W-Pos173 ELEVATED INTRACELLULAR cAMP REDUCES INTERCELLULAR COMMUNICATION BETWEEN PARTURIENT RAT UTERINE SMOOTH MUSCLE CELLS. W.C. Cole & R.E. Garfield, Dept. of Neurosciences, Mcmaster University Health Sciences Centre, Hamilton, Ontario, Canada L8N 3Z5.

The onset of parturition in rats is associated with a dramatic increase in gap junctions (GJs) between uterine smooth muscle cells (Garfield et al., Science 198:958, 1977). This increase in junctions is accompanied by a rise in intercellular communication (Cole et al.,Biophys.J. 41:84a). Our previous studies suggest that the permeability of the junctions can be altered by elevated Ca++ or inhibition of calmodulin (Cole & Garfield, Biophys.J. 45:23a). In this study we have tested whether the exchange of small molecules between myometrial cells is influenced by intracellular cAMP levels. One portion of strips of longitudinal myometrium from parturient rats was exposed to ^{3}H-2-deoxyglucose (2DG) and the longitudinal distribution and apparent diffusion coefficient (Da) for this tracer determined following a five-hour period for longitudinal diffusion. The distribution and Da for 2DG was significantly ($p<0.05$) reduced in tissues treated with dibutyryl cAMP (1mM)($Da=0.33\pm0.09X10^{-6}cm^{2}sec^{-1}$, $n=8$) or 8-bromo-cAMP (0.1mM)($Da=0.66\pm0.03X10^{-6}cm^{2}sec^{-1}$, $n=6$) compared to controls ($Da=1.4\pm0.06X10^{-6}cm^{2}sec^{-1}$, $n=6$). Similarly, stimulation of adenylate cyclase activity by forskolin (1μM) reduced the distribution of 2DG compared to controls. These observations suggest that elevated cAMP reduces metabolite coupling and are consistent with a role for this secondary messenger in the control of cell-to-cell communication in the myometrium. Moreover, it is possible that receptor-mediated alterations in adenylate cyclase activity may also influence coupling. Supported by MRC of Canada, a Canadian Heart Foundation traineeship to W.C.C. and an Ontario Heart Foundation Fellowship to R.E.G.

W-Pos174 EVIDENCE OF ION-DYE COMPETITION FOR OCCUPANCY WITHIN INTERCELLULAR CHANNELS. P.R.BRINK, S.W. JASLOVE AND L. BARR. Anatomical Sci. SUNY at Stony Brook. Septal membranes of the Earthworm median giant axon were voltage clamped using the double clamp method. The current passing electrode of one clamp was filled with a fluorescent dye, 6-carboxyfluorescein (CFL) or dichlorofluorescein (2CLFL). Both probes are normally permeant to septal junctions. Under voltage clamp, the holding potentials of both cells were set at a voltage hyperpolarized relative to normal resting potential (transjunctional potential=0), thus allowing dye to fill the cell. The dye took about 5-15 minutes to reach the septum in high enough concentration to show any effects. I-V curves were taken before and after dye outlined the septum. With 2CLFL we found no effect on junctional conductance. With CFL, however, there was a reduction in junctional conductance, which was highly variable (10-50%). Hyperpolarization of the adjacent cell resulted in an increase in conductance back to the level before CFL was present. Return to the holding potential making transjunctional potential=0 resulted in a return to the suppressed junctional conductance. Fast I-V curves of dye-blocked septa were linear over a large voltage range, as has previously been shown for non-injected septa (Verselis and Brink, Biophysical J., 45:145). These results suggest that CFL moves through junctional channels slowly, interfering with the transport of faster, current-carrying ions.

W-Pos175 CYTOSKELETAL COMPONENTS DEPENDENT INTERACTIONS BETWEEN TWO BILAYER MEMBRANES. P. Vassilev, M. Kanazirska and H. Ti Tien, Membrane Biophysics Lab, Department of Physiology, Michigan State University, East Lansing, MI 48824.

The cytoskeletal structures and particularly the microtubules are involved in many cellular mechanisms such as cell motility and excitability. They play an important role on the structural-functional organization of the cells by coupling and coordinating the events occurring in different membranes and membrane-bounded compartments. Evidence shows that they can be tightly associated to cytoplasmic, vesicular and other membranes but so far their ability physically to interconnect membranes and to transmit signals between them has not been examined. Different types of double bilayer lipid membrane (BLM) systems have been employed in this study to verify the validity of this hypothesis. In one of the used techniques, formed BLMs were located in one plane while in another one they were opposed against each other. After formation of the BLMs with or without tubulin, electric pulses with amplitude of 40-80 mV and duration of 30-100 ms were applied across one of the BLMs. The appearance of potential displacements on the other membrane were interpreted in terms of intermembrane coupling. Only the polymerized tubulin was able to mediate such a coupling at small distances between the BLMs. In the absence of GTP or in the presence of colchicine and other inhibitors tubulin could not induce a similar effect when the membranes were not in close contact. Experiments on the influence of different cytoskeletal components suggest that the cytoskeletal structures can mediate intermembrane coupling. An underlying mechanism involving transmission of electrical signals, conformational changes is considered in connection with the observed effect. (Supported by NIH GM-14971-18)

W-Pos176 RECONSTITUTION OF LENS FIBER CELL GAP JUNCTIONS: FORMATION OF PERMEABLE LIPOSOMES CONTAINING MIP26 AND CYTOCHROME C. David A. Rintoul and Marty M. Gooden, Biology Division, Kansas State University, Manhattan, KS 66506

Girsch, et al. (J. Memb. Biol., in press 1984) have reported that the putative gap junction protein (major intrinsic protein, MIP26) from bovine lens can be incorporated into multilamellar liposomes, yielding a preparation which functionally mimics the behavior of cellular gap junctions. These workers assayed for liposome permeability by monitoring changes in optical density of the liposome suspension in sucrose solutions of varying tonicity. We have used the methods of Girsch, et al. to incorporate bovine MIP26 protein into multilamellar or unilamellar liposomes containing cytochrome C. In the presence of ascorbate, oxidized cytochrome C, trapped in MIP26/brain phospholipid liposomes, is reduced. No cytochrome reduction is observed in control liposomes containing no MIP26. Our results confirm and extend the experiments of Girsch, et al., and enable us to define the membrane lipid and protein requirements for functional reconstitution of the lens fiber cell gap junction protein. Using this method, which requires much less protein and lipid than the liposome swelling assay, we can reconstitute MIP26 from normal and cataractous human lenses in order to examine the proposition that gap junction activities are compromised in the cataractous lens fiber cell.

W-Pos177 RYANODINE INHIBITS A CALCIUM CONDUCTANCE OF SKELETAL HEAVY SARCOPLASMIC RETICULUM (SR). F.A. Lattanzio, Jr., H. Walters-Khan, R.G. Schlatterer, L.J. Thompson & J.L. Sutko. Depts. Physiology & Internal Medicine, Univ. Texas Health Science Center, Dallas, TX 75235.

Ryanodine modulates SR calcium release in fast twitch skeletal and cardiac muscles through an apparent change in the conductance of SR membranes for calcium. To investigate the nature of this action, we determined the effects of ryanodine on passive calcium movements across SR membranes isolated from rabbit fast twitch skeletal muscle using the protocols of Meissner (J. Biol. Chem. 259:2365, 1984). Ryanodine inhibited the net passive movement of calcium into heavy SR vesicles in a concentration-dependent manner (0.03-300 μM), but did not significantly alter that of a light SR membrane fraction. Atomic absorption measurements demonstrated that this involved the net uptake of calcium and not calcium-calcium exchange. The ryanodine effect on passive loading was not affected by extravesicular calcium (0.125-5 mM), but was inhibited by magnesium (0.01-0.1 mM) in a complex way. Magnesium concentrations greater than 1.0 mM directly inhibited the calcium fluxes. Ryanodine's action was temperature sensitive, being marked at 37°C and greatly reduced at 25°C. Ryanodine (10-300 μM) also markedly augmented ATP-dependent calcium accumulation and blocked both passive and calcium-stimulated calcium effluxes from heavy, but not light, SR vesicles. Ruthenium red (0.5-10 μM) had effects on these calcium movements which were similar to those of ryanodine. We conclude that the heavy fraction of SR membranes contain a calcium conductance which can be blocked by ryanodine and ruthenium red and that this conductance may participate in calcium fluxes across this membrane in intact muscles. Moreover, ryanodine was effective at submicromolar concentrations, which approaches its potency in intact tissues. Supported by NSF PCM8402100, NIH 26810.

W-Pos178 **IN SITU PHOSPHORYLATION AND FUNCTIONAL MODIFICATIONS OF CARDIAC SARCOPLASMIC RETICULUM AND MYOFILAMENTS.** **E.G. Kranias, J.L. Garvey*, R.D. Srivastava* and R.J. Solaro, University of Cincinnati, College of Medicine, Cincinnati, OH 45267**

Adrenergic stimulation alters functional dynamics of the heart by mechanisms most likely involving cAMP-dependent protein phosphorylation. In vitro studies indicate that the myofilaments and sarcoplasmic reticulum (SR) may act as effectors of the adrenergic stimulation. Studies are presented here in which we tested whether alterations in functional properties of SR and myofibrils occur by in situ phosphorylation. Rabbit hearts were perfused with Krebs-Henseleit buffer containing ^{32}P-orthophosphate, freeze-clamped in a control condition or at the peak of the inotropic response to isoproterenol, and myofibrils and SR were prepared from the same hearts. Stimulation of the hearts with isoproterenol was associated with increases in: a) cAMP levels, b) protein kinase activity ratio, c) phosphorylation of myofibrils and d) phosphorylation of SR. The main phosphoproteins in situ were troponin I (TnI) and C-protein in myofibrils and 11,000 and 24,000-dalton proteins in SR. The 11,000 and 24,000 dalton phosphoproteins were interconvertible and represented most likely the monomeric and dimeric forms of phospholamban. Stimulation of ^{32}P-incorporation in myofibrils was associated with a decrease in Ca^{2+} sensitivity of the myofibrillar Mg^{2+}-ATPase activity. Stimulation of ^{32}P-incorporation in phospholamban, was associated with an increase in the initial rates of oxalate facilitated Ca^{2+} transport, assayed using SR vesicles in either microsomal fractions or homogenates from the perfused hearts. These findings provide evidence that phosphorylation of TnI, C-protein and phospholamban in the intact cell is associated with functional alterations of the myofibrils and SR which may be responsible in part for the effects of catecholamines on the mammalian myocardium. (Supported by NIH grants HL22231, HL26057, HL22619, HL00775)

W-Pos179 INTERACTION OF GLYCERALDEHYDE PHOSPHATE DEHYDROGENASE AND ALDOLASE WITH MICROSOMAL SUBFRACTIONS OF SKELETAL MUSCLE. A. M. Corbett, A.H. Caswell, R.M. Kawamoto, F. Lugo-Gutierrez, and J-P Brunschwig. Department of Pharmacology, University of Miami School of Medicine, P.O. Box 016189, Miami, FL 33101.

We have isolated a protein from the soluble fraction of skeletal muscle which stimulates the formation of triad junctions from isolated transverse tubules and terminal cisternae. This protein has subunit M_r = 34,000 and native M_r = 140,000 and has been identified as glyceraldehyde-3-phosphate dehydrogenase (GAPD). Enzymic activity of GAPD is inhibited when it binds to microsome subfractions. Proteins have been extracted with detergent and salt, from a preparation containing triads and separated by hydroxyapatite chromatography. Calsequestrin shows a specific and potent inhibition of enzyme with a stoichiometry of 0.8 mole calsequestrin/mole tetramer GAPD. The inhibition is relieved in the presence of $Ca\,Cl_2$. The GAPD competes for the same binding site on microsomal fractions as another extrinsic protein of subunit M_r = 38,000. We have identified the latter protein as aldolase. Aldolase activity is also potently inhibited by calsequestrin. Both GAPD and aldolase are predominantly localized in terminal cisternae and T-tubules with a lower but significant content in longitudinal reticulum and "light" terminal cisternae. The ultrastructural distribution of GAPD in isolated organelles and intact muscle has been investigated by electron microscopy using antibodies to the enzyme and protein A labelled with colloidal gold. Supported by NIH training grant HL-07188 , NIH grant AM-21601 and Muscular Dystrophy Fellowship (to RMK).

W-Pos180 A NUCLEOTIDE STIMULATED CALCIUM CONDUCTING CHANNEL FROM SARCOPLASMIC RETICULUM INCORPORATED INTO PLANAR LIPID BILAYERS. Jeffrey S. Smith, Roberto Coronado and Gerhard Meissner (Intr. by James R. White), Depts. of Biochemistry and Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514.

A nucleotide stimulated divalent cation conducting channel from rabbit skeletal muscle sarcoplasmic reticulum (SR) was incorporated into PE:PS (1:1) planar lipid bilayers using heavy SR "calcium release" vesicles. Vesicles were fused to the cis side of planar bilayers formed in asymmetric choline chloride (0.25 M cis, 0.05 M trans-ground). The resulting conductance was anionic with a reversal potential, Erev=+35 mV. To record divalent cation channels, the chloride containing buffer was replaced by perfusion with 0.25 M Hepes - 0.125 M Tris (cis) and 0.25 M Hepes - 0.05 M Ba or Ca (trans). Following perfusion, the bilayer conductance was minimal. Addition of 1mM ATP or AMP-PCP to the cis chamber induced a 10-100 pS divalent cation conductance with a Erev=> +50 mV. Single channel transitions, with a unitary conductance of 10 pS, could be resolved in 50 mM Ba++. The channels were inhibited by cis-Mg++ and selective for Ba++ and Ca++ versus Tris+ or Na+ with a permeability ratio, P++/P+ > 30. The pharmacological similarities, specifically, nucleotide stimulation and cis-Mg++ inhibition, lead us to believe that this channel is identical to the calcium release channel studied by radioisotope efflux measurements in our laboratory. Supported by NIH grant AM 18687.

W-Pos181 OXIDATION IN SARCOPLASMIC RETICULUM Nancy M. Scherer and David W. Deamer. Dept of Zoology, University of California, Davis, CA 95616

Previously we have shown that oxidation of lobster SR resulted in a decline in activity of the Ca^{2+}-ATPase and an increase in Ca^{2+} permeability. Enzyme inhibition did not arise from lipid peroxidation, but by modification of an essential SH group. We have further clarified the site of this SH and the type of modificaiton.

The essential SH appears to be located at the phosphorylation site based on substrate protection and binding of the fluorescent ATP analogue, TNP-AMP. Lack of correlation of the K_a for Ca^{2+} with the degree of inhibition indicates the primary lesion does not occur at the Ca^{2+} binding site; nor does inhibition result primarily from aggregation of SR proteins as judged by PAGE. The mechanism of inhibition could involve thiol/disulfide exchange, allowing regulation by cytosolic redox levels. Two observations argue against this mechanism: inhibition due to added oxidant was not reversible by DTT, and arsenite was protective. Oxidation to sulfenic acid is consistent with these findings. However, the slow, low-intensity oxidative stress which occurred during storage of SR could be reversed by DTT so redox regulation *in vivo* may occur. Moderate oxidation caused a 3- to 4-fold increase in Ca^{2+} permeability as measured by ^{45}Ca flux into nanomolar external Ca^{2+}, but Ca electrode measurements at micromolar external Ca^{2+} did not show an increased permeability after oxidation. Thus, care must be taken when comparing the two methods. The SH oxidants, Ag^{+} and chloromercuriphenylsulfonic acid, inhibited Ca^{2+} transport and stimulated release. The rate of release was greater than expected from inhibition of transport indicating that the Ca^{2+} release channel may also be gated by the oxidation state of a SH. Supported by NSF PCM 80-01153 and Jastro Shields, UCD.

W-Pos182 ACTIVATION OF THE Ca^{2+}-Mg^{2+} ATPase PUMP OF BOVINE CARDIAC SARCOLEMMA Deborah A. Dixon Department of Pharmacology, University of Miami School of Medicine, Miami, FL 33101

The Ca^{2+}-ATPase of bovine cardiac sarcolemma has a dual requirement for Ca^{2+}. This cation is required for transport and ATPase activity; it is also required for activation of the pump. When the enzyme is preincubated with 2 mM Mg-ATP in the presence of $[Ca^{2+}]_o = 1 \times 10^{-7}$ M, and Ca^{2+} is added, Ca^{2+} uptake occurs after a lag. The lag time is a measure of the rate of Ca^{2+}-dependent processes responsible for transforming the enzyme into its more active state. The rate of transport after the lag is a measure of the properties of the activated enzyme and its extent of activation. Transport was measured using chlorotetracycline fluorescence which we have shown to be proportional to $[Ca^{2+}]_i$. Uptake medium contained 250 mM sucrose, 20 mM Hepes buffered at pH 7.4 at 37°C. The $[Ca^{2+}]_o$ in all experiments was buffered at 3×10^{-7} M using CaEGTA. To this medium was added either calmidazolium (30 μm) or C subunit of cAMP dependent protein kinase (10 μm). Both calmodulin and cAMP dependent protein kinase were shown to activate the pump. Endogenous calmodulin is sufficient to fully activate uptake since the addition of purified calmodulin had no effect on either lag time or V_o. However, in the presence of calmidazolium, a calmodulin antagonist, lag time increased ∿ 45% over control value while V_o decreased to ∿ 54% of control. Addition of the C subunit in the presence of calmidazolium activated the ATPase, decreasing lag time to ∿ 27% of control while increasing V_o ∿ 36% over control. A slight activation by C subunit was seen in the absence of calmidazolium. These results demonstrate a role for calmodulin and membrane protein phosphorylation with allosteric activation of the Ca^{2+} pump. Supported by USPHS Grant GM23990 and Training Grant HL07188.

W-Pos183 THE INTERACTION OF cAMP-DEPENDENT PROTEIN KINASE AND CALMODULIN WITH SKELETAL-MUSCLE SARCOLEMMA. James R. Mickelson and Charles F. Louis. Department of Veterinary Biology, University of Minnesota, St. Paul, Minnesota 55108. (Sponsored by Murray D. Rosenberg).

Skeletal-muscle sarcolemma (SL) vesicles were isolated from LiBr-extracted muscle membranes by sucrose-gradient centrifugation. The sealed inside-out nature of these SL preparations has been described previously (Mickelson et al. Biophys. J. 45, 79a; 1984). The addition of 0.01-0.10 mg/ml cAMP-dependent protein kinase (cAMP-PK), in the presence of $MgCl_2$ and cAMP, resulted in the phosphorylation of a number of SL proteins; the major substrates had MWs of 26.5, 43, 66, 125, 160 and 245 KD. Calmodulin, by stimulating an endogenous protein-kinase, also promoted SL phosphorylation; these major substrates had MWs of 21.5, 27.5, 66, 102, 125, 160 and 245 KD. This calmodulin-dependent protein kinase (CM-PK) had a K 1/2 for Ca^{2+} of approx. 0.5 μM and a K 1/2 for calmodulin of approx. 0.1 μM. Affinity labeling with ^{125}I-calmodulin, in the presence of $CaCl_2$ and $MgCl_2$, demonstrated a skeletal-muscle SL calmodulin-binding component of MW 160,000. cAMP-PK (0.1 mg/ml), in the presence of cAMP, lowered the K 1/2 for Ca^{2+} of SL ATP-supported calcium uptake from 0.88 μM to 0.7 μM, while calmodulin lowered this K 1/2 for Ca^{2+} to 0.67 μM; when added together the K 1/2 was lowered to 0.51 μM. The Vmax of calcium uptake (control value of 10.0 nmoles Ca/mg/min) was stimulated 28% by cAMP-PK, 25% by calmodulin, and 52% by the simultaneous addition of both compounds. The Hill coefficient (n = 1.8) was unaffected by any treatment. These results indicate that skeletal-muscle SL calcium-transport could be regulated by either cAMP-PK or CM-PK promoted phosphorylations, or by a direct interaction of calmodulin with a calcium-pump protein. (Supported by the Muscular Dystrophy Association and NIH GM31382).

W-Pos184 CAFFEINE INHIBITION OF Ca^{++} ACCUMULATION IN SKINNED FIBERS. M. Sorenson and H.S.L.Coelho. Departamento de Bioquímica, Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro RJ 21.910, Brasil.

To complete a study of caffeine effects on calcium accumulation by the sarcoplasmic reticulum(SR) in isolated "skinned"fibers, extensor digitorum longus muscles of rabbits from a Brazilian supplier were used. Fibers were stored at -20ºC in 50% glycerol-R after being skinned by overnight exposure to R (K propionate, EGTA, MgATP, pH 7.0, 0ºC). Calcium accumulation rates and capacities in the presence of oxalate were measured by monitoring light scattered from Ca oxalate crystals formed inside the SR. In the absence of caffeine, Ca accumulation at 25ºC from a buffered Ca^{++} solution containing 5 mM oxalate (free Ca^{++} 0.4 uM) was about 50% slower than that measured previously in fibers from New Zealand rabbits under the same conditions, although Km values (0.6 vs 0.75 uM) and Hill n values (1.9 vs 1.7) for Ca^{++} were similar in the two populations. Caffeine inhibition of the rate of Ca uptake was also similar, increasing from 30% inhibition with 0.5 to 1.0 mM caffeine to a plateau of 60 - 80% inhibition in the presence of 2.5 and 5 mM caffeine. Values of Km and n were unaltered by 2.5 mM caffeine. However, the addition of 2.5 mM caffeine after Ca oxalate accumulation had reached its maximum rate was much less inhibitory (20-30% vs 60-80%) than when caffeine was added before formation of Ca oxalate crystals could occur. Thus caffeine does not affect the interaction of Ca with the high-affinity sites of the SR Ca pump, but inhibition appears to be enhanced when free Ca^{++} inside the SR is high. This observation is consistent with the idea that caffeine primarily increases efflux rather than decreases influx.
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W-Pos185 REGULATION OF CARDIAC SARCOPLASMIC RETICULUM FUNCTION BY A CALCIUM-CALMODULIN-DEPENDENT PROTEIN KINASE. B.A. Davis and E.G. Kranias, Department of Pharmacology and Cell Biophysics, University of Cincinnati, College of Medicine, Cincinnati, Ohio 45267

Cardiac sarcoplasmic reticulum (CSR) contains an endogenous calcium-calmodulin-dependent protein kinase (Ca-CAM-PK) and a substrate, termed phospholamban (PL). PL was phosphorylated by Ca-CAM-PK using either A) 0.5 mM ATP or B) 5.0 mM ATP (which results in 50% greater Pi incorporation). Phosphorylation of PL using 0.5 mM ATP (Condition A) resulted in a 2-4 fold stimulation of calcium transport at low concentrations of calcium (<3 μM) but did not result in enhancement of Ca^{2+}-ATPase activity. Ca-CAM-dependent phosphorylation of PL, using 5.0 mM ATP (Condition B) was also associated with stimulation of the rate of calcium transport but, in addition, induced a 50% increase in the Ca^{2+}-ATPase activity. Thus, both Conditions A and B produced similar effects on calcium transport (2-4 fold) but only Condition B produced stimulation (1.5-fold) of ATPase activity. These data indicate that the efficiency of the Ca^{2+} pump is enhanced by phosphorylation in the presence of either 0.5 mM or 5.0 mM ATP. Analysis of the coupling ratio (CR) (Ca^{2+} transported/ATP hydrolyzed) as a function of calcium demonstrated that the CR is a variable quantity, dependent upon calcium and under the regulation of PL phosphorylation. The calcium concentration yielding 1/2 maximal activation of the CR was pCa 6.2 for control and pCa 6.4 for phosphorylated vesicles (under Condition B) indicating an increase in the calcium sensitivity associated with Ca-CAM-dependent phosphorylation. Therefore, the mechanism of the enhanced rate of calcium transport by CSR associated with phosphorylation of PL, appears to be related to an increase in the coupling between calcium transported to ATP hydrolyzed. (Supported by NIH grants HL26057, HL00775, and A.J. Ryan Fellowship to B.A.D.)

W-Pos186 TENTATIVE IDENTIFICATION OF THE TRANSVERSE TUBULE MAGNESIUM ATPASE. Roger A. Sabbadini, A. Stephen Dahms and Michael Moulton. Departments of Biology and Chemistry and Molecular Biology Institute, San Diego State University, San Diego, CA 92182.

Isolated transverse tubule (TT) membranes were analysed for their content of loosely-associated and integral membrane proteins. Of particular interest was the identification of the Mg-ATPase protein. Loosely-bound and trapped proteins, many of which were 'sarcoplasmic' enzymes, could only be released from the TT by low levels of Triton-X100 under conditions which induced membrane leakiness and high salt buffer access to membrane vesicle interiors. Approximately 60% of loosely-bound protein was extracted with Triton, resulting in a 2-fold enrichment of Mg-ATPase activity. Concommitantly, chiefly one core protein possessing a Mr of 102 KDa was enriched suggesting that it is responsible for the Mg-ATPase activity. Since about 80% of the membrane lipid was associated with this protein, we conclude that the Mr 102 KDa TT component is a major integral protein of the TT. Occasionally, Triton-purified TT membranes possessed a protein of Mr 25 KDa. However, the content of this component was variable and dependent upon isolation conditions, especially conditions which minimized proteolysis. Since the Mg-ATPase activity was enriched in parallel with enrichment of the Mr 102 KDa protein and not dependent on the presence of the Mr 25 KDa protein, it is likely that the Mr 102 KDa protein is the Mg-ATPase of TT membranes. Con-A stimulates the Mg-ATPase activity of native TT vesicles and is required to prevent Triton-induced inactivation of the ATPase during puri- fication. Since the 102 KDa TT protein is the major 125I-Con A binding protein of TT membranes, it is likely that the Con A effect is exerted through its action on the 102 KDa component. Supported by the Muscular Dystrophy Assoc.

W-Pos187 DRUG-INDUCED CALCIUM RELEASE FROM TRIADIC SR. P. Palade, Department of Physiology and Biophysics, The University of Texas Medical Branch, Galveston, Texas 77550.

Conditions have been developed for spectrophotometric monitoring, with arsenazo III or antipyrylazo III, of net Ca^{++} release from isolated SR that has been preloaded with Ca^{++} in the presence of the Ca^{++}-precipitating anion pyrophosphate (7.5 mM) and 1 mM MgATP. Under these conditions purified rabbit skeletal muscle triadic SR (J. Cell Biol. 96, 1008, 1983) can be preloaded to > 4 μmol Ca/mg SR without any subsequent spontaneous Ca^{++} release. Rapid Ca^{++} release can be initiated by addition of ≥ 5 μM free Ca^{++}, ≥ 2.5 mM caffeine, ≥ 400 μM halothane, ≥ 2 μM ryanodine, ≥ 700 μM menthol, ≥ 40 μM thymol, ≥ 40 μM quercetin, ≥ 100 μM tetraϕboron, ≥ 50 μM ketoconazole, ≥ 7 μM miconazole, ≥ 30 μM W-7, ≥ 10 μM TFP, ≥ 10 μM chlorpromazine, ≥ 80 μM palmitate, or ≥ 0.1 μM Ag^{+}. These compounds may be subdivided into at least two categories on the basis of pharmacologic sensitivity. Releases elicited by 10 μM free Ca^{++}, 10 mM caffeine, 1 mM halothane, 30 μM ryanodine, 1 mM menthol, 300 μM thymol, 300 μM quercetin, 100 μm ketoconazole, 30 μM miconazole, 100 μM W-7 or 1 μM Ag^{+} amount to ≥ 35% of the preloaded Ca^{++}, and release rates (1-6 μmol/mg/min, measured accurately without need for rapid mixing equipment) are highly sensitive to block by ruthenium red (IC_{50} ≤ 20 nM) and tetracaine (IC_{50} ≤ 200 μM). Releases elicited by 100 μM TFP, 300 μM chlorpromazine, 300 μM palmitate or 30 μM Ag^{+} are much less sensitive to these blockers (IC_{50} for ruthenium red ≥ 1 μM; IC_{50} for tetracaine > 3 mM), as if another Ca^{++} efflux pathway of considerable magnitude was opened. On the basis of their pharmacologic sensitivity, both types of pathways appear distinct from that which mediates phosphate-supported spontaneous release from light SR (J. Biol. Chem. 258, 12434, 1983).

W-Pos188 CALCIUM OXALATE AND CALCIUM PHOSPHATE CAPACITIES OF CARDIAC SARCOPLASMIC RETICULUM. Joseph J. Feher and Grayson B. Lipford, Dept. of Physiology and Biophysics, Med. Coll. of Virginia, Richmond, VA 23298

Both oxalate-supported and phosphate-supported calcium uptake by canine cardiac sarcoplasmic reticulum (CSR) vesicles initially increase linearly with time but fall to a steady-state level within 20 minutes. The cause of this behavior was investigated by determining the amount and rate of calcium released upon addition of 5mM EGTA. The amount of rapidly releasable calcium was zero until a threshold calcium uptake of about 4-6 μmol calcium oxalate or calcium phosphate per mg CSR protein. After that, the amount of rapidly releasable calcium continued to increase with calcium oxalate load to reach a maximum of more than 23 μmol/mg. The amount of rapidly releasable calcium phosphate was fairly constant at about 0.7 μmol/mg. Addition of 500μM ryanodine caused a marked increase in the threshold for the rapidly releasable fraction of calcium. Transmission electron micrographs of control vesicles showed that the vesicles are heterogeneous with respect to size, relatively few of the vesicles load with calcium oxalate, and the vesicles that loaded were quite full even at low overall calcium oxalate loads. In the presence of ryanodine, most of the vesicles showed calcium oxalate loading and thus each vesicle was less full at the same overall calcium oxalate load. These observations support the view that oxalate- and phosphate-supported calcium uptake is limited by vesicle rupture and that ryanodine stimulates calcium uptake by closing a calcium channel in a subpopulation of vesicles that otherwise would not accumulate calcium.

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W-Pos189 OXIDATION OF A SULFHYDRYL GROUP CAUSES RAPID CALCIUM RELEASE FROM SARCOPLASMIC RETICULUM VESICLES. Jonathan L. Trimm, Guy Salama*, Jonathan J. Abramson. Portland State University, Department of Physics, P.O. Box 751, Portland, OR 97207, and *Department of Physiology, University of Pittsburgh, Pittsburgh, PA 15261.

We see rapid release of Ca^{2+} from actively loaded SR vesicles upon the addition of cysteine or cysteamine (∿5-10μM) and $CuCl_2$ (∿1μM). Using an ATP regenerative system of Phosphoenolpyruvate and Pyruvate Kinase, the initial rate of Ca^{2+} efflux is 2-3 n moles Ca^{2+}/mg-sec with as much as 60% of the accumulated Ca^{2+} effluxing. The initial rate is strongly dependent on magnesium concentration. Upon the addition of tetracaine (<.5mM) or ruthenium red (<2μM), most of the released Ca^{2+} is reaccumulated by the vesicles. This effect is not observed at these concentrations with either cysteine or $CuCl_2$ alone. The oxidized form of cysteine, cystine, has no effect in the presence or absence of $CuCl_2$.

This data indicates that $CuCl_2$ is causing the oxidation of an essential sulfhydryl group on the Ca^{2+} release protein with externally added cysteine, to form a mixed disulfide.

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W-Pos190 "TRAPPING" A STRUCTURALLY DISTINCT INTERMEDIATE IN THE CA^{2+} UPTAKE REACTION OF THE SARCOPLASMIC RETICULUM BY TIME-RESOLVED X-RAY DIFFRACTION. D. Pascolini[1], L.G. Herbette[2], V. Skita[1], F. Itshak[1], A. Scarpa[1] and J.K. Blasie[1], [1]Depts. of Chemistry and Biochemistry/Biophysics, University of Pennsylvania, Philadelphia, PA 19104 - [2]Depts. of Medicine and Biochemistry, University of Connecticut, Farmington, CT 06032.

Time-resolved x-ray diffraction of oriented multilayers of isolated SR membranes at 0°C has detected significant changes in the membrane profile structure that are associated with the phosphorilation of the Ca^{2+}ATPase. The Ca^{2+} uptake reaction, as determined utilizing flash photolysis of caged-ATP and double-beam spectrophotometric techniques is biphasic with a fast phase identified with the formation of E~P, and a slow phase with Ca^{2+} transport across the membrane. Only the fast phase is apparent at 0°C in a time interval of several seconds. Data collection times corresponding to this time interval were adopted for the time-resolved x-ray diffraction studies at 0°C which utilized x-ray synchrotron radiation and the flash-photolysis of caged-ATP. The profile structure of the SR membrane in the resting state at 0°C was compared to the one at 7°C: model calculations indicate a redistribution of protein density along the profile with more protein mass occuring on the extravesicular surface at 0°C than at 7°C. The changes in the average electron density profiles detected with time-resolved diffraction studies at 7 and 0°C are only qualitatively similar. Model calculations indicate that although in both cases the changes arise from redistribution of protein mass from the extravesicular surface to the interior regions of the membrane profile, at 7°C the redistribution is spread along all the interior profile, whereas at 0°C the changes are more localized to only the outer phospholipid headgroup and hydrocarbon core regions. Support: NIH HL-18708,-32588.

W-Pos191 EFFECT OF HISTIDINE MODIFICATION ON THE CA^{2+} ACTIVATION OF SR ATPASE. C. Coan, University of the Pacific, 2155 Webster St., San Francisco, CA 94115

It has been established for sometime that modification of histidyl residues on the SR ATPase inhibits Ca^{2+} uptake and concomitant ATP hydrolysis. Two histidyl residues and one lysyl residue appear to be readily modified with ethoxyformic anhydride (Tenu, J. P., Ghelis, C., Saint Leger, D., Carrette, J. and Chevallier, J. (1977) J. Biol. Chem., 251, 4322). It was futher demonstrated that the loss of activity directly follows modification of the histidyl residue. We use iodoacetamide spin labeled SR (ISL·SR) to investigate the effect of histidine modification on the Ca^{2+} activation of the ATPase. ISL·SR is sensitive to a conformational change in the enzyme active site which is associated with the acquisition of high phosphate affinity, apparently through the formation of an activated complex of the enzyme with Ca^{2+} and substrate. The EPR spectrum explicitly demonstrates the requirement for cooperative binding of 2 Ca^{2+} at high affinity (μM) sites in establishing the activated conformation. Following histidine modification the Ca^{2+} effect on the enyzme conformation, normally associated with the high affinity binding curve, is shifted two to three orders of magnitude. Studies are currently underway to determine whether or not the histidine modification affects Ca^{2+} binding per se or the coupling of Ca^{2+} binding to enzyme activation.

W-Pos192 RECONSTITUTION OF CALCIUM PUMPING FUNCTION FROM CARDIAC SARCOPLASMIC RETICULUM (C-SR). M. Inui, B. K. Chamberlain, A. Saito and S. Fleischer Department of Molecular Biology, Vanderbilt University, Nashville, TN 37235

Ca^{2+} pumping in C-SR is modulated by the action of Ca^{2+}-calmodulin and cAMP-dependent kinases. To gain insights into the mechanism by which Ca^{2+} transport of C-SR is regulated, we initiated studies to reconstitute functional canine C-SR membrane vesicles. C-SR were solubilized with Triton X-100 in 0.8 weight ratio to protein. The solubilized sample was nonmembranous as viewed by electron microscopy. Removal of the detergent by Bio Beads SM-2 and washing resulted in the reconstitution to form membranous vesicles. The reconstituted vesicles were capable of energized Ca^{2+} accumulation with a good rate and efficiency (oxalate-facilitated Ca^{2+} accumulation = 1.65±0.31 μmol/min.mg, ATPase activity = 2.39±0.25 μmol/min.mg, efficiency of Ca^{2+} and ATP = 0.69±0.09 (n=5)). The reconstituted vesicles were phosphorylated by an exogenous cAMP-dependent protein kinase to almost the same extent as that in the original C-SR. However, phosphorylation of phospholamban had no effect on Ca^{2+} accumulation of the reconstituted vesicles. When the Ca^{2+} concentrations at which the half-maximal Ca^{2+} accumulation is obtained (K_{Ca}) were compared, cAMP-dependent phosphorylation of phospholamban in the original vesicles decreased K_{Ca} from 1.5 μM to 0.85 μM. On the other hand, K_{Ca} of the reconstituted vesicles was 0.5 μM and remained unchanged by phosphorylation, suggesting that Ca pump in the reconstituted vesicles was already fully activated. These results indicate that dephospho-phospholamban acts as a suppressor of the Ca pump of C-SR and that phosphorylation of phospholamban serves to reverse the suppression.

(Supported by NIH AM 14632 and a Muscular Dystrophy Fellowship to M.I.)

W-Pos193 DISSOCIATION OF SOLUBILIZED DIMERIC SARCOPLASMIC RETICULUM ATPase INDUCED BY PRESSURE. Sergio Verjovski-Almeida, Eleonora Kurtenbach and Gregorio Weber. Dept. of Biochemistry, Inst.Biomed.Sci., Federal University of Rio de Janeiro, Brazil and Dept.of Biochemistry, University of Illinois at Urbana, USA

Sarcoplasmic reticulum ATPase was solubilized with nonionic detergent dodecyl octaethylene glycol monoether ($C_{12}E_8$) under conditions which were known to yield dimeric functional ATPase (Silva and Verjovski-Almeida, Biochemistry 22:707, 1983). Polarization of fluorescence of tryptophan residues and of a pyrene butyryl probe covalently linked to the ATPase were measured. Pressures in the range of 1 bar to 2 kbar promoted dissociation of the ATPase dimers. The pressure required for half-dissociation (p 1/2) varied with ATPase protein concentrations in the range of 8 to 20 μg/ml. The volume changes upon dissociation were in the range of -155 ml/mole to -196 ml/mole. An increase in [Ca^{2+}] from 50 μM to 5 mM and in pH from 6.8 to 8.5 increased p 1/2 whereas a decrease in pH to 6.0 was of no effect. The activity of the ATPase decreases with increasing pressures in the range of 1 bar to 0.8 kbar; the lower pressures required suggest that during catalysis the equilibrium is shifted towards the monomer. Complete reversibility of the pressure effects on fluorescence polarization was obtained, indicating that reassociation took place after releasing the pressure. However, the reversibility of the activity was dependent on the presence of high (millimolar) [Ca^{2+}] in the assay. This suggests that monomerization and decrease in affinity of the enzyme for Ca^{2+} during catalysis both act in parallel to enhance the instability of solubilized enzyme under pressure. Supported by FINEP/CNPq (Brazil) and MDA (USA).

W-Pos194 SOME NEW ASPECTS IN TRANSIENT KINETICS OF CA-UPTAKE BY THE SARCOPLASMIC RETICULUM László Mészáros and Noriaki Ikemoto, Dept. Muscle Res., Boston Biomed. Res. Inst. and Dept. Neurol., Harvard Med. Sch., Boston, Mass. 02114

Transient kinetics (first 200 msec) of Ca-uptake and formation of the phosphorylated intermediate (EP) of the Ca-ATPase enzyme in Sarcoplasmic Reticulum (SR) have been studied. Using the technique of EGTA quenching to terminate the Ca-uptake in a rapid-multimixing quench apparatus (Froehlich-Berger model) we found that the uptake time-course shows a multiphasic character; the first fast phase ($k_{app.}$ = 120 s^{-1}) is followed by a plateau and later by a second fast phase ($k_{app.}$=40 s^{-1}). The amount of Ca uptake in each of these fast phases was about equal to EP_{max}. The results suggest that the two Ca transport sites are not identical: one is occluded (*) upon addition of ATP and the other is accessible to EGTA until the bound Ca is translocated, as indicated in the following scheme:

$$E\,Ca \xrightarrow{ATP} {}^{*}EP\,Ca \xrightarrow{ADP} {}^{*}EP\,Ca \longrightarrow {}^{*}EP\,{}^{*}Ca \xrightarrow{2Ca^{2+}} EP \longrightarrow E+P_i$$

In another set of experiments we followed the Ca movement in the same time range and under the same conditions using stopped-flow spectrometry of Arsenazo III, a Ca-indicator dye. It was found that in parallel with EP formation, a rapid Ca binding (10-12 nmole/mg SR prot.) takes place. Both the rate and the amount of this Ca binding to SR had ATP- and Mg-dependence identical to those of EP formation, indicating that an extra Ca binding to SR Ca binding sites different from the transport sites is induced by EP formation. Supp. by grants from NIH (AM 16922) and MDA.

W-Pos195 DRUG "FLIP-FLOP" BETWEEN MONOLAYERS OF A MEMBRANE BILAYER AS DEMONSTRATED BY NEUTRON DIFFRACTION. Leo G. Herbette[1,2,3], David Chester[1] and David G. Rhodes[1,3].
U. of Conn. Health Center, Departments of Medicine[1] and Biochemistry[2], Farmington, CT 06032. and Dept. Biology, Brookhaven National Laboratory[3], Upton, NY 11973.

Nimodipine, a 1,4-dihydropyridine (DHP) calcium channel antagonist which associates non-specifically with skeletal sarcoplasmic reticulum (SR), was obtained in deuterated form for experiments to determine the site of this nonspecific association. 5×10^{-5} M protonated or deuterated nimodipine was added to dispersions of sealed SR membrane vesicles which were then sedimented to form membrane multilayers. Neutron diffraction patterns from these multilayers were used to generate difference profiles representing the position of the drug molecules relative to the SR membrane structure. The drug appeared at a position corresponding to the outer knob region of the Ca-ATPase and at two positions symmetrically disposed within the lipid bilayer, a greater portion appearing in the inner monolayer. It has been shown for the SR membrane that the inner monolayer contains approximately 8% more lipid than the outer monolayer (Herbette *et al.* (1984) ABB 234 235-242). Since nimodipine and other DHP's exhibit very high partition coefficients, it has been suggested that the partitioning of these drugs in a lipid bilayer may be an integral part of the approach of these drugs to their active site on sarcolemmal membrane calcium channels. That these drugs appear in the inner membrane, and can therefore "flip-flop", allows for the additional possibility that the DHP binding site on the calcium channel protein could be at the level of the inner monolayer. (Supported by NIH HL-32588, NIH HL-07420 and the American Heart Association. LGH is a Charles E. Culpepper Foundation Fellow)

W-Pos196 CALMODULIN DEPENDENCE OF CALCIUM TRANSPORT BY ISOLATED TRANSVERSE TUBULE VESICLES. Ana Maria Garcia and Cecilia Hidalgo. Boston Biomedical Research Institute, Dept. Muscle Research. Boston, Ma 02114.

Transverse tubule (T-tubule) vesicles isolated from rabbit skeletal muscle are capable of transporting Ca^{2+} with high affinity ($K_m = 5 \times 10^{-7}$ M), at a rate of 10 nmol $mg^{-1} min^{-1}$, in an ATP dependent fashion. Addition of a specific inhibitor of calmodulin stimulation, compound 48/80, strongly inhibited the rate of transport at sub-maximal [Ca^{2+}], suggesting the presence of endogenous calmodulin. The inhibition by compound 48/80 was reversed by addition of exogenous calmodulin. Upon removal of the endogenous calmodulin by treatment with 5 mM EGTA and 0.6 M KCl, the T-tubule vesicles displayed calcium transport with lower affinity than the intact vesicles ($K_m = 1 \times 10^{-6}$ M). However, upon addition of calmodulin, the K_m for Ca^{2+} decreased to 3×10^{-7} M. The rate of transport at saturating [Ca^{2+}] was independent of the presence of calmodulin and it was comparable to the rate of transport of untreated vesicles. Thus, calmodulin increases the affinity of the T-tubule transport system for Ca^{2+}. Studies on intact vesicles indicated that at least 80% of the vesicles are tightly sealed and are oriented with the cytoplasmic side to the outside. Therefore, it appears that this calcium transport system may play a role in removing excess calcium from the sarcoplasm to the T-tubule lumen. Supported by NIH grant HL23007 and a MDA postdoctoral fellowship to A.M.G.

W-Pos197 SULFHYDRYL REAGENT DYES TRIGGER THE RAPID RELEASE OF Ca^{2+} FROM SARCOPLASMIC RETICULUM VESICLES (SR). Guy Salama, Alan S. Waggoner and Jonathan Abramson. University of Pittsburgh, Department of Physiology; Carnegie-Mellon University, Fluorescence Center, Pittsburgh, PA 15261 and Portland State University, Physics Department, Portland OR 97207

Silver ions and other sulfhydryl reagents have been recently shown to trigger the rapid release of Ca^{2+} from SR. Moreover, Ag^{+} was found to act on sulfhydryl group(s) of an SR protein which may be the physiological site for Ca^{2+} release (G. Salama and J. Abramson, JBC. 259:11363 1984). In principle, reactive sulfydryl dyes containing an iodoacetyl-group attached to various cyanine types of chromophores would form covalent bonds with sulfhydryl groups on the SR protein and thereby trigger Ca^{2+} release. Several dyes were tested for their ability to trigger release, three dyes RGA 563, RGA 412 and DCR 44, like Ag^{+}, induced rapid Ca^{2+} release. SR isolated from rabbit white skeletal muscle were suspended at 0.2 to 1 mg prot./ml in 100 mM KCl, 1-5 mM $MgCl_2$, 30 mM Imidazole, pH 6.7 at 23°C. Ca^{2+} in the extravesicular space was measured through the differential absorption changes of antipyrylazo III at 720-790 nm. Additions of Ca^{2+} then ATP initiated active Ca^{2+} transport by SR and produced large Ca^{2+} gradient. Subsequent additions of these sulfhydryl reagent dyes (5 to 50 uM) triggered rapid Ca^{2+} release which could be blocked by prior additions of either ruthenium red (5-25 uM) or tetracaine (0.2 to 1mM). These fluorescent dyes were used to label and isolate SR proteins and may help identify the physiological Ca^{2+} release "channel."

This work was supported and may help us identify and characterize the physiological Ca^{2+} release "channel". Supported by NIH RO1-NS18590 and K04 NS00909 to G.S., NS 19353 to A.W., and AHA and Alaska AHA Affiliate to J.A.

W-Pos198 EFFECT OF RUTHENIUM RED ON SKINNED SKELETAL MUSCLE FIBERS OF THE RABBIT. Pompeo VOLPE, and Giovanni SALVIATI. Centro di Studio per la Biologia e Fisiopatologia del CNR, Istituto di Patologia Generale, Università di Padova, Padova, Italy.

Ruthenium red (RR) is a known inhibitor of Ca^{2+} release from isolated sarcoplasmic reticulum (SR) (Miyamoto, H., and Racker, E. J. Membr. Biol. 66, 193-201, 1982), and a blocker of Ca^{2+}-gated Ca^{2+} channels selectively localized in purified terminal cisternae (Chu, A., Volpe, P., Costello, B.R., and Fleischer, S. Biophys. J. 45, 317a, 1984). The action of RR on Ca^{2+} loading rate into, and Ca^{2+} release from SR of chemically skinned skeletal muscle fibers of the rabbit, was investigated. Ca^{2+} loading in the presence of precipitating anions, i.e., 10 mM pyrophosphate, was monitored by a light scattering method. Ca^{2+} release was indirectly measured by following tension evoked by 5 mM caffeine. Stimulation of Ca^{2+} loading rate by 5 uM RR depended upon free Ca^{2+} of the bathing medium, being maximal around pCa 5.2 and absent above pCa 5.6. Caffeine-induced tension was antagonized by RR: IC_{50} for the rate of rise of tension was 0.5 uM, that for the extent of tension was 4-5 uM. These results suggested that RR inhibits Ca^{2+} release from SR of skinned fibers and that stimulates Ca^{2+} loading into SR by closing Ca^{2+}-gated Ca^{2+} channels. (Supported by institutional funds from the Consiglio Nazionale delle Ricerche and, in part, by funds from the Dino Ferrari Foundation to Prof. A. Margreth)

W-Pos199 THE BINDING OF VANADIUM (V) OLIGOANIONS TO SARCOPLASMIC RETICULUM. Peter Csermely, Sandor Varga and Anthony Martonosi, Dept. of Biochemistry, SUNY Upstate Med. Ctr., Syracuse, N.Y. 13210

The binding of monovanadate and the di-, tetra- and decavanadate anions to sarcoplasmic reticulum vesicles was measured by equilibrium sedimentation and ^{51}V-NMR spectroscopy. The affinity of vanadate binding and the number of moles of V bound increase with the size of the oligomers. The binding data can be rationalized in terms of one binding site per ATPase molecule for monovanadate and two sites per ATPase for decavanadate. Monovanadate and decavanadate solutions produce similar inhibition of Ca^{2+}-stimulated ATP hydrolysis, but decavanadate is particularly effective in promoting the crystallization of Ca-ATPase at low V concentration (10-100 μM) in a Ca^{2+}-free medium. The Ca-ATPase crystals formed with mono- and with decavanadate are similar in appearance and both types of crystals are disrupted by 5 mM Ca. Labeling of the Ca-ATPase with fluorescein isothiocyanate (FITC) at near 1:1 molar ratio blocks half of the decavanadate binding without effect on the binding of monovanadate. The inhibition of ATPase activity by FITC is nearly completely prevented by 1 mM decavanadate, while monovanadate was ineffective in blocking the reaction of FITC with the microsomes. FITC had no effect on the crystallization of Ca^{2+}-ATPase induced by either mono- or decavanadate. These observations imply that in addition to a kinetically stable E_2-vanadate intermediate, coordination of decavanadate to additional site(s) may contribute to the stabilization of the conformation required for crystallization of the Ca^{2+}-ATPase. The decavanadate binding site(s) may overlap with the binding site for FITC, but the decavanadate binding inhibited by FITC is not required for crystallization of Ca-ATPase. (Supported by grants from the NIH (AM 26545), NSF (PCM 8403679) and the Muscular Dystrophy Association.)

W-Pos200 TWO SITE CARRIER MODEL FOR CA^{2+} IN THE SARCOPLASMIC RETICULUM. L. Noodleman and J.B. Bassingthwaighte; Bioengineering WD-12, U. of Washington, Seattle, WA 98195.

Unidirectional and net transmembrane fluxes are analyzed for a carrier with two sequential binding sites. The carrier is assumed available for binding at a single side of the membrane at any given time, and traverses the membrane with differing permeabilities depending on the number of substrate ions attached. Since the carrier concentrations on the inside and outside membrane surfaces establish a steady state depending on the free substrate concentrations at <u>both</u> interfaces, the transmembrane substrate fluxes are related to both substrate concentrations in a complex way. In addition, the binding affinities can differ for the inside and outside membrane surfaces as would result, for example, from an active transport mechanism. Fluxes are examined over a wide range of substrate concentrations and parameter values, comparing these results with experimental data. The model is general; it not only allows competition and inhibition but also countertransport. Substrate inhibition occurs when the carrier transport rate is diminished by filling both sites. The time scale for the approach of carrier concentration to steady state is also explored. Changes of substrate and inhibitor concentrations induce transients in carrier availability, but these are estimated to be so rapid that steady-state equations can be used normally. (Supported by NIH grants HL19139 and RR01243)

W-Pos201 INTRACELLULAR FREE MAGNESIUM IN FROG SKELETAL MUSCLE FIBERS MEASURED WITH ION-SELECTIVE MICROELECTRODES. Alvarez-Leefmans, F.J., Gamiño, S.M., *Giraldez, F. and +González-Serratos, H. Dept. of Pharmacology. CINVESTAV-IPN. P.O. Box 14-740, Mexico 07000 D.F.; *Fac. Med. Valladolid, Spain and +Dept. of Biophysics University of Maryland, Baltimore, MD 21201.

Several muscle functions are critically dependent on the levels of intracellular free magnesium ($[Mg^{2+}]_i$). Reported values of $[Mg^{2+}]_i$ in skeletal fibers vary from 0.2 to 6 mM depending on technique and investigator. We used Mg^{2+}-selective microelectrodes (MgSE) with liquid sensor containing the neutral ligand ETH-1117, to measure directly $[Mg^{2+}]_i$ in frog (R. pipiens or R. montezumae) sartorius muscle fibers. MgSE show significant interference from K^+ at concentrations found intracellularly. Therefore $[Mg^{2+}]_i$ and $[K^+]_i$ were simultaneously measured in the same fibers to evaluate K^+ interference on MgSE response. Na^+ interference on MgSE is relatively unimportant at $[Na^+]_i$ between 5 to 10 mM. To estimate such interference we measured $[Na^+]_i$ and found it to be 6.2±0.4 mM (n=20 fibers from 5 muscles). Consequently, the MgSE calibration solutions contained (mM): K^+, 90, 110 or 130; Na^+, 6.5; Hepes, 5 (pH=7.1) and variable concentrations of Mg^{2+} (0, 0.2, 0.5, 1.0, 2.0, 5, 10). From 19 fibers (6 muscles) equilibrated in frog Ringer the following results were obtained (mean±SE): $[Mg^{2+}]_i$ = 0.80±.07 mM; $[K^+]_i$ = 91.7±2.7 mM, resting potential (Vm) = -79.3±0.4 mV while from 15 fibers (4 muscles) equilibrated in Ringer containing 0.5 mM Mg^{2+}: $[Mg^{2+}]_i$ = 1.69±0.21 mM; $[K^+]_i$ = 115.5±0.1 mM and Vm = -83.0±0.7 mV. The values reported here for $[Mg^{2+}]_i$ are distinctly lower than those reported by others using MgSE. Our results further indicate that $[K^+]_i$ is related to $[Mg^{2+}]_i$ and that muscles maintained in ordinarilly used frog Ringer i.e. without Mg^{2+}, might have significantly lower $[K^+]_i$ and $[Mg^{2+}]_i$ than previously thought.
(Supported by CONACYT, Mexico. HGS also supported by NIH R01 NS17048-0251).

W-Pos202 INTRACELLULAR DIFFUSIBLE AND TOTAL CONCENTRATIONS OF K, NA, MG AND CA IN FROG MUSCLE. David Maughan, Department of Physiology/Biophysics, University of Vermont, Burlington, VT 05405

A microsampling and X-ray spectroscopic method (Maughan & Lechene, Fed. Proc. 39: 1732, 1980) has been used to measure the fraction of the total intracellular potassium, sodium, magnesium and calcium that is freely diffusible under conditions approximating those in skeletal muscle *in vivo*. Single fiber segments from freshly isolated frog semitendinosus muscle were manually skinned under oil. Droplets (0.2 nl) of isosmotic sucrose solution were placed on each fiber and allowed to equilibrate with the cell fluid. X-ray spectroscopic analysis of droplets from 11 relaxed fibers yielded mean extrapolated equilibrium values of 82.0 mM [K], 7.6 mM [Na], 5.8 mM [Mg], and 1.0 mM [Ca]. X-ray spectroscopic analysis of whole muscle extracts yielded (in mmol/liter cell water, after subtraction of extracellular fraction) 172.2 mM [K], 20.4 mM [Na], 13.9 mM [Mg], and 3.2 mM [Ca]. Thus, about one half of the total intracellular K or Mg, and one third of the total intracellular Na or Ca, is freely diffusible and equilibrates with a microdroplet. These diffusible fractions include the primary monovalent cations K+ and Na+, and the important regulatory divalent cations Ca2+ and Mg2+ and their ionic complexes (mostly Ca-parvalbumin and Mg-ATP). The non-diffusible fractions include both mono- and divalent ionic species restricted to the vicinity of fixed anionic charges on structural proteins and species incorporated into fixed proteins and organelles. These preliminary results suggest that, under conditions approximating those in skeletal muscle *in vivo*, more intracellular K, Na, Mg or Ca is restricted than has been usually assumed. Supported by AHA 79-165 and NBREM (Harvard), C. Lechene, Director.

W-Pos203 INTRACELLULAR pH AND INTRACELLULAR SODIUM DIFFER SIGNIFICANTLY IN RAT SOLEUS AND EXTENSOR DIGITORUM LONGUS MUSCLES. J.H. Pizzonia, M.A. Gendreau, R.M. Gundel, D.G. Brunder, J.J. Oleynek, and R.D. Moore. SUNY, Plattsburgh, N.Y. and Dept. of Physiology and Biophysics College of Medicine, University of Vermont, Burlington, Vt.

Baseline intracellular pH (pH_i) and intracellular sodium (Na_i) levels are significantly greater in soleus muscles in comparison with extensor digitorum longus (EDL) from the same rat. Intracellular pH was measured in vivo in male Sprague-Dawley rats from the distribution of DMO after a 90 minute equilibration with [^{14}C]DMO (J.Gen.Physiol. 1983, 82:15a). Back extapolation of Na^+ washout in Na^+:K^+ free Mg^{++} Ringer containing 1mM ouabain (Fed.Proc. 1979, 106(2):375-378) at 25°C was used to estimate the extracellular space (ECS); this method also gives the Na_i concentrations. In paired muscles from 12 rats the pH_i measured 7.054 ±.015 (mean,±SE) and 7.000±.015 for the soleus and EDL respectively. This difference is significant (P<.01). The extracellular pH (pH_o) measured 7.437±.005. Na_i concentration measured 13.1±.53 mEq/Kg-wet-wt. for the soleus and 9.46±.64 mEq/Kg-wet-wt. in the EDL. This represents a 32% difference in concentration that is significant (P<.001). Soleus muscle represent 10% of the total skeletal muscle of the rat and is composed of 90% slow-twitch red fibers. Slow-twitch fibers are characterized by large numbers of mitochondria and a preference for oxidative (aerobic) enzymes for the derivation of energy. EDL muscle represents 90% of rat skeletal muscle and is composed of 50% fast-twitch red fibers and 50% white fibers Fast-twitch muscle fibers are characterized by high actin-activated myosin ATPase activity and a preference for glycolytic (anaerobic) enzymes for the derivation of energy. A change in the H^+ and Na^+ ion gradients across the plasma membrane may play a role in maintaining the metobolic needs of two different types of rat skeletal muscle. [Supported by NIH grant AM21059.]

W-Pos204 EFFECTS OF METABOLIC INHIBITORS ON CALCIUM FLUX AND RELAXATION PROPERTIES IN VENTRICULAR MUSCLE CELLS S. Biedert, D.S. Miura, and S.C. Quay From the Departments of Neurology and Clinical Neurophysiology, University of Heidelberg, FRG

Metabolic inhibitors (MI) have been shown to shorten cardiac action potentials and to decrease amplitude of contraction. To study effects of oligomycin (OLI), 0.2 mcg/ml, and 2-4 dinitrophenol (DNP), 10^{-4} M, on intracellular calcium (Ca_i) and protein phosphorylation, we used spontaneously beating cultures of 8-10 day old chick embryonic ventricular cells. Addition of MI showed initial transient increase in contraction amplitude and prolonged relaxation phase due to a transient increase in Ca_i. The increment in Ca_i caused a decreased Ca influx as determined by the use of ^{1}radiotracers. Effects of MI on protein phosphorylation was then determined. DNP decreased Ca-Mg ATPase, cyclic nucleotide phosphodiesterase (CNP), actin, and phospholamban phosphorylation. OLI decreased troponin, cNP, actin, and a regulatory protein phosphorylation. Although these decreases in Ca movement may be attributed to decreases in the Ca gradient due to a release of Ca from subcellular compartments, the role of specific protein phosphorylation in the determination of Ca_i movements appear important in determining electrophysiologic and contraction characteristics of tissues.

W-Pos205 COOLING CONTRACTURES AND THEIR RELATIONSHIP TO INTRACELLULAR CA STORES IN RABBIT PAPILLARY MUSCLES. John H.B. Bridge* (Intr. by Kenneth W. Spitzer), Cardiovascular Research and Training Institute, University of Utah, Salt Lake City, Utah 84112.

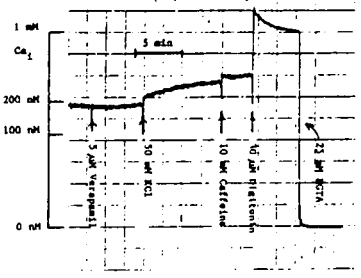
Isolated rabbit papillary muscles were mounted in a rapid flow chamber and were cooled abruptly from 30°C to between 1 and 3°C (τ=200 msec). Solution of the heat equation revealed that the muscle core temperature lagged the chamber temperature by <65 msecs. Cooling unstimulated muscles elicited a transient contracture. Its magnitude was greatest immediately following a series of twitches and progressively declined if preceded by increasing rest intervals ($t_{1/2}$=1.62 mins). Caffeine (10.0mM) applied during the rest interval significantly increased the rate at which contractures declined with rest ($t_{1/2}$=0.72 mins). Preliminary measurements on pairs of muscles from the same heart indicate that quiescent muscles contain approximately 210 µmoles/Kg tissue wet weight less intracellular Ca than muscles twitched at 30/min. Low Na solutions applied during the rest interval prevent the rest induced loss of contracture tension. Cold contractures are assumed to be due to an elevation of myoplasmic free Ca. Rest therefore appears to reduce the availability of activating Ca. The availability of extracellular Ca does not change in any obvious way with rest consequently some intracellular store of Ca must diminish or otherwise become unavailable for activation. Since caffeine releases Ca from the SR the results suggest the involvement of SR in activating cold contractures and that SR calcium diminishes with rest. Intracellular Ca measurements suggest that SR Ca is lost by the cells during rest. Low Na solutions applied during rest are expected to prevent removal of Ca from the cell via Na/Ca exchange and consequently prevent rest induced decline in cold contracture as observed.

W-Pos206 STUDIES ON THE INTRACELLULAR Ca SOURCES FOR AFTERCONTRACTIONS AND DELAYED AFTERDEPOLARIZATIONS; A COMPARISON WITH Ca SOURCES FOR TWITCHES. G.N. Tseng, A.L. Wit. (Intr. by B.F. Hoffman) Department of Pharmacology, Columbia University, New York, N.Y. 10032

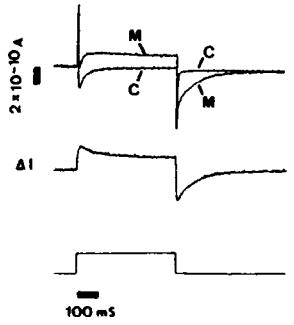
In canine atrial tissue from the coronary sinus, aftercontractions (AC) and delayed afterdepolarizations (DAD) appear when intracellular Ca is elevated by catecholamines. The Ca sources for, and the contribution of sarcoplasmic reticulum (SR) to the AC and DAD are unclear. To differentiate these sources, we measured changes in AC, DAD and twitch tension (TT, used as an index of SR Ca) in small coronary sinus preparations superfused with norepinephrine, using a fast flow system to change solutions rapidly. Changes in AC and DAD could be dissociated from changes in TT under the following conditions: (a) 60 sec perfusion with nominally Ca-free medium or TTX (10 uM) decreased AC and DAD more rapidly and to a greater extent than TT. (b) Exposure to caffeine (0.625 - 5mM) during a 25-50 sec quiescent period caused a concentration-dependent increase in DAD and AC of the first following beat, whereas the TT increased slightly at low concentrations up to 2.5mM and decreased at higher concentrations. (c) Lowering external Na to 25% increased resting tension and AC but decreased TT. DAD first disappeared and then reappeared. (d) 2-10 sec rest periods interpolated among trains of beats at 1 sec interval increased TT but decreased AC and DAD in a time-dependent manner; these effects could be partially inhibited by caffeine (1 mM). We conclude that AC and DAD are caused by similar internal Ca sources, which are not identical to those for TT. An important part of the Ca sources for AC and DAD can be readily regulated by Ca fluxes across the cell membrane.

W-Pos207 SOURCES OF Ca^{2+} DURING MEMBRANE DEPOLARIZATION BY HIGH CONCENTRATIONS OF KCl IN ISOLATED RAT VENTRICULAR MYOCYTES. By V.K. Sharma, A. Uglesity, S.P. Banerjee, and S-S. Sheu. Department of Pharmacology, University of Rochester, Rochester, NY 14642.

Recent studies suggest that the depolarization-induced Ca^{2+} influx through voltage-sensitive Ca^{2+} channels and Na-Ca exchange is responsible for the K-contracture in cardiac muscle. However, direct measurements of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in heart are still difficult and elusive. Using the new fluorescent Ca-indicator quin 2, we investigated the sources of Ca^{2+} during exposure of isolated rat ventricular myocytes to high concentrations of KCl. At the resting state $[Ca^{2+}]_i$ was 168 ± 16 nM (SEM, n=14). Increasing $[K^+]_o$ from 5 mM to values in the range of 25-75 mM caused stepwise increases in $[Ca^{2+}]_i$, which exhibited a biphasic nature. A large, rapid increase was followed by a smaller, slower component which eventually leveled off. Caffeine (10 mM) induced a 1.3-2 fold increase in $[Ca^{2+}]_i$, both before and after K-depolarization As shown in Fig., verapamil (5 μM) caused no change in the $[Ca^{2+}]_i$. However, it did block the rapid, but not the slow, component of the increase in $[Ca^{2+}]_i$ induced by K-depolarization. Similar results were obtained in the presence of 200-250 nM nimodipine. Several preliminary experiments indicated that norepinephrine (10^{-6}M) and isoproterenol (10^{-4}M) do not cause alterations in $[Ca^{2+}]_i$, before or after K-depolarization. These results are consistent with the idea that there exist at least two sources that supply the $[Ca^{2+}]_i$ increase due to K-depolarization.



W-Pos208 DOES Na^+ LOADING REVEAL A Na^+/Ca^{++}-EXCHANGE CURRENT IN SINGLE FROG ATRIAL CELLS? A. Uehara and J.R. Hume, Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824. Membrane currents were recorded in isolated single frog atrial cells using a suction-micropipette voltage clamp technique before and after intracellular Na^+ loading by either intracellular dialysis or after exposure to the sodium ionophore, monensin (M). Both experimental interventions (in the presence of TTX 3 μM) produced similar changes in membrane currents and contraction (Fig.): large increases in contraction were accompanied by an increase in outward current during depolarizing voltage clamp pulses, followed by an inward current upon return to the holding potential (-90 mV) which relaxed monoexponentially. Both inward and outward currents induced by Na^+ loading were inhibited by lanthanum, insensitive to tetrodotoxin or calcium channel antagonists, but were sensitive to changes in either external sodium concentration or internal calcium concentration. These changes in membrane current resemble: (i) the "creep currents" previously observed in cardiac Purkinje fibers during exposure to low potassium solutions (Eisner and Lederer, J. Physiol. 294: 255-277, 1979) and (ii) currents generated by a theoretical model of an electrogenic Na^+/Ca^{++}-exchange mechanism (Mullins, Am. J. Physiol. 236: C103-C110, 1979).



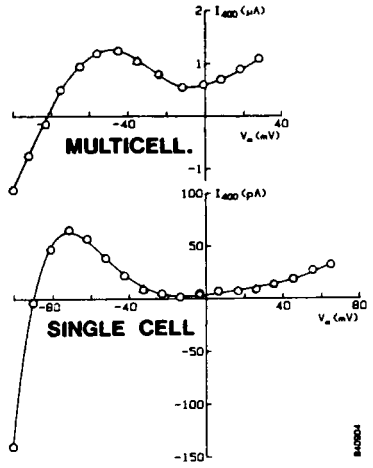
Supported by N.I.H. grant HL30143 and a grant from the American Heart Association of Michigan.

W-Pos209 Experimental and Theoretical Studies of the Electrogenic Na^+/Ca^{2+} Exchanger in Bullfrog Atrial Myocytes. D.L. Campbell, E.F. Shibata, K. Robinson, and W.R. Giles. Dept. Medical Physiology, University of Calgary, Calgary, Canada T2N 4N1.

Electrogenic Na^+/Ca^{2+} exchange has been postulated to be an important regulator of $[Ca^{2+}]_i$ and tension in cardiac muscle (Chapman, R.A. (1979). Prog. Biophys. Mole. Biol., 35:1-52; Mullins, L.J. (1981). Ion Transport in Heart. Raven Press, N.Y.). An electrogenic Na^+/Ca^{2+} exchanger will generate a current, I_{ex}, across the sarcolemma; it may therefore be measurable using the voltage clamp technique. Using the Mullins (1981) formalism we have developed a computer model which simulates the behavior of I_{ex} under voltage clamp conditions (I_{Ca} flowing, calcium-'buffering' proteins calmodulin and troponin present) in a single isolated cell from bullfrog atrium (Campbell, Robinson, and Giles. (1984) Biophys. J., 45:54a). These calculations show that during a depolarizing voltage clamp pulse to 0 mV I_{ex} is very small compared to I_{Ca}; however, upon repolarization I_{ex} may produce relatively large (30-50 pA) and slowly decaying (τ = ≈ 50 msec) 'inward tails'. The model also predicts that these I_{ex}-mediated 'slow tails' will increase in amplitude in response to clamp pulses of increasing duration (i.e. even though I_{Ca} is simultaneously decreasing). During experimental measurements of either I_{Ca} or I_{Sr} in these single atrial cells, 'slow tails' may also be observed. The model predictions and experimental findings are similar in showing that these tails (i) are approx. the same size, (ii) decay relatively slowly (time constant approximately 50 msec) and (iii) increase in initial amplitude with increasing pulse duration, suggesting that these 'slow tails' do not represent the Ca^{2+} channel deactivation. It is therefore possible that the 'slow tails' are generated by an electrogenic Na^+/Ca^{2+} exchanger mechanism. (Supported by DHHS HL-27454, and the Canadian MRC and CHF.)

W-Pos210 ELECTRICAL MODELS RELATING MEMBRANE CURRENTS AND POTENTIALS OF MULTICELLULAR HEART STRIPS TO THOSE OF SINGLE HEART CELLS. Leslie Tung, Univ. of Pennsylvania, Philadelphia, PA

With the recent development of the suction pipette voltage clamp (VC) technique, membrane currents can be recorded from single heart cells. Compared on the right are the membrane current-voltage (I-V) relations obtained from a frog ventricular myocyte (lower graph) and a frog ventricular strip (upper graph, obtained by the single sucrose VC technique). These 400 ms I-V relations, measured with identical $[Ca]_o$ and in the presence of TTX, are not scaled versions of one another. The slope of the multicellular I-V relation at rest is less steep than that of the single cell, and may be due to accumulation/depletion of extracellular K^+. Furthermore, the membrane current of the single cell is nearly zero at potentials between -20 and 0 mV, but for the multicellular preparation is typically half that obtained at -60 mV. To explain this discrepancy, a mathematical cable model was used to simulate the macroscopic currents measured in the multicellular preparation under voltage clamp conditions, given the electrical characteristics of the basic cellular unit. As a result, the observed discrepancy between the two I-V relations could be explained in terms of an electrotonic interaction between (relatively inexcitable) cells in the sucrose region and cells in the physiological node.



W-Pos211 ELECTRICAL PROPERTIES OF SUBENDOCARDIAL PURKINJE FIBERS FROM DOG VENTRICLE. T.M. Argentieri, L.H. Frame*, T.J. Colatsky, Dept. of Medicine, U. of Penn, Philadelphia, PA 19104

Subendocardial Purkinje fibers (SPF) may be implicated in the genesis of arrhythmias following coronary occlusion. The electrical properties of SPF were examined in the present study using standard microelectrode (μE) techniques. Segments of myocardium containing well-defined SPF-networks $\leq$ 200μ in diameter and several cm. in length were dissected from the septal region of dog ventricle and superfused with 4mM-KCl Tyrode's solution. The SPF network was very thin, as evidenced by our inability to record SPF-like action potentials (APs) from more than 2-3 cell layers before encountering ventricular muscle (VM) during progressive advancement of the μE. Current injection revealed coupling to be very good between adjacent layers in SPF but poor or absent between SPF and VM. Steady-state longitudinal voltage distribution was exponential within 100 μ of the current electrode, even in the presence of 1 mM $BaCl_2$ which increased Rin, as predicted by one-dimensional cable theory. Passive membrane properties for SPF are shown below. These results suggest that SPF are electrically similar to free running fibers, and amenable to voltage clamping under appropriate conditions.

MEASURED					CALCULATED				
λ	Rin	Θ	τ_m	τ_{AP}*	Ri	Rm	Cm	Cm(AP)*	τ_{AP}*
1.3±0.3mm	101±9KΩ	2±0.3M/s	5.9±0.5ms	94±4μs	503Ωcm	1.7KΩcm^2	3.5μF/cm^2	2.6μF/cm^2	72μs

(*)- Determined from the foot of the action potential

W-Pos212 NA CURRENT KINETICS IN ATRIAL AND VENTRICULAR MYOCYTES OF ADULT CATS. Christopher H. Follmer, Robert E. Ten Eick, and Jay Z. Yeh. Dept. of Pharmacology, Northwestern Univ. Med. School, Chicago, Illinois 60611. (Intr. by Kevin Chinn).

The atrial muscle action potential duration (APD) is shorter than that for ventricular muscle. A steady state Na current (I_{Na}) has been implicated to contribute to the APD in ventricular tissue. The short atrial APD may result from the absence of such a current flowing during the plateau phase. Using the single suction pipette voltage-clamp technique we characterized I_{Na} in dissociated cardiac myocytes from adult cat atrium and ventricle. Slow inward current and K^+ currents were blocked by adding 1 mM $CoCl_2$ and 1 mM $BaCl_2$ to the external solution as well as by using CsF filled electrodes. Also all currents analyzed were TTX subtracted currents. Analysis of I_{Na} evoked by voltage-clamp steps from -90 mv to test potentials (-40 to +20) showed atrial and ventricular cells to be similar: activated about -60 mv, reached a maximum at -20 mv and reversed at +60 mv. The time course of I_{Na} inactivation for atrium and ventricle were best fitted to a biexponential process. Both the fast and the slow inactivation time constants were about one-half those for ventricle. Both time constants in each tissue type are voltage-dependent, being faster with greater depolarizations. Recovery of I_{Na} from inactivation for both types of tissue was also voltage-dependent and best fitted to a biexponential function. The steady state inactivation parameters (h_∞) were not significantly different: h_∞=1.0 at -130 mv, 0.5 at -86 mv and 0 at -40 mv. A steady state non-inactivating TTX sensitive current was not detected in either tissue type. Our results suggest that atrial I_{Na} decays faster than ventricular I_{Na} and most likely does not contribute significantly to the atrial action potential plateau. In contrast, the slowly inactivating I_{Na} may contribute to ventricular action potential plateau.

W-Pos213 KCl COTRANSPORT IN CULTURED HEART CELLS MEASURED WITH ION-SENSITIVE MICROELECTRODES. S. Liu, R. Jacob, D. Piwnica-Worms, M. Lieberman. Department Physiology, Duke Univ. Med. Ctr., Durham, NC 27710 (Intr. by L. Mandel)

KCl cotransport was previously established in polystrand cultures of chick embryo heart cells (JGP 84:38a, 1984) and is now further investigated using K^+- and Cl-sensitive microelectrodes. Resting levels of activities were a^i_K=104.7±0.9mM (n=83), a^i_{Cl}=36.2±1.4mM (n=25). Because of intracellular anion interference, an apparent residual a^i_{Cl} of ≃ 10 mM was recorded in Cl-free solution: subtracting this residual gave a corrected activity (a^i_{Cl}*) of 26.9±1.9 mM (n=13). Values for Cl reversal potential were calculated either from a^i_{Cl}(E_{Cl}) or from a^i_{Cl}* (E^*_{Cl}). Polystrands gained K and Cl in 133K+127Cl for which the inward Cl chemical potential ($-\mu_{Cl}$) is greater than the outward K chemical potential (μ_K). Furosemide (3×10^{-4}M), an inhibitor of KCl cotransport, inhibited both K and Cl uptake by the same amount as did balancing μ_{Cl} and μ_K by reducing Cl to 25mM. In 0K+127Cl +DIDS (10^{-4}M), an inhibitor of Cl/HCO_3 exchange, polystrands lost K and Cl: furosemide attenuated the K and Cl losses by equal amounts. Taking the loss of cell volume into account (calculated from polystrand diameter), furosemide-sensitive net K and Cl efflux in 0K were both ≃ 5 pmole·cm^{-2}·sec^{-1}. The furosemide-sensitive changes in a^i_K and a^i_{Cl} in 133K and 0K support a 1:1 stoichiometry. Membrane potential (E_m) in 0K+DIDS rapidly depolarized to ≃ -40 mV and then gradually hyperpolarized. With 1mM Ba also present (to block the gradual hyperpolarization), E^*_{Cl} became negative to E_m by 8 and 36mV respectively by 15 min. At this time, despite an inward Cl electrochemical gradient, cells were still losing Cl, clearly demonstrating coupled movements of K and Cl. Supported in part by NIH grants HL07101, HL27105, HL17670 and GM07171.

W-Pos214 EFFECTS OF NOREPINEPHRINE AND ANALOGUES OF CYCLIC AMP ON INTRACELLULAR SODIUM ION ACTIVITY AND TWITCH TENSION OF CANINE CARDIAC PURKINJE FIBRES. M.S.Pecker, W.-B. Im, J.K. Sonn, C.O. Lee. Department of Physiology, Cornell Univ. Med. College, New York, NY 10021

In further studies of the effect of norepinephrine (NE) on intracellular sodium ion activity (a^i_{Na}), the effect of NE in the presence of tetrodotoxin (TTX) or strophanthidin and the effect of analogues of cAMP were assessed in canine cardiac Purkinje fibres electrically driven at 1 hz. TTX (5×10^{-6}M) lowered a^i_{Na} by 1.1±0.6 mM (mean ± S.D., n=4), from control levels of 8.5±1.3 to 7.4±1.1 mM. Addition of NE (10^{-6}M) lowered a^i_{Na} by an additional 1.0±0.2 mM to a level of 6.5±1.0 mM. In the presence of 16.2 mM K^+, NE ($1\text{-}5\times10^{-6}$M) lowered a^i_{Na} by 0.7±0.5 mM (n=5). Pretreatment with strophanthidin (5×10^{-6}M) in the presence of high $(K^+)_o$ prevented NE from lowering a^i_{Na} (n=4).

We also tested the effects of two analogues of cAMP. Exposure to dibutyryl cAMP (3-5 mM) for 6 to 10 minutes increased twitch tension by 94±67% (range 16-220%) (n=16) and lowered a^i_{Na} by 0.9±0.3 mM (n=11), from control levels of 7.1±1.3 mM to 6.2±1.2 mM. 8 p-chlorophenylthio-cAMP (10^{-4}-5×10^{-4}) for 6 - 10 minutes increased twitch tension by 100±63% (range 9-200%) (n=25), and lowered a^i_{Na} by 1.0±0.4 mM (n=18) from control levels of 8.4±2.7 mM to 7.4±2.5 mM. A transient fall in twitch tension to levels below control values occurred during the washout of either analogue. This averaged 23±8% below control values with dibutyryl cAMP and 28±8% with 8 p-chlorophenylthio-cAMP. Diastolic tension fell during exposure to both analogues. Thus, the effects of both analogues on a^i_{Na} and twitch tension were similar.

Overall these results are consistent with the hypothesis that NE stimulates the Na-K pump in cardiac Purkinje fibres. Further, the effects of NE are qualitatively similar to those of analogues of cAMP. Supported by USPHS HL 21136 and the N.Y. Heart Association.

W-Pos215 MEASUREMENT OF INTRACELLULAR K^+, Na^+ AND Cl^- ACTIVITIES IN VENTRICULAR MYOCYTES ISOLATED FROM RABBIT HEART. **Michel Désilets and Clive Marc Baumgarten, Dept. of Physiology and Biophysics, Medical College of Virginia, Richmond, VA 23298.**

Enzymatically isolated cardiac myocytes are becoming widely used for studying membrane events. If the isolation procedure alters intracellular ion content, comparisons with intact tissues may be confounded. Therefore, intracellular K^+, Na^+ and Cl^- activities were determined in single cells from rabbit ventricular septum (131 x 23 μ). Cells were bathed in Tyrode's containing 2.5 mM Ca^{++} and 5 mM K^+. Simultaneous impalements with a liquid ion-selective microelectrode (ISE) and a suction pipette were made. The suction pipettes were usually filled with 150 mM KCl or 500 mM K glucuronate. Comparison of E_m to that obtained with 3 M KCl-filled pipettes indicated that 150 mM KCl and 500 mM K glucuronate electrodes underestimated E_m by 5 and 3 mV, respectively. During ISE impalements, the corrected E_m averaged -85 ± 1 mV ($\bar{X}$ ± SE, n = 30). Bath K^+ was varied (1.5, 5 and 15 mM) to evaluate the E_m response of the ISE, a^i_K was 120 ± 5 mM (n = 12), and E_K was -93 ± 1 mV. After correction for extended-tip shunting, a^i_{Na} was 11 ± 2 mM (n = 9), and E_{Na} was 64 ± 4 mV. With 150 mM KCl electrodes, a^i_{Cl} was 38 ± 4 mM (n = 4) and E_{Cl} was -26 ± 3 mV. To check for diffusion of Cl^- from the E_m electrode, K glucuronate electrodes were used (Cl-ISE were about 13-times less sensitive to glucuronate than Cl^-). In this case, a^i_{Cl} was 30 ± 4 mM (n = 5) and E_{Cl} was -32 ± 3 mV. Values for a^i_K, a^i_{Na} and a^i_{Cl} in isolated cells appear to be greater than those reported for intact ventricular preparations. These differences may result from the isolation procedure, peri-electrode leaks, increased interfering ions in isolated cells or diffusion of electrolyte from the E_m electrode. Supported by NIH HL-24847. MD is an MRC fellow. CMB is an EI of AHA.

W-Pos216 CYTOPLASMIC K+ AND NA+ ACTIVITY IN SUBENDOCARDIAL CANINE PURKINJE FIBERS FROM ONE DAY OLD INFARCTS USING DOUBLE-BARREL ION SELECTIVE ELECTRODES: COMPARISON WITH MAXIMUM DIASTOLIC POTENTIAL. K.P. Dresdner, R.P. Kline and A.L. Wit, Pharmacology Department, Columbia University, College of Physicians and Surgeons, New York 10032

Double-barrel microelectrodes selective for K+ (Corning #477317) or Na+ (Fluka #ETH227) were used to measure maximum diastolic potential (MDP) simultaneously with cytoplasmic K+ activity (aKi) or Na+ activity (aNai) in ischemic Purkinje fibers (IPF) surviving in strips of subendocardium removed from one day old infarcted left ventricle caused by complete anterior descending coronary artery occlusion. IPF had depressed MDP which continuously improved over 3-4 hours during superfusion in oxygenated Tyrodes (4 mM K+, 2.8 mM Ca^{+2}) from -48.4±15.0 mV (n=25 impalements in N=4 hearts) to -72.9±9.8 mV (n=112, N=8) compared to MDP of Purkinje fibers in noninfarcted hearts (NPF) which were stable at -84.0±4.4 mV (n=103, N=14). K+ equilibrium potential (Ek) of IPF likewise improved from -78.5±7.3 mV to -91.5±5.3 mV, compared to NPF Ek of -95.9±4.7 mV (n=56, N=6). MDP change was about 2 times greater than Ek change, suggesting possible membrane conductance changes. IPF aNai (9.7±3.9 mM; n=27, N=6) initially was higher than NPF aNai (6.8±2.1 mM; n=47, N=8), and continued to increase during MDP recovery. In borderzone and recovered IPF (defined as MDP negative to -80 mV), aNai = 12.6±5.0 mM (n=46, N=5) and aKi = 99.9±19.1 mM (n=81, N=8, Ek of -94.1±5.1 mV). Compared to NPF, aNai gain (5.8 mM) is more nearly equal to aKi loss (7.0 mM) in recovered than in depressed IPF (aNai gain = 2.9 mM; aKi loss = 44.2 mM) assuming constant intracellular mean Na & K activity coefficients = 0.74. MDP depolarization per se during ischemia may counter aNai gain while promoting aKi loss. (Supported by PPG HL 30557 and HL 0727).

W-Pos217 INTRACELLULAR CHLORIDE ACTIVITY IN STIMULATED PAPILLARY MUSCLE INCUBATED IN SERUM. J.-P. Caillé, Dép. de Biophysique, Université de Sherbrooke, Sherbrooke, Québec, Canada.

An intracellular Cl ionic activity higher than predicted by a passive distribution exists in different cardiac cells superfused with "physiological saline". In order to measure the Cl ionic activity with experimental conditions similar to the conditions "in vivo", this study was realised with stimulated rabbit papillary muscle incubated in serum. The papillary muscles were isolated from the right ventricle and immediately transfered to a small chamber filled with serum. The muscle was electrically stimulated ($1Hz$) and the contraction recorded with a transducer. The chloride-sensitive microelectrodes were of the liquid-ion exchanger type (Corning #477913). The intracellular chloride-sensitive microelectrode potential recorded during the diastole permitted to determine the intracellular activity. The membrane potential (diastole) measured with microelectrodes, filled with 3M KCl was - 83.5 ± 0.5 mV (50) for these cardiac cells. At this frequency and under those conditions the intracellular chloride activity was 7.2 ± 1.2 mM (48). After a correction for the interference of the cytoplasmic constituents which was approximated to 4 mM the equilibrium potential for Cl (E_{Cl}) was evaluated to - 84.0 mV. These results suggest that with the conditions used in this study the distribution of Cl across the cellular membrane of these cardiac cells is at equilibrium with the membrane potential. This research was supported by the "Fonds de recherche en santé du Québec", "Fondation du Québec des Maladies du Coeur" and "Conseil de recherches médicales du Canada".

W-Pos218 EFFECT OF D600 ON TONIC CONTRACTIONS IN FROG MYOCARDIUM. Magda Horackova, Department of Physiology and Biophysics, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4H7.

Preparations (100-150 um in diameter and 2-3 mm long) of frog atrial muscle were stimulated at 0.33 Hz under voltage-clamp (double-sucrose gap), and the resulting membrane currents and the phasic and tonic contractions, were recorded simultaneously. It has been suggested earlier that the tonic contractions are regulated by an electrogenic Na^+-Ca^{2+} exchange (Horackova and Vassort, J. Gen. Physiol: 73:403, 1979). We investigated the effect of D600 on i) the tonic contractions elicited by long depolarizing pulses of amplitude close to calcium equilibrium potential and ii) the tonic contractions (and the corresponding positive inotropic effect - PIE) elicited by veratrine (Horackova and Vassort, Pflüg. Arch. 352:291, 1974). Within 5 min after the application of 1 uM D600, the calcium inward current (I_{si}) and the related phasic tension were reduced to <30% of their initial values; at the same time the tonic contractions were reduced to only 50-70% of control, but longer exposures (>40 min) reduced them further. Exposures to 10 uM D600 inhibited tonic contractions to <10% of control within 5 to 20 min. After addition of 50 uM D600, the tonic contractions and the increased Na entry due to the slow inactivation of I_{Na} elicited by 7.5 ug/ml veratrine were inhibited within 5 min to 20%; however, within 4 min of D600 being removed, veratrine's effects again fully developed. The results indicate that even in uM concentrations, D600 affects the tonic component of the contractile activity (and thus probably the Na^+-Ca^{2+} exchange) in cardiac muscle: D600 also inhibits veratrine's PIE (the $[Na]_i$-dependent tonic contractions) and veratrine's effect on the fast, TTX-sensitive Na^+ channel. Supported by grants from MRC of Canada (MT-4128) and Grant-In-Aid from the Nova Scotia Heart Foundation.

W-Pos219 EFFECTS OF CAFFEINE ON THE ELECTRICAL AND CONTRACTILE PROPERTIES OF ISOLATED FELINE VENTRICULAR MYOCYTES. Anthony Bahinski, Vuong DuThinh, Steven R. Houser. Dept. of Physiol., Temple Univ. Sch. of Med., Phila., Pa. 19140.

Recent studies suggest that the calcium released from sarcoplasmic reticulum (SR) during systole might influence the plateau phase of the action potential (AP) by alterations of slow inward current (I_{si}), an electrogenic Na/Ca exchanger ($I_{Na/Ca}$) and/or a Ca activated (non-specific) inward current ($I_{Na/K}$:see Noble, J. Physiol. 353,1-50,1984) The objective of the present experiments was to examine the effects of caffeine (C;which alters Ca release and reuptake by SR) on the electrophysiological and contractile properties of isolated feline ventricular myocytes. In these experiments C caused a dose dependent prolongation of AP duration (APD), an increase in the AP plateau height (APPH) but no significant change in the maximum diastolic potential (MDP;see Table). C also caused the magnitude and duration of shortening to increase in a dose dependent manner. Recent voltage clamp experiments showed that C increased the slow component of I_{si}. The effects of C on contraction are consistent with the idea that C increases cytosolic Ca (Ca_i) during systole. The C-related alterations in APPH and APD are explained best by a Ca_i induced (by C) change in $I_{Na/Ca}$.

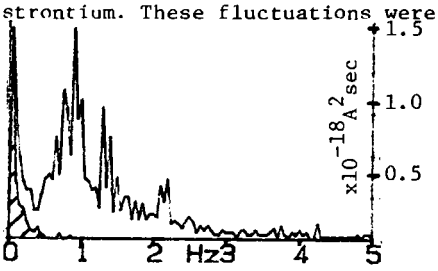
	CONTROL	0.5 mM	2.0 mM	5.0 mM	10.0 mM
MDP (mV)	-82 +/-1	-82 +/-1	-82 +/-1	-82 +/-1	-81 +/-1
APPH (mV)	+21 +/-2	+22 +/-2	+32 +/-3	+41 +/-3	+42 +/-2
APD(80%) (msec)	309 +/-18	311 +/-26	424 +/-39	524 +/-45	563 +/-68

W-Pos220 TEST OF THE "SIZE/SOLUBILITY" HYPOTHESIS IN DOG PURKINJE FIBERS: USE-DEPENDENT BLOCK OF $\dot{V}max$ BY CALMODULIN INHIBITORS. N.K. Jurkiewicz, L.B. Bird and T.J. Colatsky, Cardiac Diseases Unit, Wyeth Labs, Inc., Philadelphia, PA 19101.

Previous studies suggest that the use-dependent blocking (UDB) properties of Class I antiarrhythmic agents are largely determined by the molecular weight (MW) and lipid solubility (log P) of the drug (see K.R.Courtney, J.Mol.Cell.Card. 12: 1273-1286, 1980). We have recently reported a similar correlation between MW, log P (octanol:water) and the ability of various Class I agents to block upstroke velocity ($\dot{V}max$) in dog Purkinje fibers (Circ. 70: II-272, 1984). This correlation was tested using W-7 and W-5, two naphthalene sulfonamide derivatives known to inhibit calmodulin-dependent processes with IC_{50}'s of 20-30 μM and 200-300 μM, respectively. W-7 is larger (MW= 341 vs. 306) and more lipid soluble (log P= 1.46 vs. 0.13) than W-5, but differs from the latter only in the presence of a Cl-substituent on the naphthalene ring. Both compounds depressed $\dot{V}max$ at 3-30 μM when action potentials were elicited either following a 20 sec pause (tonic block, TB) or during stimulation at 3 Hz (UDB), but the observed potency was much greater than expected for simple amide-linked agents of comparable size and hydrophobicity. W-7 was ~6.6x more potent than W-5 in producing TB (IC_{25}= 10 vs. 66 μM), but showed a weaker tendency to produce UDB (IC_{25}= 23 vs. 10 μM), than predicted from considerations of MW and log P. These data suggest that the "size/solubility" hypothesis may not be adequate to explain the $\dot{V}max$ blocking effects of certain drugs, and that TB and UDB may involve different molecular mechanisms. Moreover, the data caution against assuming a "specific" antagonism of calmodulin by W-7 when intact cardiac preparations are used.

W-Pos221 RYANODINE INHIBITS CURRENT FLUCTUATIONS IN CARDIAC PURKINJE FIBERS. J.L. Kenyon, B.N. Maddux, R.J. Bauer, and J.L. Sutko. Departments of Pharmacology, Physiology and Internal Medicine, University of Texas Health Science Center, Dallas, TX 75235

We used the two microelectrode voltage clamp method to record membrane currents from shortened segments of calf cardiac Purkinje fibers and we measured isometric force development. Sodium pump blockade (1 μM strophanthidin) caused spontaneous synchronized fluctuations of current and force that had resonant frequencies around 1 Hz. This resonance was shifted to higher frequencies by depolarization (Kass and Tsien, Biophys.J. 38:259 1982). 1 μM ryanodine abolished both the current and the force fluctuations. The figure shows current spectral densities recorded at -60 mV in the presence of strophanthidin and after the addition of 1 μM ryanodine (cross hatched). We observed similar fluctuations in the presence of strophanthidin when extracellular calcium was replaced by strontium. These fluctuations were also abolished by 1 μM ryanodine. These results are similar to observations of a ryanodine inhibition of intracellular calcium fluctuations measured with aequorin (Orchard, et al. Nature 304: 735 1983; Wier, et al. P.N.A.S. 80:7367 1983) and they suggest that the fluctuations in current and force are secondary to fluctuations in intracellular calcium or strontium that are mediated by the sarcoplasmic reticulum and that their abolition is a consequence of a ryanodine inhibition of sarcoplasmic reticulum calcium release. Supported by NIH HL26528, AHA 81-815, NSF PCM 8402100.



W-Pos222 EFFECT OF COPPER AND PENICILLAMINE ON THE ELECTRICAL PROPERTIES OF FROG ATRIAL FIBERS. Z. Jarmoc, K.-S. Tan, and C.E. Challice, Departments of Pharmacology and Physics, The University of Calgary, Calgary, Alberta, Canada T2N 1N4.

Copper is an essential trace element in mammalian life, but if accumulation occurs, damage to liver and brain can ensue (Wilson's disease). The effect may be relieved by penicillamine. The mechanism of toxicity and of relief are both unknown. The effects of Cu^{++} at different concentrations, followed by administration of penicillamine, on the electrical properties of frog atrial fibers has been studied to obtain information on transmembrane ionic currents under these conditions. <5 μM Cu^{++} produced depolarization of the resting potential (which was both time and concentration dependent) and also a decrease in action potential (AP). The effect on duration of AP was biphasic: an increase at <40 μM Cu^{++}, a decrease at >40 μM Cu^{++}. Washout failed to restore normal properties; rather deterioration continued. Following 40 μM Cu^{++} and washout, 0.2 mM penicillamine restored AP amplitude and increased duration, the latter also being restored following extended washout (>1 hr). Penicillamine concentrations were critical. Penicillamine clearly restores the fast inward current in the AP and increases the slow inward current. Since washout does not remove the former effect the penicillamine presumably has a sequestration effect on the Cu. An increase in duration is produced also by penicillamine alone. Its effect on the Cu^{++} is complex and is in the process of being evaluated by means of a detailed analysis of the variation in the transmembrane ionic currents.

Supported by the Natural Sciences and Engineering Research Council of Canada.

W-Pos223 PHASE RESETTING AND BIFURCATION IN THE VENTRICULAR MYOCARDIUM. Young Seek Lee and Teresa Ree Chay, Department of Chemistry and Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260.

With the dynamic differential equations of Beeler and Reuter (1977, J. Physiol. 268: 170-210), we have studied the oscillatory behavior of the ventricular muscle fiber stimulated by a depolarizing applied current I_{app}. The dynamic solutions of BR equations revealed that as I_{app} increases, a periodic repetitive spiking mode appears above the subthreshold I_{app}, which transforms to a periodic spiking-bursting mode of oscillations, and finally to chaos near the suprathreshold I_{app} (i.e., near the termination of the periodic state). Phase resetting and annihilation of repetitive firing in the ventricular myocardium were demonstrated by a brief current pulse of the proper magnitude applied at the proper phase. These phenomena were further examined by a bifurcation analysis. A bifurcation diagram constructed as a function of I_{app} revealed the existence of a stable periodic solution for a certain range of current values. Two Hopf bifurcation points exist in the solution, one just above the lower periodic limit point and the other substantially below the upper periodic limit point. Between each periodic limit point and the Hopf bifurcation, the cell exhibited the coexistence of two different stable modes of operation: the oscillatory repetitive firing state and the time-independent steady state. As in the Hodgkin-Huxley case, there was a low amplitude unstable periodic state, which separates the domain of the stable periodic state from the stable steady state. Thus, in support of the dynamic perturbation methods, the bifurcation diagram of the BR equations predicts the regions where instantaneous perturbations, such as brief current pulses, can send the stable repetitive rhythmic state into the stable steady state.

W-Pos224 MEASUREMENT OF K^+ AND Cl^- CHANNELS IN RAT CULTURED VASCULAR SMOOTH MUSCLE CELLS. Richard Shoemaker, John Naftel, and Jerry Farley. Dept. of Physiology, U. of Alabama in Birmingham, Birmingham, AL 35294, and Depts. of Anatomy and Pharmacology and Toxicology, Univ. MS Med. Ctr., Jackson, MS 39216.

Cultured rat common carotid smooth muscle cells from a clonal line (9th to 12th passage) were used to measure single channel conductances by use of the patch clamp technique (Hamill et al., Pflugers Arch 391: 85, 1981). The cells were grown on plastic coverslips in Dulbecco's MEM with 10% fetal bovine serum at 37°C, 95% air - 5% CO_2. Cells were used 4 to 10 days after plating. To increase the patch seal resistance and % of patches that were usable, the cells were placed in 140 mM KCl and 5 mM HEPES for 1 hr prior to each experiment. This treatment caused the cells to round up. The solutions used in the experiments were near isotonic and the Na^+, K^+, and Cl^- contents were varied to help identify the channel types (the bath solution was 11°C). The data from 45 inside-out patches showed three K^+ and two Cl^- channels. The K^+ channels were: (1) Ca^+ activated K^+ channels, the most prevalent with a conductance of ≃ 80pS and fast flickering kinetics; (2) outward rectifying K^+ channels, g ≃ 45pS; (3) inward rectifying K^+ channels, g ≃ 52pS, that opened only at voltages more negative than -50mV. Two types of Cl^- channels were seen: (1) a large (flickering) Cl^- channel, g > 150pS, that activated with time at depolarizing voltages; (2) a small flickering Cl^- channel, g ≃ 12pS. Primary cultures of smooth muscle cells taken from rat common carotid were also patch clamped using either the cell-attached, or the inside out configuration. Similar K^+ and Cl^- channels were seen in these patches. (Research supported by: NS17789, DAMD17-83-C-3248.)

W-Pos225 IONIC CURRENTS IN AN ISOLATED SMOOTH MUSCLE CELL. P. G. Stein and P.A.V. Anderson, C. V. Whitney Laboratory and Dept. of Physiology, University of Florida, St. Augustine, FL 32086.

Whole-cell currents in smooth muscle cells isolated from the ctenophore Mnemiopsis were studied using either 2 electrode or patch-pipette voltage clamps. Five separate currents have been distinguished. These are: 1) I_{in} carried by Ca^{2+} (or Na^{+} in the absence of Ca^{2+}) and responsible for the rising phase of the action potential. This I is voltage activated above -40 mV, reaches a peak in 1-1.5 msec and decays as a single exponential (τ=2-10 msec) to a non-zero steady state. I_{in} inactivates completely at holding potentials above -20mV. 2) A large, Ca^{2+}-dependent I_K which activates with a τ=60 msec, inactivates completely with time (τ=1-2 sec) and can be blocked by 2mM Cd^{2+}. This I is maximum near +30 mV and diminishes as Em is increased or decreased. 3) A rapid, transient voltage-activated I_K similar to I_A in other cell types. This I activates with τ=1-3 msec at potentials of -50 mV or greater, and inactivates completely with time (τ=40-60 msec). 4) A smaller, voltage-activated I_K which appears to be the delayed rectifier current and is active at less negative holding potentials, activates slowly (τ=40-60 msec) but does not inactivate with time. Preliminary evidence suggests that these voltage-activated I_K currents exhibit different pharmacological properties. 5) An I carried by Cl^{-} which is eliminated in Cl-free medium. (Funded by the Whitehall Foundation & PHS GM09920-01).

W-Pos226 MYOGENIC ELECTRICAL CONTROL ACTIVITY OF HUMAN AND DOG COLON SMOOTH MUSCLE IS ASSOCIATED WITH CALCIUM CHANNEL ACTIVATION. Jan D.Huizinga, Nicolas E.Diamant and Taher Y. El-Sharkawy. (Intr. by E.E. Daniel). Dept. Neurosciences, Intestinal Diseases Research Unit,McMaster University,Hamilton,Ontario,Canada L8N 3Z5 and Dept. of Physiology,University of Toronto,Toronto, Ontario,Canada.

In the small intestine, the myogenic electrical control activity is likely associated with repetitive activation of sodium channels while the spiking activity is associated with calcium ions. It is the calcium channel which is highly sensitive to neurotransmitters and gut peptides. We found that in the colon, calcium ions are also involved in the generation of the control activity. Strips of both the human and dog colon circular and longitudinal muscle layers were both studied with the sucrose gap technique at 37°C. The electrical control activities of both muscle layers of the human colon and the longitudinal muscle layer of the dog colon were abolished by the calcium entry blocker D600 ($2X10^{-7}$M). In the dog colon, only drug induced prolongations of the plateau potentials of the control activity were sensitive to D600 ($5X10^{-8}$M) and low concentrations of Mn (0.1 mM). $BaCl_2$ (1mM) mimicked in both species maximal drug activated activity which is in the circular muscle: control activity of prolonged duration, at frequencies between 1 and 3 cpm, with intense spiking activity superimposed. In the longitudinal muscle it is control activity at about 30 cpm with spiking activity. Thus, colonic electrical control activity is associated with calcium channel activation. This, apparently, makes the control activity sensitive to interaction with neurotransmitters and gut peptides which can cause profound alterations in the patterns of colonic motility. (Supported by MRC Canada, and the Foundation for Ileitis and Colitis).

W-Pos227 SPECTROSCOPIC STUDIES OF ANISOTROPIC ROTATIONAL MOTIONS OF MUSCLE PROTEINS.
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Many biological macromolecules, including several muscle proteins, are either known or are presumed to be shaped such that different axes may have widely differing rates of rotation. Until recently, techniques for more precisely characterizing the shape and rotational properties of large irregular proteins or biopolymers have been limited. It has been proposed that fluorescence recovery spectroscopy or "pump-and-probe" techniques can be applied to monitor anisotropic rotational motions, including those occurring on a long time scale (W.A. Wegener, Biophysical Journal, vol. 46, in press). For example, two different rotational motions of a fluorescent or phosphorescent label can be quantitated if its transition dipoles for absorption and emission are sufficiently different.

Our preliminary findings, including polarization measurements of eosin in viscous media and eosin-5-maleimide covalently attached to F-actin, indicate that eosin-based labels have (at least) two absorption transition dipoles, one of which is nearly at right angles to the emission dipole. The results suggest that such probes are suitable for monitoring anisotropic motions of an elongated polymer such as F-actin. Quantitative measurements exploring this approach with muscle proteins will be reported.

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W-Pos228 EPR AS A PROBE OF ROTATIONAL DYNAMICS IN CROSSLINKED ACTO-S-1: EFFECTS OF NUCLEOTIDES.
Eric C. Svensson, Lance B. Augustin, and David D. Thomas, Dept. of Biochemistry, University of Minnesota Medical School, Minneapolis, MN 55455

We have used saturation transfer electron paramagnetic resonance (ST-EPR) to study the rotational dynamics of crosslinked acto-S-1 (XLAS-1), in which the S-1 was spin-labeled at the SH1 thiol. Previous work with spin-labeled myofibrils by Thomas et al. (1980, Biophys. J., 32:873-890) showed that changes in the rotational dynamics of the S-1 region of myosin (the myosin head) occur in the presence of nucleotides. These changes are complex, however, because myosin heads can either be attached to or detached from actin during their ATPase cycle. Relaxed myofibrils and purified myosin have been studied as models of the detached state (Thomas et al., 1980, Biophys. J., 32:873-890). In the present work, we are using S-1 covalently crosslinked to actin with 1-ethyl, 3-(3-Dimethylaminopropyl)carbodiimide (EDC) as a model of the attached state of myosin to actin. The method of crosslinking used was similar to that of Mornet et al (1981, Nature, 292:301-306). Our results indicate that in the absence of nucleotide, the rotational mobility of XLAS-1 is the same as that of non-crosslinked acto-S-1. This suggests that the crosslinking process does not alter the conformation of the acto-S-1 complex. In the presence of 5 mm ATP and 5 mm Vi, or 5 mm AMPPNP, the rotational mobility of the XLAS-1 was significantly greater, but not as great as that measured in relaxed myofibrils. This indicates that a conformational change occurs in the XLAS-1 complex in response to nucleotide, but that this change is more restricted than in relaxed myofibrils.

W-Pos229 MYOSIN CROSSBRIDGE DYNAMICS IN FILAMENTS ARE INFLUENCED BY MG^{++}: A SATURATION TRANSFER EPR AND TRANSIENT ABSORPTION STUDY.
R.D. Ludescher, S.L. Johnson, T.M. Eads, and D.D. Thomas. Dept. of Biochemistry, University of Minnesota Medical School, Minneapolis, 55455.

The disposition of myosin crossbridges with respect to the filament backbone appears to be modulated by Mg^{++}. Proteolytic digestion, chemical crosslinking, sedimentation velocity, and light scattering suggest that 5-10mM Mg^{++} induces the crossbridges to collapse against the filament backbone. The effect is most pronounced at high pH (>8.0). We have studied the dynamics of the S-1 region of myosin in filaments using two complementary techniques that are sensitive to microsecond motions: saturation transfer EPR (STEPR) and transient absorption anisotropy (TAA). A sulhydryl group of S-1 (SH1) was labeled with either iodoacetamide spin label or eosin-5-iodoacetamide. Changes in the low-field region of the STEPR spectrum induced by changing the [Mg^{++}] from 0.1 to 10mM (at pH 8.2 and 60mM ionic strength) are interpreted as reflecting a two-fold increase in the effective correlation time of the probe. The absorption anisotropy decays biexponentially to a constant value (r_{oo}). Increasing the [Mg^{++}] over the same range results in an approximately two-fold increase in r_{oo}. This is consistent with a restriction of the S-1 motion induced by high [Mg^{++}]. We are correlating the two techniques using a theory for the interpretation of STEPR spectra of spin labels moving in an anisotropic environment and employing the angular ranges measured in the TAA experiments. Studies at higher ionic strength and at pH 7.0 indicate that r_{oo} is a sensitive measure of the disposition of myosin crossbridges in filaments. These results are consistent with a model in which crossbridges are in equilibrium between a dynamically disordered state and a more restricted state, with an equilibrium constant that is dependent on Mg^{++} and other ligands, even in the absence of actin.

W-Pos230 LINEAR DICHROISM OF RHODAMINE-LABELED TnC INCORPORATED INTO SKINNED SKELETAL FIBERS. L.D. Yates*, T.P. Burghardt#, J. Borejdo# and A. Gordon*. Physiology and Biophysics, Univ. of Washington, Seattle, WA 98195* and CVRI, Univ. of California, San Francisco, CA 94143#.

Vertebrate skeletal muscle is activated when calcium binds to troponin, a regulatory protein of the thin filament. We have investigated thin filament activation in rabbit psoas muscle using linear dichroism of iodoacetylrhodamine labels attached to CYS-98 of TnC, a subunit of troponin. Calcium or magnesium binding to the labeled TnC produced a 1-3 nm blueshift with no change in fluorescent intensity. Experiments are now underway to determine the effect of calcium binding on the orientation and motion of the rhodamine probe. The presence of the label at CYS-98 reduced the affinity of TnC for TnI as judged by TnI affinity chromotography. However, incorporation of the labeled TnC into partially TnC-depleted skinned fibers resulted in recovery of tension and the force-pCa parameters (Hill coefficient N=3.30 and pCa at half maximal tension pK=5.70) to control values obtained with incorporation of unlabeled TnC (N=3.35, pK=5.69). Under relaxed conditions, the probe shows a high degree of order as measured by linear dichroism with the order parameter b=0.52 (as defined by Burghardt, et al, Proc. Natl. Acad. Sci. 80:7515 [1983]). Probe order decreased, b=0.30, in the presence of calcium (non-overlap conditions). Probe order also decreased when fibers were allowed to go into rigor, b=0.32, (overlap) and further decreased to b=0.13 when calcium was present. Increasing calcium from relaxing to activating levels resulted in monotonically decreasing probe order: at maximum activation it was intermediate, b=0.23, between the disorder found in rigor and rigor-calcium. Thus, the probe may be disordered by either calcium or cross-bridge interaction. This was supported by grants from NIH HL-16683, HL-07090, HL-31962, NS-08384 and MDA.

W-Pos231 RADIAL MOVEMENT OF CROSS-BRIDGES OF ISOLATED THICK FILAMENTS IN ATP-FREE MEDIA. S.F. Fan, M. M. Dewey and D. Colflesh. Dept. of Anatomical Sciences, SUNY at Stony Brook, NY 11794.

With the dynamic light scattering method we have shown that Ca^{2+} activates the energy requiring cross-bridge motions in isolated thick filaments from Limulus (J. Mol. Biol. 166:329 '83). We have also shown that if ATP is removed from the suspending medium, a motion with different characteristics develops. Such motion develops whether Ca^{2+} is present or not and is suppressed by trifluoperazine (TFP). It differs from the Ca^{2+} activated motion in that (1) an energy supply is not required; (2) it is insensitive to heat (42°C, 10 min.) treatment; and (3) it is also insensitive to phenylmethylsulfonyl fluoride (Biophys. J. 45:253a '84). Electron micrographs of negatively stained thick filaments in ATP-free medium show that the majority of cross-bridges extend out from the backbone of the filaments and optical diffraction patterns from these filaments lack the cross-bridge layer lines. These results strengthen our earlier speculation that the motion observed is the passive thermal motion of the cross-bridges as they swing out from the filament backbone. In polyelectrolyte gel systems such as muscle, the Donnan potential is a measure of the fixed electrostatic charge of the system. The negative Donnan potential of glycerinated muscle fibers from Limulus is increased by the removal of ATP when no Ca^{2+} is present as has been reported to occur in vertebrate muscle (Scordilis et al., PNAS USA 72:1325 '75; Elliot et al., J. Gen. Physiol. 84:40a '84) but is decreased in the presence of Ca^{2+}. TFP has no effect on the Donnan potential measured in the absence of Ca^{2+}. Thus, it seems that the charge changes measured are the result of conformational changes in proteins relevant to radial movement of cross-bridges; but the charge is not the cause of the movement. Supported in part by NIH grant GM 26392.

W-Pos232 MASS MEASUREMENT OF ISOLATED THICK FILAMENTS FROM LIMULUS STRIATED MUSCLE BY STEM MICROSCOPY. M. M. Dewey, D. Colflesh, B. Gaylinn, Dept. of Anatomical Sciences, SUNY at Stony Brook, NY 11794 and J. Wall and J. Hainfield, Brookhaven National Laboratory, Upton, NY 11973.

Limulus thick filaments shorten in situ in physiological conditions and in vitro. We have employed the STEM NIH Biotechnology Resource at Brookhaven National Laboratory to measure the mass per unit length of isolated thick filaments in both the long and shortened states. This technique has been used previously to weigh thick filaments (Reedy et al., J. Mus. Res. and Cell Mot. 2:45, '81). Thick filaments were isolated from muscle on glycerol step gradients, freed of background protein on a molecular exclusion column (BioGel A-5m), and freeze dried. Filaments isolated in EGTA relaxing solution averaged 4.0 microns long and weighed 16,703 dalton/A SD=846, N=142. Filaments dialyzed to pCa 3.5 shortened to 3.2 microns and weighed 19,111 daltons/A SD=1,538, N=267. Thus the total mass of the thick filament is unchanged upon shortening (668±34 megadaltons long, 612±54 megadaltons shortened). This suggests a model where Limulus thick filaments shorten by rearrangement of filament components in an incremental way with no change in the apparent axial diffraction pattern (Wray et al., J. Mol. Biol. 88:823, '74). Our measurement of the paramyosin and myosin content in this muscle by one and two-dimensional SDS gel electrophoresis as standardized to protein dry weight gave a mass ratio (PM/M) of 0.47 (Biophys. J. 45:230a, '83). Using this protein composition we calculate that a filament with four myosins per crown (146 A would weigh 18,822 daltons/A while one with three myosins per crown would weigh 14,116 daltons/A.) Thus our data can be modeled by a shift in the average number of myosins per crown from 3.5 in longer filaments to 4.0 in shortened thick filaments. NIH grants GM26392 (Dewey) and RR01777 (Wall).

W-Pos233 CALMODULIN-INDEPENDENT CA^{2+} REGULATION OF THICK FILAMENTS FROM LIMULUS STRIATED MUSCLE. Bruce Gaylinn, Maynard M. Dewey, and Shih-fang Fan, Dept. of Anatomical Sciences and Ben Chu, Chemistry Dept., State University of New York at Stony Brook, Long Island, NY 11794.

Limulus muscle possesses a calmodulin-dependent myosin light chain phosphorylation system (J. Sellers, J. Biol. Chem. 256:9274, 1981). Our data indicate that the thick filaments also possess a calmodulin-independent calcium activating system. By quasi-elastic light scattering (QELS) we have shown that Ca^{2+} can activate an energy-requiring motion of cross-bridges in isolated thick filaments from Limulus striated muscle (Biophys. J. 41:261a, 1983; 45:253a, 1984; J. Mol. Biol. 161:329, 1983). ^{32}P labeling shows that myosin light chain phosphorylation activity seen in homogenates is not present in our isolated thick filament preparations. The anti-calmodulin agent R24571 (calmidazolium) has no effect on calcium activated thick filament motions. Trifluoperazine (TFP) does block these motions, but its effects are not reversed by calmodulin and are probably through some other mechanism as both R24571 and TFP completely block the binding of ^{125}I-calmodulin to Limulus muscle proteins on polyacrylamide gels. Computer fitting of the calcium dependence of thick filament motions as detected by QELS suggests a single class of high affinity Ca^{2+} binding sites ($K' \cong 1.0 \times 10^5 M^{-1}$). ^{45}Ca binding to isolated thick filaments shows only this same binding affinity ($K' \cong 1.1 \times 10^5 M^{-1}$) plus non-specific binding. This kind of high affinity calcium binding in the presence of magnesium is known for a number of muscle proteins such as troponin C, molluscan myosin and calmodulin. The calcium binding capacity and protein composition of our thick filament preparations give the number of binding sites as ≅2,500 per thick filament or 2.3 ± 0.5 per myosin molecule. Supported in part by NIH grant GM 26392.

W-Pos234 COMPARATIVE STUDIES ON LIMULUS AND MERCENERIA PARAMYOSINS. S. M. Goldfine, M. M. Dewey, and B. Walcott. Department of Anatomical Sciences, S.U.N.Y., Stony Brook, N. Y. 11794.

Paramyosin is the protein which forms the core of invertebrate thick filaments. DeVillafranca and Hynes (Comp. Biochem. Physiol. 47B:9, 1974) showed Limulus paramyosin to differ from the molluscan molecule with regard to molecular weight, amino acid composition, and degree of helicity. Despite these differences some investigators have found these two types of paramyosin to be antigenically similar. Using both a different anti-Limulus paramyosin and a different protocol we were unable to demonstrate this cross reactivity (Goldfine, et. al. Biophys. J. 41:101a, 1983). We have now extended this observation by raising antibodies to paramyosin purified from a molluscan catch muscle: the white adductor of Merceneria. This antiserum does not cross react with purified Limulus paramyosin when tested by ELISA, "Western blots", or by the indirect immunofluorescent staining of paramyosin paracrystals. In order to determine if there are two completely different isoforms of this protein we have carried out one dimensional comparative peptide mapping of V8 protease digested (Cleveland, et. al. J. Biol. Chem. 252:1102, 1977) or CNBr cleaved (Gross and Witkop J. Am. Chem. Soc. 83:1510, 1961) Limulus telson muscle and Merceneria red and white adductor muscle paramyosins. The resultant peptides were analyzed by electrophoresis either on Laemmli (Nature 227:680, 1970) or Swank and Munkres (Anal. Biochem. 39:462, 1971) style polyacrylamide gels. The results obtained show from 30 to 60% of the polypeptides obtained from Limulus and Merceneria paramyosins have the same molecular weights. The red and white adductor paramyosins were found to be identical. Two dimensional peptide mapping is currently being done to verify these results. (Supported by NIH grants: GM26392 & NS19350.)

W-Pos235 STRUCTURAL CHANGES ACCOMPANYING PHOSPHORYLATION OF TARANTULA MUSCLE THICK FILAMENTS Roger Craig*, Raul Padron# and John Kendrick-Jones+. *Anatomy Department, University of Massachusetts Medical School, Worcester, MA 01605, #IVIC - Biofisica, APDO 1827, Caracas 1010 A, Venezuela, and +MRC Laboratory of Molecular Biology, Hills Road, Cambridge, England.

The thick filaments in tarantula muscle homogenates in relaxing conditions have non-phosphorylated regulatory light chains as shown by urea/glycerol gel electrophoresis and ^{32}P autoradiography. The crossbridges have a helical arrangement in which the heads from axially adjacent levels of bridges interact with each other (Padron et al. (1984), Biophys. J. 45, 10a). When the level of free Ca^{2+} in the filament suspension is increased from 10^{-8} to $10^{-4}M$, the light chains are rapidly phosphorylated by the endogenous myosin light chain kinase and calmodulin. Electron microscopy of negatively stained filaments reveals that light chain phosphorylation is accompanied by a loss of the helical ordering of the crossbridges and that the bridges sometimes project further from the filament backbone. A similar change in structure occurs when phosphorylation is accomplished in the absence of Ca^{2+} ($<10^{-8}M$) by addition of a Ca^{2+}-insensitive kinase to the filament suspension, suggesting that phosphorylation, and not Ca^{2+} per se, is the essential factor in this change. We suggest that in the relaxed state, when the regulatory light chains are nonphosphorylated, the myosin heads are held down on the filament backbone, possibly by head-head interaction and/or interactions of the heads with the tails of axially neighboring myosin molecules. Phosphorylation of the light chains alters these interactions and the crossbridges become more loosely associated with each other and/or with the filament backbone. In the intact muscle, this may facilitate actin-myosin interaction during contraction.

W-Pos236 ALUMINUM DISRUPTED BINDING OF MELITTIN TO CALMODULIN. Christopher Weis and Alfred Haug, Departments of Physiology, Microbiology and Pesticide Research Center, Michigan State University, East Lansing, MI 48824.

Upon binding stoichiometric amounts of aluminum ions, pronounced conformational changes are produced in calmodulin (CaM) (M = 17000), a key regulatory protein. These changes have been reported to involve the alpha helical content, the hydrophobic surface exposure, and the electrophoretic migration. These physico-chemical changes of the protein are reflected in calmodulin dependent enzymatic processes. To get further insight into the nature of the changes which occur in the aluminum-induced calmodulin, we used the small protein melittin (M = 2800) as a structural probe. Melittin is known to bind tightly and specifically to Ca-calmodulin and has been suggested as a model for investigating the interaction between calmodulin and its target enzymes (Biochem. J. (1983) 209, 269).

Our investigations have shown that the specificity of melittin binding to aluminum-induced calmodulin is altered. Circular dichroism studies indicate that the induction of alpha helix in the melittin molecule produced by native calmodulin is considerably reduced upon association of melittin with the aluminum induced CaM. Tryptophan fluorescence emission spectra of the melittin molecule show an increased intensity and blue shift upon complex formation with native calmodulin. However, melittin titrated with the aluminum-induced calmodulin shows neither a blue shift or an increase in fluorescence intensity. Polarization anisotropy of tryptophan fluorescence is found to be reduced in the Al-CaM/melittin complex and the relative strength of melittin binding as calculated for these experiments is considerably reduced. (This work supported by NSF grant #PCM-8314662 and a gift from Pioneer Hybrids International).

W-Pos237 CALCIUM-DEPENDENT PHOSPHORYLATION OF X-PROTEIN AND RED C-PROTEIN IN SKELETAL MUSCLE. Krystyna Kasman & Carl Moos, Biochemistry Department, SUNY, Stony Brook, New York 11794.

We previously reported that when crude C-protein, separated from myosin by DEAE-Sephadex chromatography, is incubated with ^{32}P-ATP in the presence of $MgCl_2$, $CaCl_2$ and phosphate buffer at pH 7, ^{32}P is incorporated into a 140,000 dalton protein [Kasman & Moos, Bioph. J. 41, 99a (1983)]. Hydroxylapatite (HAp) chromatography showed that the labeled protein is X-protein, not white muscle C-protein. The X-protein incorporates up to 2-3 moles ^{32}P per mole, and all the ^{32}P is on serine residues. Red muscle C-protein, which is very similar to X-protein, can be similarly phosphorylated in crude red C-protein preparations. HAp-purified X-protein and red C-protein alone are not phosphorylated under these conditions. A kinase which phosphorylates both proteins is eluted from HAp in a peak distinct from X-protein and red and white C-proteins. This phosphorylation is unaffected by cAMP, but it requires Ca ions. The Ca-dependence appears to involve calmodulin because some kinase preparations require added calmodulin for maximum activity. This kinase does not phosphorylate either intact myosin or isolated P-light chains of myosin. When red muscle slices are incubated with inorganic $^{32}P_i$ and C-protein is extracted and purified on HAp, ^{32}P is observed in the C-protein peak. An SDS gel autoradiogram of this peak shows radioactivity coinciding with the red C-protein band. This indicates that C-protein can be phosphorylated in intact red muscle. (Supported by NIH Grant AM28028, NSF Grant PCM 8317038, and Amer. Heart Assoc. Grant 83-1137.)

W-Pos238 A FLUORIMETRY STUDY OF N-(1-PYRENYL)IODOACETAMIDE-LABELLED BOVINE BRAIN S-100 PROTEINS. R.S. Mani and C.M. Kay, Medical Research Council of Canada Group in Protein Structure and Function, Dept. of Biochemistry, University of Alberta, Edmonton, Canada T6G 2H7

S-100 proteins undergo a conformational change upon binding Ca^{2+} and Zn^{2+}. The sulfhydryl fluorophore N-(1-pyrenyl)iodoacetamide was used to label the sulfhydryl groups in S-100a and S-100b proteins. Among the three sulfhydryl groups in S-100a only one, located at the C-terminal end of the β-chain, was labelled in native solvents, and in the case of S-100b also a single sulfhydryl group (residue 84) was labelled out of the two sulfhydryl groups in the β-chain. When the proteins were excited at 344 nm, the emission maximum occurred at 386 nm. Addition of Ca^{2+} to S-100a resulted in a 30% quenching in fluorescence intensity at 386 nm whereas with S-100b there was a 5% increase in fluorescence intensity. In S-100a and S-100b proteins, the probe responds differently to Ca^{2+} ions. Titration of labelled S-100a with Ca^{2+} yielded a dissociation constant (K_D) of 3×10^{-5} M and in the presence of 90 mM KCl the protein has a lower affinity towards Ca^{2+}. Addition of Zn^{2+} to S-100a caused a 15% increase in fluorescence intensity while with S-100b the observed increase was 40%. Titration of S-100 proteins with $ZnSO_4$ yielded a dissociation constant of about 9×10^{-6} M. Acrylamide quenching studies with S-100a revealed the probe to be more accessible to acrylamide in the presence of Ca^{2+}, whereas with S-100b the probe was less accessible in the presence of Ca^{2+}. The observed metal induced conformational changes around the labelled sulfhydryl group located at the C-terminal hydrophobic region may be physiologically significant since Ca^{2+} is required for the binding of S-100 protein to liposomes and this interaction is believed to result from the Ca^{2+} induced exposure of hydrophobic sites at the C-terminal end.

W-Pos239 PHOSPHORYLATION OF SKELETAL MYOFIBRILLAR PROTEINS BY cAMP-DEPENDENT PROTEIN KINASE. Megan S. Lim and Michael P. Walsh, Department of Medical Biochemistry, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

The role of cAMP-dependent protein kinase in skeletal muscle function was investigated by studying the phosphorylation of myofibrillar proteins by the catalytic subunit (C subunit) of the enzyme. Two major substrates of the cAMP-dependent protein kinase were observed in the myofibrils having the following molecular weights: 143,000 daltons and 33,000 daltons. The former has been identified as C-protein. The identity of the lower molecular weight protein is unknown. It has been determined by SDS-urea polyacrylamide gel electrophoresis that this protein is not a subunit of tropomyosin. C-protein is a component of skeletal muscle myofibrils found specifically in the A-band of the thick filaments. The function of C-protein is yet unknown; however, it is believed to play a role in modulating myosin cross bridge-actin interactions in skeletal muscle. In addition cardiac C-protein has been reported to be phosphorylated by cAMP-dependent protein kinase. We have observed phosphorylation of C-protein by C subunit in rabbit slow- and fast-twitch and canine slow-twitch skeletal myofibrils as well as in cardiac myofibrils. Purified C-protein from rabbit and canine skeletal muscle was also phosphorylated by C subunit to the extent of 0.45 mol P_i/mol C-protein. Pure C-protein inhibited the actin-activated myosin Mg^{2+}-ATPase activity by 50% in in vitro systems reconstituted from the purified contractile and regulatory proteins of both skeletal and smooth muscle. Phosphorylation of C-protein did not significantly affect this inhibition. Treatment of skeletal myofibrils with C subunit similarly had no significant effect on ATPase activity. (Supported by grants from the MRC and the AHFMR.)

W-Pos240 M-BAND PROTEINS IN A-SEGMENTS AND NATIVE THICK FILAMENTS. John L. Woodhead[+], Donald A. Winkelmann and Susan Lowey. [+]Dept. of Anatomy, The Medical College of PA, Phila. PA 19129 & Rosenstiel Center, Brandeis University, Waltham MA 02254.

We have observed that Triton skinned chicken pectoralis myofibrils readily separate into A-segments in the presence of 2mM Mg-ATP after incubation with monoclonal antibody (12C5.3) against myosin S-1 when using an antibody:myosin mole ratio of 1:1. In the absence of antibody, but in the presence of ATP, the A-bands fall apart into individual thick filaments. Therefore, the antibody appears to form cross-links between myosin heads in adjacent thick filaments thereby preventing separation of the filament array when the actin-myosin interactions are reduced by ATP. Negatively stained A-segments were shown by electron microscopy to be well-ordered rectangles 1600nm in length in which individual thick filaments were usually clearly discernible. We could not detect the presence of residual thin filaments within the isolated A-segments. A marked feature of these A-segments was the well-defined H-zone traversed by an M-band in which five striations were often visible. A-segments were also produced by cross-linking myofibrils within the M-band with polyclonal antibody against M-protein, but the degree of order was reduced. Antibodies specific for M-protein and creatine kinase were used to locate these proteins in the M-band of A-segments and in the bare zone of isolated native thick filaments. Electron microscopic examination of the A-segments showed that M-protein is located throughout the entire M-band whereas creatine kinase is restricted to a narrower region. These M-band proteins were also shown to remain bound to isolated thick filaments and appear to be responsible for the thickening observed in the middle of the bare zone. (Supported by grants from NIH, NSF and Muscular Dystrophy Association).

W-Pos241 EXCIMER FLUORESCENCE FROM PYRENE LABELLED PLATELET TROPOMYOSIN. L.D. Burtnick[*], D.I.H. Stewart[+] and L.B. Smillie[‡], Department of Chemistry, University of British Columbia, Vancouver, Canada V6T 1Y6[*] and Department of Biochemistry, University of Alberta, Edmonton, Canada T6G 2H7[‡]

N-(1-pyrene)iodoacetamide (PIA) reacts with cysteine residues on horse blood platelet tropomyosin (P-TM) to give an average extent of incorporation of 1.12 pyrene groups per P-TM chain. Control experiments with cardiac muscle tropomyosin (C-TM) suggest that cys-153 of P-TM, the homolog of cys-190 of muscle tropomyosins, reacts only weakly with PIA under the conditions we employ. This evidence plus analysis of tryptic peptides of pyrenyl-P-TM (Py-P-TM) and observed differences in the emission properties of Py-P-TM and Py-C-TM suggest that cys-246, the penultimate residue on each chain, is the major site of reaction of P-TM with PIA. The selectivity of the reaction with PIA, the high proportion of the total fluorescence from Py-P-TM that is due to pyrene excimers and the pretransition observed in thermal denaturation of Py-P-TM all suggest that the C-terminus of P-TM is not held in a rigid coiled-coil but has more flexibility than would be permitted by such a structure. The label at cys-246 also reports on end to end overlap interactions that occur between adjacent tropomyosin molecules. Addition to a Py-P-TM solution at low ionic strength of unlabelled P-TM, C-TM or of a carboxypeptidase A treated nonpolymerizable derivative of C-TM results in a reduction of the relative contribution of excimers to the total fluorescence from the sample. Addition of salt greatly reduces the effects of these tropomyosin species on the Py-P-TM emission spectrum.

(Supported by MRC Canada, NSERC Canada and the B.C. Heart Foundation.)

W-Pos242 TIME-RESOLVED FLUORESCENCE ANISOTROPY DECAY OF THE TRYPTOPHAN OF SKELETAL TROPONIN I AND ITS COMPLEX WITH TROPONIN C. Chien-Kao Wang[a], Iain Johnson[b], Tony Ruggiero[b], Danni Harris[b], and Herbert C. Cheung[a]. (a) Department of Biochemistry, Univ. of Alabama in Birmingham, Birmingham, AL 35294 and (b) Department of Chemistry, Univ. of Oregon, Eugene, OR 97403

Time-resolved emission anisotropy decay of the single tryptophan of skeletal troponin I (TNI) was measured by using a synchronously pumped mode-locked cavity-dumped dye laser as the excitation source. With 300 nm excitation three lifetimes were resolved by the method of moments: τ_1 in the range 1 - 2 ns; τ_2, 4 - 5 ns; τ_3, 7 - 9 ns. The anisotropy decay exhibited two rotational correlation times. At 19°C, $\phi_1 \sim 0.8$ ns, $\phi_2 \sim 30$ ns. ϕ_1 increased by a factor of less than 2 while ϕ_2 increased by a factor of 3 when the temperature was lowered to 2°C. ϕ_2 reflects the overall protein motion and is a factor of 3 - 4 larger than expected of an equivalent sphere with 20% hydration, suggesting TNI to be relatively asymmetric. ϕ_1 could arise from a rapid motion of the indole ring within a cone of semiangle 43°. In the absence of Ca^{2+} addition of troponin C (TNC) to TNI had no effect on ϕ_1, but in the presence of Ca^{2+} the addition resulted in a decrease of ϕ_1 to ~ 0.5 ns at 19°C, corresponding to a semiangle 37°. This fast motion appeared to be sensitive to Ca^{2+} binding to TNC in the TNI·TNC complex. The limiting anisotropy r_o of TNI was 0.26, which was little affected by TNC with or without Ca^{2+}, suggesting some motion of the tryptophan at times too short to be resolved. The very fast motion was not eliminated in the protein complex as r_o was still smaller than that expected for tryptophan in the absence of rotational motion. (Supported in part by NIH AM-25193).

W-Pos243 INTERACTION OF TROPONIN AND TROPOMYOSIN, R.H. Ingraham and C.A. Swenson, Department of Biochemistry, University of Iowa, Iowa City, Iowa 52242.

We have measured the thermodynamic parameters for the interaction between troponin (Tn) and tropomyosin (TM) at 25 °C using calorimetric and spectroscopic methods. The solution conditions were chosen to simulate a 'turned on' state, 1 mM Ca^{2+}, a 'turned off' state, 3 mM Mg^{2+} and 1 mM EGTA, and a reference state, 2 mM EDTA. Other solution components were 0.3 M KCl, 5 mM dithiothreitol and 0.01% NaN_3. Measurements of the equilibrium constants were made using TM labeled at 28% of the Cys-190 sites with dansylaziridine. The proximity of the probe to the region on TM where Tn binds resulted in a large enhancement of the fluorescence but necessitated a correction for probe effects. Using this correction, the association constants for binding to labeled Tm were found to be 2-3 times larger than for binding to unlabeled TM. The enthalpies were measured in a reaction calorimeter, and are corrected for proton effects. The thermodynamic parameters are:

State	$Kx10^{-5}(M^{-1})$	$\Delta G°$(Kcal/mole)	$\Delta H°$(Kcal/mole)	$\Delta S°$(cal/deg-mole)
Mg^{2+}	4.2	-7.7 (-10.1)[a]	-25.4	-59 (-51)[a]
Ca^{2+}	7.5	-8.0 (-10.4)	-22.1	-47 (-39)
EDTA	9.5	-8.2 (-10.6)	-23.5	-51 (-43)

a. ( ) denotes unitary quantities.

Comparison will be made with similar results on TnT and TnT fragment interactions with TM. The Tn-Tm interaction is a critical part of the interactions which regulate skeletal muscle contraction. Earlier studies suggested only small differences in free energy between 'turned on' and 'turned off' states. These studies show that if these changes are part of the regulatory signal they are small in terms of their enthalpic and entropic components as well. Supported by NIH Grants AM-27554 and HL 14388.

W-Pos244 INTERACTIONS OF SKELETAL TROPONIN T AND TROPONIN I PROBED BY SPECIFIC ANTI-SUBUNIT ANTIBODIES. Kimbrough D. Warber, Priscilla F. Strang and James D. Potter., Dept. of Pharmacology, Univ. of Miami School of Medicine, Miami, FL 33101

To obtain information about the specific sites of interaction between the three subunits of rabbit skeletal troponin (STn) - STnI, STnT, and STnC - we have begun studies utilizing antibodies raised against these individual subunits. Whole polyclonal IgG from the serum of goats immunized with purified STnI or STnT has been prepared. Binding of the antigen-specific IgG's to immobilized purified subunits has been demonstrated utilizing the enzyme-linked immunosorbent assay (ELISA) technique. ELISA experiments carried out with the whole troponin complex (STn) showed similar binding which was independent of the presence or absence of Ca^{2+}. To date, however, we have been unable to demonstrate binding (using modified ELISA, immunofluorescence, and myofibrillar ATPase assays) of these antibodies in the more complex intact myofibrillar system under a variety of conditions, including the presence or absence of Ca^{2+}. Since previous studies, both from this laboratory and from many others, have not shown a specific problem with antigen availability in myofibrils, we tentatively conclude that the reactive determinants in the isolated subunits and STn are somehow sequestered in intact myofibrils. Experiments utilizing thin filament protein reconstitutions of increasing complexity as antigen, along with the analysis of monoclonal antibodies as probes, are under way and will be discussed. Supported by NIH RO1 AM 33427 02 and HL07188.

W-Pos245 MEASUREMENT OF FLUORESCENCE AND TENSION DEVELOPMENT IN SKINNED SKELETAL MUSCLE FIBERS RECONSTITUTED WITH TnC_{DANZ}. Henry G. Zot, Konrad Guth*, and James D. Potter, Dept. of Pharmacology, Univ. of Miami School of Medicine, Miami, FL 33101, *Universitat Heidelberg, II. Physiologisches Institut, Heidelberg FDR

Fibers taken from the rabbit psoas muscle were mounted on a force transducer and skinned by incubation in 1% Brij 35. The fibers were extracted in 2mM EDTA, pH 7.8. Extraction produced a loss in the maximum tension developed by the fibers. Tension was restored to the fibers by incubation with TnC labelled with the fluorescent compound dansylaziridine (TnC_{DANZ}), which has previously been shown to undergo an increase in fluorescence when Ca^{2+} is bound at the Ca-specific sites. The emitted fluorescent light of the mounted fiber was collected through the objective of a fluorescence microscope. The fibers were bathed in a solution containing 7mM EGTA, 2mM MgATP, 3mM Mg, 70mM K, 15mM creatine phosphate, 15u/ml CPK, adjusted to pH 7.00 with propionic acid and imidazole, μ = 0.15. The addition of Ca^{2+} produced a positive tension and fluorescence increase such that each addition resulted in paired fluorescence and tension points in the steady-state. The Ca-titration curve for tension development was found to be approximately 0.5 pCa units to the right of the curve for the fluorescence change. The curves appear to be parallel with Hill coefficients near 1.0. Myofibrils prepared from rabbit back muscle similarly extracted and reconstituted demonstrated an approximate 0.5 pCa unit difference in fluorescence and ATPase curves when measured in solutions of similar composition. A model consistent with the shift of this magnitude would be one in which the actual Ca^{2+} binding curve occurs midway between the tension and fluorescence curves with tension resulting from Ca^{2+} binding to both Ca-specific sites while the fluorescence change is produced by Ca^{2+} binding at either Ca-specific site. Supported by NIH HL226193A and HL07188.

W-Pos246 DEFINITIVE ASSIGNMENT OF THE ^{113}Cd RESONANCES IN TROPONIN C. Priscilla F. Strang+, Paul D. Ellis* and James D. Potter+. *Department of Chemistry, Univ. Carolina, Columbia, S.C. 29208 and +Department of Pharmacology, Univ. of Miami School of Medicine, Miami, FL 33101.

Previous results (Ellis, P., Strang, P., and Potter, J.D. J.B.C. 259, 10348, 1984) have tentatively assigned the two sharp resonances at -107.8 and -112.7 ppm in the ^{113}Cd NMR spectrum of cadmium saturated TnC to the high affinity Ca^{2+}-Mg^{2+} sites and the two resonances at -103.1 and -109.8 ppm to the Ca^{2+}-specific sites, based on binding and competition studies. These assignments have been confirmed using two fluorescent derivatives of TnC, TnC_{DANZ} and TnC_{IAANS}. The fluorescence of TnC_{DANZ} responds primarily to Ca^{2+} binding to the two Ca^{2+}-specific sites of TnC. When TnC_{DANZ} was titrated with Cd^{2+} the same change in fluorescence was seen as with Ca^{2+} and the Cd^{2+} dependence of this change yielded an apparent K_{Cd} of $\sim 10^3 M^{-1}$. This is consistent with our previous assignment of the two low affinity Cd^{2+} sites being the Ca^{2+}-specific sites. The fluorescence of TnC_{IAANS} responds to Ca^{2+} binding at both the Ca^{2+}-Mg^{2+} and the Ca^{2+}-specific sites of TnC. When TnC_{IAANS} was titrated with Cd^{2+}, the fluorescence change associated with the Ca^{2+}-Mg^{2+} sites correlated with the high affinity Cd^{2+} binding sites, whereas the fluorescence change associated with the Ca^{2+}-specific sites correlated with the low affinity Cd^{2+} binding sites. Both results are consistent with our previous assignments. Supported by NIH GM26295.

W-Pos247 COMPARISON OF THE EFFECT OF $[Mg^{2+}]$ ON THE Ca-DEPENDENT ATPase AND TENSION DEVELOPMENT OF FAST SKELETAL MUSCLE. Anita S. Zot and James D. Potter. Dept. of Pharmacology, Univ. of Miami School of Medicine, Miami, FL 33101.

Previous studies have shown that while free Mg^{2+} concentration affects the Ca-dependence of tension development in cardiac skinned fibers (Donaldson et al., J. Gen. Physiol. 71, 645, 1978), free Mg^{2+} has been shown to have little or no effect on the Ca-dependence of rabbit myofibrillar ATPase (Potter and Gergely, J.B.C. 250, 4628, 1975). This apparent conflict was investigated by measuring Ca-dependent ATPase in rabbit myofibrils and Ca-dependent tension development in rabbit adductor skinned fibers at two different $[Mg^{2+}]$. Myofibrillar ATPase measurements were done in buffers identical to those used by Potter and Gergely ($[Mg^{2+}]$ = 31.5μM and 2.15mM) and in buffers essentially the same as those used by Donaldson et al. ($[Mg^{2+}]$ = 200μM and 5mM). Tension was also measured in buffers essentially the same as those used by Donaldson et al. Results show that the myofibrillar ATPase Ca-titration curve at higher $[Mg^{2+}]$ is slightly to the right of the low $[Mg^{2+}]$ curve in both buffer systems. Calculation of K'_{Mg} gives the same value ($\sim 120 M^{-1}$) in both buffer systems. The tension Ca-titration curve at high $[Mg^{2+}]$ is also to the right of the low $[Mg^{2+}]$ curve, but the shift is slightly greater than that seen with the ATPase. The K'_{Mg} calculated from the tension data ($\sim 495\ M^{-1}$) is larger than that from the ATPase data, but still small. These data indicate that $[Mg^{2+}]$ has a small but detectable effect on both both ATPase and tension development in rabbit fast skeletal muscle that cannot be explained by Mg binding to the Ca-Mg sites of TnC, but is consistent with a possible indirect effect of Mg on the Ca^{2+}-specific sites (Potter et al. Fed. Proc. 40, 2653, 1981). Supported by NIH HL226193A, HL07188 and the Lucille P. Markey Charitable Trust.

W-Pos248 ELECTRICAL PROPERTIES OF CRAYFISH PHOTORECEPTOR CELLS. A. Picones and H. Arêchiga. Departament of Physiology and Biophysics. Centro de Investigación del IPN. Apartado Postal 14-740, México, D.F. 07000, MEXICO.

The electrical properties of retinula cells of Procambarus clarkii in the dark and during the light response were characterized. Intracellular potential recording and simultaneus constant current injection were made through a bridge amplifier. Microelectrode resistance ranged from 50 to 90 MΩ. The resting potential of the 15 reported cells was -52.3 ± 4.4 mV ($\bar{x}$ ± S.E.). The current-voltagerelationship was linear in the studied range of injected currents (± 1.0 nA). For the hyperpolarizing currents we found an input resistance of 28.1 ± 5.4 MΩ. The voltage transients in response to hyperpolarizing current pulses showed a simple exponential relationship, suggesting isopotentiality at the cell interior. The analisis of the dV/dt vs. time curves confirmed this result. The time constant was 69.5 ± 10.3 msec. The calculated total membrane capacity was 3.2 ± 0.6 nF. The specific membrane capacity was 3.2 ± 0.5 μF cm^{-2}. A value of 69.4 ± 10.3 KΩ μF was calculated for the membrane resistance. The time course of the membrane conductance increment in response to a relatively intense light stimuli was described by two exponential components (typical values for time constants: 16.0 and 0.4 sec). For low intensity stimuli only the slow component was present. The maximun membrane conductance change increased with the stimulus intensity.

W-Pos249 CABLE PROPERTIES OF NEUROSECRETORY CELLS OF THE CRAYFISH EYESTALK.
U. García, A. Picones, L. Rodríguez-Sosa and H. Arêchiga. Department of Physiology and Biophysics. CINVESTAV, Apdo. Postal 14-740, 07000 México, D. F.

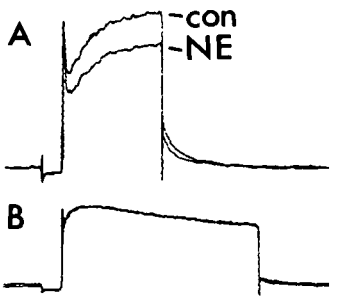
The passive membrane properties of X-organ cells of Procambarus clarkii were studied in the isolated eyestalk. The cells were identified by intracellular injections of lucifer yellow (hyperpolarizing pulses of 0.5 sec, 1 nA, 1 Hz for 2 hours). Constant current pulses applied through a bridge amplifier were injected into the soma to determinate the steady state current-voltage relationship. The resting membrane potential was -50.5 ± 3.6 mV, n=19 ($\bar{x}$ ± S.E.). A linear relationship was usually found within a broad range of the injected hyperpolarizing currents. The calculated input resistance was 73.4 ± 10.6 MΩ. Two time constants describe the course of voltage response to hyperpolarizing pulses. From these data and using the Rall's model, the electrotonic length (L) and the ratio of input conductance of the process to that of soma (ρ) were 1.51 ± 0.63 and 3.62 ± 1.08 (n=9) respectively. The morphometric analysis of X-organ cells showed a high variability in size and shape with major and minor axes of 33.4 ± 5.7 μm and 23.7 ± 1.6 μm (n=36) respectively. Taking these values the calculated membrane specific resistance (Rm) was 24.16 KΩ cm^2 and the membrane specific capacity was 7.9 μF/cm^2.

W-Pos250 THE PRE- AND POST-SYNAPTIC CONTRIBUTION OF ADENOSINE RELEASE IN THE RABBIT SYMPATHETIC GANGLION. M. Bencherif, R. Rubio and R.M. Berne, Dept of Physiology, University of Virginia, School of Medicine, Charlottesville, Virginia 22908, U.S.A.

As a step toward understanding adenosine metabolism and its role in neurotransmission, we studied the site of origin of the purines released from the sympathetic superior cervical ganlion of the rabbit. The ganglia were preincubated with ^{3}H-adenosine, washed and continuously superfused with an oxygenated Krebs-Henseleit solution. The overall release of ^{3}H-purines was determined by wash out. The electrical stimulation of the presynaptic branch resulted in a 90% increase above resting level of the purines released, whereas the presence of a combination of synaptic blockers (atropine 2μM, curare 150μM and phentolamine 10μM) resulted in only 57% increase over the control. Selective stimulation of the postsynaptic branch induced a 65% enhancement of the label efflux. In other experiments in which we incubated ganglia with ^{3}H-adenosine and ^{14}C-choline, we have found that there was a concurrent release of ^{3}H-purines and ^{14}C-acetylcholine during electrical stimulation. Interestingly, only the stimulation-induced ^{14}C-ACh was blocked when a Ca++ free solution was used. Moreover, the presence of phenylephrine (10^{-5}M) induced a transient increase of the ^{3}H-purines released without an associated neurotransmitter release. Taken together, these results indicate that the purines released during electrical stimulation are not the sole result of transmitter release and that a postsynaptic component of the release can be isolated. However a presynaptic contribution to the release of purines cannot be ruled out although this release does not seem to be associated with the release of the cholinergic vesicles. Support by grant HL 10384

W-Pos251 NOREPINEPHRINE REDUCES A VOLTAGE-DEPENDENT OUTWARD CURRENT IN HIPPOCAMPAL PYRAMIDAL CELLS. R. Numann and R.K.S. Wong (Intr. by Mae Huang). Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550.

Experiments were performed on acutely isolated pyramidal cells from adult guinea pigs. In TTX-containing solution (1 µgm/ml), voltage-clamped currents evoked by depolarizing steps from -35 to +20 mV (holding potential -50 mV) were outward (see Fig. A). Essentially three components can be distinguished, (a) a transient outward current with an inactivation time constant of about 20 ms. This component is usually partially inactivated at the holding potential but is revealed by prehyperpolarization. Additional outward currents can be differentiated by their sensitivity to Ca^{++}. Mn^{++} substantially reduces a "noisy" delayed outward current (C-current). The remaining delayed component shows slow inactivation (B). Norepinephrine (NE; 10 µM) significantly reduces the C current (Fig. A, peak current 740 pA control; 600 pA in NE. Pulse duration 500 ms). Fig. B shows that in the absence of Ca^{++} (0.75 mM Mn^{++}) NE is no longer effective (Peak current 920 pA). This suggest that NE selectively supresses a C current. Single channel recordings also reveal that NE suppresses an outward channel current with a conductance of 92 pS. These outward channel currents appear to be Ca^{++} dependent since they are blocked when 0 Ca^{++}, EGTA solutions are introduced in an inside-out recording configuration. These actions of NE are mimicked by isoproterenol. (Supported by NS13778 and the Klingenstein Foundation).



W-Pos252 EVIDENCE FOR A NA-CA EXCHANGE MECHANISM IN APLYSIA NEURONS USING NA AND CA SENSITIVE MICROELECTRODES. S. Levy and D.L. Tillotson. B.U. Med. School, Boston, MA 02118.

In nerve cells modulation of some membrane conductances is primarily mediated by a transient increase in intracellular free Ca concentration (Ca_i), mainly caused by influx through voltage-dependent Ca^{2+} channels. Over any long period of time, Ca_i must be in a steady state, which implies that all Ca^{2+} that enters the cell must be extruded. Two such long term extrusion mechanisms are membrane processes: the Ca^{2+} pump and the Na-Ca exchanger. The Na-Ca exchange mechanism uses the Na^+ gradient as a source of energy to extrude Ca^{2+}. To determine whether the Na-Ca exchange mechanism contributes to Ca^{2+} extrusion, Na_o and Ca_o were varied and Na_i was measured using Na selective microelectrodes based on the neutral ligand ETH 227. The Na electrodes were made as described elsewhere (Gorman et al., J. Physiol. 353:127, 1984). Selected neurons of the abdominal ganglion of Aplysia were first voltage clamped at -40 mV, then penetrated with the calibrated Na electrode. The mean resting Na_i was 12.9 mM $\pm$ 3.6 S.E.(n=7) in cells bathed in normal artificial seawater (NSW: Na 500, Ca 10, Mg 50, Tris 10, pH 7.8). Reducing Na_o to 5 mM (Tris substituted) decreased Na_i to 2.0 mM $\pm$ 1.11. Keeping Na_o at 5 mM and increasing Ca^{2+} to 20 mM (Mg substituted) further decreased Na_i to 1.35 mM $\pm$ 0.63. Since at Na_o=5mM, the driving force for Na ions was still inward, and since increasing Ca_o decreased Na_i, it is concluded that the decrease in Na_i is due to Na/Ca exchange. In spite of the decrease in Na_i, Ca_i, as measured with Ca selective microelectrodes, did not increase. It is possible that the rise in Ca_i, brought about by the decrease in Na_i is taken up by the Ca^{2+} pump or by mitochondria (Chapman et al., J. Physiol. 343:253, 1983). Supported by NIH grant NS18813.

W-Pos253 IONIC AND METABOLIC CORRELATES OF VOLUME ALTERATIONS IN CULTURED ASTROCYTES. James E. Olson, Raman Sankar, and David Holtzman. Dept. of Psychiatry and Neurology, Tulane University School of Medicine, New Orleans, LA 70112.

Cerebral astrocytes may be important in the regulation of extracellular water activity and the resolution of edema in the brain. We have studied the ionic mechanisms of astrocyte volume changes and their relation to oxidative metabolism. Astrocytes from primary culture swell initially in hypo-osmotic NaCl media to a greater extent than that predicted by osmotic theory. This increased swelling may result from the inhibition of a Na-dependent, ATP-independent component of respiration which is coupled to the reduction of intracellular osmoles under iso- and hyper-osmotic conditions (Olson and Holtzman, Brain Res 246:273, 1982). These observations have now been extended in kinetic studies of mechanisms of cellular volume changes. A regulatory volume decrease is observed during the first 30 min following the initial hypo-osmotic-induced swelling in astrocytes. Subsequent return to iso-osmotic conditions at this time results in cell shrinkage to a smaller volume than control cells not exposed to hypo-osmotic media. Thus, the volume recovery occurs coincident with an apparent reduction in the intracellular osmolarity or activation of a contractile process which increases cell surface tension. The regulatory volume decrease in astrocytes is blocked by 1 mM ouabain and by agents which inhibit electron transport.

We propose that energy-dependent, ionic processes in the astrocyte are involved in the control of extracellular water activity in the brain.

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W-Pos254 OLIGODENDROCYTE-SUBSTRATUM INTERACTION MODULATES THE EXPRESSION OF A CLASS OF GLYCOPROTEINS. S Szuchet, SH Yim, and PE Polak, Dept. of Neurology, University of Chicago, Chicago, Illinois 60637

We have shown that oligodendrocytes (OLG) undergo changes in their lipid and glycolipid metabolism after cell attachment to a competent substratum and during cell growth; we have also shown that protein metabolism is affected. We were therefore interested in following the fate of glycoproteins during similar events. For this we labeled cultured OLG with 5μCi/ml L-[5,6^{3}H]fucose (50 Ci/mmol) after 3-5 (floating) and 9-56 (attached) days in vitro for 20 h. Supernatant was removed, cells were washed, harvested, and sonicated. Proteins were resolved on discontinuous SDS-PAGE using 7-25% linear gradient gels followed by autoflurography and data analyzed as described (Yim and Szuchet, Trans. ANS 1984 15:231). The uptake of [^{3}H]fucose by OLG increased with time in vitro to a maximum at ∿35 days and then declined. Eight major fucosylated components ranging in mol.wt. from 113K down to 78K and some minor bands were detected. A change in the protein profile took place following cell attachment to a substratum and during cell growth (membrane synthesis). These changes can be characterized as qualitative and quantitative. The modulation in the expression of glycoproteins that followed the transition from the non-attached to the attached state involved the appearance of a new component (90K±2K), the enhancement and interconversion of another (78K→80K). Two components became conspicuous at this time and remained so throughout. It is postulated that these components may be involved in cell-substratum and cell to cell interactions.
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W-Pos255 KAINIC ACID RESPONSES OF PIRIFORM CORTEX NEURONS SHOW UNUSUAL DEPENDENCE ON EXTERNAL [CA++] CONCENTRATION. D. O. Carpenter, J. M. H. ffrench-Mullen* and N. Hori*, Wadsworth Laboratories, New York State Department of Health, Albany, New York 12201

The vertebrate central nervous system contains at least three types of receptors for the excitatory amino acids, characterized by receptors for N-methyl aspartate (NMA), quisqualic acid (Quis) and kainic acid (KA), (Watkins, et al., A. Rev. Pharmacol. 21:165: 1981). The NMA response is blocked by high Mg++ and is potentiated by zero Mg++ solutions. Nowak et al. (Nature 307:462:1984) have shown that Mg++ acts as a channel blocker of NMA responses. We have studied sensitivity of pyramidal neurons of rat piriform cortex brain slices to NMA, Quis and KA, and have compared the sensitivity of these responses to changes of both Mg++ and Ca++. As others have shown, when Mg++ in the perfusing medium was increased (2x) the NMA response was depressed or blocked, while when Mg++ was omitted from the medium the response was facilitated. The Quis and KA responses were affected to a much smaller degree. When Ca++ was omitted in the presence of normal Mg++ the KA response, and to a lesser degree the responses to both NMA and Quis, were dramatically potentiated. In 2x Ca++ solutions the KA response was usually totally blocked. The conductance increases associated with the KA and Quis responses and the conductance decrease associated with the NMA response were not altered in zero Ca++–zero Mg++ solutions. The potentiation of the KA response in zero Ca++ solutions was often so great that attempts to elicit as many spikes in normal medium resulted in irreversible cell death. These results support the hypothesis that KA activates a channel which is partially permeable to Ca++, and that Ca++ acts as a partial channel blocker similar to the actions of Mg++ at NMA receptors. KA neurotoxicity may reflect the results of Ca++ entry to a degree that overwhelmed Ca++ buffering capacity and leads to irreversible changes.

W-Pos256 MULTIPLE-SITE MONITORING OF ACTIVITY IN CULTURED NEURONS. David W. Tank$^+$ and Zahur Ahmed*, $^+$Department of Molecular Biophysics , AT&T Bell Laboratories, Murray Hill, NJ 07974 and *Division of Neurobiology, SUNY Buffalo, Buffalo, NY 14214.

An optical imaging system for use with fluorescent voltage-sensitive dyes and a compatible cell stimulation and recording system using microelectrode arrays are being developed for use in monitoring and modifying the electrical activity in small networks of tissue-cultured neurons. An inverted microscope is used to project the real fluorescence image of a small network of dye-stained cultured cells onto the polished face of an hexagonal closepacked array of 256 1-mm diameter polystyrene optical fibers. The opposite ends of the individual fibers are connected to low-noise photodiode/amplifier detectors. Dissociated primary cultures of rat diencephalon neurons (10-15μ diameter) have been used in initial experiments. Cells were stained with the styryl dyes RH237 or RH421 (the gifts of Amiram Grinvald) and cell fluorescence was excited with the 546 nm line from a stabilized Hg lamp. Action potentials could be optically monitored ($\Delta F/\Delta V$ = .5-1%/100mV) in detectors located over the images of cell bodies. In control experiments, the observed time course of the optical signal was identical to concurrent membrane potential measurements obtained using whole-cell patch recording techniques. Culture chambers containing a lithographically-defined array of microelectrodes have been constructed for use with the imaging system. Sixty 10μ diameter gold electrode pads are located in a 0.5 mm diameter area at the center of the chamber floor. Wires buried beneath an insulating layer of silicon nitride or a silicone polymer connect the bath exposed pads to external electronics through a multi-pin chip carrier. Site specific stimulation of cells can be obtained during optical monitoring of activity.

W-Pos257 Adenosine activates a potassium conductance in cultured mouse striatal neurons.
L.O. Trussell and M.B. Jackson, Dept. of Biology, U.C.L.A., Los Angeles, CA 90024.

Adenosine is reported to have an inhibitory effect on the electrical activity of numerous tissues, including smooth and heart muscle cells as well as central and peripheral neurons. We have examined the action of adenosine on neurons cultured from the embryonic mouse striatum using whole-cell patch clamp recording techniques. Picospritzer application of 50 uM adenosine or 2-chloroadenosine induced an outward current and an increase in membrane conductance in cells internally perfused with KCl or K_3Citrate (29/30 cells). In contrast, when cells were internally perfused with cesium salts, adenosine evoked an inward current (5/6 cells). For cells filled with K^+ as the major cation, I-V curves of the adenosine-induced current revealed 1) a current reversal near the estimated K^+ reversal potential and 2) a marked voltage dependence, such that the adenosine-induced current was reduced at voltages more positive than -40mV, despite an apparently larger driving force. The response to adenosine was reversibly blocked by 1 mM theophylline. We conclude that adenosine activates a voltage-dependent K-conductance mechanism by acting on a specific membrane receptor. As expected from an outward current, adenosine caused a membrane potential hyperpolarization and blocked spontaneous action potential activity in current clamped neurons. The activation of this K-conductance may require components of the cytosol since the response is lost during cell perfusion, with the washout time increasing with the pipette-cell access resistance. In contrast, responses to GABA or glutamate are present long after the adenosine response has been lost. We are currently exploring ways to prevent or slow the loss of the adenosine response during internal perfusion.

W-Pos258 MEASUREMENT OF THE RATE OF QUANTAL SECRETION INDUCED BY BLACK WIDOW SPIDER VENOM (BWSV) AT THE ENDPLATE.
R. Fesce, J.R. Segal[+], B. Ceccarelli[++] and W.P. Hurlbut. Rockefeller Univ., NY, NY. [+]Veterans Admin. Medical Center, NY, NY. [++]Dept. Pharmacol, Univ. Milan, Italy.

BWSV causes frog motor nerve terminals to secrete acetylcholine until their quantal store is exhausted; the total number of miniature endplate potentials (mepps) recorded under this condition gives an upper limit for the store of quanta in resting terminals. We applied a modification of classical fluctuation analysis (Segal et al., Biophys. J., in press) to measure mepp rate. The variance, skew and power spectrum of the fluctuating endplate voltage are used to determine the waveform, amplitude and rate of occurrence of the mepp; advantages of this procedure are that it is insensitive to slow, spurious changes in resting potential and less sensitive to nonlinear summation of mepps, and it can be applied to filtered records. In Ca^{2+}-free solutions BWSV induces secretion of 0.5-1 $x10^6$ quanta and mepp rate falls to about 10/s within 20 m. With Ca^{2+}, the power spectra deviate from white behavior at low frequencies and the intervals between extracellularly recorded mepps are not exponentially distributed, indicating that mepp rate is not stationary. In theory, a time varying rate, r(t), adds to the variance and skew frequency components which are limited to the bandwidth of r(t) and can, in principle, be filtered out. The filtering procedure was verified by analyzing computer generated records in which simulated mepps occurred in bursts. At junctions treated with BWSV in the presence of Ca^{2+} secretion was more prolonged and mepp rate often remained well over 100/s for a whole hour. The total secretion was comparable to that in Ca^{2+}-free solution, but since the terminals were not yet exhausted some recycling may have occurred. Supported by the Veterans Admin. (JRS), an MDA grant (BC) and PHS grant NS 18354 (WPH).

W-Pos259 DISTRIBUTION-FREE QUANTAL ANALYSIS USING POWER CEPSTRA.
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Classical methods for quantal analysis of synaptic transmission usually require the assumption (or demonstration) of an underlying quantal release distribution; for example, Poisson or binomial. We will discuss a novel distribution-free method for determining the quantal amplitude from excitatory postsynaptic current (epsc) amplitude histograms by calculating the power cepstrum.

The power cepstrum is commonly employed in acoustics and geophysics for determining echo times in noisy environments. To apply cepstral analysis to quantal analysis, we consider the epsc amplitude histogram to consist of an initial impulse (the amplitude histogram of single-quantum epscs) followed by echoes in the form of the amplitude distributions of multiple-quanta epscs. The power cepstrum is calculated by taking the power Fourier transform of the amplitude histogram, amplitude-compressing the transform by calculating its logarithm, and taking a second Fourier transform. The major peak of the cepstrum corresponds directly to the quantal amplitude. This method requires no prior knowledge of, or assumptions about, the underlying quantal release statistics and is capable of detecting quantal amplitudes under conditions when conventional methods fail. We believe cepstral-based quantal analysis may be widely applicable in preparations where quantal analysis has not been possible due to a lack of information regarding release statistics. We will show examples of the use of cepstra with simulated data and with epscs obtained from the mossy fiber excitatory synaptic input in the hippocampal slice preparation. (Supported by NS15772, NS11535, NS18295 and a McKnight Found. Neurosci. Devel. Award)

W-Pos260 **PYRENE ACYL-CoA AND ACYLCARNITINE BINDING TO LIVER Z PROTEIN: MEMBRANE INTERACTION.**
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Liver fatty acid binding protein (Z) was isolated according to Trulzch and Arias (Arch. Biochem. Biophys. 209, 1981:443). Solution accessibility of pyrenedodecanoic acid (PDA), pyrenedodecanoyl-CoA (PDCoA) and pyrenedodecanoylcarnitine (PDC) to nicotinamide decreases in the presence of Z, indicating that carnitine esters as well as PDA and PDCoA bind to Z. Z does not bind 5-(dimethylamino)-napthalene-1-sulfonic acid, a probe for the medium chain fatty acid binding site of albumin. Polarity of Z fatty acid binding site(s) was determined with n-(9-anthroyloxy)stearate(n-AS). Z binding site polarity for acyl chain methylenes decreases in the order 16-AS>12-AS>3-AS>7-AS, with dielectric constants: 44.7, 6.36, 2.46 and 1.22, respectively. Z protein removes both PDC and PDCoA from palmitoyloleoylphosphatidylcholine vesicles in a concentration-dependent manner. The effect of Z on membrane-bound PDA, PD-CoA and PDC was tested in rat liver mitochondria, microsomes, and plasma membranes. PDC and PDCoA are more easily removed from plasma membrane than from mitochondria or microsomes by Z. Z (1-5 μM) enhances microenvironmental levels of PDC on microsomes. Interaction of PDA with cellular membranes is dependent upon the concentration of Z, with enhancement of microenvironmental PDA on plasma membranes at 2 μM Z. The findings suggest modulation of fatty-acid membrane interactions by Z. Supported by the National Institutes of Health (HL 30186).

W-Pos261 MECHANISM OF CRYOPRESERVATION BY POLYMERS T. Takahashi, A Hirsh, E.F. Erbe*, M.F. Hammett, R.L. Steer* and R.J. Williams. American Red Cross Research Laboratories, Bethesda, Maryland; *Plant Virology Laboratory, U.S. Dept. of Agriculture, Beltsville, Maryland.

In order to understand the mechanism of cryoprotection by polymer solutions, we cryopreserved human monocytes in various polymer solutions without penetrating cryoprotectants. Hydroxyethyl starch (HES), dextran (m.w. 10,000-500,000), Ficoll and polyvinylpyrrolidone (PVP) protect cells during slow freezing (2.5°C/min) to -196°C at initial concentrations above 15% (w/w) only if followed by rapid warming. Under these conditions, about 60-70% of the cells retained normal membrane integrity and normal phagocytosis and chemotaxis. When cells suspended in 20% HES were extracellularly frozen, cooled to various temperatures slowly and then plunged into LN_2, survival after rapid warming increased to the maximum whenever slow cooling was extended below -20°C. By comparing freezing, osmotic stress and calorimetric results, we have determined that cells in HES solution may in part be protected by an equilibrium glass transition of the extracellular medium at -20°C. Electron microscope observations of intracellular ice crystal formation as a function of slow warming led us to conclude that those polymers which protect cells from freezing do so by the following mechanisms: (1) they allow the cells to moderately supercool between 0°C and ~-20° C, reducing osmotic stress; (2) below -20°C, injurious water loss from the cells is eliminated by a solid amorphrous solution between the extracellular ice and the cells which maintains osmotic stress at a tolerable level; (3) at <-70°C a small amount of intracellular ice forms but the viscosity of the moderately concentrated intracellular contents is such that, unless the system is slowly warmed through the temperature range between -60°C and -20°C, further ice growth is prevented.

W-Pos262 REPAIR MECHANISMS OF TRYPTOPHAN BY ANTIOXIDANTS AND TRYPTOPHAN METABOLITES

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Peroxy radicals and many other strongly oxidizing intermediates, such as alkoxy radicals, bromine atoms etc., are capable of oxidizing amino acids in proteins. These one electron transfer processes are responsible for damage to proteins and inactivation of protein functions, e.g. enzymatic catalysis. Formation of indole free radical intermediates takes place in the reaction of $Br_2^{\cdot}$ and $^{\cdot}N_3$ with tryptophan. The kinetics of these reactions has been studied by pulse radiolysis of appropriate model systems. The R-$\dot{\text{I}}\text{ndH}^+$ and R-$\dot{\text{I}}\text{nd}$ radical forms of Trp have been found to react efficiently ($k = 10^6 - 10^9$ dm^3 mol^{-1} s^{-1}) with a variety of electron donors such as phenolic antioxidants, ascorbate and promethazine, which restitute the parent molecule. On the other hand, reactions of these radicals with oxygen and H-atom donors such as sulfhydryls could not been measured ($k<10^5$ dm^3 mol^{-1} s^{-1}). Relative unreactivity in the latter case has been explained by the resonant structure of the indole radicals. A new class of antioxidants, i.e. 5-hydroxyindole derivatives are good electron donors and could act as excellent repairing agents of the damaged proteins. Since some of the 5-hydroxyindole derivatives are metabolites of tryptophan, and because some of them also act as neurotransmitters, their role as antioxidants in the maintenance of integrity of neuro-membrane proteins will be discussed.

W-Pos263 SYSTEMS FOR ENDOTHELIAL TRANSPORT AND REACTION IN BLOOD-TISSUE EXCHANGE. J.B. Bassingthwaighte; Center for Bioengineering WD-12, University of Washington, Seattle, WA 98195.

The explosive accumulation of evidence for the central role of the endothelial cell on exchange between blood and tissue shows that transformation is as important as transport. An example is the stimulus by acetyl choline to produce a factor which relaxes smooth muscle. Another is the sidedness of endothelial cells, for example, the albuminal surface transporting adenosine more rapidly than the luminal surface. Such situations can be modeled quantitatively by developing multi-models which account for transport, reaction or transformation, and accumulation for several related molecules. Rapid events require the use of spatially-distributed convection-diffusion-permeation models which can account for heterogeneity of flows and for at least 2 cell types. Use of Lagrangian-flow algorithms for the capillary-tissue units provides accurate and efficient execution of such complex systems models. (Supported by NIH grants RR01243 and HL19139)

W-Pos264 THE MALE CONTRACEPTIVE GOSSYPOL: MEMBRANE ACTIONS OF THE GOSSYPOL TAUTOMERS. Juan Reyes, Steven D. Wyrick*, Laura Borriero and Dale J. Benos. Department of Physiology and Biophysics, Laboratory of Human Reproduction and Reproductive Biology, Harvard Medical School, Boston, MA 02115 and *Department Medicinal Chem. and Natural Products, School of Pharmacy, UNC, Chapel Hill, NC 27514

The experimental male contraceptive gossypol is a polyalcohol binaphtalene aldehyde that can exist as keto, enol and hemiacetal tautomers in aqueous solution. Gossypol in aqueous solution uncouples mitochondrial oxidative phosphorylation, decreases the interfacial potential of lipid bilayers, and monolayers, and induces proton transport in lipid bilayers (Reyes et al., 1984, J. Biol. Chem., 259:9607). Comparative studies of the effects of hemiacetal and keto-enol gossypol on the interfacial potentials of lipid monolayers, the conductance of phospholipid bilayers and mitochondrial oxidative phosphorylation indicate that: a) the actions of gossypol on lipid interfacial potentials, bilayer conductance, and mitochondrial oxidative phosphorylation cannot be due to the hemiacetal tautomer; b) the uncharged keto-enol gossypol does not modify the interfacial potential of lipid monolayers; c) the polyvalent enol gossypol moiety can interact with the membrane/solution interface to produce the reported effects on interfacial potentials; and d) the gossypol keto-enol tautomerism, acid-base equilibrium and the permeabilities of the charged and uncharged keto-enol gossypol moieties across lipid membranes determine the property of gossypol to act as a proton carrier in biological membranes. Supported by NIH grants AM 25886, HD 12353, and by funds from the Rockefeller Foundation.

W-Pos265 Effect of Adriamycin, Daunorubicin, Adriamycinone, N-Dodecanoyl Daunorubicin and Fe^{2+} complex of Adriamycin on Myocardial Cell Membranes and Sarcoplasmic Reticulum. A. ASZALOS*, J. BRADLAW, E. REYNALDO, G. YANG and T. BEELES. FDA, Washington, D.C. and USUHS, Bethesda, MD. (Intr. by A. Szabo).

Adriamycinone, Fe^{2+} complex of adriamycin, and n-dodecanoyl daunorubicin, prepared in our laboratory, in addition to adriamycin and daunorubicin were studied for effects on myocardial cell membranes and sarcoplasmic reticulum. Myocytes were isolated from neonatal rat heart ventricles. Sarcoplasmic reticulum vesicles were isolated by the method of Duggan and Martonosi (J. Gen. Physiol. 56, 147, 1970). Treatment by all these agents, except adriamycinone, for 24 h at a concentration of $3X10^{-9}$mole/10^6cells, influenced the beat rate of myocytes and stopped the beat. ESR measurements indicate that the fluidity of myocyte membranes decreases instantaneously upon treatment either with adriamycin or its Fe^{2+}complex or with daunorubicin. Fluorescence depolarization measurements support these findings. These agents did not influence Ca^{2+}uptake of sarcoplasmic reticulum vesicles. It is concluded that adriamycin, daunorubicin and their derivatives are incorporated into myocyte membranes and disrupt the beating mechanism through effects mediated by membrane components.