length and tension oscillations were not correlated with variations in stiffness. Tension and stiffness were normalized to the values at 180 mM ionic strength. The results demonstrate a very close correlation between isometric tension and stiffness at physiological and high (180-300 mM) ionic strengths. At 125 mM the increase in tension was significantly greater than the increase in fiber stiffness. This result suggests that the force per crossbridge increases significantly at low ionic strength in these maximally activated fibers. Thus, relative isometric tensions at ionic strengths of 180 mM and above do reflect the relative number of force generating crossbridges but this is not the case at low ionic strengths. Supported by NIH.

T-Pos173 EFFECTS OF AMPLITUDE OF LENGTH OSCILLATION ON DYNAMIC STIFFNESS OF RABBIT PAPILLARY MUSCLE IN ACTIVE CONTRACTURE: CORRELATION WITH MODEL PREDICTIONS.
William Hunter, Toshimitsu Shibata, Roger Hajjar, Elaine Silverman. Department of Biomedical Engineering, School of Medicine, Johns Hopkins University, Baltimore, MD 21205

Most measurements of dynamic stiffness of cardiac (or skeletal) muscle have limited the amplitude of sinusoidal length oscillation to 1% or less. We wondered whether mechanical properties measured with such small perturbations would be valid over a larger range of motion, where a larger proportion of cross-bridges would be shifting their site of attachment. Hence, we compared the dynamic stiffness of steadily activated papillary muscles for 1%, 2%, 4%, and 6% peak-to-peak length oscillations. We measured the length of the central half of the muscle, between two pins inserted across it. Ba^{++} (0.5 mM) replaced Ca^{++} in the bath to achieve a steady level of activation. For all amplitudes of oscillation, force remained essentially sinusoidal at all frequencies (.05 - 30 Hz), except near 1 Hz, where stiffness was minimum. Here, force became more distorted the larger the amplitude. Magnitude and phase of dynamic stiffness were nearly identical at frequencies up to 2 - 3 Hz. Above this, larger amplitudes of oscillation showed progressively smaller modulus and less positive phase shift. Also above 2 - 3 Hz, the mean level of force during oscillation progressively decreased with increases in either amplitude or frequency. Such trends in behavior are predicted by a model that includes a nonlinear "uncoupling" property in which the rate of loss of force generators is proportional to the magnitude of velocity (irrespective of direction). Consistent with the data, the model also exhibits "resonance-like" behavior near the frequency of minimum stiffness. These results (even at 1% oscillation amplitude) are thus not consistent with a description based on independent exponential rate processes.

T-Pos174 CONTRACTION AND CATCH IN SKINNED ABR MUSCLES FROM MYTILUS. P. Bryant Chase, Dept. of Biol. Sci., Neurobiology Section, USC, Los Angeles, California 90089.

Contraction and relaxation were studied in two skinned bundle preparations of Mytilus' anterior byssus retractor (ABR) muscle, a catch muscle. Thin bundles (about 140 μm dia.) were treated with either (i) EDTA (10 mM; 25 mM EGTA; no added divalent cations (M^{++}); pH 7.0; 50 mM BES; 10 min) or (ii) detergent (usually 0.1% w/v saponin; 25 mM EGTA; pMg 2.3; pH 7.0; 50 mM BES; 15 min).

M^{++} sensitivity of isometric force generation was determined in bundles freshly skinned with detergent. pK and n, Hill equation parameters, were obtained using a nonlinear least-squares regression algorithm. For Ca^{++}, pK = 6.11 ± 0.04 and n = 2.56 ± 0.15 (N=4); for Sr^{++}, pK = 4.67 ± 0.09 and n = 1.59 ± 0.16 (N=5); for Ba^{++}, pK = 4.36 ± 0.09 and n = 1.92 ± 0.06 (N=5). Conditions were: 10 mM EGTA, pMg 2.3, and 2 mM MgATP; free [ion]s were calculated as described in Chase (Biophys. J. 45: 349a, 1984).

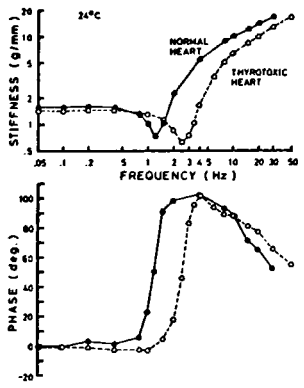
Addition of 10 μM cAMP significantly lowered the ABRM's M^{++} sensitivity by 0.15 pCa units, 0.25 pSr units, or 0.30 pBa units. This effect of cAMP was irreversible for detergent-skinned muscle; maximal stimulation by either Ca^{++} or Ba^{++} resensitized EDTA-treated muscle.

For contraction in pCa 5, $t_{\frac{1}{2}}$ = 12.3 ± 4.1 s (N=8 detergent-skinned bundles) vs. 44.6 ± 17.6 s (N=10 EDTA-treated bundles). There was a greater difference for relaxation in pCa 8: EDTA-treated bundles appeared to enter catch (Chase & Abbott, Soc. Neurosci. Abs. 10: 783, 1984). Although diffusion was apparently slowed in EDTA-treated muscles, inadequate Ca buffering was not responsible for catch tension: (i) little tension redevelopment followed quick release, and (ii) relaxation rate was not increased by increasing [EGTA] to 25 mM.

Supported in part by a USC Pre-doctoral Fellowship and by Sigma Xi, the Research Society.

T-Pos175 THYROXINE TREATMENT OF RABBITS SHIFTS FREQUENCY SPECTRUM OF MYOCARDIAL DYNAMIC STIFFNESS TO HIGHER REGION. Toshimitsu Shibata, William C. Hunter, and Kiichi Sagawa. Dept. of Biomedical Engineering, The Johns Hopkins University, Baltimore, MD 21205

The influence of thyroid hormone to increase the rate of both shortening and force development in myocardium potentially involves changes in both excitation-contraction (E-C) coupling and myofilament mechanics. To specifically test only the myofilament component, we bypassed E-C coupling by replacing calcium with barium in the fluid bathing an excised rabbit papillary muscle to produce a stable contracture. We compared 6 muscles from rabbits pretreated with thyroxine (0.15-0.20 mg/kg/day, for 2 weeks) to 15 normal muscles. We excluded the effect of damaged ends by measuring the length of a central segment between two tungsten pins (50μ, diam) inserted into the muscle. Applying small sinusoidal length oscillations (1% Lmax), we measured the dynamic stiffness ($S=\Delta F/\Delta L$) at frequencies from 0.05 to 50 Hz. The figure shows representative plots of the stiffness modulus and the phase shift of force to length oscillations. Both spectrum curves from thyrotoxic myocardium were parallel shifted to a higher frequency region than normal. The frequency where stiffness modulus is minimum was 2.6 Hz (± 0.2 SD) in thyrotoxic myocardium, which was significantly higher ($P<0.001$) than that of normal myocardium (1.2 Hz, ± 0.3). These results confirm that the mechanical processes of the myofilaments themselves have a higher rate in thyrotoxic myocardium and are consonant with the biochemical finding that thyrotoxic myocardium has a great amount of myosin isozyme with a higher ATPase activity. We also examined other cardiac tissues known to have different myosin ATPase activities (rabbit atrial trabeculae and neonatal rabbit papillary muscles). These showed similar shifts in the frequency spectrum of stiffness to regions higher than those for adult rabbit papillary muscles.



T-Pos176 FROG MUSCLE FIBER DYNAMIC STIFFNESS DETERMINED USING NONLINEAR SYSTEM IDENTIFICATION TECHNIQUES. Ian Hunter, Dept. of Physiology and Biomedical Engineering Unit, McGill University, Montreal, Canada H3G 1Y6.

The dynamic stiffness of muscle has previously been determined using linear identification techniques involving either sinusoidal or PRBS length perturbations. Given the nonlinearities observable in the muscle length and tension step responses, the adequacy of these linear techniques is questionable. In this study the dynamic stiffness (from 1 to 1000 Hz) of muscle fibers (Rana temporaria tibialis anterior) was determined using linear stochastic identification methods and a new approach that does not assume linearity. Stochastic length perturbations covering a range of amplitudes (about a mean sarcomere length of 2.2 μm) were imposed during tetanic stimulation. Estimates of the fiber dynamic stiffness, obtained using linear identification methods, accounted for >95% of the tension variance only when the peak-to-peak length perturbations were <0.5%. Even for these small length changes the coherence of the dynamic stiffness dropped off below 10 Hz. In contrast the nonlinear identification technique accounted for most of the tension variance over the full range of %length changes studied. These results suggest that linear techniques are not appropriate for the study of the mechanical behavior of muscle at lower frequencies, and are not sufficient for characterizing larger amplitude responses. Nonlinear identification techniques show promise in both these respects. (Supported by the MRC)

T-Pos177 A BUILDING NOISE CANCELLATION TECHNIQUE IN MYOFIBRIL TENSION FLUCTUATION MEASUREMENTS. Tatsuo Iwazumi, Dept. of Physiol. & Biophys., Univ. Texas Med. Br., Galveston, TX 77550

I reported previously (Biophys. Soc. Abstr. 45: 158a) that during steady contractions elicited by Ca^{++} healthy sarcomeres did not exhibit tension fluctuations even at the noise floor of the system (0.5 ng/$\sqrt{Hz}$). However, due to the extraordinary sensitivity of the force transducer, there were building vibration noises whose spectrum obscured the data from 2 Hz to 30 Hz with a peak at about 12 Hz at a level of 25 ng/$\sqrt{Hz}$. Although this peak value was much lower than the theoretical fluctuation rms value predicted according to the cross-bridge mechanical cycling, it was felt necessary to reduce the building noises as much as possible for a definitive demonstration of the absence of tension fluctuations. Since the system has two identically shaped transducers, one for length control and another for force, any low frequency environmental vibrations are felt in-phase by these two transducers whereas the myofibril contractile force is applied to them out of phase. Therefore, after proper sensitivity equalization, the sum of signals from the length and force transducers produces a force signal with greatly reduced building noises. The noise peak was reduced by 26 db resulting in the noise peak of only 1.25 ng/$\sqrt{Hz}$. The same noise reduction scheme can be applied during length perturbations. The response of the length transducer was generated by a dynamic simulator, and the noise component was extracted by subtracting the simulated response from the position signal. This method allowed tension fluctuation measurements during slow stretch and release of the myofibril length. Supported by Muscular Dystrophy Association.

T-Pos178 WIDE SPECTRUM OF FIBER-TYPES BY CONTRACTION SPEEDS OF SKINNED FIBERS FROM A MIXED MUSCLE OF HAMSTERS. J. Gulati, S.P. Scordilis† and A. Babu (Int. by T. Robinson).
Albert Einstein College of Medicine, Bronx NY 10461 & †Smith College, Northampton MA 01063.

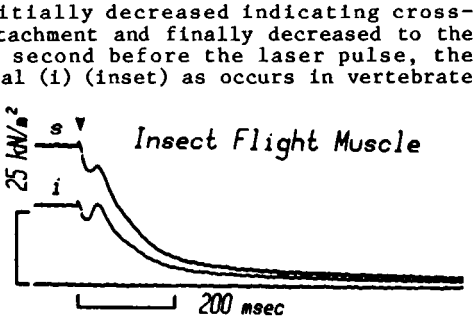
Many contemporary biochemical and structural studies suggest a spectral diversity of fiber types rather than the traditional slow-I, fast-IIA & -IIB types (D. Pette, Plasticity of Muscle, 1980; B. Eisenberg, Handbook of Physiology, V. 10, 1983). To study this further we measured two physiological properties of single fibers from a mixed muscle of adult hamsters: (1) the unloaded shortening speed V_{max}, (2) pCa- and pSr-force plots. These were correlated with (3) SDS polyacrylamide gel pattern of the same fiber for identifying the myosin light-chains by silver staining. Flexor digitorum muscle was used. We find a wide, 10-fold, range of unloaded speeds (0.4 Lo/sec to 5 Lo/sec) for different fibers. The distribution of speed values is practically continuous over this range, suggesting a wide spectrum of fiber types. pCa-force plots were similar for the various fibers. But the pSr-plots clustered in two groups. Fibers with $V_{max} < 1.4$ Lo/sec were by 1 pSr unit more sensitive (S-type, pSr_{50}=5.65) than fibers with $V_{max} \geq 1.4$ Lo/sec (F-type, pSr_{50}=4.73). F- to S-type Sr-transition was found to be matched by the shift in light chain patterns on gels: S-type fibers ($V_{max} < 1.4$ Lo/sec) had two bands of 27K and 19K daltons (characteristic of slow-twitch myosin), F-type fibers ($V_{max} \geq 1.4$ Lo/sec) had three bands (25K, 18K & 16K). We conclude that the mechanisms that set the V_{max} for adult fibers have a critical transition, marked both by the shift in Sr-response and in the light chains. The results point to a dual mechanism for the intrinsic control of contraction speed in vertebrate muscle , where (1) the spectrum of speed may reflect the wide multiplicity of phenotypes of actomyosin with (2) the particular type of myosin light chain pattern regulating whether the speed varies in the lower or the upper ranges (MDA & Blakeske Fund at Smith).

T-Pos179 THE EFFECT OF CA^{+2} ON THE MAXIMUM SPEED OF SHORTENING (V_{max}) IN SKINNED MUSCLE FIBERS. Rome LC, Striz S, Stephenson DG, Julian FJ. Dept Anes Res, Brigham & Women's, Boston.

The reliability of the "slack test" has been improved by photographic calibration and it provides an accurate measure of V_{max} (Rome et al. Biophys J, 45 (2), 345a 1984,1985). The "photo-calibrated slack test" was used to re-examine the effect of $[Ca^{2+}]$ on V_{max}. The experiments were performed at sarcomere lengths (sl) of 2.1-1.8um where resting tension was negligible and with "calcium jump solutions" with which no "residual tension" was observed. V_{max} of mechanically skinned fibers of the m. iliofib. of R pip. at 7.5°C was 2.38 ± SE=0.23,n=6 at 20-35% activation and 3.57 ML/s ± SE=0.52,n=6 at 45-60% activation. These values are statisically different from one another ($p<.05$) and from a value of 6.12 ML/s ± SE=0.44,n=10 at 80-100% activation found previously at sl = 2.3-1.9um. An effect of $[Ca^{2+}]$ on the speed of shortening was also demonstrated using force clamps. Force clamps down to loads of 1 mg were completed in 2-3ms. At loads of 0.1-0.3 P_o, the initial velocity of shortening was clearly lower at low $[Ca^{2+}]$. At larger loads, the difference was smaller. It was not possible to clamp loads below 0.05P_o at submaximal activation and thus to accurately determine V_{max} and to quantitate the effect of $[Ca^{2+}]$ on V_{max} by force clamps alone. A confounding aspect of these experiments was that as the experiment progressed, the initial speed of shortening for contractions at low loads actually increased. For example, if a series of force clamps were performed at low $[Ca^{2+}]$ and then the $[Ca^{2+}]$ was raised and another series performed, we always observed an increase in speed. When the $[Ca^{2+}]$ was then lowered to the original level, however, the speed often remained high. This suggests that some irreversible damage occurs upon repeated exposure to high $[Ca^{2+}]$. This may be responsible in part for the conflicting results found in the past. NIH Grants AM07046 (LCR), HL30133 (FJJ).

T-Pos180 **RELAXATION OF INSECT ASYNCHRONOUS FLIGHT MUSCLE BY PHOTOLYSIS OF CAGED ATP** M. Yamakawa, K. Güth*, M.G.Hibberd and Y.E.Goldman, Department of Physiology, University of Pennsylvania, Phila., PA 19104 and Physiological Institute II*, University of Heidelberg, Heidelberg, F.R.G.

We compared the kinetics of cross-bridge detachment in asynchronous insect flight muscle with previous measurements on vertebrate skeletal muscle. Single glycerol-extracted fibers from dorsal longitudinal flight muscle of *Lethocerus indicus* were mounted between a force transducer and length driver with foil clips and nitrocellulose glue. The fiber was put into a rigor solution with 10 mM caged ATP and 10-50 mM reduced glutathione, and then relaxed with 0.82 mM ATP liberated by laser photolysis of caged ATP (inset arrow). Tension initially decreased indicating cross-bridge detachment, then increased indicating transient reattachment and finally decreased to the relaxed level. If the fiber was stretched (s) by 0.4% one second before the laser pulse, the tension trace did not converge with that of an isometric trial (i) (inset) as occurs in vertebrate muscle. These results may indicate that in insect flight muscle, reattachment of cross-bridges depends on the pre-photolysis strain or that a population of cross-bridges detaches more slowly than the predominant rate. Stretch activation (~30 µM Ca^{2+}, 5 mM ATP) was suppressed after several photolysis trials. Transient cross-bridge reattachment and non-convergence of the traces persisted in photolysis-relaxation trials after stretch activation had apparently disappeared. This suggests that these properties are not directly related to stretch activation. Supported by the MDA, NATO grant 547/82, and NIH grants HL15835 to the Penna. Musc. Inst. and AM00745.



T-Pos181 THE MAXIMUM SPEED OF SHORTENING IN INTACT AND SKINNED MUSCLE FIBERS. Rome LC, Striz S, Stephenson DG, Julian FJ. Dept Anes Res Labs, Brigham & Women's Hosp, Boston, MA.

By using improved techniques, we have shown that unloaded speed of shortening (V_u) and the speed of shortening at loads approaching zero (V_{iso}) are the same in intact R. temp fibers. (Rome et al., Biophys J, 45 (2) 345a 1984). In this study we tested this in mechanically skinned R. pipiens fibers (pCa=5.5-4.4). Force steps were typically performed down to loads of 0.02 P_o. The force-velocity curve of the skinned fibers differed in shape from that of the intact fibers. At high loads, (0.8 P_o) the data from intact fibers was non-hyperbolic, whereas data from the skinned fiber fell on the hyperbolic Hill curve. At low loads, the force velocity data fell above the hyperbola in both. In intact fibers, V_{iso} was measured from the linear extrapolation of low loads (0.005 P_o to 0.02 P_o) to zero load. Various hyperbolic fits to the force-velocity data ($P < .05\ P_o$ and $P > .8\ P_o$ excluded) underestimated V_{iso} by 7-20%. Because of the difficulty in obtaining lightly loaded force clamps ($0.01 < P/P_o < .05$) in the skinned fibers, V_{iso} was estimated by extrapolation of the Hill curve. At 7.5°C, a/P_o was 0.11 ± 0.01 (9), (mean ± SE, (n)) and V_{iso} was 5.87 muscle length/s ± 0.38 (9), (approx 6% underestimation of V_{iso} as determined by linear extrapolation). The slack test was calibrated by taking a series of photographic exposures of the fiber at various times following each length step. V_u = 6.12 muscle lengths/s ± 0.44 (10). V_u/V_{iso} = 1.05 ± 0.03 (10) and where V_{iso} could be determined by linear extrapolation V_u/V_{iso} = 0.99 ± 0.018 (5). We conclude that there is a unique maximum speed of shortening in single fibers. Discrepancies previously observed were probably due to inaccuracies in the estimation of V_u and V_{iso}. Supported by NIH Grants AM07046 (LCR) and HL30133 (FJJ).

T-Pos182 DAMAGED MYOFIBRILS PRODUCE LARGE TENSION OSCILLATIONS AND SPONTANEOUS FLUCTUATIONS. J.P. Payne, G.H. Templeton, and T. Iwazumi*. Dept. of Physiology, UTHSCD, Dallas TX 75235 and *Dept. of Physiology and Biophysics, UTMB, Galveston, TX 77550

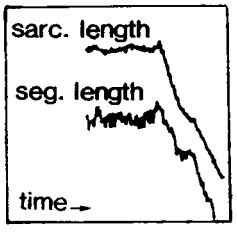
There is at present two conflicting reports concerning the stochastic behavior of actively contracting muscle. Borejdo and Morales (Biophys J '77,20:315) observed spontaneous steady-state isometric tension fluctuations in HS-treated fibers using instrumentation having a noise floor of approximately 0.7 ng/$\sqrt{Hz}$ and a bandwidth of 0-4 Hz. Iwazumi (Biophys J '83,41:263a), however, failed to discern tension fluctuations in isometrically contracting single myofibrils with instrumentation of comparable sensitivity but a bandwidth of 0-1 kHz. Using the methodology developed by Iwazumi, the discrepancy between these findings can be partially resolved by observations made from damaged myofibrils. These were visually distinguished from healthy ones as the former had focal defects in the A-bands or nonuniform sarcomere lengths and produced less active tension with longer rise times. Upon activation (pCa 6.5-5.0), damaged myofibrils developed regions which independently shortened and lengthened, producing extreme variations in sarcomere positions. Active tension displayed 1 Hz oscillations, 5-10% of the mean in amplitude. Digital spectral analysis showed spontaneous tension fluctuations which had pseudo-second order kinetics, a roll-off between 10-20 Hz, and a relative deviation of 2-3%. With successive activations, the striation pattern became grossly distorted with overstretched and torn sarcomeres mixed with hypercontracted ones. When structural damage became severe enough that sarcomere position oscillations no longer occured, no tension fluctuations were detectable. It is concluded that isometric tension fluctuations occur only in damaged muscle where the structural and/or functional integrity is not uniform along the length of the preparation.

T-Pos183 SIMULTANEOUS SEGMENT LENGTH AND SARCOMERE LENGTH MEASUREMENTS CONFIRM STEPWISE SHORTENING. H.L.M. Granzier and G.H. Pollack, Division of Bioengineering, WD-12, University of Washington, Seattle, WA. 98195.

Stepwise shortening has been observed so far with four different methods - laser diffraction, high-speed cinemicrography, on-line striation analysis, and segment length measurement. In order to exclude the possibility that each one has its own independent artifact that might give rise to "apparent" stepwise shortening, we studied the same region of a fiber with two different methods.

Segment length was measured on-line as the distance between thin hairs attached to the upper surface of the fiber (Granzier et al., Biophys. J. 45:350a, 1984). Sarcomere length was measured on-line by laser diffraction; the first order was projected onto a photodiode array, and the median position of the first order was calculated.

The two methods were employed either simultaneously or alternating back and forth. Though minor differences were often observed, the patterns of shortening were qualitatively similar. In preliminary results from six fibers, we found that ten of the 15 clear steps observed with the segment length method were also observed with the laser diffraction method. The figure shows an example.



These results indicate that stepwise shortening is not a methodological artifact; it appears to be a genuine feature of muscle shortening.

T-Pos184 SHORTENING-INDUCED DEPRESSION OF CONTRACTILE FUNCTION IN SKELETAL MUSCLE. Peter L. Becker and Richard A. Murphy, Dept. of Physiology, Univ. of Virginia, Charlottesville, VA. 22908.

Active shortening decreases the contractile function of skeletal muscle beyond that predicted by length-tension considerations. Is this effect due to 1) an alteration of crossbridge properties, 2) a decrease in activation ($[Ca^{2+}]$ or Ca^{2+} sensitivity) or 3) an alteration in sarcomere length distributions? Hypotheses 2 and 3 both predict that the normalized force-velocity (F-V) relation will have the same shape and V_o, while hypothesis 1 predicts a different shape and, most likely, a different V_o. Hypotheses 1 and 2 both predict an eventual recovery with continued stimulation. Data was obtained from electrically-stimulated, 1-2 day old chick anterior latissimus dorsi muscles at 37°C. Force was recorded during isovelocity releases at a variety of velocities with and without an immediately preceeding "conditioning" shortening of 7% L_o at 2% V_o. The ratio of the conditioned-muscle force to the control force at any velocity tended to be constant, and any variability in this ratio was independent of velocity. This indicates that the normalized F-V relation was unchanged as a result of shortening, although F_o was decreased. V_{us}, as measured by the "slack" method, was unchanged following shortening (101.9 ± 2.6% of control), which was consistant with the F-V data. To detect any recovery of contractile ability with continued stimulation, an interval was allowed between the end of the conditioning shortening and the onset of the isovelocity release. No recovery was observed with up to 6 seconds of additional stimulation. The constant shape of the normalized F-V relation after shortening and the lack of recovery argue against the hypotheses that shortening-induced depression is due to either 1) an alteration of crossbridge chemistry or 2) an alteration in the degree of activation. These observations are consistant with the hypothesis that shortening-depression of mechanical function results from a heterogeneous redistribution of sarcomere lengths. Supported by NIH grant 5 P01 HL19242.

T-Pos185 THE EFFECT OF SARCOMERE LENGTH ON Ca^{2+} SENSITIVITY AND Ca^{2+}-BINDING IN DETERGENT-EXTRACTED CARDIAC MUSCLE BUNDLES. Polly A. Hofmann and Franklin Fuchs, Department of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261

Recent work on skinned cardiac muscle bundles (Hibberd & Jewell, 1982) has indicated that there is a rightward shift in the force-pCa relationship when sarcomere length is decreased from optimal length to a length along the ascending limb of the length-force curve. This study was designed to test the hypothesis that this shift is related to a length-dependent change in the Ca^{2+}-troponin affinity. Measurements were made of the force-pCa and bound Ca^{2+}-pCa relationships of bovine, detergent-skinned muscle bundles at sarcomere lengths 2.4 um and 1.7 um. A shift occurred in the pCa necessary for half maximal force from pCa 5.75 to pCa 5.4, respectively. Studies done in rigor using a double isotope technique indicate that in the shorter sarcomere preparation Ca^{2+} saturation was reduced by 7-31% in the pCa 5-6 range. The reduced Ca^{2+} binding at short sarcomere length was also observed during ATP-induced force generation. These data provide evidence that the length-dependent variation in force could be accounted for by length-dependent changes in Ca^{2+} - troponin affinity. (Supported by grants from the NIH and the Western Pennsylvania Heart Association).

T-Pos186 MECHANICAL AND STRUCTURAL PROPERTIES OF GLYCERINATED SINGLE FIBERS FROM LIMULUS MUSCLE. Susan H. Gilbert, Dept. of Anatomical Sciences, SUNY at Stony Brook, Stony Brook, NY 11794

The thick filaments of the striated muscle from the telson of the horseshoe crab Limulus shorten under conditions that activate the muscle (e.g., Brann et al., 1979), suggesting the presence of a unique mechanism of chemical to mechanical energy conversion. To explore the relationship between sarcomere structure and mechanical properties, glycerinated single fibers from this muscle are being studied. Usable preparations were obtained only when the initial skinning solution contained 50% glycerol. Fibers that pulled loose during glycerination in solutions with potassium had short sarcomeres and A bands (4.1 and 3.5 μm, respectively) and produced very little tension. Tension was monitored by a photoelectric transducer in one end of a chamber mounted on an inverted polarizing microscope. Sarcomeres and A bands were measured from videotapes of the microscope images. Activation (by (mM): 5 ATP, 6 Mg++, 120 potassium propionate, 10 imidazole; pCa 6.2, pH 7.0) produced tensions as high as 300 kN/m^2, achieved in 10-15 sec and maintained for as long as 150 sec. The sarcomeres were not isometric. Sarcomeres and A bands in the center of the fiber shortened (e.g., from 6.9 and 4.8 to 4.1 and 3.1 μm, respectively), while sarcomeres at the ends lengthened. Sometimes A bands in the long sarcomeres shortened. Sarcomere and A-band shortening in central sarcomeres were reversed early in an experiment by exposure to relaxing solution (like activating solution but with 5 mM EGTA and no free Ca++). With many activations fibers developed less tension and showed progressive heterogeneity of sarcomere length, shorter A bands, increased resistance to stretch in relaxing solution and diminished 'active state' during activation. Supported by NSF PCM 8209033.

T-Pos187 'THIXOTROPIC' BEHAVIOR AT JOINTS HAS ITS ORIGIN IN SHORT RANGE STIFFNESS OF RELAXED MUSCLE. Allen W. Wiegner, Clinical Neurophysiology Lab., Massachusetts General Hospital, Boston, MA 02114

Lakie et al. (J Physiol 353: 265, 1984) have described thixotropic effects at a number of human joints. That is, when the relaxed joint is subjected to a small sinusoidal torque, the amplitude of the steady-state response is increased up to several fold by a transient larger perturbation. The original, stiffer state is restored by several seconds of inactivity. We have previously found thixotropic behavior in the intact ankle joint of the rat as well as in the isolated ankle joint and isolated muscles acting at the ankle (Soc Neurosci Abstr 8: 329, 1982). In further experiments with a rat soleus muscle preparation (intact circulation, nerve cut), we now find the transition between stiff and loose states to be at the same magnitude of stretch (0.3% of muscle length) as the limit of short range stiffness (SRS) of the passive muscle. In a typical muscle (length = 36mm) SRS ranged from 32 g/mm at a stretch rate of 3.7 mm/sec to 20 g/mm at a stretch rate of 0.037 mm/sec. By stimulating the muscle and applying a ramp stretch at varying intervals (0.3 - 10 sec) thereafter, we have measured the time course of development of SRS. With shorter intervals, SRS declines to a value intermediate between the SRS and longer range stiffness values measured after a very long interval. Cooling from 34°C to 15°C has converted a relatively elastic SRS response to ramp stretches to a strongly viscoelastic response with increased stress relaxation, suggesting a temperature dependent rate of separation of the attachments responsible for the SRS. Although SRS effects are small, SRS may be of physiological significance in that it reduces minute fluctuations in muscle length which might otherwise generate "afferent noise" from muscle spindles.

T-Pos188 FURTHER EVIDENCE THAT CALCIUM BINDING TO THE ACTIVATING SITE IN MUSCLE DEPENDS ON ACTIVE CROSS-BRIDGE ATTACHMENT. A.M. Gordon and E.B. Ridgway, Department of Physiology and Biophysics, University of Washington, Seattle, Washington 98195; and Department of Physiology and Biophysics, Medical College of Virginia, Richmond, Virginia 23298.

When a barnacle single muscle fiber which has been micro-injected with the Ca luminescent protein, aequorin, is stimulated under voltage and length control and allowed to shorten during the declining phase of the resultant Ca transient, extra light (Ca) is observed (Gordon and Ridgway, Europ. J. Cardiol. 7:27-34, 1978). This extra Ca probably comes from the activating sites since the time course of the amplitude of the extra light (Ca) for a shortening step is intermediate between the free Ca and muscle force (Ridgway and Gordon, J. Gen. Physiol. 83:75-103, 1984). The amplitude of the extra light (Ca) increases with the size of the shortening step but saturates for steps greater than 8-9% of the initial length. Step stretches often decrease the light but have a much smaller effect than comparable shortening steps. The extra light (Ca) for a given amplitude shortening step varies with the active force as the active force is increased or decreased by changes in stimulus intensity or initial muscle length. It also increases with the re-development of force following a shortening step initiated during contraction. However, the extra light for a shortening step is decreased over the control value by a stretch of the contracting fiber to a high force level immediately preceeding the shortening step. Thus, the extra light is correlated with the number of attached cross-bridges and not with the length change per se. The data is consistent with the hypothesis that attached cross-bridges increase Ca binding to the activating sites nearby so that detachment of, or decreased strain in, these cross-bridges brings about dissociation of Ca from the activating sites. This work was supported by NIH grants NS-10919 and NS-08384.

T-Pos189 "REPRIMING" OF THE CONTRACTILE RESPONSE TO ION SUBSTITUTION IN SKINNED SKELETAL MUSCLE FIBERS. Michael Fill and Philip M. Best, Department of Physiology and UICOM, University of Illinois, Urbana, IL 61801.

The ability of skinned fibers to respond to repeated stimulation by ion substitution without intervening exposure to a high calcium loading solution has been studied. Single fibers from frog Semitendinosus muscle were skinned (sarcolemma manually removed) and then stimulated by substituting choline Cl for K propionate in the bathing solution (K x Cl product constant at 360 mM). Solutions contained 125 mM total monovalent cation, 2 mM MgATP, 1 mM Mg, 5 mM creatine phosphate, 15 U/ml CPK, 50 μM EGTA (pCa=7.3) and about 30 mM MOPS buffer (pH=7.0 at 20°C), I=0.15. After skinning, fibers were placed in K propionate solution and were never exposed to high EGTA or elevated calcium. Therefore, the isometric tension transients that followed ionic substitution were taken to indicate release of endogenous levels of calcium from the SR. Fibers were stimulated to release calcium by a 10 sec. exposure to the choline Cl solution and then returned to K propionate solution. Immediately following a release, fibers were insensitive to a repeated stimulation via ionic substitution even though the SR contained significant calcium ion as indicated by the large tension responses that could be induced by caffeine. Fibers gradually regained their ability to respond to ionic substitution. Recovery of the contractile response was a function of the time elapsed from the previous stimulation. The half time for recovery was approximately 30 seconds; with full recovery taking approximately 3 minutes. This behavior of skinned fibers stimulated by ionic subsitution is reminiscent of the repriming of contractile activation in intact cells stimulated by elevated potassium ion. Supported by HHS AM 32062 and the MDA.

T-Pos190 ARE THE CONTRACTILE ABNORMALITIES IN PRESSURE OVERLOAD HYPERTROPHY PRODUCED BY CHANGES IN INTRACELLULAR CALCIUM HANDLING? STUDIES WITH AEQUORIN. Judith K. Gwathmey and James P. Morgan. Harvard Medical School, Beth Israel Hospital, Boston, MA 02215.

The development of pressure overload hypertrophy (H) of mammalian working myocardium is associated with a decline in contractile function and prolongation of the isometric contraction (T). Evidence suggests that these changes may be due to abnormalities in intracellular Ca^{++} handling; we tested this hypothesis in control (C) ferrets and ferrets in which right ventricular (RV) H was induced by banding the pulmonary artery. The heart wt vs body wt ratio (0.52 vs 0.40), RV wt vs body wt ratio (0.16 vs 0.09) and fiber dia (18.9 vs 13.0 μ) were greater in H than C ($p<.001$). Papillary muscles from C (n=14) and H (n=14) $\leq$ 1 mm dia were chemically loaded intracellularly with the Ca^{++} indicator aequorin (Pflügers Arch 1984;400:338). The aequorin signal (L) (i.e., intracellular Ca^{++} transient) and T were recorded. Muscles were stimulated at 3s intervals in a modified Krebs solution containing 2.5 mM Ca^{++} and bubbled with 95% O_2/5% CO_2 to pH 7.4 at 30°C. T development in H was less than in C (15.1 vs 37.1 mN/mm^2); this does not appear primarily due to diminution of the Ca^{++} transient since the relative amplitudes of L in H (determined by the method of fractional luminescence) are similar to C. Moreover, H did not achieve C levels of T in response to maximally effective $[Ca^{++}]_o$. On the other hand, the times to peak T and 80% relaxation were significantly prolonged in H ($p < .001$) and correlated with a prolongation in the time course of L, probably reflecting a decreased rate of uptake (and perhaps release) of Ca^{++} by stores. Thus, the prolonged time course, but not the diminished maximal T, in H can be attributed to changes in the Ca^{++} transient. (Support: HL31117 and HL07374).

T-Pos191 HALOTHANE ALTERS TETANI OF SKELETAL MUSCLES FROM PIGS SUSCEPTIBLE TO MALIGNANT HYPERTHERMIA (MH). Esther M. Gallant and Virginia M. Goettl, Dept. of Veterinary Biology, U. of Minnesota, St. Paul, MN 55108.

As already known, halothane potentiates twitches of normal and MH-susceptible (MHS) muscles and depresses tetani of MHS muscles only. However, time courses of these responses ± halothane have not previously been clearly established. Bundles of muscle cells, intact from tendon-to-tendon, were placed between parallel Pt plate electrodes for massive transverse stimulation (twitch-1 ms pulses, 0.05 Hz; tetani-200 Hz, 300 ms trains). Responses were recorded on magnetic discs and displayed on a digital oscilloscope (expanded scale) for determination of time intervals. Time from stimulus onset to beginning of force did not differ for normal (4.8 ± .1 ms) and MHS (5.1 ± .1 ms) muscles and was unaltered by halothane (2.5%). Rise times for twitches and tetani of normal (33.0 ± .3; 56 ± 3 ms) and MHS (32.8 ± .6; 53 ± 1 ms) muscles were not different and were unaltered by halothane. Half-relaxation times for twitches and tetani of normal (24.0 ± .7; 32 ± 1 ms) and MHS (23.4 ± .6; 30 ± 1 ms) muscles were not different. However, 1/2-relaxation times for MHS tetani were significantly ($p < .0025$) increased (43 ± 3 ms) after 15 min of halothane while normal tetani were not altered. The changes in tetani of MHS muscles exposed to halothane resemble fatigue thought to result from increased H^+ (J. Gen. Physiol. 72:593). MHS muscles produce excessive amounts of H^+ when exposed to halothane (Br. J. Anaesth. 50:799). Alternatively, diseased muscles low in parvalbumin also have delayed relaxation (PNAS 81:4814). Twitch potentiation with no change in time to peak could result from recruitment of fibers within the bundle or from a more complete activation of individual fibers. Supported by the AHA, Minnesota Affiliate.

T-Pos192 THE EFFECTS OF EXTERNAL ON POTASSIUM CONTRACTURES IN TONIC FIBERS OF THE FROG. M. Huerta, J. Muñíz & E. Stefani. Department of Physiology and Biophysics. CINVESTAV-IPN Apdo. Postal 14-740, 07000 México. D.F.

Frog skeletal muscles possess different types of extrafusal muscle fibers: twitch and slow or tonic. Twitch fibers generate a transient contracture in high K^+. In contrast, tonic fibers give a maintained K^+ contracture. The role of external Ca^{2+} during maintained contractures remains unclear. Recent studies with voltage clamp have shown the presence of a slow and sustained inward Ca^{2+} current in tonic muscle fibers (Huerta & Stefani, Biophys. J., 41: 60a, 1983). Therefore, external Ca^{2+} could play a role in maintaining the tension during K^+ contractures. Tension was isometrically recorded from tonic bundles of cruralis muscle from Rana pipiens. The control saline was (mM): NaCl 117.5, KCl 2.5 and $CaCl_2$ 1.8 (pH 7.4). The experiments were performed at room temperature (20-22°C). Maintained K^+ contracture (20-120 mM) were highly dependent on external of external Ca^{2+}, since, the exposure of the muscle to Ca-free saline (1 mM EGTA + 3 mM Mg^{2+}) practically abolished the maintained phase of the K^+ contracture. Similar results were found when calcium channel blockers (nifedipine 1 μM, diltiazem 1 μM) were added to the control saline. In addition, when external Ca^{2+} was omitted from the contracture fluid, the tension relaxed spontaneously and it was re-established when external Ca^{2+} was restored. The replacement of Ca^{2+} for a non-permeating divalent cation (Ni^{2+}) prevented tension development. This effect was reversible. The present results indicate that the sustained phase of tension in tonic fibers K^+ contractures depend on the presence of Ca^{2+} in the external solution. (Supported by grant PCCBEU-020187 from CONACyT, México).

T-Pos193 CONTRACTILE FAILURE WITH FATIGUE OR HYPOXIA: STUDIES WITH SKINNED SKELETAL AND CARDIAC MUSCLE FIBERS. R.E. Godt, K.J. Fender, G.C. Shirley & T.M. Nosek. Dept. of Physiology; Medical College of Georgia; Augusta, GA 30912.

Contractile force of striated muscle declines markedly with fatigue or hypoxia. Under these conditions intracellular ATP levels stay nearly constant at the expense of phosphocreatine (PC). Concommitantly inorganic phosphate (Pi) and ADP concentrations increase considerably and pH decreases somewhat. In an effort to determine the influence of these changes on the contractile apparatus, we performed parallel experiments with chemically-skinned single fibers from rabbit psoas and small bundles from guinea pig papillary muscle. Maximal calcium-activated force (pCa4) is strongly inhibited by Pi (1-30 mM), with the effect being considerably greater in cardiac muscle, confirming recent observations by J.C. Kentish (personal communication) in rat skinned cardiac fibers. A decrease in pH from 7 to 6.65 inhibits maximal force in cardiac muscle by 17% but has little effect on that of skeletal muscle. Increasing ADP (0.35-3 mM) increases force slightly in both muscles. When PC decreases (0-15 mM), force increases but only when active creatine kinase (CK) is present. (Changes in PC are without effect in the absence of exogenous CK when endogenous CK is inhibited with DNFB). Thus the decrease in PC *per se* in fatigued or hypoxic muscle cannot explain the drop in force. These data suggest that the inhibition of force in intact muscle with fatigue or hypoxia is due in large measure to the inhibitory effect of Pi on force generation by the contractile machinery. This is compounded in cardiac muscle by an additional inhibition caused by decreasing pH. (Support: GA Heart Assoc. and NIH/AM 31636).

T-Pos194 MECHANICAL AND ENERGETIC BEHAVIOR OF FROG SKELETAL MUSCLE IN HYPOTONIC SOLUTION. D.M. Burchfield and J.A. Rall. Dept. Physiol., Ohio State University, Columbus, OH 43210.

Experiments were designed to examine the effects of hypotonicity (0.67 x normal ringer made by dilution) on the behavior of frog semitendinosus muscle at 0°C. Tetanus force at a resting sarcomere length of 2.4 μm increased by 20 ± 5% (n=11) and kinetics of force development and relaxation accelerated. Steady rate of energy liberation (S.R.E.L.) increased by 10 ± 2% but correction must be made for the increased heat capacity of muscle after soaking in hypotonic ringer. The change in heat capacity of muscle plus adherring fluid plus thermopile was determined by electrical calibration (Peltier technique). This heat capacity increased by 8 ± 2.5% in hypotonic ringer. Assuming that the muscle (blotted) is 80% of the total heat capacity, and that the heat capacity of adherring fluid and thermopile are unaltered in hypotonic ringer, the calculated increase in heat capacity of the muscle is 10%. In parallel experiments where one muscle of a pair was soaked in normal ringer and the other in hypotonic ringer and both blotted and then weighed, muscles soaked in hypotonic ringer weighed 11 ± 0.9% (n=15) more than those in normal ringer. Thus the corrected S.R.E.L. was 20 ± 5% greater in hypotonic ringer. Effects on Ca^{2+} cycling energy liberation were determined by measuring S.R.E.L. in muscles stretched to a resting sarcomere length of 3.8 μm. Surprisingly, corrected S.R.E.L. (after subtraction of stimulus heat) decreased by 30 ± 7% (n=5). Assuming that Ca^{2+} cycling energy liberation is not altered by muscle length, effects of hypotonicity on rate of energy liberation by cross-bridges can be estimated as the difference in S.R.E.L. at 2.4 μm and 3.8 μm. S.R.E.L. by the cross-bridges increased by 43 ± 9% in hypotonic ringer. In conclusion, hypotonic ringer (0.67 R) has opposite effects on rate of energy liberation by the cross-bridge cycle and Ca^{2+} cycle. (Supported by NIH grant AM20792).

T-Pos195 REPRIMING OF THE LABILE MAINTENANCE HEAT AND UNEXPLAINED ENTHALPY IN FROG SKELETAL MUSCLES AT 0°C. E. Homsher, J. Lacktis, A. Wallner, and G. Zohman. Dept. of Physiol., Med. School, UCLA, Los Angeles, Ca. 90024.

The time course of heat production, [H(t)], in an isometric tetanus can be described by: $H(t) = LMH \cdot (1-\exp-[k \cdot t]) + B \cdot t$, where t is the stimulus duration (s), B, a steady rate of energy liberation, LMH, the labile maintenance heat (~30 mJ/g) and k, a rate constant (~1 s^{-1}). Energy balance studies have shown that during the first 3-5 sec of a tetanus, 30-40 mJ of heat/g of muscle is produced, the unexplained enthalpy (UE), which is not accounted for by the high energy phosphate hydrolysis during the tetanus. As LMH and UE are of similar magnitude and are reduced by prior activity, they are hypothesized to be manifestations of the same process. To test the hypothesis, the UE and LMH produced by sartorii in a 6 s *conditioning* tetanus and a 6 s *test* tetanus 6, 30, 60 or 300 s after the conditioning tetanus were measured. If the hypothesis is correct, the UE and LMH production in each of the test tetani (repriming) will be the same. While the UE and LMH produced in the conditioning tetanus and a test tetanus 6 sec later are not different from each other, at 30 s UE is completely restored while LMH is less than 45% restored. UE repriming appears to be a single exponential process having a time constant of 4-10 s, while LMH repriming is biphasic with rate constants of 10 and 500 s. Thus UE represents but one of at least two components that constitute the LMH. (Supported by NIH grant AM 30900).

T-Pos196 REACTIVITY OF PHOSPHOCREATININE, A NEW HIGH ENERGY COMPOUND IN MUSCLE. M.R. Iyengar, D. W. Coleman, Dept. Animal Biology, University of Pennsylvania and T.M. Butler, Dept. of Physiology, Thomas Jefferson University, Philadelphia, PA (Intr. by Dr. S. Davidheiser).

N-phosphocreatinine (PCrn) is spontaneously converted to phosphocreatine (PCr) and creatinine (Crn) at nearly identical rates at pH 7.4 and 38°C. Changes in pH, temperature and in the type of buffer anions alter the relative rates of the two reactions and thus effectively direct the product formed. The effect of pH on the reactions of PCrn in combination with the titration curve of PCrn indicates that $PCrn^{2-}$ forms PCr exclusively and that $PCrn^{1-}$ is rapidly and nearly completely converted to Crn. The apparent K_{eq} for the PCrn $\rightleftharpoons$ PCr reaction is between 200-300 at pH 9.0. The ΔG^{o} for the formation of PCr from PCrn (pH 7.0) is -2.5 K Cal/mole. ΔG^{o} for the hydrolysis of the phosphate bond in PCrn is about -13 K Cal/mole. This demonstrates that PCrn is an unusually high energy phosphate. PCrn has been found to be present in methanol-EDTA (pH 7.0) extracts of freshly frozen rabbit white muscle. Identification of PCrn in muscle extracts is based on (i) identity of retention time with authentic PCrn by HPLC; (ii) identity of the u.v. absorption spectrum of the peak with that of pure PCrn anel (iii) conversion of the putative PCrn peak material to Crn on acidification. The PCrn content of rabbit white skeletal muscle is 0.05 µmole/g wet weight. Our results show that about 25% of total Crn in muscle is produced via PCrn as an intermediate and provide an explanation for the observed physiological variability of Crn output.