W-Pos242 THE EFFECT OF PHOSPHATE ON THE RATE OF FORCE RECOVERY IN RABBIT PSOAS MUSCLE FIBERS.
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The rate of force recovery either after a period of shortening at maximum velocity or after a rapid restretch following such a shortening has been measured over a range of phosphate concentrations. Force recovery after a restretch under sarcomere length control is well fitted by a single exponential in the absence of added phosphate and reasonably well fitted in the presence of phosphate. Under our experimental conditions (6 mM magnesium acetate, 5 mM ATP, 10 mM CaEGTA, 50 mM imidazole, 87 mM potassium acetate, pH 7, 5 ^{o}C) the average tension was 1.1 kg/cm^2 and the average rate of force recovery fitted to a single exponential was 4 s^{-1}. Phosphate at 10 mM reduces the tension by 50 % but only increases the rate of force recovery by 45%. We have also investigated the effect of phosphate on the recovery in the presence of MnATP and although the rate of recovery is significantly faster than with MgATP, the effects of phosphate are very similar. The constraints imposed by these data on models in which the rate of force recovery is limited either by the force generating step itself (Hibberd & Trentham, 1986 Ann. Rev. Biophys. 15, 119) or a step preceding the force generating step (Brenner & Eisenberg, 1986, PNAS 83, 3542) will be discussed. This work has been supported by the Medical Research Council and the Muscular Dystrophy Association of America.

W-Pos243 THE EFFECT OF SHORTENING ON THE PHOSPHATE RELEASE STEP OF THE ACTOMYOSIN ATPase MECHANISM. E. Homsher and J. Lacktis, Physiol. Dept., School of Medicine, UCLA, Los Angeles, CA 90024.

It is thought that the release of inorganic phosphate (Pi) from the AM·ADP·Pi complex is associated with the generation of force during the crossbridge cycle. This concept is supported by the observation [Dantzig, et al, Biophys. J. 51, 3a, (1987)] that the rapid photogeneration of 1 mM Pi from caged phosphate (c-Pi) in an isometrically contracting glycerinated muscle fiber produces a sudden sustained decrease in force equivalent to 0.1 P_o. For the reaction shown below the rate of force decline (λ) at 10 ^{o}C is 38±2 s^{-1} and $\lambda = k_{+1} + k_{-1}$(Pi).

$$AM\,ADP\,Pi \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} AM'ADP + Pi \xrightarrow{k_n}$$

In isometric contractions the AM'ADP state should constitute a significant fraction of the attached crossbridges, while during steady state shortening the AM'ADP should be less populated while those after k_n will be more heavily populated. Thus the amplitude of the force reduction following flash photolysis of c-Pi in a shortening muscle should be less than that observed in isometric contractions. To test this prediction, glycerinated rabbit psoas muscle fibers were activated (pCa=4.5) at 10 ^{o}C in a contraction solution containing 5mM c-Pi at an initial sarcomere length of 2.7 um. After force became maximal, the fibers were allowed to shorten. During steady state shortening, 1 mM Pi was photogenerated by a pulse of light from a dye laser. The size of the Pi-induced force reduction was smaller the greater the shortening velocity. λ increased with shortening velocity and was 96±4 s^{-1} at a velocity of 0.2 muscle lengths /s. The results suggest that the fraction of crossbridges in the AM'ADP state is reduced as shortening velocity increases. Studies of the dependence of λ on [Pi] suggest that during shortening, the increase in λ is primarily a result of an increase in k_{-1}. Supported by NIH grant AM30988-06.

W-Pos244 **THIN FILAMENT ACTIVATION BY PHOTOLYSIS OF CAGED-CALCIUM IN SKINNED MUSCLE FIBERS FROM THE FROG.**
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We have used the photolabile calcium chelator nitr-5 to determine the calcium dependence of the activation process in single skinned fibers from the semitendinosus muscle of the frog, *Rana temporaria*. After equilibration in 3mM nitr-5 (pCa 6.5), laser flash photolysis (75mJ in <200ns at 347nm giving 50% photolysis of nitr-5) caused a rise in Ca^{2+} to pCa<5.8,sufficient to produce full isometric force (150 kN/m^2) with a $t_{1/2}$=40 ± 2ms, (s.e.m., n=8; pH7.0, pMg2.7, I=0.2M, 13°C). This rate is similar to that observed by Kress *et al.* (J. mol. Biol. **188**, 325, 1986) in tetanically stimulated frog sartorius at 14°C ($t_{1/2}$=32.5ms). As the laser pulse intensity and hence the Ca^{2+} released was decreased, the rate of force development remained constant until less than 50% maximal force was reached. This finding suggests that *in vivo* the rate of force rise is not limited by the availability of calcium in the vicinity of the thin filaments and hence by calcium release from the sarcoplasmic reticulum. Under similar conditions the force rise following photolysis of 2mM caged-ATP (pCa≤4.5) had a $t_{1/2}$ of 22 ± 2ms (n=8) at 13°C. In this case the thin filaments are activated by rigor cross-bridges and calcium, prior to photolysis. Therefore the difference in the rate of force generation observed with caged-calcium compared with caged-ATP ($t_{1/2}$ 40 *vs.* 22ms) must reflect the time course of thin filament activation and/or cross-bridge attachment. (Supported by N.I.H. R01-AR37701-021, M.D.A. and S.E.R.C.)

W-Pos245 X RAY DIFFRACTION OBSERVATIONS FROM FROG MUSCLE IN CAFFEINE RCC. A.A. Stewart**, Y Maeda**, C.C. Ashley*. *University Laboratory of Physiology, Parks Road Oxford OX 1 3PT U.K. **EMBL c/o DESY Notkestrasse 85 Hamburg F.D.R.

Rapid cooling contracture (RCC) (Conway and Sakai, 1960 PNAS 46, 897-903) gives graded contractions which are stable over the period required for X ray exposure and can be recorded using a wire per wire electronic counter (Yu, Hart and Podolsky, 1979 JMB, 132, 53-67). The caffeine concentrations used were in the range 0.5-2.5 mM producing forces ranging between 0 and 100% of electrically stimulated force. A rapid flow of cold saline cooled the frog semitendinosus muscle within 0.5s to less than 1°C from about 20°C. The flow was then reduced and maintained for 40s. The muscle was stimulated electrically early in the contracture (3s 10v 25Hz) to measure the response at full activation. The response of a muscle to a given caffeine concentration is repeatable and the caffeine sensitivities of a pair of muscles from the same animal are similar. We have investigated the relationship between the change in the second actin layer line (A4) and isometric tension at a range of levels of activation both at rest length (2.6um) and at highly stretched lengths (4.0um). The force development in the RCC has been plotted against the change in the intensity of the 2nd actin 1.1, both the tension and the X ray change were expressed as a fraction of the changes recorded during maximal electrical stimulation at the beginning of that contracture. The relation in relative terms appears unaltered by muscle stretch although the change in the 2nd actin 1.1. on maximal activation is reduced to approx. 60% of that at rest length. We find a linear relation between force and the change in the second layer line intensity. A.A.Stewart is an EMBL predoctoral fellow. Supported by MDA and NIH AR37701/021.

W-Pos246 THE EFFECT OF HIGH pH AND CROSSLINKING ON THE FILAMENT LATTICE OF VERTEBRATE STRIATED MUSCLE.

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Small angle X-ray diffraction has been used to study the effects of crosslinking with dimethyl suberimidate (DMS) on the filament lattice of rabbit psoas muscle at normal and high pH in the relaxed and rigor states. In both states, the lattice swells slightly at pH 8.5. This swelling could be caused by increased electrostatic repulsion from increased charge, an increased charge diameter or both. An increased charge diameter could result from the swinging out of HMM S-2 at high pH. This swinging out appears to be inhibited by crosslinking with DMS to the filament backbone. We observed a slight lattice swelling in rigor muscle and a strong sampling of myosin layer lines at pH 8.5 indicating an increase in three dimensional order. Crosslinking rigor muscle at normal pH caused slight but significant lattice shrinkage but attempts to relax the crosslinked muscle appeared to be unsuccessful in that they caused no change in lattice spacings or 11/10 intensity ratios. Relaxed muscles at pH 8.5 gave diffuse or no diffraction patterns. Attempts to crosslink muscle in relaxing solution with or without osmotic compression of the lattice, gave no evidence of crosslinking at either pH. In contrast to controls, muscles treated with DMS at normal pH and transferred to relaxing solution at pH 8.5 were stable and swelled like rigor muscle. Thick filaments appear to be unstable in relaxing solution at pH 8.5, but can be stablized by DMS. This finding is consistent with the recent suggestion of Ueno and Harrington (Biochemistry 26:2584) that DMS does not bind S-1 to the backbone or to actin but may link myosin tails to the thick filament backbone.

W-Pos247 EFFECTS OF PHYSIOLOGICAL ADP LEVELS ON SKINNED MUSCLE FIBER CONTRACTION. P.B. Chase and M.J. Kushmerick, Dept. of Radiology, Brigham & Women's Hospital, Boston, Mass. 02115

At millimolar concentrations, ADP has been reported to increase the steady-state isometric force of Ca-activated skinned muscle fibers (e.g., Kawai, J Mus Res Cell Motil. 7:421, 1986; Cooke & Pate, Biophys J. 48:789, 1985); it has also been reported to decrease both shortening velocity (Cooke & Pate, 1985) and the rate constants associated with oscillatory work (Kawai, 1986). ADP was increased by adding only ADP to the bathing solution. Without PCr or CK, substantial radial gradients of ATP and ADP would also be present in an active fiber.

Our approach has been to study ADP effects by shifting the CK equilibrium towards higher ADP via increased Cr. Cr in activating solution (pCa 4.75; (mM) 5 MgATP; 15 PCr; 290 U/ml CK; 12°C) was 70 μM (HPLC: Waters column #80002); ADP was << 1 μM (HPLC: Vydac column #303NT405). Increasing Cr to 50 mM increased ADP to 70 μM, which is above the mitochondrial K_m. ADP slightly decreased isometric force (by about 4% maximum). Maximum shortening velocity (determined by both the slack test and extrapolation of isotonic shortenings to 0 load) decreased as ADP increased (by about 15% maximum).

These results show that (a) changes in ADP over the physiological range have little effect on contractile function; and (b) experiments need to be designed in which ADP and ATP are buffered simultaneously in active fibers in order to adequately test hypotheses involving [ATP] and [ADP], as was found to be the case with pH (Chase & Kushmerick, Biophys J. 51:476a, 1987).

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W-Pos248 BINDING OF Ca^{2+} TO SKINNED MUSCLE FIBERS AT SHORT SARCOMERE LENGTH: COMPARISON OF SKELETAL AND CARDIAC MUSCLE. Franklin Fuchs, Margaret E. Whaley, and Polly A. Hofmann. Dept. of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

The Ca^{2+} sensitivity of the cardiac contractile system is diminished as the sarcomere length is reduced along the ascending limb of the length-force curve (Hibberd and Jewell, 1982). This effect has been attributed to a reduced Ca^{2+}-troponin C affinity at shorter lengths, the latter mediated by length-dependent variation in the number of attached cross-bridges (Hofmann and Fuchs, 1987). Skinned rabbit psoas fibers also show a reduced Ca^{2+} sensitivity in the same sarcomere length range but indirect evidence suggests that it is not based on changes in the Ca^{2+} binding properties of troponin C (Allen and Moss, 1987). In this study a double isotope technique was used to compare the bound Ca^{2+}-pCa relationships (in rigor) of bovine ventricular fibers and rabbit psoas fibers subjected to chemical skinning with Triton X-100. Comparing cardiac muscle at sarcomere lengths 2.4μm and 1.7μm, there was no significant difference in Ca^{2+} binding over the pCa range 8.0-6.0, but in the pCa range 6.0-5.0, in which the regulatory site of troponin C is titrated, there was 15-25% less binding at the shorter sarcomere length. Comparing skeletal muscle at sarcomere lengths 2.4μm and 1.4μm, there was no discernible difference in Ca^{2+} binding over the pCa range 7.5-5.0. Thus different mechanisms may mediate length-dependence of Ca^{2+} sensitivity in skeletal and cardiac muscle. Supported by the Am. Heart Assoc. and NIH (AM10551).

W-Pos249 EVIDENCE FOR MYOSIN-LINKED Ca^{2+} REGULATION OF CONTRACTION IN VERTEBRATE SKELETAL MUSCLE. Joseph M. Metzger and Richard L. Moss., Department of Physiology, University of Wisconsin, Madison, WI 53706.

Techniques were developed to assess changes in the rate constant for tension redevelopment, k_{tr} (modified from Brenner and Eisenberg, PNAS 83, 3542, 1986), in skinned single fibers from fast-twitch rabbit psoas and rat superficial vastus lateralis muscles due to variations in the level of thin filament activation. In control fibers k_{tr} increased in a sigmoidal manner as $[Ca^{2+}]$ was increased from submaximal (pCa 6.2: k_{tr} = 3 sec^{-1}) to maximal activating levels (pCa 4.5: k_{tr} = 20 sec^{-1}). When the extent of thin filament activation was altered independently of $[Ca^{2+}]$ by extraction of various amounts of troponin C the relationship between k_{tr} and Ca^{2+} was unchanged, whereas steady state isometric tension became less sensitive to Ca^{2+}. In fibers from which whole troponin was partially extracted (to achieve low level Ca^{2+}-insensitive activation) and TnC extracted from the remaining troponin complexes, tension was identical in the presence and absence of Ca^{2+}; however, k_{tr} was found to increase in the presence of Ca^{2+}. In other fibers in which myosin LC_2 content was reduced by 50%, k_{tr} was unchanged at pCa 4.5; however, there was an elevation in k_{tr} at submaximal $[Ca^{2+}]$. Addition of exogenous LC_2 to these fibers led to a partial reversal of this effect at submaximal $[Ca^{2+}]$, and the degree of reversal was consistent with the extent of LC_2 recombination. A model involving a direct effect of Ca^{2+} on the rate of cross-bridge attachment will be discussed. Specifically, LC_2 may be repressive to cross-bridge attachment, and this repression is removed either by Ca^{2+} binding to LC_2 or by extraction of LC_2. Supported by NIH.

W-Pos250 TENSION RESPONSES OF FROG SINGLE MUSCLE FIBERS TO SOLUTIONS OF DECREASED TONICITY. Bernard. H. Bressler and Kevin Matsuba, Department of Anatomy, University of British Columbia, Vancouver.

Active isometric tension of skeletal muscle has been shown to vary as an inverse function of the tonicity of the bathing solution. This study provides further insight into the nature of the contractile change by comparing changes in isometric tetanus tension to the instantaneous stiffness of muscle fibers in solutions of reduced tonicity. Single muscle fibers were isolated from frog (Rana temporaria) semitendinosus muscle. Tetanus tension and fiber stiffness were measured in solutions of NR and compared to responses in 0.7NR and 0.5NR. Solutions were made hypotonic by dilution of 10x concentrated Ringer (NR). In both dilutions of isotonic Ringer maximum isometric tetanus tension increased significantly (Student's T-test, $p \leq 0.05$) compared to NR. Tension did not change from that measured in NR when the isotonicity of the solution was restored with sucrose. The increase in tetanus tension recorded in hypotonic Ringer was not accompanied by a corresponding change in the measured stiffness. This suggests that the potentiated tension resulted from an increased tension per cross-bridge rather than an increase in the number of attached bridges. This could be the result of myosin heads being bound to actin at low intracellular ionic strength even in the resting fiber. (Supported by the Medical Research Council of Canada).

W-Pos251 THE STIFFNESS OF WEAKLY ATTACHED CROSS-BRIDGES IN RELAXED FROG MUSCLE FIBRES.
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Tension transients of single skinned frog muscle fibres in response to small length changes, completed within 40 μs, have been measured over the first 5 ms with microsecond time resolution. The responses could be described with a model in terms of identical segments connected in series. The segments contained an undamped and 2 (or 3) damped elastic elements. This modelling technique together with measurements of equatorial X-ray diffraction patterns enabled us to determine mass shift, changes in filament spacing and changes in elastic components of relaxed fibres incubated in solutions of varying ionic strength or Dextran concentrations (conditions: pH 7.0, temp. 4°C, sarc. length 2.15 μm). The stiffness increment of relaxed fibres, which occurs if fibres are incubated at low ionic strength, can be attributed to an increased amount of weakly attached cross-bridges (mass shift measurements support this idea) and a decreased filament spacing (the stiffness increment of relaxed fibres incubated in solutions with varying Dextran concentrations is proportional with the decrement of filament spacing). In case of frog fibres it is concluded that only at ionic strength conditions lower than 50 mM the stiffness contribution of weakly attached cross-bridges is larger than the stiffness increment due to decreased filament spacing. This means that the total stiffness attributable to weakly attached cross-bridges in low ionic stength relaxed fibres is less than a tenth of the total stiffness attributable to rigor cross-bridges. As a result, combinations of stiffness of weakly attached cross-bridges, as found in low ionic strength relaxed fibres, and rigor cross-bridges cannot account for cross-bridge stiffness as found in Ca(2+)-activated fibres.

W-Pos252 MECHANICAL ASSAY OF ROLE OF CALCIUM DURING ISOMETRIC RELAXATION.
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Free $[Ca^{++}]_i$ returns to baseline levels before twitch relaxation. We sought a mechanical assay to probe for the presence of Ca++ bound to TnC during relaxation. At various times into an isometric twitch we applied a length impulse (8-10% L_{max}) sufficient to break all crossbridge connections. A 'deactivation curve' (DC) was obtained by plotting peak force redeveloped after the impulse (F_r, at time t_r) relative to peak control twitch force F_p vs time of impulse t_i (i.e., F_r/F_p vs t_i). A 'modulation curve' (MC) was obtained by plotting F_r relative to F_c (control force at time t_r) vs t_i. DC indicates the presence of Ca++ bound to TnC; if no Ca++ were bound, no force would redevelop after the impulse. MC shows the relative importance of calcium to total force; MC=1 suggests that Ca++ is bound to all TnC's allied with active crossbridges. We obtained these curves in 3 rabbit papillary muscles, both before and after addition of ryanodine (Ry) to inhibit SR Ca++ uptake. Before Ry, MC and DC decreased throughout the twitch, approaching zero slightly after peak force. After 2uM Ry, DC was right shifted and shallower, suggesting that Ca++ stayed bound to TnC. This prolonged mechanical effect of bound Ca++ is consistent with studies showing a protracted free Ca++ transient. With Ry, MC exhibited a tri-phasic behavior. As force increased, MC decreased from 1 to 0.3-0.5. Near peak force, MC started to rise again, reaching a plateau between 0.6 and 1, implying that most, but not all, TnC sites associated with active crossbridges had bound Ca++. These results suggest that in rabbit papillary muscle, relaxation is not controlled by decline of $[Ca^{++}]_i$ (DC = 0, MC = 0). However, if SR calcium uptake is deranged, relaxation can be limited by $[Ca^{++}]_i$ (MC => 1). 5T32GM07057, R01-HL38488.

W-Pos253 PARTIAL EXTRACTION OF MYOSIN LC_2 ALTERS THE Ca^{2+} SENSITIVITIES OF ISOMETRIC TENSION AND MAXIMUM SHORTENING VELOCITY (V_{max}) IN RABBIT SKINNED SKELETAL MUSCLE FIBERS.
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Previously, we have shown that extraction of LC_2 from skinned muscle fibers results in a decrease in V_{max} during maximal activation (JBC, 257: 8588, 1982). In the present study, we have examined the effects of extraction of ~50% endogenous LC_2 upon tension and V_{max} as functions of pCa. Measurements of isometric tension and V_{max} were obtained at various pCa's: (1) prior to LC_2 extraction, (2) after LC_2 extraction and (3) in some cases, after LC_2 recombination. Extraction was done in a solution of 20 mM KCl, 20 mM EDTA and 5 mM imidazole (pH 7.0) at 30-34°C for 120'. After extraction, the fibers were briefly bathed in relaxing solution containing TnC and whole Tn. LC_2 extraction resulted in reversible increases in tensions at pCa > ~5.8. This effect suggests that LC_2 extraction facilitates cross-bridge attachment to actin, corresponding to an increase in the attachment rate constant, f, in A.F. Huxley's (1957) model. With regard to V_{max}, plots of slack test data from untreated fibers consisted of a single straight line for pCa's < 5.9. At pCa's > 5.9, these plots were biphasic, exhibiting an initial phase of high velocity shortening and a subsequent phase of low velocity shortening. V_{max} in the high velocity phase was relatively insensitive to changes in $[Ca^{2+}]$ while V_{max} in the low velocity phase decreased as $[Ca^{2+}]$ was reduced. The LC_2 extraction procedure uniformly depressed velocities in the high velocity phase, but appeared to have no effect on the low velocity phase. This suggests that LC_2 extraction decreases the rate of cross-bridge detachment (g) from actin. Supported by NIH.

W-Pos254 **EFFECTS OF P_i ON THE CALCIUM DEPENDENCE OF FORCE AND STIFFNESS IN GLYCERINATED RABBIT PSOAS FIBERS.** Donald A. Martyn and Albert M. Gordon, Center for Bioengineering and the Department of Physiology and Biophysics, University of Washington, Seattle, WA 98195

Force (*F*) and stiffness (*k*) were measured in glycerinated psoas fibers at various calcium levels with 0, 10 and 20 mM P_i in the bathing solutions. Bathing solutions contained (in mM) 135 potassium propionate, 1 Mg^{2+}, 4 Na_2ATP, 15 creatine phosphate, 10 EGTA, variable amounts of pH buffer (MOPS) and 10 μ/ml creatine phosphokinase. The pH was 7.0 and temperature 10°C. Calcium levels were established by adding various amounts of $CaCl_2$. All solutions contained 4% dextran T-500. Fiber *k* was measured by imposing sinusoidal length changes (0.1-0.2%) at 1 kHz and measuring the resulting *F* oscillations. P_i was found to effect the relation between maximum *F* and *k*, and to decrease the sensitivity of both *F* and *k* to calcium. Maximally activated *F*, as a fraction of the *F* obtained with no P_i (+/- SEM), was found to be .84 +/- .03 and .77 +/- .02, in 10 and 20 mM P_i, respectively. On the other hand, maximal *k*, as a fraction of that obtained with 0 P_i, was 1.03 +/- .06 and .95 +/- .05, at 10 and 20 mM P_i, respectively. Increasing bathing solution P_i caused a greater decrease in *F* than *k*. The Hill equation parameters (pK and n) for *F*-calcium (as pCa) relations obtained at each P_i level were 6.18 +/- .01, 2.64 +/- .12 (0 P_i), 6.09 +/- .01, 3.84+/- .23 (10 mM P_i), and 6.0 +/- .01, 3.4 +/- .17 (20 mM P_i), while corresponding values for *k*-calcium relations were 6.26 +/- .006, 3.31 +/- .15 (0 P_i), 6.17 +/- .009, 4.8 +/- .44 (10 mM P_i) and 6.09 +/- .006, 3.83 +/- .18 (20 mM P_i). Increasing P_i caused a similar decrease in calcium sensitivity for *F* and *k*. The results also indicate that at each P_i *k* was about .08-.09 pCa units more sensitive to calcium than *F*. The results suggest that under these experimental conditions attached crossbridges can exist in both force and nonforce producing states, and that the calcium dependence of these states may be different. This work is supported by PHS grant #HL 31962 and NS 08384.

W-Pos255 THE EFFECT OF PHOSPHATE ON MUSCLE ISOMETRIC TENSION. E. Pate, K. Franks, and R. Cooke. Dept. of Mathematics, W.S.U., Pullman WA, and Dept. of Biochem. and CVRI, UCSF, San Francisco, CA.

Previous work has shown that increasing concentrations of orthophosphate decrease active, isometric tension in glycerinated, rabbit psoas fibers. The precise functional relation between [Pi] and tension has remained unresolved. This is in part due to limited variation in [Pi] in previous studies, and in part due to contaminating [Pi] in experimental buffers, arising primarily from added ATP and creatine phosphate. Using the sucrose phosphorylase / sucrose, enzymatic system to accurately define [Pi] buffer concentrations, and [Pi] concentrations ranging from 200 μM to 80 mM, we find that at both pH 7 and pH 6.2, isometric tension decreases linearly with the log [Pi]. The slope of the linear fits shows tension decreases by 25% (relative to the value at pH 7, 200 μM Pi) for each 10-fold increase in [Pi]. The slopes are approximately equal at both values of pH. These observations imply that changes in isometric tension are linearly related to changes in the free energy of hydrolysis of MgATP when [Pi] is varied. The linear dependence of isometric tension on log [Pi] is shown to arise naturally from models of cross-bridge kinetics that involve a transition from a weakly-bound, actomyosin.ADP.Pi state to a strongly-bound and elastically distorted actomyosin.ADP state. Supported by USPHS AM30868 and NSF DCB8511082.

W-Pos256 CONTRACTION OF MUSCLE FIBERS GENERATED BY DIFFERENT NUCLEOSIDE TRIPHOSPHATES. E. Pate and R. Cooke. Dept. of Mathematics, W.S.U., Pullman, WA, and Dept. of Biochem. and CVRI, U.C.S.F., San Francisco, CA.

We have examined the ability of a series of nucleoside triphosphates to support contraction of permeable, fast (psoas) and slow (soleus) skeletal muscle fibers. Millimolar concentrations of all ligands were used, along with a creatine kinase, creatine phosphate regeneration system. In fast skeletal muscle, a number of nucleotides (GTP, ITP, ε-ATP) supported weak contractions with isometric tensions (Po) less than 20% that observed in the presence of similar concentrations of ATP, and maximum contraction velocities (Vmax) less than 10% of ATP. CTP supported tensions comparable to that with ATP and a Vmax 50% that of ATP. UTP yielded tensions 75% of ATP and Vmax 20% of ATP. More limited data show that several nucleotides that do not work well in fast fibers can support reasonable tensions and velocities in slow fibers. Both GTP and 2-aza-ε-ATP support isometric tensions similar to that produced by ATP, with values for Vmax that are only sightly depressed.

The data on the fast muscle fibers can be compared to the kinetic data on the actomyosin-S1 ATPase measured by H. White, X. Wang, and B. Belknap (see abstract, this meeting). In general those nucleotides which only poorly supported contractions in the fibers, also displayed an altered, non-hyperbolic dependence of the NTPase on actin concentration. For these nucleotides, both mechanical data and acto-S1 kinetics are consistent with the inhibition of at least one transition between states in which the myosin head is attached to actin. Supported by USPHS AM30868 and NSF DCB8511082.

W-Pos257 VELOCITY OF SHORTENING AT SHORT SARCOMERE LENGTHS IN INTACT SKELETAL MUSCLE FIBERS.
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The velocity of unloaded shortening (V_u) is independent of the degree of overlap of thick and thin filaments at sarcomere lengths (SL) greater than 2.2 μm (Biophys. J. 51:219a, 1987). The purpose of the present experiments was to determine the relationship between V_u and filament overlap at SL less than 2.2 μm. Experiments were performed on intact single fibers from the tibialis anterior muscle of the frog (R. temporaria). The relationship between velocity and SL was assessed by measuring, over the SL range from 2.2 μm to 1.55 μm: 1) force generated during shortening at constant velocities between 90% and 100% of V_u; 2) shortening velocity during force clamps at low loads; and 3) unloaded shortening velocity using step length releases (slack test). Force generated during shortening at constant velocity was constant between SL of 2.2 μm and 1.85 μm but declined as shortening continued. Under force clamp conditions, shortening velocity was constant between SL of 2.2 μm and 1.85 μm, then declined with decreasing SL. The relationship between step size and slack time was linear between SL of 2.2 μm and 1.85 μm but a deviation in the direction of decreasing velocity was detected at SL less than 1.80 μm. We conclude that, during shortening at high velocities, the force generated externally by intact skeletal muscle fibers is constant between the SL of 2.2 μm and 1.85 μm but falls as shortening continues. Although the classical SL-tension relation does not show a change in slope at 1.85 μm, current estimates of filament lengths indicate that overlapping thin filaments meet crossbridges in the opposite half-sarcomere at this length. Supported by NIH grants AR07972 (DRC) and HL35032 (FJJ).

W-Pos258 PARVALBUMIN CONCENTRATION IN SKELETAL MUSCLE FIBERS OF THE FROG. Tien-tzu Hou, Leo J. D'Anniballe and Jack A. Rall. Department of Physiology, Ohio State University, Columbus, OH 43210.

Parvalbumin (PV) is an intracellular calcium-binding protein which may function to promote relaxation in fast contracting skeletal muscles. Quantitative analysis of the role of PV requires knowledge of its concentration in myoplasmic water. PV concentration was determined in single fibers (N=18) isolated from tibialis anterior muscles of the frog, R. temporaria. Amounts of the 2 isoforms of PV (PVa and Pvb) characteristic of frog skeletal muscle were determined in each fiber by comparison to purified standards by SDS polyacrylamide gel electrophoresis and densitometry. Volume was determined in fibers stretched to a resting sarcomere length of 3.3 um (or greater) as the product of length times cross-sectional area, assuming circular cross-sectional shape. Fraction of fiber volume occupied by myoplasmic water was estimated according to Baylor et al (J. Physiol. 344, 625 1983). Total PV concentration = 1.0 ± 0.07 mmol/L myoplasmic water with a coefficient of variation (S.D./mean X 100) of 31%. PVa represents 63 ± 3% of the total PV concentration.

In order to develop a more convenient method to compare PV content in different muscles, PV content was determined in fiber bundles as amount (ug) per dry weight (g). Total PV content in bundles of tibialis anterior muscles is 43 ± 6% less in R. pipiens than in R. temporaria (N = 6 bundles each). This relationship is similar to the ratio of amount of labile maintenance heat (about 0.5) in the two frog species. (Supported by NIH AM20792.)

W-Pos259 CARDIAC HYPOTHERMIA: ^{31}P and ^{1}H NMR STUDIES OF HUMAN MYOCARDIAL TISSUE.
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Contractile force recovers well in human atrial trabeculae after prolonged exposure to temperatures of 12° and 20°C; however, after temperatures of 1°C or 4°C resting force increases and recovery of contraction is poor[1]. The effects could be explained by an inadequate rate of ATP regeneration at very low temperatures. We have used ^{31}P and ^{1}H NMR to monitor ATP and lactate production as a function of temperature in human atrial appendages removed during bypass procedures. Spectra were recorded for up to 16 hrs at 1, 4, 12 and 20°C in either Bruker AM-360 or MSL-300 spectrometers. Samples were also quick frozen for biochemical assay. On removal from the patient, the estimated ATP content of atrial myocytes (50% of total appendage) was 3.4 ± 0.8 μmol/g wet weight. Myocyte CrP content was very, low but so also were P_i and total creatine (4 μmol/g wet wt). The rate of decrease of ATP and corresponding increase of P_i are greater at 12 and 20°C than at 1° or 4°C. The rate of glycolysis correlates positively with temperature. The intracellular pH decreased at a rate of 1 pH unit for 20 μmol lactate per g total tissue to reach a value of 6.5 after 6 hrs at 12°C or 20°C and 7.0 at 4°C. We conclude that the increased resting force and failure to recover contraction at very low temperatures is not due to a loss of ATP. An alternative explanation may be a failure of atrial myocytes to maintain low intracellular free Ca^{++} at low temperatures.

1. Keon, W.J., Hendry, P.J., Taichman, G.C. & Mainwood, G.W. (submitted).

W-Pos260 FUNCTIONAL STATE OF MYOFIBRILS, MITOCHONDRIA AND BOUND CREATINE KINASE IN CARDIOMYOPATHIC HAMSTERS. V.I. Veksler, ; R. Ventura-Clapier, ; P. Lechêne, ; G. Vassort. Introduced by K. SCHWARTZ.

The functional state of myofibrils and mitochondria as well as creatine kinase which binds to these organelles was investigated in cardiac skinned fibres of hereditary cardiomyopathic hamsters (CHF146) as compared to normal golden hamsters. Triton X-100 skinned fibres were used to investigate the mechanics of the contractile machinery while the saponin-skinned fibres were used to evaluate the respiratory properties of the total tissue mitochondria. Resting tension, maximal Ca-activated tension, stiffness, and rate of tension recovery after quick stretch were not significantly altered in diseased animals while a slight increase in Ca sensitivity was observed. Functional activity of myofibrillar creatine kinase was decreased in cardiomyopathy. The ratio of maximal ADP stimulated respiratory rate to the respiratory rate in the absence of ADP was unchanged in myopathy as compared to age-matched controls. However, creatine stimulated respiratory rate was lower in myopathic animals as compared to controls. These results suggest that hereditary cardiomyopathy is associated with alterations in the myocardial creatine kinase system while myofilaments and mitochondria preserve their basic functional properties.

W-Pos261 ION-MAN COMPETITION: IN SEARCH OF THE BEST SALT FOR ADJUSTING IONIC STRENGTH IN SKINNED SKELETAL MUSCLE EXPERIMENTS. Mark A. Andrews, Thomas M. Nosek & Robert E. Godt. Dept. of Physiology & Endocrinology, Medical College of Georgia; Augusta GA 30912.

It is well known that increased ionic strength ($\Gamma/2$) decreases the maximal calcium activated force (Fmax) of skinned striated muscle fibers. However, the extent of decrease is dependent upon the major salt used to adjust $\Gamma/2$ (Gordon et al. J. Gen Physiol. 62:550, 1973). We reexamined this effect on chemically skinned rabbit psoas fibers using a wide variety of salts including those commonly used in skinned fiber experiments. The control solution contained (mM): 1 Mg^{2+}, 1 MgATP, 15 Na_2Phosphocreatine, 5 EGTA, 20 imidazole, 0.25 mg/ml creatine kinase, pH 7, 22°C, $\Gamma/2$=90. As expected, Fmax decreased as $\Gamma/2$ was increased by addition of uni-univalent salts to control solution, although the extent of decrease depended upon the specific salt utilized. Over the likely physiological range of $\Gamma/2$ (165-240 mM), potassium salts decreased Fmax in the potency sequence: methanesulfonate (MS) < lactate ≈ acetate ≈ Cl ≈ isethionate < propionate < nitrate < perchlorate (added as Na-salt), a sequence mirroring the classical Hofmeister series for destabilization of protein structure (von Hippel & Schleich Acc. Chem. Res. 2:257, 1969). Cations (as MS-salts) over this range of $\Gamma/2$ decreased Fmax in the sequence: choline ≈ tetramethylammonium (TMA) < K ≈ Na. The specific salt effects are significant. For example, at $\Gamma/2$ of 240 mM, Fmax with choline-MS is decreased to 79% of control, with KCl to 60%, and with Na-perchlorate to but 12%. Thus, on these grounds, TMA- or choline-MS is to be preferred in mixing solutions for skinned fibers. (Support: NIH AR 31636 & HL/AR 37022)

W-Pos262 SUSTAINED POWER OUTPUTS BY FAST AND SLOW SKELETAL MUSCLES OF THE MOUSE. Susan V. Brooks, John A. Faulkner, and Doris A. McCubbrey. Department of Physiology and Bioengineering Program University of Michigan, Ann Arbor, Michigan 48109.

The hypothesis was tested that during a 30 minute time period of repetitive shortening contractions with the muscles in situ, fast extensor digitorum longus (EDL) muscles of the mouse develop a higher sustained power than slow soleus muscles. A frequency-power relationship was determined for maximum power during single contractions at 35±1°C. With fiber length (L_f) optimized for force development, a Cambridge 300-H ergometer produced ramped displacements from 105% of L_f to 95% of L_f. Power was calculated as velocity times the average force during shortening. Maximum powers were 221±22 w/kg for EDL muscles at 350 Hz and 89±5 w/kg for soleus muscles at 250 Hz. Both muscles sustained power best at 150 Hz. Single contraction at 150 Hz produced powers of 164±10 w/kg for EDL and 62±5 w/kg for soleus muscles. Each muscle was stimulated at 150 Hz repetitively at increasing train rates and shortened 10% L_f at optimal velocity (approximately 19±2 mm/s for EDL and 8±1 mm/s for soleus). EDL muscles (n=10) began repetitive contractions at a train rate of 2 Hz with increments of 1 Hz every 10 min until power decreased. For soleus muscles (n=6), train rates began at 0.25 Hz with increments of 0.25 Hz. The power developed by EDL muscles plateaued at 9.1±0.4 w/kg at a train rate of 7 Hz and that of soleus muscles reached a maximum of 7.2±0.5 w/kg at a train rate of 3 Hz. Neither EDL nor soleus muscles could sustain these power outputs when initiated as a step function. We accept our hypothesis and conclude that the power outputs sustained for 30 min by fast muscles of the mouse are significantly greater than those of slow muscles. Supported by NIH grant AG 06157.

W-Pos263 MUSCLE SOUNDS OCCUR AT THE RESONANT FREQUENCY OF SKELETAL MUSCLE

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Acoustic and force signals were recorded simultaneously during maximal isometric twitches of frog gastrocnemius muscles. The muscles were suspended between a Cambridge 305 servomotor and a fixed post, in a temperature controlled bath of frog Ringers. Stimulation was via the sciatic nerve. Acoustic signals were transduced with Bruel and Kjaer model 8103 hydrophones having bandwidths of 0.1 Hz - 150 kHz ± 3 dB. Acoustic signal instantaneous frequencies were calculated using time-frequency transformations (e.g., Wigner Transform or Exponential Distribution).

Imposing sinusoidal length changes on a muscle via the servomotor produced transverse standing waves when the frequency of length change matched the muscle's resonant frequency or a harmonic of the resonant frequency. During a tetanic muscle contraction the resonant frequency initially increased and then became constant as the force plateau was reached. The resonant frequency at a particular time during contraction was approximately equal to the peak instantaneous frequency of the acoustic signal at that same time. Therefore, the acoustic signal can be used as a noninvasive monitor of muscle resonant frequency during contraction. Since the resonant frequency is highly dependent on stiffness, muscle sounds may offer a noninvasive measure of muscle stiffness during contraction. This work was supported by NIH grant #NS01017 and the Grass Foundation.

W-Pos264 ACTIN SELF-ASSEMBLY AS A FUNCTION OF pH. Fei Wang, Rosemary Sampogna, and Bennie R. Ware, Department of Chemistry and Graduate Biophysics Program, Syracuse University, Syracuse, New York 13244-1200.

Actin self-assembly has been characterized using the technique of fluorescence photobleaching recovery (FPR). The objective of these experiments has been to examine the dependence of the assembly parameters on the pH of the medium over a pH range that might be encountered in cell cytoplasm. Trace quantities of fluorescein-labeled actin were included with normal column-purified muscle actin and FPR measurements were made as a function of time throughout the assembly process in media of varied pH. Assembly has been conducted under varying ionic conditions at each pH. The data establish that the actin self-assembly process is faster at reduced pH and that the final extent of assembly is considerably greater at reduced pH, particularly when the assembly is conducted in the absence of excess divalent cation. Separate assays of critical concentration using the pyrenyl actin assay confirm these results, but this assay appears to be less vivid at reduced pH, particularly in the absence of excess divalent cation.

W-Pos265 DOSE DEPENDENCE OF CYTOCHALASIN D ON ACTIN ASSEMBLY. Else Urbanik and Bennie R. Ware, Department of Chemistry, Syracuse University, Syracuse, New York 13244-1200.

The dose dependence of the effects of cytochalasin D (CD) on the assembly of column-purified skeletal muscle actin has been investigated using the fluorescence photobleaching recovery (FPR) technique. The specific activities detected are the reduction of the fraction of actin incorporated into filaments, the relative lengths of filaments as estimated from their translational diffusion coefficients, and the acceleration of the kinetics of actin assembly. These distinct activities exhibit significantly different dose dependences. (1) The activity of CD in reducing the fraction of actin incorporated into filaments is maintained essentially independent of concentration down to CD:actin ratios of 1:100, then decreases down to the lowest detectable activity at a ratio of about 1:1,000. (2) The increase of the steady-state filament diffusion coefficients is seen at much higher dose rates and is still increasing, though with decreased slope, at the highest CD:actin ratio studied, which was 2:1. (3) Acceleration of the kinetics of assembly does not appear to be a sensitive function of the dose rate and has been observed at CD:actin ratios down to 1:200. The data presented constitute additional evidence for different affinities of CD associated with its distinct molecular activities in regulating actin assembly and provide the most specific physical determination of the associated affinities.

W-Pos266 CYTOCHALASIN D-INDUCED ACTIN NUCLEATION PRODUCES AN EARLY BURST OF ATP HYDROLYSIS. J.E. Estes, H.J. Kinosian, L.A. Selden, L.C. Gershman. Research and Medical Services, Veterans Administration Medical Center, Albany, N.Y. and Departments of Physiology and Medicine, Albany Medical College, Albany, N.Y. 12208.

We published previously that Mg-actin nucleates more readily than Ca-actin (Selden et al (Biochem. Biophys. Res. Comm. 116:478-485, 1983). Since it is known that cytochalasin D (CD) accelerates actin nucleation, we used pyrene-labeled monomeric actin to observe the time course of CD-induced Mg-actin polymerization in experiments similar to those of Goodette and Frieden (J. Biol. Chem. 261:15974-15980, 1986). We confirmed their finding of a rapid (<1 sec) CD-induced fluorescence intensity increase and that Mg^{++} must occupy the high affinity site for this effect. However, as long as Mg-actin is used, the effect is induced equally well by KCl or $CaCl_2$ in the presence of CD. Measurement of ATP hydrolysis following the addition of CD and salt indicated that Ca-actin showed minimal ATP hydrolysis regardless of the type of polymerizing salt used, but Mg-actin showed an "early burst" of ATP hydrolysis approximately stoichiometric with actin concentration which was then followed by steady-state hydrolysis. This initial P_i burst was dependent only on the tightly-bound cation and not on the type of polymerizing salt. These results suggest that during the very early period after CD addition, the entire Mg-actin monomer population has participated in the actin nucleation reaction and that nucleation is regulated by the tightly-bound divalent cation. Supported by the Veterans Administration and NIH grant #GM-32007.

W-Pos267 DIVALENT CATION EXCHANGE ON ACTIN. L.C Gershman, L.A. Selden, H.J Kinosian and J.E.Estes. Research and Medical Services, Veterans Administration Medical Center, Albany, and Departments of Medicine and Physiology, Albany Medical College, Albany, NY 12208

The actin molecule contains one high-affinity binding site for a divalent cation with a dissociation constant in the nanomolar range at pH 7. We have recently measured the dissociation rate constants for Ca^{++} (k_{-Ca}) and Mg^{++} (k_{-Mg}) from actin as well as the equilibrium dissociation constants and using these values we have estimated the association rate constants for Ca^{++} (k_{Ca}) and Mg^{++} (k_{Mg}) to actin (Estes, J.E., Selden, L.A., and Gershman, L.C., J. Biol. Chem. 262, 4952-4957, 1987). In the same study we also demonstrated that the "slow change" in 1,5-I-AEDANS-actin fluorescence parallels, and thus essentially monitors, divalent cation exchange. Using 1,5-I-AEDANS-actin, we have investigated divalent cation exchange on actin over a wide range of concentrations of free Ca^{++}, [Ca], and free Mg^{++}, [Mg]. As long as the ionic strength is held reasonably constant, the measured apparent rate constants for divalent cation exchange k_{app} fit the theoretically expected values for competitive exchange at a site having simple first-order binding kinetics:

$$k_{app} = k_{-Ca}/\{1+(k_{Mg}[Mg]/k_{Ca}[Ca])\} + k_{-Mg}/\{1+(k_{Ca}[Ca]/k_{Mg}[Mg])\}$$

We conclude that the model presented appropriately describes high-affinity divalent cation binding to actin. Supported by the VA and NIH grant GM-32007.

W-Pos268 IONIC STRENGTH EFFECTS ON HIGH AFFINITY DIVALENT CATION BINDING TO ACTIN. L.A. Selden, H.J. Kinosian, J.E. Estes, and L.C. Gershman. Research and Medical Services, Veterans Administration Medical Center, Albany, and Departments of Medicine and Physiology, Albany Medical College, Albany, Ny 12208.

The high-affinity binding of divalent cations to actin weakens with increasing pH from pH 7-8, suggesting a charge effect on the high-affinity binding (Estes, J.E., et al, J. Biol. Chem. 262, 4952-4957). Here, we report the effects of ionic strength on high-affinity divalent cation binding using 1,5-I-AEDANS-actin. The "fast-change" in 1,5-I-AEDANS-actin fluorescence increases with increasing $CaCl_2$, $MgCl_2$, or KCl concentrations in a manner suggestive of cation binding as first repoŕted by Carlier et al (J. Biol. Chem. 261, 10778-10784). The rate constant k_{-Ca} for dissociation of Ca^{++} from actin, measured from the "slow change" in 1,5-I-AEDANS-actin fluorescence, is increased at high $CaCl_2$ or KCl concentrations. The rate constant k_{-Mg} for dissociation of Mg^{++} from actin is similarly increased at high $CaCl_2$ or KCl concentrations. With increasing KCl concentration, k_{-Mg} appears to increase approximately linearly with KCl concentration, whereas k_{-Ca} appears to be linearly related to the induced fluorescence "fast-change". We conclude that the tight binding of divalent cations to actin is weakened at high ionic strength , probably as a result of charge neutralization by specific low-affinity divalent cation binding and/or nonspecific cation binding. Supported by the VA and NIH grant GM-32007.

W-Pos269 PROBE DIFFUSION THROUGH FILAMENTOUS ACTIN SOLUTIONS Jay Newman, Nicholas Mroczka, and Kenneth L. Schick, Physics Department, Union College, Schenectady, N.Y. 12308.

The diffusion coefficients of polystyrene latex sphere probes in solutions of polymerized actin were measured using dynamic light scattering. Four different probes with radii, R, ranging from 0.05 to 0.5 μm were separately used in actin solutions with concentrations, c, ranging from 1.5 to 21 μM which had been polymerized with either 1 mM $MgCl_2$, 1 mM $CaCl_2$, or 100 mM KCl. Under all conditions, and at four different scattering angles in the range 30 to 90°, the measured average diffusion coefficients, D, of the probes in polymerized actin solutions were systematically smaller for samples of increased actin concentration or of increased probe radius. Control experiments indicated that the probes did not bind to the actin. These data for Mg- or Ca-polymerized actin were found to be quite well summarized by the scaling relation $D/D_o = \exp[-a R^{\delta} c^{\nu}]$, where D_o is the measured diffusion coefficient of the probes in water (and, as measured as well, in the starting actin solutions prior to polymerization with added salt), with values of $\delta \sim 0.75$ and $\nu \sim 1$. With probes in an actin solution at 35 μM, we observed large slow fluctuations in the scattered intensity which were strongly correlated with fluctuations in the D values. Under these conditions a quasi-static diffraction pattern was observed at very low angles indicating the presence of long-range ordering. After a single slow inversion of the sample cell both the large fluctuations and the diffraction pattern vanished, as did the large apparent macroscopic viscosity commonly seen in undisturbed concentrated polymerized actin solutions. These results will be discussed in terms of weak long-range interactions as well as filament entanglements. We thank J.E.Estes & L.A. Selden for purified actin and NSF for grant DMB-8607031.

W-Pos270 AN IMMUNOCHEMICAL STUDY OF THE N-TERMINAL SEGMENT OF ACTIN AND ITS ROLE IN MACROMOLECULAR INTERACTIONS. G. Das Gupta, J. White, J.C. Bulinski, and E. Reisler, Department of Chemistry and Biochemistry, Department of Biology, and the Molecular Biology Institute, University of California, Los Angeles, CA 90024.

The role of the N-terminal segment of actin in actin-actin and actomyosin interactions was studied by using polyclonal site specific antibodies prepared against a synthetic peptide spanning the first 7 N-terminal residues of α-actin from skeletal muscle. Affinity purified IgG and Fab fragments showed high reactivity towards actin and specificity for its N-terminal segment (Miller, et al., Biochemistry 26, 6064-6070 (1987)). It is shown now in competitive ELISA experiments that the site specific Fab fragments have the same reactivity towards G- and F-actin forms. Consistent with this, light scattering measurements and fluorescence experiments using pyrene labeled actin did not reveal any significant effect of Fab on the nucleation and elongation of actin filaments. In contrast to Fab, the bivalent IgG showed greater affinity for F- than for G-actin and promoted the polymerization of the monomeric actin. These results indicate that the N-terminal segment of actin does not reside near nor affects the actin-actin interface. Although the actin antibodies do not block acto-S-1 interactions in the absence of nucleotides (Miller, et al., 1987), we find now that the Fab fragments completely inhibit the actin activated ATPase of S-1. The mechanism of this inhibition is currently under investigation.

W-Pos271 ACTOBINDIN IS A POTENT INHIBITOR OF THE NUCLEATION PHASE OF THE POLYMERIZATION OF ACTIN. Peter K. Lambooy and Edward D. Korn, Laboratory of Cell Biology, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892.

Actobindin is a recently discovered dimer of a 12,500-dalton polypeptide (P.K. Lambooy and E.D. Korn (1986) J. Biol. Chem. 261, 17150-17155). It interacts with G-actin on a 1:1 molar basis with a K_D of about 5 μM; it does not interact detectably with actin filaments. We have now found that actobindin is a more potent inhibitor of the early phase of actin polymerization than can be explained by its interaction with G-actin. Substoichiometric concentrations of actobindin (relative to G-actin) dramatically inhibit the rate of spontaneous polymerization, primarily by inhibiting the nucleation step. Actobindin inhibits the spontaneous polymerization of ADP-G-actin, as well as of ATP-G-actin, and is effective when polymerization is initiated by $MgCl_2$, KCl or $MgCl_2$ + KCl. Also, nanomolar levels of actobindin inhibit the rates of elongation of covalently cross-linked actin dimers and trimers added to 1 μM G-actin. However, similar concentrations of actobindin do not affect the rates of elongation of F-actin and sonicated F-actin. The rates of hydrolysis of ATP by G-actin and F-actin and the ATP hydrolysis that accompanies actin polymerization are unaffected by actobindin. We speculate that actobindin, in addition to binding monomeric actin, also interacts strongly with actin nuclei, perhaps by trapping or destabilizing them. The ability of actobindin to inhibit polymerization of actin de novo at concentrations too low to inhibit the rate of elongation of actin filaments could serve an important regulatory function in cells.

W-Pos272 LENGTH DISTRIBUTION IN F-ACTIN DETERMINED FROM FLUORESCENCE RESONANCE ENERGY TRANSFER MEASUREMENTS ON TREADMILLING FILAMENTS. Chris M. Coppin and Paul C. Leavis, Dept Physiology, Tufts Univ. Med. School and Dept Muscle Research, Boston Biomedical Research Inst., Boston, MA 02114.

Actin polymerization is accompanied by the hydrolysis of bound ATP, the resulting ADP remaining bound to the protein while the phosphate is released. Polymerization stops when the actin monomer concentration drops below the so-called critical concentration (cf Oosawa and Kasai, in Subunits in Biological Systems, Timashef and Fasman, Eds., Dekker, NY 1971). However, steady-state ATP hydrolysis continues as ATP-actin units in the solution exchange with ADP-actin units in the filament, a process known as treadmilling (cf Wegner, A. J. Mol. biol. 161; 607, 1982). We have employed fluorescence energy transfer to study the dynamics of treadmilling. G-actin was labelled with either fluorescein maleimide as a donor fluorphor or eosin maleimide as an acceptor employing a modification of the method of Taylor et al (J. Cell Biol. 89; 362, 1981). The labelled solutions were allowed to separately polymerize to F-actin and were mixed in the presence of ATP in a 1 fluorescein-F-actin to 5 eosin-F-actin ratio at a final concentration at which treadmilling occurs. The incorporation of donor-labelled actin into acceptor-labelled filaments and vice versa during treadmilling was followed by monitoring the time-course of donor quenching. The second derivative of the kinetic data, N(t), is the distribution of filaments ending a complete treadmilling cycle in time t. This method provides an easy alternative to such techniques as quasi-elastic light scattering and electron microscopy in determining a continuous distribution of filament lengths. (Supported by grants from NIH (HL-20464) and the Muscular Dystrophy Association)

W-Pos273 SUBTILISIN CLEAVAGE OF ACTIN AFFECTS BOTH ACTIN-ACTIN AND ACTIN-S-1 INTERACTIONS. Deborah Schwyter and Emil Reisler, Department of Chemistry and Biochemistry and the Molecular Biology Institute, University of California, Los Angeles, CA 90024

In an effort to study the structurally and functionally important sites of actin, we have compared the properties of actin nicked with subtilisin to those of intact actin. Proteolysis with subtilisin carried out at 23°C at 1:1000 (w/w) subtilisin to monomeric actin ratio introduces a nick between met_{47} and gly_{48}. SDS-PAGE shows that 9K and 35K actin fragments are generated under such conditions with virtually no other degradation products detectable, thus, yielding an improved preparation over that of chymotryptically split actin (Konno, K. Biochem 26, 3582-3589 (1987)). According to Western blot analysis, the two fragments remain associated with each other during repeated polymerization/depolymerization cycles. The polymerization of nicked actin has been monitored by both light scattering and pyrene fluorescence intensity measurements. These studies reveal that the polymerization of nicked actin by KCl, Mg^{2+}, and S-1 is reduced when compared to that of intact actin and requires longer nucleation times. Ultracentrifugation experiments have shown that the critical concentration for polymerization of nicked actin by 2mM Mg^{2+} is increased 2-fold over that for intact actin. The assembled form of nicked actin activates S-1 ATPase reaction with a 40-fold increase in K_m. These results suggest that the site of subtilisin cleavage may be important in actin-actin and actin-S1 interactions.

W-Pos274 MEASUREMENT OF THE STATISTICAL PROPERTIES OF GEL NETWORKS BY THE SCALE-VARIANCE OF TRACER DIFFUSION. Li Hou and Frederick Lanni. Center for Fluorescence Research in Biomedical Sciences, and Department of Biological Sciences. Carnegie-Mellon University, Pittsburgh, PA 15213.

In measurements of diffusion by fluorescence recovery after photobleaching (FRAP), only long-range tracer motion within the specimen contributes to the time-dependent signal, the scale being determined by the dimensions of the pattern photochemically imprinted on the specimen initially. In order to fully understand the results of intracellular FRAP experiments in which tracer proteins and inert polymers have been used (Luby-Phelps et al (1987)PNAS 84:4910, (1986)J Cell Biol 102:2015, (1985)J Cell Biol 101:1245) we have studied tracer diffusion of size-fractionated ficoll in model agarose gels. Our data can be understood in terms of several factors; the size-dispersity of the ficoll fraction incorporated into the agarose melt prior to gelation, and the average pore size (or mesh) and percolation cutoff of the agarose network. Our goal is to derive statistical measures of gel structure from the average FRAP diffusion coefficient and mobile fraction as a function of tracer size, FRAP pattern size, and gel network density. Measures of interest would include average mesh, pore size distribution, and connectivity. As an independent indicator of average mesh, we are measuring the angular-dependence of Rayleigh scatter from rapidly-quenched agarose gel specimens. Feke and Prins ((1974)Macromol 7:527-530) have shown that in such gels there is a dominant correlation length for mass distribution, which should translate into a well-defined mesh or narrow pore-size distribution. When the same specimens are used in FRAP experiments, a direct comparison can be made. These methods can be extended to the study of bulk gels reconstituted from cytoskeletal and cytomatrix components. Supported by NIH grant GM34639

W-Pos275 Do Glycolytic Enzymes Bind to the Actin Cytoskeleton in Living Cells? L.J. Pagliaro and D.L. Taylor, Center for Fluorescence Research in Biomedical Sciences, Carnegie-Mellon University, Pittsburgh, PA 15213.

Biochemical studies of glycolytic enzymes have indicated that they exist exclusively in the soluble phase of cytoplasm. There is, however, evidence that some glycolytic enzymes may exist in the solid phase of cytoplasm. We have tested this hypothesis directly, using a fluorescent analog of aldolase, labeled with carboxytetramethyl-rhodamine succinimide ester, and fluorescence recovery after photobleaching (FRAP) measurements in vitro and in vivo. Optimally labelled analog (D:P=2.6) maintains the enzymatic and actin binding activities of native aldolase in vitro, as well as tetrameric conformation. In order to establish the validity of using FRAP to study an enzyme in vivo, we have established that aldolase analog retains most of its activity after the bleaching doses used. In addition, we have demonstrated that the actin binding activity of aldolase in vitro is inhibited by its substrate, fructose 1,6-diphosphate, with specificity. When we microinjected Swiss 3T3 cells with aldolase analog and performed FRAP measurements in vivo at 37°C, we found that there was significant spatial variation in both its diffusion coefficient and immobile fraction. The perinuclear diffusion coefficient is $\approx 1.5 \times 10^{-7}$ cm^2/sec, with a ≈25% immobile fraction, while the peripheral diffusion coefficient is about three fold slower, with no immobile fraction on the recovery timescale we used. Imaging of microinjected living cells shows clear exclusion of 150,000MW fluorescein-dextran from stress fibers, but only very slight exclusion of the aldolase analog. These data, together with earlier published results, suggest that aldolase exists in both the fluid and solid phases in living cells, and may be bound to actin in vivo.

W-Pos276 HIGH RESOLUTION IMMUNOCYTOCHEMICAL LOCALIZATION OF DESMIN IN ISOLATED SKELETAL MUSCLE MYOFIBRILS. M.J. Mailloux, P.M. Charest and P.A. Rogers. Laval University Hospital Research Center, Sainte-Foy, Québec, Canada G1V 4G2.

Desmin is one of the major intermediate filament (IF) subunit proteins in mammalian skeletal muscle fibers. Evidence from several laboratories suggests that desmin-containing IFs are found primarily between or close to the Z-lines of adjacent myofibrils. The objective of the present series of experiments was to localize desmin in isolated myofibrils using immunoelectron microscopy. Immunolabelling of isolated rat skeletal muscle myofibrils with desmin antibodies and a gold conjugated second antibody revealed that: 1) a significant amount of desmin labelling was located between the Z-lines of the same sarcomere, 2) a portion of the IFs labelling with desmin antibody existed as bundles of diameters ranging from 30-80 nm, and 3) KI extraction of myofibrils resulted in the translocation of desmin-containing IFs to the remaining Z-line structure revealing a residual IF network connecting the Z-line structures. It is concluded that desmin-containing IFs are only a part of the exosarcomeric cytoskeletal structure in myofibrils. These results and those of other studies suggest that the cytoskeletal structure of the muscle cell is more complex than previously thought.

W-Pos277 SMOOTH MUSCLE AND NON-MUSCLE ACTINS ARE NOT SEGREGATED INTO SEPARATE SUBPOPULATIONS OF NATIVE THIN FILAMENTS IN SWINE CAROTID ARTERY.
Jean S. Drew and Richard A. Murphy, Department of Physiology, School of Medicine, University of Virginia, Charlottesville, VA 22908.

Smooth muscles contain both smooth muscle and non-muscle actins (α-SM, γ-SM, β-NM, γ-NM). Recent work suggests that smooth muscles contain at least two subpopulations of thin filaments: one contractile subpopulation associated with caldesmon, and one cytoskeletal subpopulation associated with filamin (Small, J.V., et al. (1986) J. Cell Biol., 102:210-220). We tested the hypothesis that smooth vs. non-muscle actins segregate into different subpopulations of thin filaments. We isolated native thin filaments from swine carotid artery (Marston, S.B. and Smith, C.W.J. (1984) J. Mus. Res. and Cell Motil. 5:559-575). Scanning densitometry of 2-D gels showed that the proportions of isoactins in isolated native thin filaments were similar to those in whole tissue homogenate ($<$5% difference for all isoforms; n=2). Colloidal gold labeling showed that smooth muscle and non-muscle actins are polymerized together in isolated native thin filaments. No segregation of isoactins was apparent when 3 polyclonal antisera identifying 1) β-NM, 2) γ-NM, or 3) α-SM, γ-SM and γ-NM actins were used. These findings are consistent with those of others showing that 1) different isoactins copolymerize in vitro; 2) transfected actins distribute similarly to native isoactins in cultured cells; and 3) different isoactins are not segregated into domains in non-muscle tissue. Supported by a National Science Foundation Graduate Fellowship and NIH grant 5 PO1 HL19242.

W-Pos278 **CHANGES IN MYOSIN HEAVY CHAINS AND ATPase ACTIVITY IN E_2 TREATED RAT UTERUS.**
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We have used SDS/PAGE to analyze the myosin heavy chain (MHC) population in actomyosin preparations from ovariectomized rat uterus following 0, 1, 2, 3 and 4 days of β-estradiol (E_2) administration (2 μg/kg). Three MHCs, which we designated $SmHC_1$ (204 kDa), $SmHC_2$ (200 kDa) and $SmHC_3$ (196 kDa) in order of increasing mobility, were present. $SmHC_3$ has been shown to possess the same mobility and to be immunologically similar to non-muscle myosin, whereas $SmHC_1$ and $SmHC_2$ cross-react with antisera to smooth muscle (tracheal) myosin. The percent of $SmHC_2$ relative to the total amount of $SmHC_1$ + $SmHC_2$ varied from 34% in ovariectomized uterus to 76% in response to 3 days of E_2 treatment. The myosin Ca^{2+}-ATPase, measured in the same actomyosin preparations in 0.5 M KCl at 30°C, increased in response to E_2 treatment and correlated ($r \geq .90$) with the increase in the relative proportion of $SmHC_2$, suggesting that $SmHC_1$ and $SmHC_2$ might represent different isoforms of smooth muscle MHC. However, examination of the MHC population in SDS extracts of uterine tissue indicated no change in the relative proportion of $SmHC_1$ and $SmHC_2$ between ovariectomized and E_2 treated uterus. This difference in percent $SmHC_2$ in SDS extracts versus actomyosin preparations from the same tissue (32.7 ± 1.5% and 70.0 ± 5.9% respectively, for 3 day E_2 treated uterus) suggested that $SmHC_1$ was converted into $SmHC_2$ by limited proteolysis during the actomyosin isolation procedure. It has been shown previously that limited proteolysis increases the myosin Ca^{2+}-ATPase activity of uterine actomyosin (Needham and Williams (1959) Biochem. J. 73:171). Moreover, the total proteolytic activity in ovariectomized uterine tissue significantly increases in response to E_2 treatment (Goodall (1965) Arch. Biochem. Biophys. 112:403). SDS extracts of uterine muscle freeze-clamped *in vivo* contain a significant amount of $SmHC_2$ (> 30%), however the functional significance of this myosin species *in vivo* remains to be determined. Supported by NIH HL 07571 (TEH) and AHA SWO (AFM).

W-Pos279 ROLE OF PROTEOLYSIS IN THE GENERATION OF MYOSIN HEAVY CHAIN SPECIES IN AORTIC TISSUE.
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We have analyzed the myosin heavy chain (MHC) population in rat, dog and rabbit aorta by SDS-PAGE. Three MHC species, which we have designated $SmHC_1$, $SmHC_2$ and $SmHC_3$ in order of increasing mobility, were present in SDS extracts and actomyosin (AM) preparations from adult rat and dog aorta. Only $SmHC_1$ and $SmHC_2$ were present in rabbit aorta. Antisera to bovine tracheal myosin cross-reacted with $SmHC_1$ and $SmHC_2$ but not with $SmHC_3$. Antisera to human platelet myosin reacted preferentially with $SmHC_3$ and cross reacted to some extent with rat $SmHC_1$ and $SmHC_2$ but not with $SmHC_1$ and $SmHC_2$ from rabbit or dog aorta, indicating that $SmHC_3$ is a non-muscle type MHC. The % $SmHC_3$ was 8.0 ± 0.3 (n=5) in rat aorta and 22 ± 1.0 (n=5) in dog aorta. The relative proportions of $SmHC_1$ and $SmHC_2$ were similar in SDS extracts and AM preparations from adult rat ($SmHC_1$ = 58%), rabbit ($SmHC_1$ = 63%) and dog ($SmHC_1$ = 58%) aortas. Incubation of homogenates of adult rat and dog aorta at 37°C for several hours in the absence of protease inhibitors but with EGTA present did not change the relative proportions of $SmHC_1$ and $SmHC_2$. However, isolation of AM from adult rat and dog aortas in the absence of EGTA and leupeptin, but with aprotinin and pepstatin present, increased the relative proportion of $SmHC_2$ to 72% in rat aorta and 87% in dog aorta compared to 45% and 47% $SmHC_2$ in rat and dog AM isolated from the same tissue in the presence of EGTA and leupeptin. These results indicate that $SmHC_2$ (200 kDa) is generated by limited proteolysis of $SmHC_1$ (204 kDa) and suggest that a Ca^{2+}-dependent proteinase is responsible. Whether the $SmHC_2$ levels in SDS extracts of aortic tissue reflect an *in vivo* population of this MHC species or are generated during the isolation procedure remains unclear. Supported by: The American Heart Association-SWO.

W-Pos280 PROTEOLYTIC STUDIES OF SMOOTH MUSCLE MYOSIN ROD FILAMENTS.
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Chymotryptic digestion studies have been used to probe the effects of pH and $MgCl_2$ on the S-2/LMM hinge in synthetic filaments prepared from the papain generated rod fragment of chicken gizzard smooth muscle myosin. The corrected rates of cleavage of the S-2/LMM hinge in rod filaments, formed in the presence of 0.1M NaCl, 0.1mM $MgCl_2$, show little sensitivity to variations in the buffered pH over the range 7.25-8.0. This is in striking contrast to the sharp pH dependence of the chymotryptic cleavage rates of the S-2/LMM hinge in skeletal myosin rod filaments (Reisler & Liu (1982) JMB 157, 659). Over the alkaline pH range from 7.25-8.0, the addition of 5mM $MgCl_2$ causes a dramatic decrease in the corrected cleavage rates. Turbidity measurements and analytical centrifugation sedimentation studies indicate that addition of 5mM $MgCl_2$ causes an increase in filament size. Further, the rates of interfilament cross-linking induced by sulfo-maleimidobenzoyl-succinimide are increased due to the presence of 5mM $MgCl_2$, suggesting a Mg^{2+}-promoted tighter packing of the smooth muscle myosin rod filaments. Steric inaccessibility of the hinge due to tighter association could account for Mg^{2+}-induced inhibition of proteolysis. Previous cross-linking studies have indicated that a similar Mg^{2+}-induced inhibition of hinge cleavage in skeletal rod filaments reflects a tightened association of the S-2 region with the filament backbone (Reisler et al. (1983) Biochem. 22, 4954). If the interpretation is valid for smooth muscle rod filaments, the pH insensitivity of the cleavage rates implies that in low [$MgCl_2$], S-2 is weakly associated with filament backbone over the pH range 7.25-8.0. Tight association of S-2 in smooth muscle myosin rod filaments in alkaline pH requires mM [$MgCl_2$]. Supported by Mt. Sinai Seed Funds.

W-Pos281 INHIBITION OF 10S-6S CONFORMATIONAL CHANGE OF SMOOTH MUSCLE MYOSIN BY A MONOCLONAL ANTIBODY Masaaki Higashihara and Mitsuo Ikebe, Dept. of Physiology and Biophysics, Case Western Reserve University, Cleveland, OH 44106

We produced monoclonal antibodies against smooth muscle myosin. One of these antibodies, MM2, recognized subfragment S-1 (S-1) and modulated several functions of smooth muscle myosin as follows: 1) KCl dependence of Ca^{2+}-ATPase and Mg^{2+}-ATPase of myosin was altered by MM2. It was shown previously that Ca^{2+}- and Mg^{2+}-ATPase of smooth muscle myosin is markedly decreased below 0.3 M KCl and this is due to the formation of 10S myosin. The decrease in the ATPase activity at low KCl was abolished by MM2. 2) MM2 activated actin-activated ATPase activity at low $MgCl_2$ concentration. 3) MM2 increased the level of actin-activated ATPase of dephosphorylated myosin at higher concentration of Mg^{2+} (6-10 mM). 4) MM2 increased the rate of phosphoyrlation and dephosphorylation of myosin about 10-fold at 85 mM KCl. 5) The resistance of 10S myosin against papain proteolysis which cleaves S-1-S-2 junction specificly was released by MM2 and the rate of digestion became similar to that of 6S myosin. 6) S-1 release by papain digestion of 6S myosin was inhibited by MM2. These results suggest that MM2 binds close proximity to the head-neck junction and blocks the conformational change of 6S myosin to 10S myosin. MM2 may provide a useful probe to determine a functional domain of 10S-6S conformational change. (Supported by N.I.H. grant AR 38431, by Syntex Scholorship Award, and by American Heart Association Northeast Ohio Affiliate. M.I. is Established Investigator of American Heart Association)

W-Pos282 INTRAMOLECULAR CROSSLINKING OF THE HEAVY CHAIN OF GIZZARD MYOSIN. Sumitra Nag, Renne Chen Lu, and John C. Seidel, Dept. of Muscle Research, Boston Biomedical Research Institute, Boston, MA 02114

The photoactivatable crosslinker 4-(2-iodoacetamido) benzophenone (BPIA) attached to SH1 of skeletal muscle myosin S1 forms a crosslink with the N-terminal 25kDa region in the absence of nucleotide and with the 25 or the 50kDa region in the presence of Mg-nucleotide upon photolysis (Lu et al., PNAS 83, 6392, 1986). When gizzard myosin S1 was labeled with BPIA, a crosslink between the N-terminal 70kDa and C-terminal 25kDa papain fragments was formed after photolysis in the presence of a nucleotide and no crosslinked product was formed if photolysis was carried out in the absence of nucleotides. To find out which segment of the 70kDa region was involved in crosslinking, gizzard myosin was labeled with BPIA, photolyzed, and digested with trypsin. The soluble fraction of tryptic digest of myosin contained 24, 50 and 68kDa fragments. A band with chain weight of 120kDa was found in the photolyzed sample accompanied by a decrease in intensity of the 50 and 68kDa fragments. When gizzard myosin was modified with BPIA, there was a loss of EDTA-ATPase and no change in the Ca-ATPase activities, indicating that probably SH1 is modified. To rule out the possibility that the other reactive thiol - SHA in the S2 region of the 68kDa region - might be modified, gizzard myosin was labeled with 1,5 IAEDANS and fluorescence was found in SH1 but not in SHA. Treatment of myosin or tryptic HMM with 1,5 IAEDANS prior the modification with BPIA prevents the formation of the 120kDa product, indicating that SH1 participates in the crosslinking with 50kDa region. Supported by NIH grants HL15391, AR28401 and RR0571.

W-Pos283 ^{1}H-NMR OF TURKEY GIZZARD MYOSIN, HMM AND S-1. Leslie E. Sommerville*, Gillian D. Henry+, Brian D. Sykes+ and David J. Hartshorne*. University of Arizona, Department of Nutrition and Food Science, Muscle Biology Group, Tucson, Arizona 85721* and University of Alberta, Department of Biochemistry, Edmonton, Alberta, Canada T6G 2H7+.

Smooth muscle myosin can undergo a transition from a folded, 10S state, to an extended, 6S state. Myosin heads in the 6S conformation are thought to be more mobile. ^{1}H-NMR was used to monitor changes in the internal motion of myosin, HMM and S-1 under a variety of ionic conditions. A significant (5%) portion of the signal from these proteins was contributed by highly mobile residues. Resonances from highly mobile residues in myosin and HMM were found to increase when myosin 10S conditions were changed to myosin 6S conditions. No increase in resonances from highly mobile residues was observed for S-1 when the same changes were made. This increase in the number of mobile residues in myosin and HMM is a direct measurement of changes in the internal mobility of these proteins. These results suggested that a region other than S-1 was involved in contributing to these changes in fast motion. The most likely area is the S-1/S-2 hinge region. Further studies are being done to identify the region of increased mobility in the 6S conformation. This work was supported by NIH Grants HL23615 and HL20984 (to D.J.H.) and MRC Grants (to B.D.S).

W-Pos284 ANALYSIS OF SMOOTH AND NONMUSCLE MYOSIN FUNCTION USING AN IN VITRO MOTILITY ASSAY. Seiji Umemoto, A. Resai Bengur and James R. Sellers, NHLBI, NIH, Bethesda, MD 20892

We have been using the Nitella-based in vitro motility assay to analyze the movement of phosphorylated smooth and nonmuscle myosin-coated beads (Meth. Enzym. 134, 531, 1986). This system appears to be an in vitro analog of the unloaded shortening velocity of muscle fibers. The rate of movement of phosphorylated smooth muscle myosin-coated beads is strongly dependent on ionic conditions going from about 0.24 μ/s at 10 mM KCl to 0.45 μ/s at 50 mM KCl. Phosphorylated turkey gizzard myosin moves at about twice the rate of phosphorylated bovine tracheal or aorta myosin, about 5 times the rate of phosphorylated human platelet myosin and about 1/10th the rate of rabbit skeletal muscle myosin. Gizzard myosin can be di-phosphorylated to 2 mol/mol LC by myosin light chain kinase (MLCK) if large amounts of MLCK are used. This has been shown to give a 2-fold increase in the actin-activated MgATPase activity over that of the mono-phosphorylated myosin (JBC 261, 36, 1986). We find that di-phosphorylated gizzard myosin moves at the same rate as does the mono-phosphorylated myosin. Protein kinase C (PKC) can phosphorylate gizzard myosin 20 kDa light chain at ser-1 or-2 and at thr-9 resulting in a decrease in the actin-activated MgATPase activity by increasing the Kapp while having little effect on Vmax (JBC 259, 8808, 1984). PKC phosphorylation of MLCK phosphorylated gizzard myosin only slightly slows bead movement. These two studies suggest that the additional phosphorylation of myosin may not affect the unloaded shortening velocity of muscle fibers. Tropomyosin (TM) activates the actin-activated MgATPase activity of phosphorylated smooth muscle myosin by about 2.5-fold due to an effect on the Vmax rather than the Km. TM addition to Nitella increases the rate of bead movement by about 40%.

W-Pos285 EVIDENCE FOR NON-MUSCLE MYOSIN IN SMOOTH MUSCLE CELLS. T.J. Eddinger, B.D. Gaylinn, P.L. Monical & R.A. Murphy. Department of Physiology, School of Medicine, University of Virgina, Charlottesville, VA 22908. (Intro. by G.G. Romero)

We have compiled evidence that non-muscle myosin heavy and regulatory light chains (LC_{20}) are present in adult smooth muscle tissues. Swine carotid smooth muscle shows three bands on SDS PAGE having apparent M_r's of approximately 200 kD. The top two bands are known to be smooth muscle myosin heavy chains (MHC's). The lower band had identical mobility with swine platelet MHC and was labeled by a polyclonal antibody raised against human platelet MHC. Using two dimensional gel electrophoresis, four spots can be observed having a M_r of approximately 20 kD and a pI between pH 4.8 and 5.0. The basic pair are smooth muscle LC_{20} (non- and phosphorylated respectively) while the other two are referred to as myosin LC "satellites". These satellites have been attributed to artifactual charge modification, proteolysis, unidentified proteins, multiple phosphorylation, and/or LC_{20} isoforms. Evidence that these satellites contain non-muscle isoforms of LC_{20} include: they copurify with myosin; they are antigenically related to but distinct from smooth muscle LC_{20}; they co-migrate with light chains found in platelets and 3T3 cells on two dimensional gels; the proportion of satellites increase in a manner similar to that described for the non-muscle isoform of both actin and MHC when vascular smooth muscle cells are grown in culture, and finally, they comprise approximately 18% of the total LC_{20} composition in swine carotid tissue. This is similar to our estimates of approximately 14% non-muscle MHC and approximately 20% non-muscle actin content in this tissue. Supported by PHS Grant 5 PO1 HL 19243 and the American Heart Association, Virginia Affiliate.

W-Pos286 CHANGES IN SMOOTH AND NON-MUSCLE MYOSIN HEAVY CHAIN CONTENT WITH DEVELOPMENT IN SMOOTH MUSCLE. T.J. Eddinger and R.A. Murphy, Department of Physiology, School of Medicine, University of Virginia, Charlottesville, VA 22908.

The content of smooth and non-muscle myosin heavy chains (MHC) in newborn (<7 days) and adult (80 - 100 kg) swine was determind using SDS PAGE. Three bands in the 200 kD M_r range were detected. Antibodies specific for smooth or non-muscle (NM) myosin confirmed that the top two bands were smooth muscle MHC (SM1 & SM2), while the lower band was NM MHC. The table shows the percent of each MHC present for these three tissues from piglets and adult swine (n > 5; ± SD).

	PIGLET			ADULT		
	SM1	SM2	NM	SM1	SM2	NM
Carotid	44 ± 2	24 ± 2	32 ± 3	41 ± 3	45 ± 2	14 ± 2
Stomach	56 ± 4	32 ± 4	12 ± 2	43 ± 5	48 ± 4	9 ± 1
Uterus	50 ± 1	27 ± 1	27 ± 1	42 ± 7	37 ± 4	21 ± 4

The smooth muscle tissue from piglet carotid, stomach and uterus had a significantly greater percent of NM MHC than did adult animals. In piglet carotid, stomach and uterus the SM1 values were greater than in adult while the SM2 values were less than in the adult. The data reveal developmental changes in MHC expression, which may contribute to reported differences in MHC ratios in smooth muscle. All tissues showed significant NM MHC expression which was greater in the young animals. With one exception (piglet stomach), the sum of SM2 and NM MHC was fairly constant (54-59%) such that decreases in NM MHC with aging were associated with comparable increases in SM2. An important question provoked by these data is whether the three MHC combine randomly to form 6 myosin variants with respect to their heavy chain composition. Supported by PHS Grant 5 PO1 HL19243 & the American Heart Assoc., Virginia Affiliate.

W-Pos287 THE EXPRESSION AND PHOSPHORYLATION OF SMOOTH AND NON-MUSCLE MYOSIN 20 kD LIGHT CHAIN IN CULTURED VASCULAR SMOOTH MUSCLE CELLS. P.L. Monical, G.K. Owens and R.A. Murphy, Department of Physiology, University of Virginia School of Medicine, Charlottesville, Virginia, 22908.

The exposure of primary vascular smooth muscle cell (VSMC) isolates to serum mitogens in culture is associated with a significant reduction in the expression of smooth muscle-specific alpha-actin and myosin heavy chain (MHC). During the log phase of cellular growth, the dominant actins and MHC expressed by these cells are non-muscle variants. With growth arrest, smooth muscle-specific alpha-actin and MHC are again synthesized. We now report that a similar pattern occurs with the expression of the 20 kD myosin regulatory light chain (LC-20). Tissues or primary VSMC isolates contain about 85-90% smooth muscle and only 10-15% non-muscle LC-20. During log phase growth in culture, smooth muscle LC-20 accounts for less than 10% of the total LC-20 expressed in rat VSMC. The proportion of smooth muscle LC-20 rises to approximately 40% after growth arrest induced either by contact inhibition in confluent cultures or by incubation of pre-confluent cultures in defined serum-free medium. Cultured VSMC retain functional Angiotensin II receptors. Cells harvested from confluent cultures show increases in LC-20 phosphorylation in response to this agonist. There is an initial phosphorylation transient which parallels changes in myoplasmic Ca^{+2} estimated by Indo-1 fluorescence in these cells (D. Dostal and M. Peach, unpublished observations). Angiotensin II stimulated phosphorylation of both the smooth muscle and non-muscle LC-20 with no evidence of differential regulation. These results provide additional evidence that contractile protein isoform expression provides a marker for cytodifferentiation in cultured VSMC. Supported by USPHS grant 5-PO1-HL19242.

W-Pos288 COMPARISON OF THE PHOSPHORYLATION RESPONSE OF SMOOTH MUSCLE AND NON-MUSCLE MYOSIN ISOFORMS IN VASCULAR SMOOTH MUSCLE. B.D. Gaylinn and R.A. Murphy. Department of Physiology, School of Medicine, University of Virginia, Charlottesville, VA 22908.

Both smooth muscle and non-muscle isoforms of myosin are present in the media of swine carotid artery (Eddinger, et al., abstract this meeting). Non-muscle isoforms of both myosin heavy chain and the 20 kD myosin light chain (NM LC_{20}) are present in comparable amounts. The non-muscle LC_{20} is the major component of the myosin light chain "satellites" observed after two dimensional gel electrophoresis and is equivalent to the LC_{20} isoform recently described by Csabina, et al. (Comp. Biochem. Physiol. 87B:271-277, 1987). The LC_{20} species from KCl-activated carotid strips quick frozen at -90°C were resolved by two dimensional gel electrophoresis and quantified by densitometry. Analysis of this data and of autoradiographs of gels from similar preparations labeled with ^{32}P demonstrates that the NM LC_{20} forms a phosphorylated-dephosphorylated pair that accounts for approximately 18% of the total LC_{20} present. Both the extent and the time course of NM LC_{20} phosphorylation parallel that of the smooth muscle LC_{20}. The data also suggests that small amounts of diphosphorylated smooth muscle LC_{20} co-migrate with the dephosphorylated NM LC_{20}. At high levels of phosphorylation a satellite spot corresponding to diphosphorylated NM LC_{20} can also be detected. Total diphosphorylated light chain species amount to < 4% of all LC_{20} when smooth muscle monophosphorylation is 60%. At any level of KCl activation approximately 7% of all phosphorylated LC_{20} is diphosphorylated. There was no evidence for differential regulation of the smooth muscle and non-muscle LC_{20} isoforms on depolarization. The low levels of diphosphorylation are not consistent with a significant functional role. Supported by USPHS 5-T32-HL07355 and 5-P01-HL19242.

W-Pos289 REGULATION OF FETAL SMOOTH MUSCLE MYOSIN BY PROTEIN KINASE C. Primal de Lanerolle, Cynthia Forgue and Masakatsu Nishikawa*, Department of Physiology and Biophysics, Univ. of Illinois, Chicago, IL and *Mie University, Mie, Japan

Phosphorylation of the 20 KD light chain (LC_{20}) regulates adult smooth muscle myosin in the following ways: phosphorylation of LC_{20} by the Ca^{2+}/calmodulin-dependent enzyme myosin light chain kinase (MLCK) stimulates the actin-activated Mg^{2+}-ATPase activity (AM ATPase activity) of adult smooth muscle myosin; the simultaneous phosphorylation of a separate site on LC_{20} by the Ca^{2+}/phospholipid-dependent enzyme protein kinase C (PK-C) attenuates the MLCK-induced increase in the AM ATPase activity of adult myosin. Fetal smooth muscle myosin, purified from the gizzards of 12 day old chick embryos, is structurally different from adult smooth muscle myosin. Phosphorylation of fetal myosin LC_{20} by MLCK results in stimulation of the AM-ATPase activity of this myosin. PK-C was found to phosphorylate 3 sites on fetal LC_{20}, including a serine or threonine residue on the same peptide phosphorylated by MLCK. Interestingly, phosphorylation by PK-C stimulates the AM ATPase activity of fetal myosin. Moreover, unlike adult myosin, there is no attenuation of the AM ATPase activity when fetal myosin is simultaneously phosphorylated by MLCK and PK-C. Our data demonstrate, for the first time, the <u>in vitro</u> activation of a smooth muscle myosin by another enzyme besides MLCK and raise the possibility of alternate pathways for regulating smooth muscle myosin <u>in vivo</u>.

W-Pos290 ISOLATION OF A cDNA CLONE CODING FOR AVIAN BRUSH BORDER MYOSIN HEAVY CHAIN. Yvette A. Preston, Ralph V. Shohet, David A. Brill and Robert S. Adelstein. (Intr. by: Mary Anne Conti) NHLBI, NIH, Bethesda, MD 20892

We screened a lambda gt11 cDNA library (from P. Matsudaira, MIT) constructed from avian brush border mRNA, using affinity-purified antibodies (from J. Sellers and S. Kawamoto, NHLBI) raised against human platelet myosin. Two cDNA clones, one of 4.1 kb and one of 4.3 kb were isolated independently and found to hybridize to each other. Both of these cDNA clones also hybridized to a 23 base oligonucleotide probe (synthesized by E. Appella, NCI) derived from the amino acid sequence of a tryptic peptide purified from the light meromyosin fragment of human platelet myosin. Northern blot analysis using poly A^+ RNA from chicken intestinal brush border and chicken kidney showed hybridization of the 4.1 kb clone to a 6.5 kb mRNA. Both cDNA clones contained multiple restriction sites for Pst I and Pvu II, as seen in other cDNA clones for the vertebrate myosin α-helical rod region. Sequence analysis of a Pst I fragment revealed 57% nucleotide similarity to rabbit cardiac α-myosin rod. The translated nucleotide sequence revealed a pattern of hydrophobic and charged amino acids similar to that found in the coiled-coil region of muscle myosin.

W-Pos291 CHARACTERIZATION OF A GENE FOR A MYOSIN-LIKE PROTEIN. J.A. Horowitz and J.A. Hammer III, Laboratory of Cell Biology, NHLBI, NIH, Bethesda, MD 20892.

We recently reported the structure and sequence of an Acanthamoeba gene encoding the 171 kDa heavy chain of myosin II (Hammer et al. J. Cell Biol. 105, 913, 1987). This genomic clone, along with numerous other non-overlapping clones, was initially identified by its hybridization with fragments of the nematode unc 54 muscle myosin gene. We report here the partial characterization and sequence of a gene contained in one of these nonoverlapping clones (λ3.5). Phage clone λ3.5 was found to hybrid select a mRNA encoding a ~170 kDa polypeptide that comigrated on SDS-PAGE with the heavy chain of purified myosin II. The λ3.5 insert hybridized to a ~5.2 kb mRNA and this hybridization was confined to a ~6 kb stretch of the ~20 kb λ3.5 insert. The sequence of the 5'-half of this 6 kb region encodes a typical myosin globular head domain (~50% similarity versus other myosins). Furthermore, the positions of the 7 introns in this region of the gene are largely conserved relative to other characterized myosin genes. Surprisingly, however, the deduced amino acid sequence of the 3' half of the 6 kb region shows no significant similarity (in any translation frame) to the sequence of the rod domain of conventional myosins (including the complete lack of the heptad repeat of hydrophobic residues that is characteristic of all α helical coiled-coils like the myosin rod). Nevertheless, all indications (including S1 nuclease mapping of several splice sites) are that this gene is transcribed in log phase amoeba. Current efforts are aimed at establishing unequivocally the carboxyl terminal amino acid sequence of the λ3.5 gene product by cloning and sequencing a corresponding cDNA.

W-Pos292 THE EFFECT OF HEAVY CHAIN PHOSPHORYLATION ON THE CONFORMATION OF THE ACTOMYOSIN IA COMPLEX. H. Brzeska, T.J. Lynch, and E.D. Korn, Laboratory of Cell Biology, NHLBI, National Institutes of Health, Bethesda, MD 20892.

Myosins IA (MIA) and IB (MIB) are monomeric proteins, composed of single heavy and light chains, whose actin-activated Mg^{2+}-ATPase activities depend on heavy chain phosphorylation. The amino acid sequence, enzymatic properties and organization of the functional regions of the NH_2-terminal 80-kDa of the MIB heavy chain are similar to those of muscle myosin subfragment-1. These similarities include the location of an actin binding site near a tryptic site 62 kDa from NH_2-terminus of the MIB heavy chain. We have now found that, in the absence of actin, trypsin cleaves the MIA heavy chain at sites 38, 64, 91 and 112 kDa from the NH_2-terminus regardless of the state of heavy chain phosphorylation. The presence of actin protects only the sites at 38 kDa and 64 kDa from trypsin cleavage. Moreover, the protection of the 38-kDa site is strongly enhanced by phosphorylation of the heavy chain. Based on the similarities between the MIA and MIB heavy chains and muscle myosin subfragment-1, we propose that the protection of the 64-kDa site by actin is due to direct actin-myosin interactions at a region near this site. The protection of the 38-kDa site may result also from direct interaction, or, perhaps more likely, from conformational changes produced in the 38-kDa region by binding of actin to the 64-kDa region. In either case, the conformation of the region surrounding the 38-kDa site of the MIA heavy chain in the actomyosin rigor complex varies with the state of phosphorylation of the heavy chain.

W-Pos293 INTRAMOLECULAR AND INTERMOLECULAR INTERACTIONS OF DICTYOSTELIUM MYOSIN. P.F. Flicker, C. Pasternak, S. Ravid, and J.A. Spudich (Intr. by R.W. Aldrich), Dept. of Cell Biology, Stanford University, Stanford, CA 94305.

Electron microscopy of rotary shadowed Dictyostelium myosin has revealed intra- and intermolecular interactions between myosin molecules. In very low ionic strength primarily parallel dimers and monomers are seen. Molecules in parallel dimers are staggered by 145Å. Antiparallel interactions between dimers occur in multiples of 145Å; contacts extend along 280-300Å or 400-450Å. The monomers at these low ionic strengths have a bend in the tail about 650Å from the end of the rod distal from the heads. Contacts within the dimer and in the bent monomers occur mainly in the center third (~ 600Å) of the rod. The terminal 300-400Å tends not to associate with other regions of the rod. Images of myosin in 60 mM KCl show thick filaments, bent monomers, and parallel dimers. Bent monomers appear to be excluded from thick filaments, suggesting that they cannot participate in filament assembly. The formation of bent monomers may be controlled by heavy chain phosphorylation. A correlation is apparent between the number of bent monomers and the amount of phosphorylation by a partially purified heavy chain kinase.

W-Pos294 CHARACTERIZATION OF A FORM OF THE BRUSH BORDER 110-kDa PROTEIN-CALMODULIN COMPLEX CONTAINING A 90-kDa FRAGMENT. J.H. COLLINS, J.P. RIEKER, AND H. SWANLJUNG-COLLINS. UNIV. PITTSBURGH SCH. MED., PITTSBURGH, PA.

The 110-kDa protein-calmodulin complex from intestinal brush border microvilli exhibits enzymatic, actin-binding and physical properties characteristic of the globular "head" region of myosin. We have isolated and identified a kinase from intestinal brush borders that catalyzes the phosphorylation of the 110-kDa protein component of the brush border 110-kDa protein-calmodulin complex. Phosphorylation exclusively at threonine apparently occurs at a single site. Calmodulin in the complex is not phosphorylated by the kinase.The activity of the kinase is completely inhibited by calcium and calmodulin. We have also isolated a form of the complex containing a 90-kDa fragment of the 110-kDa protein that is not phosphorylated by the kinase. Dissociation of approximately half of the calmodulin from the 90-kDa complex in the presence of Ca^{2+}, and actin-binding properties are similar to the 110-kDa complex. However, the 90-kDa complex shows differences in binding to an affinity column. We are investigating the enzymatic properties of the 90-kDa complex in comparison with the 110-kDa complex and brush border myosin. Supported by grants GM35448 and GM32567.

W-Pos295 THE EFFECT OF HALOTHANE ON THE BINDING OF CALCIUM BY CARDIAC TROPONIN C.
ES Casella and TJJ Blanck, Division of Cardiac Anesthesia, The Johns Hopkins University, Baltimore, Maryland 21205

The volatile anesthetic, halothane, is known to depress cardiac contractility. We hypothesized that one mechanism by which halothane causes myocardial depression involves an interference with the recognition or response of calcium sensitive proteins to calcium. Previous studies with papillary muscles and actomyosin ATPase suggest that these proteins may be an important site of action of halothane. One such protein is cardiac troponin C (CTnC). We examined the fluorescence enhancement of bovine CTnC to calcium in the presence and absence of halothane. For the control experiments (n=7), small aliquots of calcium chloride were added to a fluorescence cuvette containing a 2 ml solution of 5 μM CTnC, 5mM KCl, 25 mM Hepes, 2 mM EGTA, pH 7.5 at 25°C. Free calcium concentration was titrated from 10^{-9}M to 10^{-3}M. In the halothane experiments (n=6), 0.9 mM halothane was added to a solution with the same constituents as the control experiment, and placed in a teflon-sealed cuvette to maintain constant anesthetic concentration. The additions of calcium chloride to CTnC resulted in the same fluorescence enhancement in the presence and absence of halothane. From this data, we conclude that, under these conditions (pH 7.5, 25°C), halothane does not alter the binding of calcium by CTnC.

W-Pos296 CA^{2+}-BINDING TO TROPONIN C IN RABBIT CARDIAC MYOFIBRILS DURING DEVELOPMENT.
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The isotype population of thin filament regulatory proteins changes during cardiac development. We investigated the possible functional significance of this by measuring Ca^{2+}-binding to myofibrils isolated from rabbit hearts at 4, 22 and 90 days of age. Ca^{2+}-binding to myofibrillar TNC was measured at room temperature, over a broad range of pCa values in the presence of 5 mM MgATP, 2 mM free Mg^{2+}, pH 7.0, 0.15 M ionic strength. Myofibrillar TNC content, as determined by quantitative PAGE, was not significantly different among the preparations studied, and amounted to 0.4-0.5 nmol TNC/mg myofibrillar protein. Over much of the range of activating pCa values, Ca^{2+}-binding to myofibrils from 4 day old hearts was significantly less than that observed for 22 day and adult preparations. The data were fit to a 3 class model constrained so that $n_1 = 2n_2$, where we assign n_1 as the high affinity structural sites and n_2 the single low affinity regulatory site of cardiac TNC. Values obtained with this model were n_1=0.80 nmol/mg protein, $k_1 = 0.73 \times 10^7$ M^{-1}, k_2=1.36 x 10^5 M^{-1} for 4 day; n_1=1.01, k_1=1.60 x 10^7, k_2=3.39 x 10^5 for 22 day; n_1=0.97, k_1=1.46 x 10^7, k_2=7.85 x 10^5 for adult hearts. PAGE analysis indicates that these differences in Ca^{2+}-binding are not related to differences in TNC, but may be related to differences in the distribution of isoforms of TNT. Supported by BRSG S07 RR05408-25 and NIH P01-HL 22619.

W-Pos297 **CA-SENSITIZATION OF CARDIAC MYOFILAMENTS BY (+/-) PIMOBENDAN (UD-CG 115 BS) IS DUE MAINLY TO THE (-) OPTICAL ISOMER.**
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Racemic pimobendan is a positive inotropic drug that possesses activity as a relatively weak phosphodiesterase inhibitor. This drug also has Ca-sensitizing activity due to an enhancement of the affinity of regulatory sites on cardiac TNC for Ca^{2+}. Our aim here was to know whether this effect is different with optical isomers of pimobendan. Submaximal force developed at constant pCa by detergent-extracted heart muscle fibers was increased more greatly by the (-) isomer. For example, in the presence of 50 μM drug, force at pCa 6.25 was increased to 184% of control by the (+) isomer but to 255% of control by the (-) isomer. Using intact papillary muscles, we also measured force and parameters of slow action potentials ($\dot{V}_{max}$; action potential duration, APD; amplitude, APA) induced in 25 mM $[K]_o$. Compared to the (-) form, (+) pimobendan was relatively weak in its positive inotropic effects. In the presence of 1 μM propranolol, which we used to block possible adrenergic effects, force was increased by (+) pimobendan to 120% of control; $\dot{V}_{max}$ increased to 113% of control and APA and APD increased slightly. In the presence of 1 μM isoproterenol 50 μM (+) pimobendan did not alter SAP parameters, although force increased to 124% of control. In the presence of 1 μM propranolol 50 μM (-) UDCG increased force to 218% control. $\dot{V}_{max}$ was increased to 141%, APD to 129% and APA to 106% of control. However in the presence of 1 μM Isoproterenol, slow action potential parameters were unaffected by (-) pimobendan, although force was increased to 184% control. Our data indicate that the effect of pimobendan on the regulatory sites of TNC involves subtle structural aspects of the regulatory Ca-binding domain and is more complicated than a non-specific change in hydrophobic regions.

W-Pos298 **Ca^{2+}-DEPENDENT CONFORMATIONAL CHANGES IN CARDIAC TnT IN THIN FILAMENTS.**
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Beef cardiac TnT can be labelled with the fluorescent probe Iaans ((2-(4'iodoacetamidoanilmo) napthalene-6-sulfonic acid)) to about 0.8-1 mole Iaans/mole of TnT. When reconstituted into whole cardiac Tn, a biphasic Ca-dependent change in TnT_{Iaans} fluorescence occurred. The first phase exhibited 20% increase in fluorescence with a pCa_{50} of about 7.8, followed by 30% decrease in fluorescence with a $pCa_{50\%}$ of 6.5 (μ = 100, pH 7.0 + 1 mM $MgCl_2$). Similar changes occurred in the absence of Mg^{2+} with the decrease in the second phasic decrease reaching 20%. The first phase occurred in a Ca^{2+}-concentration range necessary to saturate the Ca^{2+}-Mg^{2+} binding sites in cardiac TnC, while the second change arise was associated with Ca^{2+}-binding to the regulatory Ca^{2+}-site on TnC in Tn (Johnson et al., 1980, J. Biol. Chem., 255, 11688). Upon complexing the reconstituted Tn_{Iaans} with Tm, the Ca^{2+}-dependent fluorescent changes were generally similar to those in Tn_{Iaans} except that the pCa_{50} of the decrease in fluorescence was shifted to the left (pCa_{50} = 7.25). Furthermore, when similar studies were performed on reconstituted thin filaments (actin Tm·Tn_{Iaans}), the second phasic decrease in fluorescence observed in Tn_{Iaans} and Tm·Tn_{Iaans} was changed into an increase amounting to about 22% with a pCa_{50} of 6.4 (μ = 120, pH 7.0). This change is similar to that arising from Ca^{2+}-binding to the Ca^{2+}-regulatory site of TnC_{Iaans} in the actin·Tm·Tn complex. Our results indicate that Ca-binding to the Ca^{2+}-Mg^{2+} sites and Ca^{2+}-regulatory site in cardiac TnC can affect the conformation of cardiac TnT and that this Ca^{2+}-dependency can be a) monitored by the Iaans probe and b) altered by thin-filament protein interactions.
Supported by a grant from American Heart Association -Ohio Affiliate (S.E-S.) and by NIH HL 22231 (RJS).

W-Pos299 DISPROPORTIONATE Ca^{2+} BINDING AND MgATPase ACTIVATION ON THE CARDIAC THIN FILAMENT.
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Cardiac troponin has only one Ca^{2+}-specific binding site, unlike skeletal muscle troponin, which has two. This single site has now facilitated detailed study of the relationship between Ca^{2+} binding and MgATPase activation. To measure Ca^{2+} binding, troponin was reconstituted from bovine cardiac troponin I, troponin T isoform T1, and troponin C modified with IAANS (Johnson, et al., [1980] J Biol Chem 255:9635). This reconstituted complex, TnIA, was combined with actin and tropomyosin (Tm), producing a thin filament with a fluorescence intensity increasing approximately 15% on addition of $CaCl_2$ (λex 332, λem 445). The Kd for this fluorescence change was 0.6 μM, at 25° C, pH 7.06, 30 mM ionic strength, 1 mM MgATP, 2.5 mM $MgCl_2$, and 0.75 TnIA:7 actin. Under these conditions Ca^{2+} activates the MgATPase rate of myosin subfragment 1 (S-1) only three-fold.

To produce a greater Ca^{2+}-sensitive activation of the S-1 MgATPase rate a higher concentration of troponin was used; slightly subsaturating TnIA was bound to actin-Tm, and then excess nonfluorescent troponin (Tn) added. This resulted in a thin filament with fluorescence properties resembling those of actin-Tm-TnIA in the absence of nonfluorescent Tn. Twice as much Ca^{2+} was needed for 50% MgATPase activation (Kapp 1.1 μM) than for producing 50% of the fluorescence change (Kapp 0.6 μM). A similar discordance between MgATPase and fluorescence occurred when the thin filaments were prepared by another method: 7 actin:2 Tm:2 TnIA:0 nonfluorescent Tn were mixed, pelleted in an Airfuge, and resuspended. These data suggest that Ca^{2+} can bind to the Ca^{2+}-specific site on the thin filament but not produce activation. Activation occurs when Ca^{2+} binding to more than one troponin produces a cooperative alteration in thin filament conformation.

W-Pos300 THE EFFECT OF BEPRIDIL ON THE STRUCTURE AND FUNCTION OF BEEF HEART CARDIAC TROPONIN C.
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Proton NMR at 360 MHz has been used to monitor the interaction between the calmodulin antagonist, bepridil and Ca^{2+} saturated beef heart troponin C (cTnC). One equivalent of the drug induces considerable changes in the aromatic region of the protein spectrum, that of the methionine methyl residues and several high field shifted methyl signals. Changes are observed in α proton signals from the small regions of β sheet structure intimately associated with Ca^{2+} binding sites probably reflecting modifications of affinity. Conditions: 25°C, 0.1M KCl, 0.05M K phosphate, 0.1mM DTT in D_2O, pH meter reading 7.0.

The dissociation of Ca^{2+} from cTnC was monitored using the fluorescent indicator Quin-2 as a Ca^{2+} chelator. The dissociation of Ca^{2+} is biphasic corresponding to the rates of release from the low affinity Ca^{2+} specific site ($150s^{-1}$) and from the high affinity Ca^{2+}-Mg^{2+} sites ($1s^{-1}$). The rate of Ca^{2+} dissociation from the low affinity Ca^{2+} specific site was reduced by bepridil in a dose dependent manner (to $50s^{-1}$ at 50uM). Under these conditions, the rate of Ca^{2+} dissociation from the high affinity Ca^{2+}-Mg^{2+} sites was unchanged. Conditions: 15°C, 0.1MKCl, 10mM MOPS, 0.3mM $MgCl_2$, 0.1mM DTT, pH 7.0. These results extend those of Solaro et al. (1986) J. Pharm. Exp. Ther. 238 502 and indicate that the mechanism whereby bepridil increases the Ca^{2+} sensitivity of cardiac myofibrillar ATPase involves increased Ca^{2+} bound to the low affinity sites of cTnC.

W-Pos301 CARDIAC REGULATORY PROTEINS IN HAMSTER CARDIOMYOPATHY. Ashwani Malhotra, and James Scheuer, Dept. of Med., Montefiore Med. Ctr., Albert Einstein Col. of Med., BX, NY.

Studies on intact myofibrils from myopathic hamsters (BIO 53: 58) suggest that the regulatory control of contractile proteins may be altered in these hamsters (Malhotra et al., JMCC 1985). Reassociation studies were conducted on cardiac myosin in the presence of relaxing factor (Tn-Tm) isolated from hearts of control (C) and myopathic (M) hamsters at 6-7 mos of age. Actomyosin ATPase activity was determined in the absence (2mM EGTA) and presence of varying concentrations of free calcium (Ca). Results are shown as: μ mole Pi per min per mg at 25°C. Means of 4-5 studies:

Cardiac Myosin	Relaxing Factor	Actomyosin ATPase Activity		Calcium Sensitivity
		+Ca(10^{-5}M)	-Ca(2mMEGTA)	
C	C	0.239	0.067	72%
C	M	0.216	0.129	40%

Rabbit muscle actin was used in all experiments. Ca-sensitivity was decreased in the actomyosin recombined with Tn-Tm from M. Tn-Tm from C and M muscles showed similar gel patterns on gradient SDS gels (5-16.5%) or (10-16.5%) with small additional protein bands in the profile from myopathic muscle. These data suggest that Ca-sensitivity of regulatory proteins from cardiac muscle have decreased inhibitory action on cardiac actomyosin ATPase activity in the presence of EGTA. These results are in concordance with intact cardiac myofibrillar ATPase data from myopathic hamster (JMCC, 1985), suggesting an alteration in the regulatory protein subunit(s).

W-Pos302 NATIVE CARDIAC THIN FILAMENTS ACTIVATE CARDIAC MYOSIN Mg-ATPase. Philip K. Ngai, Thomas Trueb and Joachim W. Herzig. Biological Research CVS, Ciba-Geigy AG, Ch-4002, Basel, Switzerland.

Cardiac and skeletal actomyosin ATPase activities have traditionally been studied using myofibrillar preparations or systems reconstituted from purified contractile and regulatory proteins. We have developed an easy and rapid procedure to isolate thin filaments from porcine ventricular muscle. The most important attribute of such a preparation is that all the essential contractile and regulatory components are present together in physiological proportion to each other and in their native state. This is in contrast to systems reconstituted from individually purified actin, tropomyosin and troponins which are prepared from either acetone or ether muscle powder. The identities of all components in our preparation are confirmed by immunoblotting techniques. In addition, the troponin C component exhibits Ca-dependent mobility shift on SDS-polyacrylamide gels, a feature characterized by other Ca-binding proteins. When reconstituted with purified myosin (also obtained via an improved procedure), we have also shown that these thin filaments are able to activate myosin Mg-ATPase in a Ca-dependent manner yielding average rates of 100 nmol Pi/min/mg. This is at least a 20-fold activation over basal rate in the absence of Ca. These data are comparable to those obtained by using detergent-treated myofibrils and are significantly higher than rates of other systems reported previously. Furthermore, the Ca-dependent activation profile of this system is also similar to that generated by using cardiac skinned fibres. Finally, the effects of several pharmacological agents (such as APP 201-533, calmidazolium, DPI 201-106, fendiline, hydralazine and mastoparan) on the Ca-dependent ATPase activities of this system are examined.

W-Pos303 **POTASSIUM IONS INHIBIT Ca^{2+}-BINDING TO SKELETAL TnC AND Ca^{2+}-ACTIVATION OF SKELETAL MYOFIBRILLAR ATPase. Saleh C. El-Saleh (Intr. by P. Sullivan, Ohio Univ., Athens, OH 45701). Dept. of Physiology & Biophysics, College of Medicine, Univ. of Cincinnati, Cincinnati, OH 45267.**

The Ca^{2+}-dependent activation of skeletal myofibrillar ATPase activity was measured at two K^+ concentrations (K^+ = 35 mM, μ = 50 and K^+ = 115 mM, μ = 120; pH 7.0). There was a 2.3 fold increase in the Ca^{2+} sensitivity of the myofibril ATPase activity when K^+ was lowered from 115 mM to 35 mM. The possibility that this enhancement of Ca^{2+}-sensitivity was due to the lowering of the ionic strength was investigated. Under conditions where K^+ was replaced by choline$^+$ (using choline chloride) the lowering of the ionic strength from μ = 120 to 50, produced only 1.53 fold enhancement of Ca^{2+}-sensitivity. Similar results were obtained using the tetramethyl ammonium$^+$ (TMA^+) cation (with tetramethyl ammonium chloride as the substituent for KCl). Fluorescent studies on the Ca^{2+}-binding to free TnC_{Danz} and to TnC_{Danz} reconstituted into thin filament under varied KCl and choline chloride conditions revealed that K^+, but not choline$^+$ can lower the Ca^{2+}-binding to TnC by 1.8-2.0 fold, suggesting a direct inhibitory effect of K^+ on the regulatory Ca^{2+}-sites in TnC. Also, these results suggest that the state of cycling cross-bridges at low ionic strength (with K^+, choline$^+$, or TMA^+) may enhance Ca^{2+}-binding to TnC in thin filaments producing the 1.53 fold enhancement of the Ca^{2+}-dependent ATPase of myofibrils. In this regard, it is interesting to note that the basal (pCa 8.0) and not the V_{max} ATPase of myofibrils at μ = 50 was increased by about 2 fold regardless of the nature of the cation present in the assay.

W-Pos304 TROPONIN I ENHANCES ACIDIC pH INDUCED DEPRESSION OF Ca-BINDING TO THE REGULATORY-SITES IN SKELETAL TROPONIN C. Saleh C. El-Saleh and R. John Solaro. University of Cincinnati, College of Medicine, Cincinnati, OH 45267-0576.

Inhibition of muscle force development by acidic pH is a well known phenomenon, yet the exact mechanism by which a decrease in pH inhibits Ca-activated force in striated myofilaments remains poorly understood. Whether or not the deactivation by acidic pH involves direct competition between Ca^{2+} and protons for regulatory binding sites on TNC or whether other proteins in thin filament regulation are important remains unclear. We measured the effects of acidic pH on Ca^{2+} dependent fluorescent changes in fast skeletal troponin C (TNC) labelled with the probe danzylaziridine (DANZ), which reports Ca^{2+}-binding to the regulatory (Ca^{2+}-specific) sites. Measurements were also made with TNC_{DANZ} complexed with troponin I (TNI) or with TNI-TNT in the whole TN complex. Our results show that a drop in pH from 7.0 to 6.5 was associated with a relatively small (1.6 fold increase) in the midpoint for the relation between free Ca^{2+} and Ca^{2+}-binding to the regulatory site on TNC_{DANZ}. However, when TNC_{DANZ} was present in its complex with either TNI alone or TNI-TNT, the increase in midpoint free Ca^{2+} was elevated by 3.5 fold. We tested whether this is due to an acidic pH induced alteration in the affinity of TNI for TNC. A decrease in pH from 7.0 to 6.5 was associated with a nearly 2 fold decrease in the affinity of TNI for TNC. We also probed the effect of acidic pH on the intrinsic fluorescence of tryptophan residues in TNI alone and TNI labeled at Cys-133 with IAF (5-iodoacetamide fluorescein). A drop in pH from 7.0 to 6.5 was associated with a 15% decrease in intrinsic fluorescence and a 30% decrease IAF fluorescence. We conclude that while protons may directly affect the regulatory sites on fast skeletal TNC, the effect of acidic pH is amplified by TNI in its interaction with TNC.

W-Pos305 CONFORMATIONAL CHANGES IN STnC DETECTED BY MONOCLONAL ANTIBODIES. Priscilla F. Strang and James D. Potter. Department of Pharmacology, University of Miami School of Medicine, Miami, Florida 33101.

Skeletal muscle TnC (STnC) contains four Ca^{2+} binding sites, two with a high affinity for Ca^{2+} that also bind Mg^{2+} competitively (Ca^{2+}-Mg^{2+} sites) and two sites of lower affinity that are specific for Ca^{2+} (Potter and Gergely (1975) J.B.C. 250, 4628). We have characterized a monoclonal antibody (B_9D_9) that was produced against STnC. The binding of this antibody to STnC is sensitive to the binding of Ca^{2+} and Mg^{2+}. As the Ca^{2+} concentration is increased the amount of antibody bound decreases with a pK of ~7.0 and correlates with Ca^{2+} binding to the Ca^{2+}-Mg^{2+} sites. In addition, saturation of the Ca^{2+}-Mg^{2+} sites with Mg^{2+} also prevents antibody binding. Thus the conformation of STnC brought about by metal binding to these sites affects the antibody binding to its epitope.

Recently Wang, et al. (J.B.C. (1987) 262, 9636) have presented evidence that STnC at low pH has an elongated structure similiar to that seen in the crystal and that at neutral pH its structure becomes more compact. We have found that in the absence of metal this antibody binds best to STnC at low pH and its binding is reduced with increasing pH. It is possible that the structural change brought about by pH may be responsible for the reduction in antibody binding. Since pH, Ca^{2+} and Mg^{2+} have the same effect on antibody binding this may mean that Ca^{2+} or Mg^{2+} binding to the Ca^{2+}-Mg^{2+} sites may also make TnC more compact. Supported by NIH AM33427.

W-Pos306 EFFECT OF S-1 ON THE STRUCTURE AND Ca^{2+} AFFINITY OF TnC IN THIN FILAMENTS. James D. Potter, Alan Mandveno and Bruce Thompson. Department of Pharmacology, University of Miami School of Medicine, Miami, FL 33101.

Previous studies by our lab (Zot, A.S. and Potter, J.D. (1986) Biophys. J. 49, 451a) have shown that rigor (-ATP) and cycling (+ATP) crossbridges (HMM) alter the structure (fluorescence) of TnC_{DANZ} incorporated into reconstituted thin filaments (RTF). In other studies (Guth, K. and Potter, J. (1987) J. Biol. Chem. 262, 13627), using TnC_{DANZ} reconstituted skinned muscle fibers, we have observed qualitatively similar changes in TnC structure with crossbridge attachment, as well as changes in the apparent Ca^{2+} affinity of the Ca^{2+}-specific regulatory sites of TnC. In rigor the Ca^{2+} affinity of these sites increased two fold and with cycling crossbridges at least ten fold. Since the later conclusion required making certain assumptions, we have attempted to confirm this effect of cycling crossbridges using RTF where no assumptions are necessary. In these studies S-1 was used instead of HMM and was found to produce similar changes in TnC_{DANZ} structure. The Ca^{2+}-dependence of TnC_{DANZ} fluorescence was studied in RTF, RTF + S-1 (-ATP) and RTF + S-1 (+ATP). The midpoint (pK) and the Hill coeff. (n) for these transitions were pK = 5.97, n = 1.20, pK = 6.18, n = 1.16 and pK = 6.82, n = 1.14, respectively. Thus in this system, rigor and cycling crossbridges increase the Ca^{2+} affinity of these sites by a factor of two and eight fold, respectively and are in agreement with our skinned fiber conclusions. The cooperative response of the fluorescence change seen in skinned fibers (+ATP) was not found in the RTF since the binding of S-1 to RTF is the same in the presence and absence of Ca^{2+} (Chalovich and Eisenberg (1982) J. Biol. Chem. 257, 2432). Supported by NIH AR 37701 and HL07188 (BT).

W-Pos307 COMPARISON OF RECOMBINANT, CHICKEN AND RABBIT TROPONIN C USING FLUORESCENT PROBES. Z. Dobrowolski, G.-Q. Xu and S.E. Hitchcock-DeGregori, Department of Anatomy, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

In order to study structure-function relationships in troponin C (TnC), we synthesized a cDNA encoding avian fast TnC and showed that the recombinant protein overexpressed and purified from E. coli is functional (Xu and Hitchcock-DeGregori, unpublished results). Here we have used two extrinsic fluorescent probes to compare recombinant TnC with chicken and rabbit fast TnCs: eosin-5-iodoacetamide (IAE) used to modify covalently Cys 101 (Cys 98 in rabbit) and 9-anthryocholine bromide (9AC), a hydrophobic probe used to measure the exposure of hydrophobic surfaces. The fluorescence intensity and spectra of IAE on all three TnCs were almost the same suggesting that the environment of the residue and probe is similar. The fluorescence intensity increased as a function of calcium showing two transitions that were comparable in all three proteins, a large increase with a midpoint of about $5x10^{-7}$M Ca^{2+}, reflecting calcium binding to the high affinity sites, and a smaller increase with a midpoint of about $1x10^{-4}$M Ca^{2+} due to calcium binding to the low affinity sites. In contrast, binding of 9AC to chicken and recombinant TnC was similar but considerably weaker than to rabbit TnC indicating that the structure of the hydrophobic surfaces differs or the exposure is lower in chicken and recombinant TnCs. The binding of 9AC to all three TnCs was much less than to calmodulin. However, the addition of EGTA (to chelate calcium) or TnI greatly reduced the fluorescence intensity in all four proteins. We wish to emphasize that recombinant and chicken TnCs were indistinguishable using these two probes and that they will serve as valid controls for analysis of mutant recombinant proteins. Supported by NIH GM36326 to SEHD and a New Jersey Heart Association Fellowship to GQX.

W-Pos308 AVIAN PECTORALIS MUSCLE (PM): MOLECULAR AND FUNCTIONAL DIVERSITY AT THE SINGLE CELL LEVEL. Peter J. Reiser, James M. Graham, Marion L. Greaser, Richard L. Moss, Dept. of Physiology and Muscle Biology Laboratory, University of Wisconsin, Madison, WI 53706.

On the basis of myofibrillar protein composition and fiber typing,other laboratories have described a small region within the avian PM known as the "red strip" (RS). This region, which is surrounded by a virtually homogenous population of fast-twitch fibers, is composed of two types of fibers which thus far have been considered to be fast-twitch and slow-twitch. The objective of the present study was to provide a correlative physiological-biochemical characterization of the muscle fibers in the RS and to compare these fibers to the fibers that comprise the major portion of the avian PM. The maximal velocity of shortening (V_{max}) and tension/pCa relationships of chemically skinned muscle fibers were measured. In addition, the protein compositions of the same fibers was determined by SDS-PAGE. The results demonstrate the presence of three distinct fiber types, each having unique physiological properties and contractile protein compositions. For example, the majority of fibers in the PM are fast with a mean V_{max} of 4.05 muscle lengths/sec (ML/s) and have a greater Ca^{2+}-sensitivity of tension development than the fibers in the RS. These fast fibers have exclusively fast-type myosin heavy and light chains (MHC, MLC) and fast-type TnT, TnI and TnC. Two groups of fibers are present in the RS: (1) a slow group (V_{max} = 0.45 ML/s) with exclusively slow-type MHC's and MLC's and slow-type TnT, TnI and TnC and (2) a group with intermediate velocities (V_{max} = 2.59 ML/s) with a unique MHC, fast-type MLC's, TnC, and TnI but with a TnT composition different from that of the fast fibers. The results from these extremely heterogeneous fibers allow for the determination of the functional significance of several myofibrillar protein isoforms found in single fibers of avian skeletal muscle.

W-Pos309 TROPONIN-C CAN BE CROSSLINKED TO TROPONIN-I BY A DISULFIDE BOND. H.-S. Park, B.-J. Gong & T. Tao. Dept. of Muscle Research, Boston Biomedical Research Institute, and Dept. of Neurology, Harvard Medical School, Boston MA 02114.

Troponin-C (TnC) and troponin-I (TnI) are the Ca^{2+} binding and inhibitory subunits of troponin, respectively. The interaction between them as a function of Ca^{2+} is crucial in the regulation of skeletal muscle contraction by Ca^{2+}. Previously we have measured the distance between Cys-98 of TnC and Cys-133 of TnI to be 34-39 Å (Tao et al. Biophys. J. 49, 142, 1986). Dobrovol'sky et al. (B.B.A. 789, 144, 1984) reported that the same cysteines can be crosslinked by 1,3-difluoro-4,6-dinitrobenzene. We now report that a variety of thio-reactive crosslinkers, including Nbs_2 can be used to crosslink the two proteins. The ternary troponin complex was treated with stoichiometric amount of bismaleimide derivatives whose maleimide groups are separated by a methylene group, phenyl group (pPDM), naphthalene group, or stilbene group (DMSDS). In all cases, substantial crosslinking between TnC and TnI was obtained when the reactions were carried out in the absence of Ca^{2+} (presence of EDTA). If either Cys-98 of TnC, or Cys-133 of TnI was alkylated, no crosslinking was obtained. Crosslinking was also obtained by incubating the ternary complex with Nbs_2 first in the presence of Ca^{2+} to prevent reaction with TnC, then in its absence to form the disulfide bond. As expected, this crosslink could be cleaved by DTT. The troponin complex containing the disulfide bond is no longer capable of regulating actomyosin ATPase activity in a Ca^{2+}-dependent manner. Our results suggest that the Cys-98 region of TnC and Cys-133 region of TnI possess considerable flexibility, so that crosslinkers varying in length from 0 to 18 Å can span the two sulfhydryls. (Supported by N.I.H. AR21673, and a postdoctoral fellowship from the AHA, Massachusetts Affiliate to H.-S. P.)

W-Pos310 A TnI-DERIVED PEPTIDE INHIBITS CALCIUM RESPONSIVENESS OF SKINNED SKELETAL AND CARDIAC MUSCLE FIBRES. J.C. Rüegg, C. Zeugner, J.E. van Eyk and R.S. Hodges II. Physiologisches Institut, Universität Heidelberg, Im Neuenheimer Feld 326, D-6900 Heidelberg and Department of Biochemistry, MRC Group in Protein Structure and Function, University of Alberta, Edmonton, Canada T6G 2H7

A peptide segment of only 12 amino acid residues of Troponin-I (TnI) is essential for the interaction of this troponin subunit with Troponin-C. We have investigated whether a synthetic peptide comprising these amino acid residues (TnI 104 to 115, cf. van Eyk J.E. and Hodges R.S.: Biophys. J. 51, 240, 1987) inhibits the calcium-induced contractile responses of chemically-skinned rabbit psoas muscle fibres or pig right ventricle fibres. Fibres were prepared by skinning with 1% Triton-X-100 in relaxing solution containing (in mM): imidazole 20, ATP 10, $MgCl_2$ 12.5, NaN_3 5, EGTA 5; pH 6.7 and then stored at -20°C for up to 5 weeks in a mixture of glycerol and relaxing solution. Fibres were then activated in a contraction solution containing (in mM): imidazole 20, ATP 10, $MgCl_2$ 12.5, NaN_3 5, CaEGTA 5, creatine phosphate 10, creatine kinase 380 U/ml; pH 6.7). 10-100 µM peptide inhibited the isometric contractile responses elicited by 2.5 µM Ca^{2+} dose dependently and reversibly, the IC_{50} being 20 µM. At 50 µM concentration, the peptide also caused a rightward shift of the calcium-force relationship in skinned skeletal fibres by 0.3 pCa units and by 0.1 pCa unit in skinned cardiac fibres. Maximal contractile force (elicited by 30 µM Ca^{2+}) was inhibited by 40%. These results are taken to mean that the TnI peptide inhibits the TnC-TnI interaction whereby causing a decrease in calcium sensitivity, but a more direct inhibitory effect on actin may also be considered.

W-Pos311 PW Brandt[+], D Roemer[+] and FH Schachat[#] IN THE ABSENCE OF CA^{2+}, ACTIVATION OF SKINNED RABBIT PSOAS FIBERS BY RIGOR CROSSBRIDGES IS COOPERATIVE AND THE INTERACTIONS EXTEND THE LENGTH OF THE THIN FILAMENT. [+]Columbia University, [#]Duke University.

Abstract: When fibers are activated by Ca^{2+} in physiological concentrations of MgATP, all the troponin-tropomyosin (Tn-Tm) units of a regulatory strand undergo a concerted transition from the "off" to the "on" position (Brandt et al, JMB, 1984, 1987). To see if the Tn-Tm units make a similar concerted transition in fibers activated by rigor crossbridges, we determine the slopes of the pS/tension relations (pS = -log[MgATP]) between pS 3 and that for maximum tension (about pS 5), in fibers from which TnC is extracted. The slope is quantified by fitting the data to a modified substrate inhibition equation with an n_S term analogous to the n_H of the Hill equation. In control fibers n_S is about 4.7; it progressively decreases with extraction of TnC, but maximum tension is unaffected. Incubation in TnC solutions restores n_S to control values. Extraction of about one TnC per strand reduces n_S just as it reduces the n_H of pCa/tension curves, thus rigor crossbridges also induce a concerted transition of all the Tn-Tm units of a regulatory strand. The 7 actins in TnC deficient units are turned "on" by rigor bridges and support tension crossbridges; this contrasts with their inactivity on Ca^{2+} activation. Our model holds that by either method of activation (rigor bridges or Ca^{2+}), the cooperative signal cannot cross TnC deficient units.

W-Pos312 A FLUORESCENT PROBE STUDY OF THE CYSTEINE ENVIRONMENTS IN MUSCLE TROPOMYOSINS. L.D. Burtnick and A. Racic, Department of Chemistry, University of British Columbia, Vancouver, British Columbia, Canada, V6T 1Y6.

A sulfhydryl-specific fluorescent reagent, 7-diethylamino-3-((4'-iodoacetylamino)phenyl)-4-methylcoumarin (DCIA), was used to label cysteine residues on tropomyosins from rabbit cardiac and rabbit skeletal muscles. The emission maximum at 486 nm, the high degree of fluorescence polarization and the limited accessibility of the bound DCIA to quenching by iodide suggest that the probe is bound in the hydrophobic cleft between polypeptide chains of the tropomyosin coiled coil, as well as being bound covalently at a cysteine residue. The labelled tropomyosins retain their abilities to bind F-actin and are able to interact with deoxyribonuclease I. They, however, show a reduced tendency to aggregate in a head-to-tail manner in low ionic strength solutions.

Supported by the British Columbia Heart Foundation and the Natural Sciences and Engineering Research Council of Canada.

W-Pos313 MYOCARDIAL AND SKELETAL MUSCLE CONTRACTION IN LOW SALT WITH CARDIAC-TnC: Sr^{2+}-ACTIVATION

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On cardiac muscle, we have previously shown (Babu et al, JBC, 262:5815,1987) that the substitution of skeletal troponin-C for the cardiac-TnC shifts the pSr-force relationship from the characteristic value of the heart muscle to that of fast-twitch skeletal fibers. These results were in contrast with repeated findings of others with substitution of CTnC for STnC in the skeletal fibers. Is it, then, that the regulatory sites on TnC dictate contraction sensitivity of cardiac muscle but not of skeletal muscle? However, we found that CTnC was unable even to restore full tension in skeletal fibers with either Ca^{2+} or Sr^{2+} activations in physiological salt concentration (180-200mM). In low salt, the effectiveness of CTnC was greatly restored, and we use low salt (100mM) here to investigate the effects on pSr-force relationship of skeletal fibers. Psoas fibers from hamsters were used. For cardiac muscle, trabeculae from the right ventricle were used. We show that (1) the pSr50 of psoas fibers, marking the position of the pSr-force relationship, is shifted to the left from 4.4 to 5.0 by low salt. Simultaneously the Hill coefficient n_H decreased from 4.4 to 2.2. With CTnC, there was a further shift in pSr50 to 5.7 and decrease in n_H to 0.9. (2) low salt also caused a shift in the pSr50 of cardiac muscle from 5.0 to 5.6 and in n_H from 3.0 to 1.3. We conclude that the TnC moiety dictates the contraction sensitivity in both skeletal and cardiac muscles. Further, the shifts in the Hill coefficient indicate that TnC also directly affects the cooperativity in the activation mechanism of the thin filament. (Supported in part by the Blakeslee Fund at MA)

W-Pos314 CALCIUM DISTRIBUTION WITHIN SARCOMERES OF SKINNED SKELETAL MUSCLE FIBERS. M. Cantino, T. Allen, and D. Johnson. (Intro. by A.M. Gordon) Center for Bioengineering and Dept of Physiology and Biophysics, University of Washington, Seattle, WA 98195

Studies by Bremel and Weber (Nature 238: 87, 1972) of calcium binding showed that rigor complexes enhance troponin's affinity for calcium in both myofibrillar and reconstituted preparations. More recent studies on skinned fibers support this conclusion, but they rely upon a fluorescent probe on troponin C to detect calcium binding. Such a probe is sensitive to cross-bridge formation even in the absence of calcium (Schultie et al., Biophys. J. 51: 25a, 1987); therefore, a direct measurement of calcium binding to troponin in regions of overlap and non-overlap of thick and thin filaments is needed. We are using the electron microprobe to determine the amounts of bound calcium present in A, I, and overlap regions in glycerinated rabbit psoas muscle. Fiber bundles are frozen rapidly in rigor solutions that contain either 10 μM (pCa 5) or 0.6 nM (pCa 9.2) free calcium. The total calcium in the solutions is kept below 40 μM and hence below the detection level of the technique. X-ray spectra from A, I, and overlap regions of selected sarcomeres are collected in sequence under identical conditions to minimize errors due to beam current drift. Calcium concentrations in the I band (in mmoles/kg dry wt) are found to be similar to those reported by Kitazawa et al. (J. Musc. Res. and Cell Motility 3: 437, 1982) under the conditions described above. Preliminary analysis suggests comparable calcium binding at pCa 5 in the overlap and the non-overlap thin filaments. Future studies will include similar analyses at intermediate levels of calcium. (Supported by NS 08384 and HL 31962.)

W-Pos315 MEASUREMENT BY NEUTRON SCATTERING OF THE DISTANCE BETWEEN TROPOMYOSIN STRANDS IN ACTIN-TROPOMYOSIN Bivin, D. B., Stone, D. B., Schneider, D.[1] and Mendelson, R., C.V.R.I. and Biochem./Biophys., Univ. of Calif. San Francisco, CA and [1]Brookhaven Nat. Lab., Upton, N.Y.

Previous work in this laboratory[1] has demonstrated that deuterated actin can be prepared from Dictyostelium discoideum (D. d.) and rendered "invisible" to neutrons by solvent contrast-matching[2]. We now report the first use of this actin in the study of the mechanism of control of vertebrate muscle contraction. The distance between the TM strands in the acto-TM complex is being measured using rabbit skeletal TM and deuterated D. d. actin.

We have developed an improved preparative procedure to more rapidly and economically produce deuterated actin. The interaction of this actin with TM and the TM-TN complex has been examined. Activation of rabbit S1 ATPase by deuterated actin complexed with TM-TN is modulated by Ca^{2+} in a manner similar to that observed with either regulated rabbit skeletal actin or regulated protonated D. d. actin. TM inhibits D. d. acto-S1 ATPase to the same degree as when rabbit actin is used. Preliminary neutron scattering experiments with 10-20 mg/ml actin and [TM]/[A] = 1/7 [5 mM imidazole, pH7, 80 mM KCl, 2 mM MgCl (TM 90% bound)] indicate that the TM strands lie at a distance of approximately 3.5 nm from the center of the F-actin helix. It appears that the eventual accuracy with which this parameter will be measured is of the order of 0.1 nm. Supported by NIH grants HL-16683 and HL-07192. [1]Stone, D. B., Curmi, P. M. and Mendelson, R. A. (1987) Meth. in Cell Biol. 28, 215-229. [2]Curmi, P. M., Stone, D. B., Schneider, D., Spudich, J. A. and Mendelson, R. A. (1985) Biophys. J. Abs. 47, 218a.

W-Pos316 ENERGY TRANSFER FROM ACTIN TO ACRYLODAN-TM MONITORS THE STATE OF TM ON THE THIN FILAMENT.
Y. Ishii and S.S. Lehrer, Department of Muscle Research, Boston Biomedical Research Institute, Boston, MA. 02114

The regulation of skeletal muscle contraction is thought to involve changes in the state of tropomyosin (Tm) on the thin filament from an inhibited to an active state. The binding profile of myosin subfragment 1 (S1) to the thin filament could be fit to a 2-state-cooperative model from which the Tm state could be inferred [Greene and Eisenberg (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 2616]. Recently we have directly monitored the state of Tm on thin filament using the monomer fluorescence of pyrene-maleimide labeled Tm [Ishii and Lehrer (1985) Biochemistry 24, 6631]. We now report measurements with acrylodan-Tm (ACTm). The fluorescence of ACTm did not change upon binding to F-actin when excited in the acrylodan absorption band. When excited in the tryptophan absorption band of F-actin there was a 15% increase in ACTm fluorescence and a few % decrease in tryptophan fluorescence of actin indicating energy transfer associated with the ACTm-actin complex. This tryptophan-sensitized AC fluorescence further increased by 15 % upon S1 binding to the complex, with a profile that followed the Tm-state change, saturating well below stoichiometric S1 binding to actin. This S1-induced increased energy transfer provides a clear indication of a change in the spatial relationship between Tm and actin associated with regulation. (Supported by NIH, NSF and MDA)

W-Pos317 A NOVEL TROPOMYOSIN-BINDING PROTEIN FROM THE HUMAN ERYTHROCYTE MEMBRANE. V.M. Fowler, Research Institute of Scripps Clinic, La Jolla, CA (Intr. by Elizabeth J. Luna).

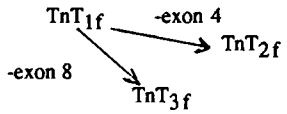
A new Mr 43,000 tropomyosin-binding protein (TMBP) has been identified in erythrocyte membranes by binding of ^{125}I-Bolton Hunter labelled-tropomyosin to nitrocellulose blots of membrane proteins separated by SDS-gel electrophoresis (Fowler, V.M. 1987. J. Biol. Chem. 262:12792-12800). This protein is not actin since ^{125}I-tropomyosin does not bind to purified actin on blots. Binding of ^{125}I-tropomyosin to this protein is specific since it is inhibited by excess unlabelled tropomyosin but not by F-actin or muscle troponins. This protein has been purified to 95% homogeneity from a 1 M NaBr extract of Triton-extracted membranes and is an asymmetric monomer (f/fo=1.5), as calculated from a Stokes radius of 3.9 nm and a sedimentation coefficient of 2.8 S. Binding of ^{125}I-tropomyosin to the purified protein saturates at one tropomyosin molecule to two Mr 43,000 TMBPs, with an affinity of about 5 x 10^{-7}M. The TMBP blocks tropomyosin binding to purified F-actin and also cosediments with an oligomeric complex of spectrin, protein 4.1, and actin isolated from a low salt extract of membranes by sedimentation on 5-20% sucrose gradients. The TMBP is present in the membrane skeleton in approximately the same number of copies as the number of short actin filaments per cell (30-40,000), and thus could function to regulate the association of tropomyosin with the erythrocyte actin and play a role in specifying the correct assembly of the spectrin-actin junctional complexes. This may be of general significance for plasma membrane structure, since immunoreactive TMBP polypeptides are present in a variety of non-erythroid cells and tissues, including skeletal muscle, brain, lens, liver, ileum brush border, and endothelial cells.

W-Pos318 ACTIN-TROPOMYOSIN CROSSLINKING WITH THE USE OF ACTIVE ESTERS. Z. Grabarek, T. Tao, Y. Mabuchi, B.-J. Gong, & J. Gergely, Dept. of Muscle Research, Boston Biomed. Research Institute; Depts. of Neurology, and Biol. Chem. and Mol. Pharmacol., Harvard Medical School, and Dept. of Neurology, Mass. General Hospital, Boston MA.

We have employed the two step zero-length crosslinking procedure (Grabarek and Gergely, this Meeting) to study tropomyosin-actin interaction in the thin filament. We have used actin fluorescently labeled at Cys-374 with 7-diethylamino-3-(4'-maleimidylphenyl)-4-methylcoumarin (CPM). Either actin or tropomyosin was activated with 1-ethyl-3-[3-(dimethylamino)propyl] -carbodiimide (EDC) in the presence of N-hydroxysuccinimide (NHS) followed by incubation with other components of the filament. In both sets of experiments SDS-polyacrylamide gels showed that, in addition to some higher molecular weight species, peptide bands corresponding to a 1:1 crosslinked actin-tropomyosin complex appeared. When crosslinking was carried out in the presence of troponin no Ca^{2+}-dependence of the actin-tropomyosin crosslinking was detected regardless whether actin or tropomyosin was activated. However myosin subfragment 1 (no nucleotide), virtually abolished the actin-tropomyosin crosslinking with or without troponin present. The electrophoretic bands were cut out and subjected to proteolysis with BNPS-skatole, CNBr and hydroxylamine (Sutoh, Biochemistry 21,3654,1982). Comparison of the fluorescent electrophoretic band patterns obtained from digestion of CPM.actin and from the CPM.actin- tropomyosin complex shows that in case of actin activation crosslinking involves the N-terminal 12-residue segment in actin while upon activation of tropomyosin the crosslinking site comprises the C-terminal segment of actin (residues 356-375). (Supported by grants from NIH: HL-05949, HL-07266, AR-21673, and MDA)

W-Pos319 ALTERNATIVE SPLICING AND THE RELATIONSHIP AMONG FUNCTIONALLY DIFFERENT FAST TNT ISOFORMS IN RABBIT SKELETAL MUSCLE. Margaret M. Briggs and Frederick H. Schachat. Dept. of Anatomy, Duke University Medical Center, Durham, NC 27710. (Intr. by K. A. Taylor).

N-terminal variants of fast TnT result from alternative splicing of 5 short 5' exons of the fast TnT gene (Breitbart et al.(1985) Cell 41, 67). In rabbit skeletal muscle there are three major fast TnT isoforms, designated TnT_{1f}, TnT_{2f}, and TnT_{3f} by their mobility on 2D gels. To determine the relationship among these N-terminal variants, we have combined amino acid sequencing with peptide mapping and immunological approaches. Comparison of the amino acid sequence of TnT_{2f} (Pearlstone et al.(1977) J.Biol.Chem, 252, 983) with that predicted from the rat gene shows that TnT_{2f} lacks the sequence corresponding to exon 4. We find that TnT_{1f} is identical to TnT_{2f} except that it contains the "missing" sequence. To characterize TnT_{3f} we have defined the epitope recognized by a monoclonal antibody that reacts with TnT_{1f} and TnT_{2f}, but not TnT_{3f}. Synthetic peptides were used to localize the epitope to an amino acid sequence encoded by exon 8. Peptide mapping and partial sequence analysis indicated that this is the only region missing in TnT_{3f}. These observations indicate that each of the three major fast TnT isoforms has a unique sequence, and that TnT_{2f} andTnT_{3f} are each related to TnT_{1f} by single alternative splicing events.



W-Pos320 DISTRIBUTION OF FURA-2 IN SMOOTH MUSCLE CELLS, Moore, E.D.W., and Fay, F.S. Dept. of Physiology, U.Mass. Med. Ctr., Worcester, MA 01655.

We have undertaken a study of the distribution of the Ca^{2+}-sensitive fluorescent dye fura-2 in smooth muscle cells. The cells were prepared daily by enzymatic dissociation of the stomach of the toad Bufo marinus. Cells were incubated at 30°C with the cell-permeant acetoxymethylester form of the dye for periods of up to 6 hours. At intervals, aliquots were removed and their fluorescence recorded on a SPEX fluorolog spectrophotometer. We also dialysed aliquots of the cells to remove unloaded dye from the preparation, or to remove dye that the cells had expelled, and compared the results to undialysed samples. We report that smooth muscle cells extrude fura-2 free acid. To assess the intracellular distribution of the remaining fura-2 we co-loaded smooth muscle cells with rhodamine 123, which stains the mitochondria, and fura-2/am, or alternatively, we co-loaded cells with $DiOC_6(3)$, a dye developed by Terasaki et al which stains the sarcoplasmic reticulum, and fura-2/am. Single cells were examined with epifluorescent illumination using a digital imaging microscope to record the cellular fluorescence to a resolution of 0.2 um. Rhodamine 123 stained the mitochondria, the nucleus and the plasma membrane. $DiOC_6(3)$ also stained the nucleus and the plasma membrane as well as the sarcoplasmic reticulum. When cells were loaded with fura-2/am so that the intracellular concentration of free acid was approximatly 200 uM, there was no distribution of fura-2 into regions high in Ca^{2+}, either the mitochondria or the S.R. Fura-2 loaded heavily into the nucleus, as could be seen in either the 340 nm or the 380 nm images, but calculation of the Ca^{2+} concentration from 340/380 ratios indicated that the nuclear Ca^{2+} concentration was sometimes higher than, sometimes lower than, and occasionally no different from the cytoplasmic Ca^{2+} concentration. Supported in part by grants from AHA and NIH (HL14523).

W-Pos321 MEASUREMENT OF CYTOSOLIC FREE CA^{2+} IN RAT UTERINE SMOOTH MUSCLE WITH AEQUORIN. Janet L. Smart, D. George Stephenson* and Fred J. Julian, Dept. of Anaesthesia Research Laboratories, Brigham & Women's Hospital, Boston, MA 02115 and *Dept. of Zoology, La Trobe University, Bundoora, Victoria 3085, Australia.

We examined changes in intracellular Ca^{2+} levels in longitudinal smooth muscle strips stimulated electrically, with oxytocin and when preparations were spontaneously active. Aequorin was incorporated into smooth muscle strips using the EGTA-loading technique (Sutherland et al., 1980 and Morgan and Morgan, 1982). The characteristics of the Ca^{2+}-dependent signals differed with the type of stimulus employed. For example, when the electrical field was high (43 V/cm) the ionized Ca^{2+} level at the peak light response was estimated to reach about 7 μM and approximately 3-5 μM when the electrical field was lower (26 V/cm). The relatively steady Ca^{2+} levels during the continuing force response were estimated to be around 1-2 μM. Spontaneous contractions showed a gradual increase in light signal preceeding force development. Maximum ionized Ca^{2+} levels during this phase ranged from 1-3 μM. Oxytocin induced a substantial but gradual rise in aequorin light emission closely following force generation and remaining high while force was maintained. These results indicate that Ca^{2+} levels achieved in rat uterine smooth muscle during contraction depend upon the type of stimulation and are of similar magnitude to those observed in other types of smooth muscle. Supported by NIH grant HL35032(FJJ) and NRSA HD07008 (JLS).

W-Pos322 EVIDENCE FOR PURINERGIC NEUROTRANSMISSION IN THE RAT INTRAPULMONARY ARTERY. T.INOUE AND M.S.KANNAN*, DEPARTMENT OF VETERINARY BIOLOGY, COLLEGE OF VETERINARY MEDICINE, UNIVERSITY OF MINNESOTA, ST. PAUL, MN 55108.

The electrical responses of the smooth muscle cells (SMCs) of rat intrapulmonary artery (PA) to electrical stimulation (ES) of the perivascular nerves were characterized using the intracellular microelectrode technique. The resting membrane potential of the SMCs was -51 mV. ES of the perivascular nerves produced excitatory junction potentials (e.j.ps.) of 1 to 8 mV in amplitude, with a duration of approximately 5 sec. The e.j.ps. were abolished by tetrodotoxin (TTX, 5 uM), but not by guanethidine, α - and β-adrenoceptor blocking agents, or atropine. In vessels obtained from reserpine-treated rats or in those incubated in vitro with 6-hydroxydopamine to produce functional sympathectomy, ES resulted in TTX-sensitive e.j.ps. The e.j.ps. were attenuated by low concentrations of α,β-methylene ATP (0.1 uM). α,β-methylene ATP selectively inhibited the ATP-induced depolarization of the SMCs, but not those induced by norepinephrine or other agonists such as 5-hydroxytryptamine. The results suggest that in the rat PA, the e.j.ps. result from the release of non-adrenergic, non-cholinergic neurotransmitter from the perivascular nerves that are not sympathetic. The most likely transmitter substance is ATP released from non-sympathetic nerve-endings.

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W-Pos323 RYANODINE DISRUPTS EXCITATION-CONTRACTION COUPLING IN TRACHEAL SMOOTH MUSCLE. W.T. Gerthoffer, K.A. Murphey and M.A. Khoyi. Department of Pharmacology, University Nevada School of Medicine, Reno, NV 89557.
Muscarinic activation of airway smooth muscle in low calcium solutions stimulates myosin phosphorylation without increasing tension (Gerthoffer, J. Pharmacol. Exp. Ther. 240:8-15, 1987). Blocking calcium influx reduced phosphorylation, but not to basal levels. It was proposed that release of intracellular calcium contributed to dissociation of phosphorylation and contraction. To test this hypothesis the effects of ryanodine were studied under similar conditions. Ryanodine inhibits calcium release from striated muscle sarcoplasmic reticulum and may also increase calcium leak from the SR depending on the experimental conditions. We tested the effects on calcium release from smooth muscle SR by stimulating tracheal muscle strips with caffeine (1 - 60 mM). Ryanodine (10^{-7} to 10^{-5} M) antagonized caffeine contraction of both canine and bovine tracheal smooth muscle in a dose-dependent fashion. Ryanodine also reduced the maximum response to carbachol with no effect on the EC50. Carbachol (10^{-6} M) and ryanodine (10^{-5} M) both significantly reduced $^{45}Ca^{2+}$ content of tracheal muscle. The effect of carbachol plus ryanodine on $^{45}Ca^{2+}$ content was not different than either drug alone, suggesting that ryanodine reduces caffeine and carbachol responses by depleting releaseable Ca^{2+} stores. Ryanodine significantly reduced calcium induced contractions and myosin phosphorylation in carbachol stimulated muscle. Therefore, some of the calcium responsible for elevated phosphorylation is released from the sarcoplasmic reticulum. (Supported by NIH grant HL 35805).

W-Pos324 EFFECT OF RELAXATION BY ISOPROTERENOL AND FORSKOLIN ON INTRACELLULAR CALCIUM IN AEQUORIN-LOADED CANINE TRACHEAL SMOOTH MUSCLE. Susan J. Gunst and Somnath Bandyopadhyay, Dept. of Physiology, Mayo Foundation, Rochester, MN 55905.

Muscle strips were suspended vertically in a tissue bath between a small clamp and a force transducer. They were loaded with the bioluminescent, cytoplasmic free Ca^{++} indicator aequorin using a modification of the method of Morgan and Morgan (J. Physiol. 357:539, 1984) and positioned over a photomultiplier tube so that the light emitted by aequorin could be measured. Muscles were contracted with acetylcholine (10^{-6} or 10^{-5} M), carbachol (10^{-7} or 10^{-6} M) or 5-hydroxytryptamine (10^{-5} M) and then relaxed with 10^{-5} M isoproterenol (ISO) or 10^{-5} M forskolin. Relaxation ranged from 50-90% of initial force. It was accompanied by a rapid decline in light indicating a fall in cytoplasmic Ca^{++}. When muscles were contracted with 30 or 60 mM K^+, ISO also induced relaxation; however, no decline in light occurred. The effect of Na^+-K^+ ATPase activity on intracellular Ca^{++} was investigated by first incubating aequorin-loaded muscles in K^+-free medium to inhibit the Na^+-K^+ ATPase. Muscles were then contracted with acetylcholine or 5-hydroxytryptamine and Na^+-K^+ ATPase activity was stimulated by the addition of 10 mM K^+. 10 mM K^+ caused relaxation (80-100% of initial force) and a simultaneous rapid fall in light. Results suggest that relaxation caused by ISO, forskolin or activation of Na^+-K^+ ATPase is accompanied by a fall in cytoplasmic free Ca^{++} in canine trachealis muscle. Supported by USPHS grant HL29289.

W-Pos325 LANTHANUM POTENTIATES THE INCREASE IN CYTOSOLIC FREE CALCIUM EVOKED BY ANGIOTENSIN IN CULTURED ARTERIAL MUSCLE CELLS. Lucinda Smith and Jeffrey Bingham Smith. Dept. Pharmacology, University of Alabama at Birmingham, Birmingham, AL 35294.

Smooth muscle cells from rat aorta were grown on cover glasses and loaded with fura-2 in order to monitor cytosolic free Ca with a dual wavelength fluorometer. Angiotensin II (ANG) rapidly increased cytosolic free Ca from 105 $\pm$ 9 to 510 $\pm$ 44 nM (n=24). The rise time (baseline to peak) was 8.6 $\pm$ 0.3 sec. Immediately after reaching the peak, free Ca fell (almost as rapidly as it rose) to a plateau value of 163 $\pm$ 9 nM, which was maintained for several min. The decrease in free Ca correlates temporally with a striking increase in 45Ca efflux and 39.6 $\pm$ 2.3 % decrease in total cell Ca (28 experiments, 3 or more replicates each). Lanthanum had no significant effect on basal free Ca, but strikingly potentiated the effects of ANG on both the peak and plateau increases in free Ca. In the presence of 1 mM La free Ca was 1375 $\pm$ 327 nM at the peak and 528 $\pm$ 66 nM (n=7) during the plateau phase of the ANG response. La had no significant effects on the basal free Ca or the increases in inositol bis- or tris- phosphate evoked by a 15 sec incubation with ANG. La strongly inhibited both the stimulation of rapid 45Ca efflux by ANG and the decrease in total cell Ca evoked by ANG. The present findings indicate that La potentiates ANG-induced increases in free Ca by blocking Ca exodus from the cells. Increased Na^+/Ca^{2+} antiport, which is abundant in these cells (JBC, 262:11988-11994), probably causes the rapid efflux of Ca since replacing extracellular Na with other monovalent cations has effects on Ca regulation which are similar to those of La. (Supported by Grants HL01671 and DK39258 from the National Institutes of Health.)

W-Pos326 SOME FACTORS INFLUENCING INOSITOL-TRISPHOSPHATE-INDUCED CA^{2+} RELEASE FROM SARCOPLASMIC RETICULUM ISOLATED FROM ARTERIAL SMOOTH MUSCLE.

George D. Ford, Department of Physiology, Medical College of Virginia, Richmond, Virginia.

Inositol-trisphosphate (IP_3) is believed to be the messenger linking α_1-adrenergic receptor stimulation with the release of Ca^{2+} stored within the sarcoplasmic reticulum of arterial smooth muscle. IP_3-induced Ca^{2+}-release from a fragmented sarcoplasmic reticulum prepared from bovine aorta was monitored using the Ca^{2+}-sensitive dye, antipyrylazo III and dual wave-length spectroscopy. The IP_3-induced Ca^{2+} release is dependent on the Mg^{2+} present in the bathing medium, particularly at submaximum doses of IP_3. Another factor is how long the FSR is allowed to take up calcium, i.e. "to load", before IP_3 is added. Generally, the longer loading is allowed to proceed before IP_3 is added, the greater the Ca^{2+}-release induced, although the fraction of IP_3 releasable Ca^{2+} as a function of total calcium loaded remains fairly constant. On the other hand, ryanodine added before the FSR loads with calcium, potentiates the amount of IP_3-induced Ca^{2+} release when IP_3 is added shortly (2 to 6 minutes) after the loading has begun. The effect of ryanodine is to increase the fraction of the total calcium load in the FSR that is sensitive to IP_3-induced release.

W-Pos327 HISTAMINE-INDUCED MOBILIZATION OF MYOPLASMIC $[Ca^{2+}]$ FROM EXTRACELLULAR AND INTRACELLULAR STORES IN VASCULAR SMOOTH MUSCLE.

CM Rembold and RA Murphy, Cardiology Div., Depts. of Int. Med. and Physiology, Univ. of VA Sch. of Med., Charlottesville, VA 22901 USA.

Myoplasmic $[Ca^{2+}]$ (as estimated by aequorin), myosin phosphorylation (MP), and isometric stress were measured in histamine stimulated swine carotid media. High dose histamine stimulation (10-100 μM) produced transient elevations in $[Ca^{2+}]$ and MP with rapid generation of near maximal stress. Low dose histamine (1 μM) produced monotonic rises in $[Ca^{2+}]$ and MP with slower stress development and lower peak stress. Histamine (100 μM) still produced a $[Ca^{2+}]$-transient in the absence of extracellular $[Ca^{2+}]$, suggesting that the $[Ca^{2+}]$-transient was derived from intracellular stores. Reintroducing Ca^{2+} to this partially Ca^{2+}-depleted preparation was associated with an initial large rise in $[Ca^{2+}]$ that was not associated with rapid stress development. We hypothesize that this represents increased $[Ca^{2+}]$ in a region associated with refilling of the intracellular $[Ca^{2+}]$ stores. After a 90 sec delay, stress developed slowly over the next 4 min and $[Ca^{2+}]$ estimates over this period were comparable to control tissues that were not $[Ca^{2+}]$ depleted. $[Ca^{2+}]$-transients were less apparent during a cumulative dose response protocol suggesting that lower doses of agonists partially released the intracellular $[Ca^{2+}]$ store. Increasing histamine beyond the concentrations inducing near maximal stress did not increase the $[Ca^{2+}]$ measured after 10 min of stimulation. Regulatory systems appear to prevent steady-state $[Ca^{2+}]$ and MP from rising to levels higher than necessary to maintain peak stress.

Supported by the Markey Charitable Trust 86-025, 1RO1-HL38918-01, and 5PO1-HL19242.

W-Pos328 DEMONSTRATION OF MULTIPLE TYPES OF CALCIUM CHANNELS IN SMOOTH MUSCLE CELLS OF THE GUINEA-PIG TAENIA COLI.

Y. Yamamoto, S. L. Hu, and C. Y. Kao, Department of Pharmacology, State University of New York Downstate Medical Center, Brooklyn, NY 11203.

Whole-cell currents recorded by the tight-seal voltage-clamp method from freshly dispersed smooth myocytes of the guinea-pig taenia coli bathed in 3 mM Ca^{2+} have shown that following a rapid activation the calcium channel inactivates with three exponential terms (Yamamoto et al., Biophysical J. 51:200A, 1987), suggesting the presence of multiple forms of Ca^{2+}-channels. To test such a possibility, Ca^{2+}-channel currents were increased by use of Ba^{2+} as the charge-carrier, and single-channel events in cell-attached patches were studied. In the whole-cell mode, replacement of 3 mM Ca^{2+} by Ba^{2+} did not affect the activation rate (2 ms to peak) but slowed the inactivation time-constants at +10 mV from 7.5, 71, and 364 ms in Ca^{2+} to 48, 275, and 1001 ms in Ba^{2+}. In cell-attached patches, at least two types of single channels were observed: one (large) had a unit conductance of 20 pS in 80 mM Ba^{2+} and 9 pS in 80 mM Ca^{2+}; the other (small), a unit conductance of 5 pS in 80 mM Ba^{2+}. These types further differ in the following properties: the large channels inactivated at V > -20 mV, and were sensitive to dihydropyridine agents, whereas the small channels did not inactivate and were insensitive to dihydropyridines. The large channels appear comparable to the L-type channel in cardiac myocytes and neurones, and the small channels appear to differ from the T-type channels. In macroscopic currents of the whole cell, the large channels predominate because of its large conductance and high opening probability, but the small channel may contribute at low membrane potentials.

Supported by NIH grant R01HD00378.

W-Pos329 CALCIUM BUFFERING CAPACITY, CALCIUM CURRENTS, AND $[Ca^{2+}]$ CHANGES IN VOLTAGE CLAMPED, FURA-2 LOADED SINGLE SMOOTH MUSCLE CELLS. Peter L. Becker, John V. Walsh, Joshua J. Singer, & Fredric S. Fay. Dept. of Physiology, U. Mass. Med. School, Worcester, MA, 01655.

Depolarization of smooth muscle leads to an inward calcium current (I_{Ca}) and to contraction. The present experiments were undertaken to investigate the effect of I_{Ca} on $[Ca^{2+}]$, focusing on the characteristics of intrinsic intracellular calcium buffers and the contributions of I_{Ca} to the changes in $[Ca^{2+}]$. $[Ca^{2+}]_i$ was monitored during voltage clamped pulses to 0 mV in single gastric smooth muscle cells from Bufo marinus. Cells were loaded with fura-2 and placed in a HEPES-buffered salt solution with 20 mM Ca^{2+} and the ratio of fluorescence at two wavelengths was measured with a high-time resolution microfluorimeter. Cells were impaled with a conventional microelectrode (20 - 60 Mohm) filled with 3 M CsCl and voltage clamped through an active bridge circuit. Voltage-clamp-induced I_{Ca} transients rapidly change the $[Ca^{2+}]_i$ and repetitive clamp pulses can elevate the $[Ca^{2+}]_i$ for prolonged periods. The rate of decline in $[Ca^{2+}]_i$ after brief or prolonged elevation was the same, indicating that Ca^{2+} buffers equilibrate at least as rapidly as fura-2. By analysing the rate of decline of $[Ca^{2+}]$ after elevation in cells loaded with different [fura-2], we calculate that the intrinsic Ca^{2+} buffering power is 5 to 10 times that of the fura-2 in our normally (~125 uM) loaded cells. Several observations suggest that I_{Ca} alone cannot account for the changes in $[Ca^{2+}]$. 1) The amount of Ca^{2+} entry calculated by integrating the I_{Ca} transient appears to be much less than that necessary to account for the observed change in the $[Ca^{2+}]$ based on even a low estimate of the Ca^{2+} buffering power. 2) Under certain conditions, we have been able to generate I_{Ca} transients that are identical and yet the $[Ca^{2+}]$ changes were different from one another. Support NIH: HL-14523, AM07807, DK31620. NSF DCB8511674. And MDA.

W-Pos330 STUDIES OF INTRACELLULAR $[Ca^{2+}]$ CHANGES DURING ACTION POTENTIALS AND VOLTAGE CLAMP PULSES IN FURA-2 LOADED SINGLE SMOOTH MUSCLE CELLS. Peter L. Becker, Fredric S. Fay, Joshua J. Singer & John V. Walsh. Dept. of Physiology, U. Mass. Med. School, Worcester, MA, 01655.

Intracellular $[Ca^{2+}]$ under both current clamp and voltage clamp was studied in single smooth muscle cells isolated from the stomach of the toad Bufo marinus. Cells were loaded with fura-2 and placed in a HEPES-buffered salt solution containing 20 mM Ca^{2+} and, for studies on action potentials, 10 mM TEA. The ratio of fluorescence at two wavelengths was measured with a high time resolution spectrofluorimeter. Cells were impaled with a single, conventional microelectrode (20 - 60 Mohm) filled with 3 M KCl or, in the case of voltage-clamp studies of Ca^{2+} current, 3 M CsCl. Voltage clamp was achieved with an active bridge circuit. The use of conventional microelectrodes, as opposed to patch pipettes, served to minimize disturbance of the cytosolic contents, thus facilitating study of the cell's Ca^{2+} handling mechanisms.

During an action potential (AP), whose duration was typically on the order of 50 - 100 msec, the rise in $[Ca^{2+}]$ began during the upstroke, ceased during the downstroke and was as much as several hundred nM. An elevation in $[Ca^{2+}]$ similar to that elicited by an AP was observed under voltage-clamp during command pulses of 300 msec duration to 0 mV from a holding potential of -100 mV. Multiple AP's or command pulses closely spaced in time led to a progressive increase in $[Ca^{2+}]$ up to ceiling of about 1.0 uM. The rate of decline in the $[Ca^{2+}]$, which was independent of the $[Ca^{2+}]$ at the onset of decay, was much slower than the rate of rise and was well described by a single exponential with a time constant on the order of 5 sec. Ca^{2+} currents of the same magnitude were obtained in the same cell at significantly different $[Ca^{2+}]$'s (150-500 nM) which were set by preceding Ca^{2+} currents.

Supported by NIH: HL-14523, AM07807, DK31620 and NSF DCB8511674 and MDA.

W-Pos331 SARCOLEMMAL CALCIUM CONTRIBUTION TO DEPOLARIZATION-INDUCED CONTRACTION OF CANINE CORONARY ARTERY: AN EM AUTORADIOGRAPHIC STUDY. Wheeler-Clark, E.S., G.B. Weiss and L.M. Buja (Dept. of Pathology, Univ. of Texas Health Science Center at Dallas, TX 75235)

Functional responses and subcellular calcium distribution were compared in canine coronary (proximal circumflex and L.A.D.) arteries. Coronary artery rings developed > 1 g isometric tension in response to solutions containing 80 mM K^+ (substituted for Na) and 0.75 mM Ca; these contractions were inhibited virtually 100% by 60 min preincubation with 0.3 μM nitrendipine. Subcellular Ca distribution was examined in similar coronary artery strips using ^{45}Ca electron microscopic (EM) autoradiography procedures described previously (Wheeler-Clark et al., J. Pharmacol. Exp. Ther. 239:286, 1986). Artery strips were loaded with ^{45}Ca in the presence or absence of nitrendipine (65 min) and/or high K^+ (final 5 min) before freezing. In control and nitrendipine (+/- high K^+) muscles, relative ^{45}Ca activities were ca. 7-fold higher for sarcolemma (SL) and 4-fold higher for the sarcoplasmic reticulum (SR) than for cytosol (CSL) or extracellular space (ECS). In comparison, the ^{45}Ca activity of the SL was reduced 75% ($P < .05$) in K^+-contracted muscles. We calculate that translocation of SL Ca could increase CSL Ca as much as 15%. We did observe a 15% increase in CSL Ca - similar to that measured by electron probe in contracted smooth muscle (Kowarski et al., J. Physiol. (Lond.) 366:153, 1985); however, this and smaller Ca changes in the SR or ECS were not significant by our standards. We suggest that the Ca required for high K^+-induced contraction of coronary arteries originates from extracellular and SL sources rather than from the SR. Also, nitrendipine prevents both the influx of extracellular Ca and the translocation of SL-associated Ca. This SL Ca is probably bound rather than diffuse double layer Ca. (Supported by P50-HL17669 & HL-31152).

W-Pos332 SINGLE POTASSIUM CHANNELS RECORDED FROM VASCULAR SMOOTH MUSCLE CELLS. E.L. Stuenkel (Intr. by J. Lemos), Dept. of Physiology, University of California, San Francisco, CA.

Isolated vasculature fragments are easily obtained by collangenase digestion of rat pancreas, which removes glandular cells. Electron micrographs show the fragments to consist of a single layer of smooth muscle cells enveloping the endothelial cells lining the vasculature lumen. Contractions are elicited in response to stretch of the vascular fragment. Three types of K selective channels have been characterized from the smooth muscle cells. Type one is a Ca and voltage sensitive K channel of large unitary conductance, (~200 pS with symmetrical 145 mM K^+). In the i-o configuration this channel is activated at $[Ca^{++}]_i$ >50 nM and with increasing positive Vm. Ionic selectivity shows failure of the channel to conduct the cations Na^+, Cs^+ or Ba^{++} or anions Cl^- and SO_4. Application of Na^+_i results in a reduced single channel conductance while Cs^+_i produces rapid channel fluctuations. A second type of K selective channel was observed that exhibits a slope conductance of ~100 pS in symmetrical 145 mM K^+. In the c-a configuration (145 mM K^+ pipette) a reversal potential of -50 to -60 mV is observed. Application of salt gradients (K^+, Na^+, Cs^+, Cl^-) show this channel to be nearly perfectly K selective. This channel is sensitive to TEA blockade. The third type of K channel exhibits a slope conductance in symmetrical 145 mM K^+ of 25 pS. This channel is also observed in c-a patches; ionic selectivity shows it to be highly selective for K and insensitive to TEA blockade. This channel exhibits voltage sensitivity. The latter two channel types occur much less frequently than the maxi $I_{K(Ca)}$ channel (i.e. type one).

W-Pos333 THE EFFECT OF RGW-2938, A POSITIVE INOTROPIC/VASODILATOR, ON CANINE TRACHEAL SMOOTH MUSCLE CONTRACTED WITH VARIOUS AGONISTS. Linda Merkel, Luz M. Rivera and Mark H. Perrone Rorer Central Research, Horsham, PA 19044. (Intr. by Theodore J. Torphy)

Isolated canine trachealis strips were challenged with four different agonists and the effect of RGW-2938 (3,4-dihydro-3-methyl-6-(1,4,5,6-tetrahydro-6-oxo-3-pyridazinyl)-2[1H]-quinazolinyl) on muscle relaxation was examined. Contractions elicited with equieffective concentrations of methacholine (0.3 μM), serotonin (3 μM) and histamine (6 μM) were almost completely reversed by RGW-2938 with an IC_{50} of 10.9±5;1.5±0.5 and 0.1±0.03 μM (N=5) respectively. On the other hand, trachealis strips contracted with an equieffective concentration of KCl (48 mM) relaxed only 10 - 20% with 0.1 μM RGW-2938 but 100% in the presence of nifedipine, a calcium channel antagonist. A pretreatment with 0.1 μM RGW-2938, however, did not have any inhibitory effect on either rate of contraction or maximal tension development in the presence of 0.3 μM methacholine. We also examined the effect of this compound on calcium release from intracellular stores. Trachealis strips were exposed to 0.3 μM methacholine, then extracellular calcium was depleted by incubation in an EGTA containing Krebs buffer for 10 min. A second challenge with methacholine yielded a transient contraction that was 41.5±4%(N=6) of the initial contraction. The presence of RGW-2938 in the EGTA Krebs buffer reduced this transient contraction significantly to 14.5±1% in a dose-dependent fashion. One possible explanation for these data is that RGW-2938 relaxes airway smooth muscle by exerting an inhibitory effect on calcium release from intracellular stores.

W-Pos334 CAGED PHENYLEPHRINE: SYNTHESIS AND PHOTOCHEMICAL PROPERTIES. Jeffery W. Walker and David R. Trentham, National Institute for Medical Research, London, NW7 1AA, U.K.

Caged phenylephrine is a photosensitive precursor of the α_1-agonist phenylephrine that is being used in studies of pharmacomechanical coupling in smooth muscle. Two diastereoisomers were formed by alkylation of the phenolic oxygen of phenylephrine hydrochloride with (2-nitrophenyl)-diazoethane. The diastereoisomers were separated on a preparative C_{18} HPLC column eluted isocratically with 3 mM P_i at pH 4.5-methanol (4:1 v/v). The more retarded isomer was used in physiological studies due to its minimal biological activity as a receptor agonist or antagonist.

The photochemical properties of caged phenylephrine were evaluated by comparison with those of caged ATP (P^3-1(2-nitrophenyl)ethyl ATP) whose photolysis mechanism was re-evaluated by monitoring the formation of the nitroso absorption band (ε = 50 $M^{-1}cm^{-1}$ at 740 nm) in addition to the release of H^+, ATP and the decay of an aci-nitro intermediate, I. The photolysis mechanism was resolvable into two rather than three steps:

$$\text{Caged ATP} \xrightarrow{>10^5\ s^{-1}} \text{I} + H^+ \xrightarrow{118\ s^{-1}} \text{2-nitrosoacetophenone} + \text{ATP}$$

at 21°C under physiological ionic strength, Mg^{2+} and pH. An oxonium ion intermediate (McCray and Trentham, Biophys. J. 51, 447a, 1986) previously proposed on the basis of a first order kinetic process is due to a further reaction of a thiol-nitrosoketone adduct. Caged phenylephrine had a quantum yield of 0.4. The photolysis kinetics were monitored spectroscopically at 380 nm and 740 nm following a laser flash at 320 nm and suggest that phenylephrine is released at 3 s^{-1} at neutral pH and 21°C. [Supported by NIH Grant HL 15835 and MRC, U.K.]

W-Pos335 $[Ca^{2+}]_i$, $[Ca^{2+}]_o$ AND FORCE IN PHASIC AND TONIC SMOOTH MUSCLE. B. Himpens, G. Matthijs and A.P. Somlyo, University of Penna School of Medicine, Philadelphia, PA.

The purpose of this study was to determine whether the greater contractile response of (depolarized) tonic than phasic smooth muscle to $[Ca^{2+}]_o$ (1) is due to differences in Ca^{2+}-metabolism or in the Ca^{2+}-sensitivity of the regulatory/contractile proteins. Force (expressed as % of response to 140mM K^+, 1mM Ca^{2+} depolarization) and $[Ca^{2+}]_i$, at various $[Ca^{2+}]_o$ (in 140mM K^+), were measured simultaneously in fura-2 loaded strips of depolarized tonic (rabbit pulmonary artery) (PA) and phasic (guinea pig ileum) (IL) smooth muscle.

In tonic smooth muscle the $[Ca^{2+}]_i$ ED_{50} for force was 140-160nM, reached at $[Ca^{2+}]_o$ = 100μM, with $[Ca^{2+}]_o$ applied either cumulatively or non-cumulatively. In phasic smooth muscle, the non-cumulative ED_{50} was 170nM, reached at $[Ca^{2+}]_o$ = 650μM. Force was even more suppressed in IL during cumulative increases of $[Ca^{2+}]_o$, and was below 50% at $[Ca^{2+}]_i$ = 220nM, reached at 1mM $[Ca^{2+}]_o$. This was not due to osmotic swelling, because increasing the osmolarity (with sucrose) further depressed force, while carbachol induced a transient peak in force and $[Ca^{2+}]_i$, before reducing the signals to a lower steady state level. We conclude: 1) $[Ca^{2+}]_i$ rises more with $[Ca^{2+}]_o$ in depolarized PA than in IL, during non-cumulative increases in $[Ca^{2+}]_o$, probably due to greater Ca^{2+} influx through voltage operated channels, and 2) the amount of force developed at a given $[Ca^{2+}]_i$ is lower in the phasic IL than in the tonic PA smooth muscle.

(1) Somlyo & Somlyo, Fed. Proc. 28:634, 1969)

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W-Pos336 EFFECTS OF C KINASE ACTIVATION ON CALCIUM-FORCE RELATIONSHIPS IN FERRET AORTA. A.L. Ruzycky and K.G. Morgan. Dept. of Med., Harvard Medical School, Boston, MA 02215

We have previously reported that, in ferret aorta, phorbol esters induce sustained contractions with no detectable increases in $[Ca^{2+}]_i$. In the present study, we used the Ca^{2+} indicator aequorin, chemically loaded into aortic strips, to further investigate the effect of C kinase activation on Ca^{2+}-force relationships. The relatively specific C_2kinase inhibitor H-7 (30 uM) caused a 20% decrease in intrinsic myogenic tone (0.1 ± 0.01 $N/m^2 \times 10^5$; n=15) in the absence of any statistically significant decrease in $[Ca^{2+}]_i$ (250 ± 20 nM compared to control of 240 ± 10 nM) in spite of the fact that further detectable decreases in $[Ca^{2+}]_i$ were observed on addition of EGTA in the absence of further decreases in active tone. When $[Ca^{2+}]_i$ was increased by maximal contractile concentrations of K^+ (to 420 ± 30 nM), the subsequent addition of 12-deoxyphorbol-13-isobutyrate (DPBA), caused a statistically significant increase in force (23 ± 2%; n=17) with no significant increase in $[Ca^{2+}]_i$ (420 ± 30 nM). Similarly, in tissues maximally contracted with DPBA (10^{-5} M), subsequent addition of K^+ (81 mM) produced a further increase in force (30 ± 4%, n=17) along with a simultaneous sustained increase in $[Ca^{2+}]_i$ to 420 ± 20 nM. Furthermore, DPBA caused a significant shift to the left and an increase in the maximum of the control (K^+ generated) Ca^{2+}-force relationship. Thus, C kinase stimulation generates additional force by recruiting a second force producing system after the first system (utilized by K^+) is saturated by $[Ca^{2+}]_i$ as well as by causing an apparent decrease in the minimal Ca^{2+} requirement for contraction. These results support the idea that C kinase activation may be involved in the maintenance of intrinsic tone in vascular smooth muscle. Support: NIH HL 31704, an AHA EI and a postdoctoral fellowship from the Mass. Heart Assoc.

W-Pos337 MYOSIN PHOSPHORYLATION AND INTRACELLULAR CALCIUM DURING ISOMETRIC CONTRACTIONS OF ARTERIAL SMOOTH MUSCLE. Meei Jyh Jiang and Kathleen G. Morgan. Harvard Medical School, Beth Israel Hospital, Boston, MA 02215.

We investigated the time course of myosin phosphorylation and $[Ca^{++}]_i$ during isometric contractions of ferret aortic strips stimulated by three agonists: K^+ (21 mM), phenylephrine (10^{-5}M) and a tumor-promoting phorbol ester, 12-deoxyphorbol 13-isobutyrate 20-acetate (DPBA, 1 uM). Two dimensional polyacrylamide gel electrophoresis was used to determine the phosphorylation levels of the 20,000-dalton myosin light chains ($LC_{20}P$). Aequorin, preloaded into aortic strips, was used as an intracellular Ca^{++} indicator. During the 30-min period of K^+-induced contractions, both $LC_{20}P$ and $[Ca^{++}]_i$ increased significantly at all time points (0.12 mol Pi/mol LC_{20} to 0.17-0.24 mol Pi/mol LC_{20} and 230 nM to 260-280 nM, respectively) as did the steady-state stress($3.24\pm0.38 \times 10^4$ N/m^2). Similarly, both $[Ca^{++}]_i$ and $LC_{20}P$ increased during phenylephrine-induced contractions ($5.11\pm0.45 \times 10^4$ N/m^2). A transient increase in both $[Ca^{++}]_i$ and $LC_{20}P$ was was observed within 30 sec, followed by a drop to suprabasal levels. DPBA induced a slow contraction ($3.85\pm0.37 \times 10^4$ N/m^2) without significantly changing either $[Ca^{++}]_i$ or $LC_{20}P$ over a 90-min period. Our results suggest that levels and time courses of $LC_{20}P$ correlate well with those of $[Ca^{++}]_i$ in all three types of contractions examined. However, $[Ca^{++}]_i$ and $LC_{20}P$ do not always correlate with force generation, suggesting that a more complex mechanism may be involved in regulating the steady-state force of smooth muscle. Support: NIH HL 31704, AHA EI and Mass. Heart Postdoctoral Fellowship.

W-Pos338 THE CALCIUM SENSITIVITY OF FORCE IN SINGLE SKINNED VASCULAR SMOOTH MUSCLE CELLS. F.V. Brozovich and K.G. Morgan, Cardiovascular Division and Department of Medicine, Beth Israel Hospital, Harvard Medical School, Boston, MA 02215.

To determine the calcium sensitivity of isolated vascular smooth muscle cells, an enzymatic isolation technique for single cells from the ferret aorta was developed. Small pieces of aorta were incubated and agitated gently, without centrifugation or pipette aspiration, in a Hanks solution containing collagenase, elastase and trypsin inhibitor, and the isolated single cells were plated onto glass coverslips. The coverslips were then placed on the stage of a Zeiss inverted microscope, and flooded with relaxing solution (pCa=8 ($-\log_{10}[Ca^{2+}]$=8), [MgATP]=3mM). Cells were then skinned for 15 minutes in relaxing solution with 30 μg/ml saponin. Smooth muscle cells isolated in this manner would stick to glass and were attached to glass microtools, one of which was connected to a force transducer (Cambridge, model 400A). Free $[Ca^{2+}]$ was controlled between 10^{-8} and 10^{-4} M using 15 mM [EGTA]. Cell length did not change during skinning, and in relaxing solution, rest length averaged 77±15 μm ($\bar{x}$±sd, n=39), which is shorter than that reported by this laboratory for isolated ferret portal vein cells; however, these single cells from the aorta displayed normal mechanical properties. Using the maximum circular cross-sectional dimension, maximal Ca^{2+} activated muscle force averaged 0.84±0.29 x10^5 N/m^2 ($\bar{x}$±sd, n=27). When the cells were activated (increasing $[Ca^{2+}]$), on average, muscle force began to develop at a pCa of 7.3 and reached a maximum level at a pCa of 6.3, similar to force vs. Ca^{2+} curves constructed using aequorin in intact strips of ferret aorta. The Hill equation was used to fit plots of relative steady state force vs. pCa, yielding an average pk=6.89±0.19, N=2.3±0.9 ($\bar{x}$±sd, n=18). Supported by the NIH HL-31704, and an AHA EI.

W-Pos339 EFFECTS OF VASODILATORS ON Ca^{2+} SENSITIVITY AND FORCE IN VASCULAR SMOOTH MUSCLE STRIPS. T. T. DeFeo & K. G. Morgan, Harvard Medical School, Beth Israel Hospital, Boston, MA 02215.
The effects of 10^{-6}M nifedipine (N), 10^{-3}M hydralazine(H), and 10^{-6}M forskolin (F) on ferret aorta force and $[Ca^{2+}]_i$ (as indicated by aequorin) were determined during contractions induced by: (1) warming from 22°C to 37°C, (2) 12-deoxyphorbol, 13-isobutyrate, 20-acetate (DPBA) and (3) potassium depolarization (K). On warming there is no significant change in $[Ca^{2+}]_i$ even though 5.9±0.8 mN of tone develops. During K, $[Ca^{2+}]_i$ rises to a sustained plateau while DPBA causes no significant rise in $[Ca^{2+}]_i$. N and H inhibited the warming contraction (Δ = -1.6±0.4 mN, and -3.1±0.6 mN respectively), while causing an associated drop in $[Ca^{2+}]_i$, (Δ =52±8nM & 45±9nM, respectively); but in the presence of F, a similar inhibition (Δ = -1.8±0.3 mN) was accompanied by no significant decrease in $[Ca^{2+}]_i$. N and H prolonged the latency for force development and increased the time to peak contraction (TPC) in the presence of DPBA, but did not cause a statistically significant change in the contraction amplitude. F prolonged the latency for force development and increased the TPC in the presence of DPBA, and also caused a 94±4.3% inhibition of the contraction amplitude. During K, all three agents caused a dose-dependent decrease in $[Ca^{2+}]_i$ coincident with decreases in steady state force. Calcium-force curves were constructed in the presence of each of these vasodilators by plotting the calibrated aequorin light signal against the resulting force. The control calcium force curve was not shifted by N or H, but was significantly shifted to the right by F. These results indicate that vasodilators can cause relaxation of vascular smooth muscle by a combination of mechanisms involving both decreases in $[Ca^{2+}]_i$ and changes in the calcium-force relationship. Support: NIH HL31704, an AHA EI and a grant from Pfizer, Inc.

W-Pos340 Ca^{2+} DEPENDENCE OF MYOSIN PHOSPHORYLATION IN TRACHEAL SMOOTH MUSCLE CELLS. D.A. Taylor and J.T. Stull, Dept. Physiol., Univ. Tx. Hlth. Sci. Ctr. Dallas, Dallas, TX 75235.

Tracheal smooth muscle cells in primary culture retain many biochemical properties of cells in tissue (Fed. Proc. 45:764, 1986). These cells provide a model for studying Ca^{2+} dependent regulation of myosin light chain phosphorylation (LCP) by myosin light chain kinase (MLCK). Agonist or Ca^{2+} ionophore stimulation of fura-2 containing cells resulted in an increase in cytosolic free Ca^{2+} concentrations (Ca_i) and in the extent of LCP. Maximal values of Ca_i were greater with ionophore than with agonists. The quantitative relationship between Ca_i and LCP was similar with all agents. Control levels of LCP (9 ± 2%) were maintained until Ca_i increased to 200 nM from a resting value of 150 + 9 nM. Half-maximal LCP (30%) occurred at approximately 240 nM Ca_i. The Hill coefficient for the relationship between LCP and Ca_i was 2.7. Thus, relatively small changes in Ca_i cause large increases in LCP.

Cyclic nucleotides cause relaxation of smooth muscle which may be mediated either through a direct effect to lower Ca_i or through an effect on the relationship between Ca_i and LCP via a change in the affinity of Ca^{2+}/calmodulin for MLCK. Pretreatment of cells with forskolin to stimulate cAMP formation or with 8-bromo-cGMP did not affect the Ca_i required for half maximal LCP. However, the temporal and maximal responses in Ca_i and LCP to histamine or ionophore were decreased. Though forskolin or 8-bromo-cGMP did not alter the Ca_i required for half maximal LCP, both nucleotides decreased Ca_i and LCP proportionately. In addition, cGMP decreased slightly maximal LCP at high Ca_i. Thus, the primary effect of both cyclic nucleotides appears to be related to decreases in Ca_i which cause a proportional decrease in LCP (Supported by HL26043).

W-Pos341 Cardiac Muscle Activation by Laser-Induced Photolysis of Nitr-5 (Caged-Ca^{2+}). R.J. Barsotti, J.C. Kentish*, T.J. Lea+ and I.P. Mulligan+. Bockus Res. Inst., Graduate Hosp., Phila., PA 19146; *Dept. of Physiology, University College London, UK; +Laboratory of Physiology, Oxford, UK.

We studied the time course of calcium activation in chemically skinned ventricular trabeculae from the guinea-pig, using the photolabile calcium chelator, Nitr-5 (Tsien & Zucker, Biophs. J. 50:843, 1986). Saponin (50 μg/ml) was used to make the sarcolemma permeable but leave the sarcoplasmic reticulum (s.r.) functional. The s.r. was loaded with Ca^{2+} in a solution containing 0.1 mM Nitr-5 (pCa 6.8, pH 7.1, I = 200 mM, 12°C). The tissue was illuminated with a pulse of laser light (200 ns, 347 nm, 70 mJ), causing the photolysis of approximately 50% of the Nitr-5 and decreasing the pCa to 6.5. This induced transient tension responses which rose with a half-times from 1.5 to 7.0 s and reached approximately 50% of the maximal tension developed at pCa 4.5. The faster responses were comparable to those reported by Fabiato (J. Gen. Physiol. 85:247, 1985) for mechanically skinned single cardiac cells. In the presence of 10 mM caffeine, the same procedure produced no tension response, indicating that 1) the Ca^{2+} released from the photolysis of Nitr-5 was insufficient to directly activate the myofibrils, and 2) the s.r. was the source of the Ca^{2+} that activated the myofibril. By comparison, in trabeculae in which the s.r. was destroyed with triton, the half-time of the tension response following the direct activation of the myofilaments by Ca^{2+} released by the photolysis of higher [Nitr-5] (2 mM, pCa 6.5) was < 1 s. The final tension was similar to that achieved by triggering calcium-induced-calcium-release (CICR) with lower concentrations of Nitr-5. We have shown that laser photolysis of caged-Ca^{2+} can be used to trigger CICR in a chemically skinned multicellular preparation of ventricular muscle. The difference observed in the rate of rise of tension in contractions induced via CICR (low [Nitr-5]) versus direct activation (high [Nitr-5]) reflects the time required for Ca^{2+} release from the s.r. and diffusion to the thin filament.

W-Pos342 Rate of Ca^{2+} release Following Laser Photolysis of a new Caged Ca^{2+}.

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We have studied laser flash photolysis (347 nm) of DM-nitrophen and its divalent cation complexes. DM-nitrophen is a photolabile substituted EDTA molecule, the affinity of which for Ca^{2+} decreases by about 5 orders of magnitude on photocleavage. The decomposition of a photochromic intermediate (possibly an aci-nitro form) was examined between 425 and 435 nm following a laser flash. Little dependence on the kinetics of decay was seen in the range pH 6.9 to pH 8.9. At pH 6.9 DM-nitrophen itself showed biphasic decay of the intermediate (half-times 23 μsec, 530 μsec). In the presence of excess Ca^{2+} these values were (30 μsec, 210 μsec), but in the presence of excess Mg^{2+} only a single component was observed (29 μsec). Photolysis of the DM-nitrophen-Ca^{2+} complex at pH 6.9 in the presence of the metallochromic indicator antipyrylazo III, measuring absorbance at 650 nm, shows an exponential rise in absorbance with a half-time $\leq$ 200 μsec. It is not clear if the increase in absorbance at 650 nm is limited by the response-time of the dye to photoreleased Ca^{2+}. However, the measurements provide a maximal estimate of the half-time for Ca^{2+} release from our caged Ca^{2+} of approx. 200 μsec. At pH 6.9 the limit on the Ca^{2+} release half-time is consistent with the rates of decay of the photochromic intermediate. The DM-nitrophen-Ca^{2+} complex can be employed as a caged-Ca^{2+} which releases Ca^{2+} in the sub-millisec time range. Supported by NIH HL15835 to the Pennsylvania Muscle Institute and NIH HL30315.

W-Pos343 Ca^{2+} MEASUREMENTS USING FURA-2 AND QUIN2 IN *BALANUS NUBILUS* SINGLE MUSCLE CELLS.

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Experiments were carried out using single muscle fibres, injected with the fluorescent Ca^{2+} indicators fura-2 and quin2, under voltage clamp control. These fibres showed a concentration-dependent suppression of the force response and a slowing of decay times of force and fluorescence (Ca^{2+}-indicator complex) signal. 600μM $quin2_4$, at which no force production was apparent, was used to measure rates of release of Ca^{2+}, as most of the released Ca^{2+} would be bound to quin2. In response to depolarizing pulses which in the absence of quin2 gave forces <1% P_o, release rates of ca. 1μM/ms were measured, which varied with depolarizing pulse amplitude. Biphasic rates of release with an initial fast phase followed by a slower phase were measured at pulse durations >ca. 1.2s. A computer model of the Ca^{2+} transient in barnacle muscle fibres used the measured rate of change of Ca^{2+} at 600μM $quin2_4$ to simulate Ca^{2+} release with simultaneous binding to TnC T (100μM) and P (200μM) sites and the Ca^{2+} pump. As force developed in the absence of buffer was <1% P_o, T site Ca^{2+} occupancy was kept below 10%, while free [Ca^{2+}] was less than ca. 1μM [Ashley et al. (1974) In: Calcium binding proteins, Drabikowski et al., eds., Elsevier]. The model indicates that 600μM quin2 causes a ca. 2.5x increase in Ca^{2+} release above the amount calculated from the model in the absence of buffer. It is suggested that one source of this extra Ca^{2+} is entry through cleft or sarcolemmal Ca^{2+} channels, as quin2 and fura-2 fluorescence transients were almost always associated with current oscillations, which were not seen in the absence of added buffer. It is unlikely that the extra Ca^{2+} flux is due to regenerative Ca^{2+} release, as the extra flux increases with increasing buffer concentration. In skinned barnacle muscle fibres, regenerative release is abolished when solution [EGTA] is raised above ca. 100μM (T.J. Lea, unpublished).

W-Pos344 MYOPLASMIC BINDING OF FURA-2 INVESTIGATED BY STEADY-STATE FLUORESCENCE EMISSION ANISOTROPY. A. Olson, M. Konishi, S. Hollingworth and S. M. Baylor.
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Fura-2 (Grynkiewicz et al. 1985) injected into frog skeletal muscle fibers appears to bind to muscle constituents of large molecular weight (Hollingworth and Baylor, 1987). In order to learn more about this binding, the fluorescence emission anisotropy (A) of Fura-2 (Williams, Fogarty, Tsien and Fay, 1985) was measured in resting fibers using a 420 nm excitation beam. A in myoplasm was 0.27, which was significantly higher than A of 0.17 for Fura-2 in Buffer (110 mM KCl, 10 mM K_2PIPES, 10 mM K_2EGTA, pH 7.0, 20°C) plus 0.655 M sucrose to adjust viscosity to 2 cP (centipoise), an upper limit of myoplasmic viscosity. The elevated value of A is consistent with a reduced rotational mobility of the dye molecules due to myoplasmic binding. At 0.5 mM Fura-2, A was also measured in Buffer plus one of the following soluble myoplasmic proteins (Sigma Chemical Co.): ALD (aldolase, 48 mg/ml; A=0.22); GAPDH (glyceraldehyde-3-phosphate dehydrogenase, 22 mg/ml; A=0.18); CPK (creatine phosphokinase, 34 mg/ml; A=0.23); PARV (parvalbumin, 18 mg/ml; A=0.14). All proteins except PARV significantly increased A compared with Buffer alone at the equivalent viscosities (1.1 -1.4 cP). In Buffer plus a mixture of ALD+GAPDH+CPK (viscosity of 2 cP), A was very similar to that measured in frog fibers, with a similarly weak dependence on Fura-2 concentration. To characterize further the properties of protein-bound dye, the fluorescence emission spectrum of 0.5 mM Fura-2 was measured (using 420 nm excitation) in the presence and absence of the ALD+GAPDH+CPK mixture. The proteins appear to increase fluorescence emission at wavelengths between 470 and 520 nm while slightly decreasing the emission at longer wavelengths. Experiments are in progress to investigate the calcium-binding properties of the protein-bound form of Fura-2. Supported by NS 17620

W-Pos345 EU 4093 LOWERS $[Ca^{2+}]_i$ IN SKELETAL MUSCLE FIBERS FROM MH SUSCEPTIBLE SWINE
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Malignant hyperthermia (MH) is a hypermetabolic disease of skeletal muscle which is associated with a defect in intracellular Ca^{2+} regulation. We have studied the effects of EU 4093 on $[Ca^{2+}]_i$ in MH susceptible pigs. EU 4093 is a new water soluble drug which is thought to have the same properties as dantrolene on $[Ca^{2+}]_i$. We used a Ca^{2+} selective microelectrode prepared and calibrated as described previously (Lopez *et al.*, Biophys J. 43:1,1983) to measure $[Ca^{2+}]_i$ in vivo, in superficial fibers of the peroneus longus muscle. The pigs were anesthetized with N_2O/O_2, fentanyl and movement prevented with pancuronium. EU 4093 was administered in sequential 0.5mg/kg doses to a cumulative dose of 0.5,1.0,1.5 and 2.0 mg/kg to each of four pigs. This lowered resting $[Ca^{2+}]_i$ from 0.40±0.01 μM to 0.21±0.01, 0.15±0.01, 0.08±0.01 and 0.04±0.01 μM respectively (Mean±SEM). EU 4093 had no significant effect on Vm at any dose. The 2 mg dose was successful in preventing a clinical MH episode in these same pigs in response to a halothane/succinylcholine challenge. Our data demonstrate that EU 4093 has a dose related effect in lowering $[Ca^{2+}]_i$ in MH susceptible pigs, similar to our earlier findings with dantrolene. (Supported by NIH GM 15904 and Conicit S1-1277, EU4093 donated by Norwich Pharmaceuticals)

W-Pos346 BUPIVACAINE DEPRESSES CULTURED HEART CELL CA^{2+} FLUX AND MEAN $[Ca^{2+}]_i$
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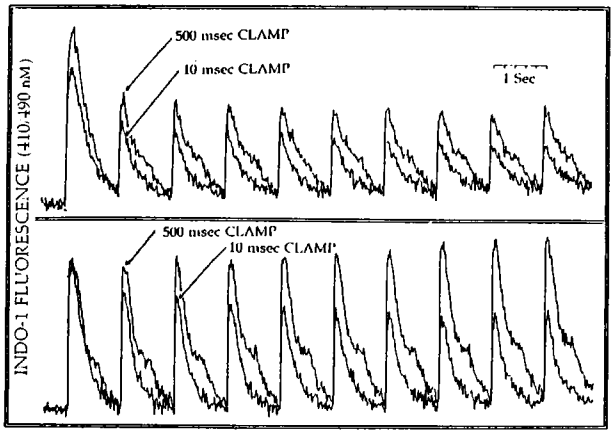
The effect of bupivacaine (5, 10 and 20μg/ml) was studied on the rapid phase of sarcolemmal $^{45}Ca^{2+}$ and on the mean intracellular free calcium concentration ($[Ca^{2+}]_i$) in monolayer cultures of spontaneously beating chick embryo ventricular cells. The cells were prepared and the rapid phase of sarcolemmal Ca^{2+} flux studied as described previously (Barry and Smith, J. Physiol. 325:243 1982) using 15, 30, 60, 120, and 300 sec time points. $[Ca^{2+}]_i$ was estimated from the 340/380 ratio emission signal of Fura 2 (10 min loading with Fura 2AM) with a Spex fluorimeter. All experiments were carried out at 37°C using HEPES buffered physiologic salt solutions, pH 7.4, containing 2% fetal calf serum. Bupivacaine caused a concentration related decrease in both the initial rate (7-25%) and 5 min (10-40%) $^{45}Ca^{2+}$ content. Under the same conditions there was a concentration dependant reversible decrease in mean $[Ca^{2+}]_i$ estimated from Fura 2 fluorescence. It is well established that local anesthetics block Na^+ channels of excitable membranes. Our observations demonstrate that the myocardial depression caused by bupivacaine is also associated with both a decrase in the rate of $^{45}Ca^{2+}$ influx and 5 min $^{45}Ca^{2+}$ content as well as a reduction in mean $[Ca^{2+}]_i$.
Supported by AHA (Mass Affiliate) PDA and NIH HL 35781 JM

W-Pos347 NEGATIVE STAIRCASE IN CYTOSOLIC Ca^{2+} IN RAT MYOCYTES IS MODULATED BY DEPOLARIZATION DURATION. Harold A. Spurgeon, Gerrit Isenberg, Antti Talo, Michael D. Stern, Maurizio C. Capogrossi, and Edward G. Lakatta. Gerontology Research Center, NIA, NIH, Baltimore, MD 21224

A negative staircase in the cytosolic Ca^{2+} transient (ΔCa_i NS) underlies the negative twitch staircase in rat cardiac muscle and myocytes. Here we show that depolarization duration modulates ΔCa_i measured as Indo-1 fluorescence (410/490 nm) in voltage clamped single rat myocytes (Hepes buffer; 23°C; stimulation from -45 to +5 mV from rest at 1 Hz). In 1.0 mM [Ca^{2+}] (top panel), ΔCa_i NS became more negative as clamp duration was reduced. Increasing bath [Ca^{2+}] to 3.0 mM (lower panel) reduced the steepness of the ΔCa_i NS with a 10 ms clamp and when clamp duration was 500 msec, ΔCa_i NS was abolished. With increasing clamp duration the ΔCa_i duration increases; this permits greater SR Ca^{2+} pumping, and minimizes loss of the SR Ca^{2+} load. Thus, the short rat action potential duration, via its effect on voltage dependent Ca^{2+} channels and Na/Ca exchange, is likely a determinant of the ΔCa_i NS characteristic of this species.



W-Pos348 **EFFECTS OF VANCOMYCIN ON SKINNED MYOCARDIAL FIBERS OF THE RABBIT.** Rashid J. Cajee, Judy Y. Su, Chia-Ying Pan-Lee, Department of Anesthesiology, University of Washington, Seattle, WA 98195

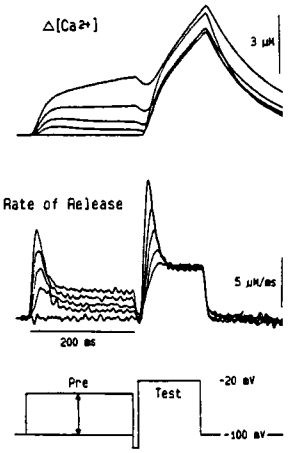
Vancomycin, an antibiotic, causes severe hypotension. Direct depression of myocardial contractility by vancomycin has been described. The mechanism of its action is not clear. Accordingly, the study was designed to examine the effects of vancomycin on the intracellular sites (Ca^{2+}-activation of the contractile proteins, and Ca^{2+} uptake and release from the sarcoplasmic reticulum [SR]) of muscle contraction using skinned myocardial fiber preparation. Right ventricular papillary muscles were isolated from rabbits killed by cervical dislocation. Pieces of the muscle were homogenized (sarcolemma disrupted) and fiber bundles were dissected and mounted on forceps, one end was attached to photodiode force transducers. The fiber bundles were activated with one of the submaximal pCa (5.6-5.0) and followed by pCa 3.8 and relaxed (pCa > 8) between contractions. Five different solutions were used to load Ca^{2+} into (uptake phase), and to release Ca^{2+} from (release phase) the SR using 25 mM caffeine resulting in a tension transient (Pflügers Arch. 380:29, 1979). Each experiment consisted of control (no drug), followed by test (with vancomycin), and finally control again. Vancomycin ($\geq$10 µM) increased the submaximal and the maximal Ca^{2+}-activated tension development of the contractile proteins. Vancomycin (>10 µM), however, decreased the caffeine-induced tension transients when present in the uptake phase and in the uptake and release phase and at higher concentration (1 mM) when present in the release phase. We conclude that the combination of vancomycin-induced increases in Ca^{2+} activation of the contractile proteins and decreases in Ca^{2+} uptake or release from the SR may result in increases or only slight decreases in myocardial contractility in isolated intact cardiac tissues. Supported by a grant from Lilly Research Labs, Indianapolis, IN, and in part by grants from NIH, #HL20754 and #HL01100.

W-Pos349 RESOLUTION OF FREE CALCIUM TRANSIENTS ASSOCIATED WITH CONTRACTION IN CULTURED NEONATAL RAT VENTRICULAR MYOCYTES. A.C. Morris, H.K. Hagler, H. Nazeran, and L. M. Buja. Department of Pathology, The University of Texas Health Science Center at Dallas. (Intr. by P.A.W. Anderson)

The purpose of this study was to measure dynamic changes in intracellular free Ca in cultured ventricular myocytes under control conditions and under Ca loading conditions induced by Na^+, K^+ ATPase inhibition. Cultured neonatal rat ventricular myocytes (M) were grown on laminin-coated glasscoverslips for three days. M were incubated for 30 min in medium 199 containing 3.0 µM fura 2/AM, pH 7.3 at 37°C and then placed in fura-free medium for 1 hour of equilibration. The coverslips were mounted in Sykes-Moore chambers and examined using a Nikon Diaphot microscope equipped with UV optics, a heated stage, perfusion pump (1.0 ml/min), and a photomultiplier tube. Ca transients associated with spontaneous contractions of M were resolved using chopped 340 and 380 nm excitation illumination produced by a Tracor Northern Fluoroplex 1000 coupled by a bifurcated quartz fiber optic to the microscope epilluminator. M exhibited a contraction rat of 80 to 100 spontaneous contractions per minute. Therefore, the excitation illumination was chopped at a rate producing 40 measures of 340/380 per second in order to properly resolve the frequency components of the contractile cycle predicted by sampling theory. Prior to treatment with ouabain (10^{-3}M), fields of regularly contracting M (n=5) had 340/380 ratios of 1.22 ± 0.16 (mean ± SD) during relaxation and ratios of 2.10 ± 0.62 at peak contraction. After 30 mintues of perfusion with 10^{-3}M ouabain, M were in a fibrillatory state with fura ratios sustained at 2.96 ± 1.83. Perfusion for 45 and 60 min produced a greater state of contracture with corresponding fura ratios of 3.31 ± 2.10 and 3.33 ± 1.26, respectively. After 60 min of Ca loading, perfusion of M with normal medium for 30 min reestablished the spontaneous contractions with ratios of 1.02 ± 0.15 during relaxation and 2.23 ± 0.9 at peak contraction. Using calibration values obtained from fura 2 in solution, the free calcium concentration in beating M ranged from 73 to 289 nM, in the fibrillatory state was 540, and after 60 min of ouabain treatment was 662 nM. These results demonstrate that free Ca transients in rapidly contracting myocytes can be resolved using fura 2 and that contracture induced by ouabain treatment is reversible.

W-Pos350 CALCIUM DEPENDENCE OF INACTIVATION OF CALCIUM RELEASE FROM THE SARCOPLASMIC RETICULUM IN FROG SKELETAL MUSCLE. B.J. Simon, M.G. Klein and M.F. Schneider, Dept. of Biological Chemistry, Univ. of Maryland School of Medicine, Baltimore MD 21201.

$[Ca^{2+}]$ transients and resting $[Ca^{2+}]$ were measured in cut segments of single frog twitch fibers voltage clamped in a double Vaseline gap chamber (10 °C) by simultaneously using the calcium indicators antipyrylazo III (AP III) and fura-2 (Klein et al., Biophys. J. 51, 199a, 1987 and ms in revision). The rate of SR calcium release (RREL) was calculated from the calcium transients and corrected for depletion of calcium from the SR (Schneider et al., J. Physiol. 392, 1987). A variable amplitude pre-pulse (bottom) was used to elevate $[Ca^{2+}]$ (top) prior to a fixed test pulse. Increasing $[Ca^{2+}]$ resulted in increasing degrees of suppression of the peak of the test RREL without any effect on its final level (middle). The relationship between $[Ca^{2+}]$ at the end of the pre-pulse and the fractional depression of the peak of the test RREL was fit better by a two-site calcium binding model for inactivation than by a single-site model. The $[Ca^{2+}]$ at which the test RREL was half-inactivated was 0.37 ± .06 μM (mean ± s.e., n=7) assuming all AP III in the fiber followed the absorbance vs. $[Ca^{2+}]$ relationship of Kovacs et al. (J. Physiol. 343, 1983) and with fura-2 calibrated to give $[Ca^{2+}]$ transients in agreement with AP III. Supported by NIH F32-AM07267 (BJS) and RO1-NS23346 (MFS) and MDA (MGK and MFS).



W-Pos351 **STIMULUS-RESPONSE COUPLING IN MAMMALIAN CILIATED CELLS: INTRACELLULAR [Ca++] TRANSIENTS DETECTED BY FURA-2.** Manuel Villalon*, Thomas R. Hinds*, and Pedro Verdugo. Ctr. for Bioeng. and Depts. of Biol. Struct. and Pharmac. Univ. of Washington. Seattle WA, 98195.

Calcium has been thought to play an important role in the control of ciliary movement in a broad variety of ciliated cells. In mammalian ciliated cells Ca^{++} depletion can cause reversible ciliary arrest (**Biophys. J.16,** 120a, 1976). When demembranated models of respiratory ciliated cells are reactivated in the presence of Calmodulin, variations in $[Ca^{++}]$ ranging from 0.1 to 1 μm can produce corresponding increases of ciliary beat frequency (**Cell Motility** Suppl. 1, 222, 1981). The stimulus-response coupling in mammalian ciliated cells is thought to be coupled by release of intracellular Ca^{++} (**Nature 283,** 764, 1980). However, direct measurements of fluctuations of intracellular $[Ca^{++}]$ associated with stimulation of ciliary activity have not been reported.

The experiments presented here were designed to measure changes in intracellular $[Ca^{++}]$ following purinergic stimulation of ciliary activity. Experiments were conducted in cultured ciliated cells of the rabbit oviduct. Changes in intracellular $[Ca^{++}]$ were measured by the fluorescent probe Fura-2. Monolayers of ciliated cells were loaded with Fura-2 AM; equilibrated in Hanks' solution, pH 7.2, 37°C for 15 min; and mounted at a 45° angle in the cuvette of a double channel spectrofluorometer. Changes in fluorescence emission were detected at 500 nm before and after stimulation with ATP, using excitation wave lengths of 347 & 380 nm. Preliminary results indicate that a 2 to 3 fold transient increase of intracellular $[Ca^{++}]$ occurs within the first 10 sec, and last approximately 1 min., after the infusion of 0.1 mM ATP in the cuvette.

It has been shown that ATP can strongly stimulate ciliary movement in mammalian ciliated cells in culture. This action is not dependent upon ATP dephosphorylation, and it is probably mediated by a purinergic receptor of the P_2 since it can be also induced by non-hydrolizable analogs of ATP (**Fed. Proc. 44,** 641, 1985). The present results provide the first direct measurement of stimulus-induced changes of intracellular $[Ca^{++}]$ in mammalian ciliated cells. These results are in agreement with our previous indirect evidence that suggest that cilio-stimulation in mammalian ciliated cells might be coupled by fluctuations of intracellular $[Ca^{++}]$ (**Nature 283,** 764, 1980).

Supported by grant HL 38494 of NIH and grant R 010-7-01 from the Cystic Fibrosis Foundation.

W-Pos352 DO Ca^{2+}-INDUCED Ca^{2+} RELEASE CHANNELS PARTICIPATE IN E-C COUPLING IN SKELETAL MUSCLE? D.G. Brunder and P.T. Palade, Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550.

If the Ca^{2+}-induced Ca^{2+} release channel is involved in the physiological mechanism of E-C coupling, specific blockers of this channel should be effective *in situ*. We have applied neomycin and gentamicin, blockers of Ca^{2+}-induced Ca^{2+} release from rabbit SR vesicles (J. Biol. Chem. 262:6149,1987), to the cut ends of single frog skeletal muscle fibers in a Hille-Campbell vaseline gap voltage clamp. Calcium release is estimated from: 1) the shortest stimulus to +130 mV from the holding potential of -90 mV that just elicits a local contraction of the fiber; and 2) direct measurement of Ca^{2+} transients with Antipyrylazo III. Block of Ca^{2+} release in response to voltage clamp pulses requires much higher concentrations (≥ 50 uM) applied to the end pools than are necessary to block release *in vitro*. Correction of the concentrations for diffusional effects and binding in the myoplasm suggests that the K_i's for gentamicin and neomycin are still 1-2 orders of magnitude higher that the K_i's observed in *in vitro* experiments with rabbit SR vesicles. Protamine at concentrations of 250 ug/ml (over 10000x the K_i in isolated SR vesicles) has no effect on Ca^{2+} release, although its apparent diffusion coefficient could be very low. Additional experiments will be necessary to differentiate among the following possibilities: 1) differences in drug sensitivity exist between channels in frog and rabbit skeletal muscle; 2) *in situ* and *in vitro* experimental conditions are sufficiently different; 3) the *in situ* gating mode is less drug sensitive than *in vitro*; or 4) the Ca^{2+}-induced Ca^{2+} release channel is not involved in the physiological release mechanism. (Supported by RO1 AR34377).

W-Pos353 GTP POTENTIATES CALCIUM RELEASE FROM SARCOPLASMIC RETICULUM

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The involvement of G proteins in excitation-contraction coupling was investigated. G proteins were identified in rat skeletal muscle subcellular fractions using Western blot analysis with rabbit antibodies raised against the beta and alpha subunits of bovine transducin. The anti-beta antibodies recognized a 35 kD protein in the plasma membrane, T-tubule and triad vesicles which comigrated with transducin beta subunit. The plasma membrane was most enriched in this protein while smaller amounts were detected in the T-tubule vesicles. Only a trace amount was observed in the triad fraction. A similar protein was also found frog transverse tubule vesicles. Antibodies to bovine transducin alpha subunit did not bind to any of the proteins in the rat skeletal muscle subcellular fractions. To determine if GTP-binding proteins play a role in excitation-contraction coupling, experiments were performed on frog skinned fibers using GTP-γ-S to activate G proteins. This analog alone was unable to induce calcium release from the sarcoplasmic reticulum even at concentrations as high as 500μM. However, the threshold caffeine concentration required to trigger calcium release is shifted to lower concentrations by preincubation of the fiber with GTP-γ-S. The amount of calcium released by a supra-threshold concentration of caffeine is irreversibly increased by pretreatment with GTP-γ-S. In conclusion, G proteins have been identified in plasma membrane and T-tubule membrane vesicles, and GTP-binding proteins appear to potentiate calcium release from the sarcoplasmic reticulum.

W-Pos354 INHIBITION OF AFTER-GLIMMERS AND AFTER-CONTRACTIONS BY SODIUM CHANNEL BLOCKADE WITH YOHIMBINE.

G. Maurice Briggs, Judith Gwathmey, and James P. Morgan. Dept. of Medicine and Cardiovascular Division, Harvard Medical School and Beth Israel Hospital, Boston, MA 02215.

Elevation of intracellular calcium is believed to result in Ca^{++} overloading of the sarcoplasimc reticulum (SR). This results in phasic release of calcium from the SR triggering delayed afterdepolarizations and aftercontractions. We used ferret right ventricular papillary muscles loaded with aequorin and stimulated at 0.33 Hz. To investigate the effect of inhibiting sodium channels on the intracellular calcium transient the intracellular calcium concentratiuon $[Ca^{++}]_i$ was elevated by exposing the muscle to 8 mM Ca^{++} and 10^{-6} M isoproterenol. Sodium channels were blocked with yohimbine in the presence of 10^{-6} M phenoxybenzamine. The addition of 10^{-4} M yohimbine resulted in an increased stimulation threshold, reduced peak and resting light as well as reduced peak and resting tension. The after-glimmers and aftercontractions were eliminated. The Fourier spectra were also investigated. We conclude that the after-glimer was eliminated by lowering the intracellular calcium concentration via enhanced sodium/calcium exchange as a result of decreased intracellular sodium. Supported by HL39091, HL31117, HL01611, grant in aid and fellowship from the American Heart Association, Massachusetts affiliate.

W-Pos355 EFFECTS OF Ca AGONISTS AND ANTAGONISTS ON E-C COUPLING IN SKELETAL MUSCLE FIBERS.

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We studied the excitation-contraction (EC) coupling effects of Ca-active drugs on cut segments of frog fast twitch fibers under voltage clamp, loaded with a Ca-sensitive dye. Charge movements, membrane Ca currents, Ca transients and Ca release flux were determined by published methods. In the presence of D600 (either 30 or 1.5 μM at 9°) successive depolarizing pulses lead to a state with: (a) **no Ca release**; (b) **no membrane Ca current**; (c) **charge movement with the properties of "Charge 2"**, that is, the same voltage distribution as in a fiber inactivated by prolonged depolarization. All changes reversed incompletely and in parallel upon hyperpolarization. The agonist dihydropyridine (+) 202 791 at 100 nM potentiated Ca currents but **inhibited Ca release** in fibers held at polarized holding potential (HP = -90 mV) by ≈ 10% and by ≈ 50% at HP = -70 mV. These results, consistent with recent observations of Melzer & Pohl (J. Physiol. 390: 151P) are interpreted with a state model of the voltage sensor of EC coupling (Brum & Rios J. Physiol. 387: 489) assuming that both drugs bind preferentially to inactivated states of the voltage sensor. The results, in particular the fact that both EC coupling and T-membrane Ca currents undergo use-dependent "paralysis" in D600, are evidence of similarity between Ca channels and the voltage sensors of EC coupling. Additional evidence will be presented (Pizarro et al., this meeting). The inhibitory effect of the agonist drug on Ca release, accompanied by increase in Ca current, confirms that the latter has no role in the early stages of EC coupling. Supported by NIH.

W-Pos356 EFFECT OF D-600 AND La^{+++} ON CHARGE MOVEMENT IN DEPOLARIZED MUSCLE FIBERS.

C. Caputo and P. Bolaños. C.B.B. IVIC, Ap. 21827. Caracas 1020A. Venezuela.

Intramembrane charge movement has been measured in cut skeletal muscle fibers from *R. pipiens* using the triple vaseline gap voltage clamp technique and the UCLA-WAD system for pulse generation, data acquisition and analysis.The internal solution contained K-Aspartate and 10 mM EGTA, while the external solution contained $(TEA)_2$-SO_4, Rb_2SO_4, $CaSO_4$, $MgSO_4$ and TTX. Charge movement signals were obtained using the P-P/4 procedure, with the pulses being subtracted at either hyperpolarized or strongly depolarized membrane potential values (subtracting holding potential, SH). In 10 normally polarized fibers (-100 mV) the following values were obtained for the charge movement parameters: Q_{max} = 45.2 nC/uF; k = 21.4 ; V = -37.1 mV. Upon depolarization, confirming the results of Brum and Rios (J. Physiol. 187:489-517.1987), we found that charge movement changed its voltage dependence, since it appeared to move at voltages more negatives than -80 mV, without a decrease of the total charge moved. In fact, in the same 10 fibers depolarized to -30 to 0 mV the above parameters had the following values :Q_{max} = 43.3 ; k = 44.4; V = -93.3 mV. Thus, depolarization results in a shift of about 50 mV of the Q-V relationship similar to that found by Bezanilla et al. (J. Gen. Physiol. 79:21-40, 1982), for the case of the sodium gating currents in squid axons, and a change of the curve slope. In the presence of 50 uM D-600 the Q-V curve is further shifted by about 40 mV toward more negative potentials, while in the presence of 100 uM free La^{+++} ions the shift is reduced by about 20 mV. In both cases the amount of total charge is diminished by 20 %.

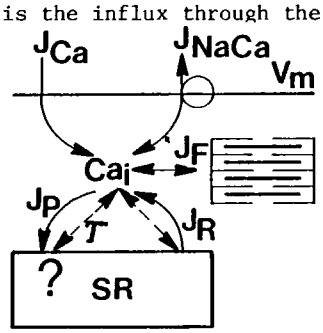
Supported by MDA and CONICIT S1-1148.

W-Pos357 THE DISTRIBUTION OF CHARGE MOVEMENT COMPONENTS IN SURFACE AND T-TUBULE MEMBRANES OF FROG SKELETAL MUSCLE FIBERS, Christopher L.-H. Huang and Lee D. Peachey, Physiological Labs., Cambridge CB2-3EG, U.K. and Dept. of Biology, Univ. of Pennsylvania, Philadelphia, PA 19104-6018.

An evaluation of possible roles for charge movement components in muscle fiber membranes would be aided by knowing the anatomical distribution of these components in surface and T-system membranes. We have determined both linear capacitance and the voltage-dependence of non-linear capacitance normalized to linear capacitance in frog sartorius muscle fibers using a three microelectrode voltage clamp at the end of the fiber. Bathing solutions were used that reduced ionic membrane currents and tubular voltage attenuation. In the linear cable analysis, we have taken into account surface membranes, T-system membrane, and the extra membrane covering the end of the fiber. When muscles were subjected to sudden withdrawal of 400 mM glycerol from a hypertonic Ringer's solution at about 10 degrees C, the reduction in linear capacitance of surface fibers and its dependence on electrical fiber diameter suggested that at least 75% of the T-system membrane became electrically inacessible. These 'detubulated' fibers showed non-linear capacitance of the type referred to as q_β and addition of tetracaine was without effect on charge movements in these fibers. In contrast, when muscles were exposed to 400 mM glycerol and it was removed gradually, linear capacitance was the same as in untreated fibers, and both q_β and the more steeply voltage-dependent and kinetically slower q_γ were present. Treatment of these fibers with tetracaine abolished the q_γ component. These results suggest a predominant localization of q_γ in T-tubules and agree with the hypothesis that q_γ represents a key step in E-C coupling in these fibers. (Supported by MDA and a Royal Society Fellowship to LDP).

W-Pos358 MODELING OF EXCITATION-CONTRACTION COUPLING IN HEART MUSCLE. V. Schouten, L. Cleemann, P. Pancha and M. Morad. University of Pennsylvania, Department of Physiology, Philadelphia, PA 19104.

It has been commonly assumed that the sarcoplasmic reticulum, SR, of heart muscle is functionally divided into two pools, an uptake pool and a release pool. According to this scheme many beat-dependent phenomena reflect slow equilibration of Ca^{2+} between these two compartments. We propose that the SR may be modeled as a single compartment if it is simultaneously assumed that intracellular Ca^{2+}, Ca_i, binds in a time dependent manner to regulatory sites of the Ca^{2+} pump of the SR, J_p. Four other Ca^{2+} fluxes contribute to the control of Ca_i: J_{Ca} is the influx through the Ca^{2+} channel, J_{NaCa} is the flux carried by the Na-Ca exchanger, J_R represents Ca^{2+}-induced Ca^{2+} release from the SR and J_F is the Ca^{2+} which binds to the contractile filaments and produces force. A normal beat produces a Ca_i transient which results in Ca^{2+} binding at the regulatory sites, J_p. This binding is accompanied by an accelerated pumping which lasts for some seconds (T) following the beat until Ca^{2+} slowly dissociate from the regulatory sites. This model has been used in computer simulations to reproduce phenomena such as rate-staircase, mechanical restitution, post extrasystolic potentiation, Ca^{2+} loading of the SR, spontaneous Ca^{2+} oscillations and Ca^{2+} overload.



W-Pos359 THE EFFECT OF 2,3-BUTANEDIONE MONOXIME ON THE INITIAL HEAT- TENSION-TIME INTEGRAL RELATION AND AEQUORIN LIGHT OUTPUT FROM FERRET PAPILLARY MUSCLES. EM Blanchard, NR Alpert (Dept of Physiol, U. of Vermont, Burlington, VT), D.G.Allen and G.L.Smith (Dept of Physiology, University College London ,U.K.)

The net heat produced by the time of mechanical relaxation of an isometric twitch of a papillary muscle, initial heat (I), is primarily due to the hydrolysis of ATP by two enzymatic processes: 1) the crossbridge cycle and 2) the active transport of calcium . One of our goals is to isolate the heat related to calcium cycling, tension independent heat (TIH), and to determine what information the TIH signal can provide about the calcium requirements for activation . The drug 2,3-butanedione monoxime (BDM) reduces I and tension-time integral (TTI) of ferret papillary muscles (0.33 Hz,30^{o} C) in a linear manner with a positive heat intercept (20% of I) at negligible levels of TTI. This result indirectly indicates that BDM (0-15 mM) selectively inhibits crossbridge cycling in the ferret papillary muscle but has little or no effect on calcium cycling. Experiments determining the effect of BDM on the free Ca^{2+} transient from ferret muscles supports this idea about the mechanism of BDM action. The light output from muscles injected with the aequorin was not affected by concentrations of BDM up to 5 mM although TTI had fallen by 70%. At BDM concentrations of 10 and 15 mM, reductions in the peak light transient (25% and 50%, respectively) were detected though these reductions in light were smaller than the reductions in TTI (96% and 100%,respectively). Even though aequorin light and TIH reflect different aspects of calcium cycling, free Ca^{2+} and total calcium, respectively, the results support the idea that BDM can selectively inhibit crossbridge cycling by a direct action on the contractile apparatus while leaving calcium cycling unaffected. Supported by PHS #28001/06/P1.

W-Pos360 EVIDENCE FOR PHYSICAL ASSOCIATION BETWEEN JUNCTIONAL SARCOPLASMIC RETICULUM RYANODINE RECEPTOR AND JUNCTIONAL TRANSVERSE TUBULAR DIHYDROPYRDINE RECEPTOR. C.M. Knudson, T. Imagawa, S.D. Kahl, M.G. Gaver, A.T. Leung, A.H. Sharp, S.D. Jay and K.P. Campbell. Department of Physiology and Biophysics, University of Iowa, Iowa City, IA 52242

The mechanism by which Ca^{2+} release from the terminal cisternae of the sarcoplasmic reticulum is coupled to depolarization of the transverse tubular membrane in skeletal muscle is not known. Schneider and Chandler (Nature 242:244, 1973) originally proposed a direct physical coupling between the protein responsible for voltage-dependent charge movement in the transverse tubular membrane and the junctional sarcoplasmic reticulum Ca^{2+} release mechanism. Recent results from our laboratory suggest that the ~450,000 Da ryanodine receptor is the junctional sarcoplasmic reticulum Ca^{2+} release channel (Imagawa et al. JBC in press). In addition, Rios et al. (Nature 325:717, 1987) have recently demonstrated that the 1,4-dihydropyridine Ca^{2+} channel blockers inhibit voltage-dependent charge movement in the transverse tubular system and therefore suggested that dihydropyridine receptor is the voltage sensor in transverse tubular membrane. If the dihydropyridine receptor is physically coupled to the ryanodine receptor then we hypothesized that under the right conditions we could isolate a complex of the dihydropyridine receptor and the ryanodine receptor using WGA-Sepharose. Initial experiments have resulted in the isolation of a complex that contained [^{3}H]ryanodine binding activity and [^{3}H]PN200-110 binding activity. SDS-PAGE analysis of this complex revealed the presence of the ~450,000 Da ryanodine receptor and all the dihydropyridine receptor subunits. Therefore, these results support the hypothesis of a direct physical coupling between the dihydropyridine receptor of the transverse tubular membrane and the ryanodine receptor of the junctional sarcoplasmic reticulum. (Supported by NIH HL39265)

W-Pos361 ULTRASTRUCTURE OF THE RYANODINE RECEPTOR FROM SKELETAL MUSCLE SARCOPLASMIC RETICULUM. Akitsugu Saito, Makoto Inui and Sidney Fleischer (Intr. by Gerald Stubbs). Department of Molecular Biology, Vanderbilt University, Nashville, TN 37235.

The ryanodine receptor has been purified from skeletal muscle sarcoplasmic reticulum (SR) and shown to be equivalent to the feet structures involved in junctional association with the terminal cisternae of SR to form the triad junction. The dimensions of the feet structure are 210 x 210 x 120 Å [M. Inui, A. Saito and S. Fleischer. J. Biol. Chem. *262*, 1740 (1987)]. This receptor is composed of a single high molecular weight polypeptide (M_R~360 KD). More recently, we have incorporated the purified receptor into bilayers and found it to have the characteristics of the calcium release channel in SR (L. Hymel, M. Inui, S. Fleischer and H.G. Schindler. PNAS, in press). New ultrastructure can now be discerned using negative staining with uranyl acetate (see photo). Extensive structural information of the SR calcium channel can be observed. A central circular pore (~40 Å) can be regularly seen in the square face. The feet structures consist of a number of subunits arranged with four-fold symmetry. (Supported by grants from NIH AM 14632, HL 32711 and the Muscular Dystrophy Association. M.I. is an Investigator of the American Heart Assoc.)

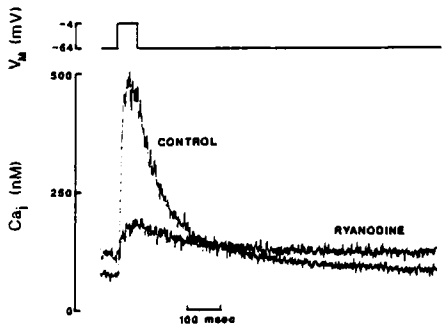
W-Pos362 VOLTAGE-DEPENDENCE OF PURIFIED RYANODINE RECEPTOR INCORPORATED INTO PLANAR LIPID BILAYERS

M. Fill, J. Ma, K.P. Campbell, T. Imagawa, C.M. Knudson, E. Stefani and R. Coronado. Department of Physiology and Molecular Biophysics, Baylor College of Medicine, Houston, TX 77030; and Department Physiology and Biophysics, University of Iowa, Iowa City, IO 52242.

We've shown elsewhere that the 450,000 Da ryanodine receptor forms monovalent and divalent-selective channels when incorporated into planar bilayers (Imagawa et al., JBC, 1987, in press). Here we investigate its voltage-dependent properties. Ryanodine receptor from rabbit skeletal muscle was artfully purified by immunoaffinity using CHAPS-solubilized triads (Campbell et al., JBC v262, 1987). Planar bilayers were formed in 50 mM NaCl at pH 7.0. Purified receptor was added to one side to a final concentration of 60 ug/ml protein, .01% CHAPS. An equal concentration of CHAPS was added to the opposite side. Capacitance (C), determined from a 10 mV step, was measured at holding potentials (V) between + and - 150 mV. In a bare bilayer, the relationship between C and V^2 was linear with a slope of 3.343 pF V-2 and symmetric about V=0 mV. With CHAPS on both sides, the relationship remained symmetrical but with a slope of 4.892 pF V-2. Purified receptor made this relationship asymmetric. At positive potentials, the average slope was approximately 6 times steeper and over this voltage range did not saturate. At negative potentials, the slope was less steep than in controls. A simple surface charge effect was ruled out. Consistent with these results, low concentrations of receptor (1-2 ug/ml) was sufficient to incorporate single channels which in most instances were voltage-gated. Supported by AHA, MDA, and NIH.

W-Pos363 **EFFECT OF RYANODINE ON INTRACELLULAR CALCIUM IN VOLTAGE CLAMPED RAT CARDIAC VENTRICULAR CELLS.** **J.R. Berlin, M.B. Campbell* and W.J. Lederer, Dept. of Physiology, Un. of Maryland Sch. of Medicine, Baltomore, MD and *Dept. of Pharmacology, Un. of Miami Sch. of Medicine, Miami, FL.**

Ryanodine has been shown to increase calcium efflux in cardiac muscle preparations (Bers & MacLeod, Circ. Res. 58, 769, 1986; Hilgemann, J. Gen. Physiol, 87, 675, 1986). To determine the reason for the increased calcium efflux, we investigated the effect of ryanodine on intracellular calcium concentration (Ca_i) in rat cardiac ventricular myocytes. Cells were voltage clamped by a single patch electrode technique. Ca_i was measured by injecting fura-2 (K+ salt) into the cell from the clamping electrode and recording fluorescence (500 nm) during illumination with 340 nm and 380 nm light. The figure shows that 25 uM ryanodine greatly decreased the amplitude of the Ca_i transient produced by a 50 msec depolarization. Ryanodine also caused a rise in Ca_i (from 60 nM to 100 nM) at the holding potential. In the presence of ryanodine, an additional tonic increase in Ca_i could be produced by trains of depolarizing pulses or by depolarizations of long duration (0.5-10 sec). This tonic rise of Ca_i decayed with a $T_{1/2}$ of approximately 1.5 sec (at -64 mV) and was abolished when D-600 (25 uM) was also present. Increased Ca_i at the holding potential could explain the reported increase in calcium efflux due to ryanodine.



W-Pos364 **THE EFFECT OF RYANODINE ON DRIVE RELATED CHANGES IN CYTOSOLIC CA2+ AND CONTRACTION IN FELINE VENTRICULAR MYOCYTES** W.H. duBell & S.R. Houser. Dept. of Physiol. Temple U. Sch. of Med. Phila., PA 19140.

Ryanodine (R) is thought to interfere with the release of Ca2+ from the cardiac sarcoplasmic reticulum (SR). Experiments were done to evaluate the effects of R on drive related changes in Ca2+ transients and contraction in isolated feline ventricular myocytes. Cytosolic Ca2+ was studied using indo-1 and contraction was measured with a video based edge detector. Cells were superfused with normal Tyrode solution (2 mM Ca2+) at room temperature and stimulated from rest at 0.2 Hz. When steady state was reached, rest periods of 15 sec, 30 sec, 1 min and 2 min were interposed. Following exposure to 1 uM R the stimulation protocol was repeated. With stimulation from rest, both Ca2+ transients and contraction showed a positive staircase and progressive decrease in duration. As rest duration was increased, the magnitude of the first postrest Ca2+ transient and twitch were decreased. In all cases a prominent slow phase of relaxation was observed in both. Following R, Ca2+ transients and twitches were significantly diminished. In addition, there was a pronounced increase in diastolic Ca2+ and a decrease in resting length. The slow phase of relaxation was eliminated and a slow increase in diastolic Ca2+ was observed between beats. These results suggest that R does not inhibit SR Ca2+ uptake but the release sites leak Ca2+ during diastole. (Supported by a grant from SE PA AHA and NIH Grants HL 33921 and HL 33648.)

W-Pos365 ENDOGENOUS OCCURRENCE OF A LYSOPOLYPHOSPHOINOSITIDE IN FROG SKELETAL MUSCLE.

N. Lagos, K. Asotra and J. Vergara, Department of Physiology, UCLA, Los Angeles, CA 90024.

One of the basic requirements for a chemical hypothesis of excitation-contraction (E-C) coupling involving inositol phosphates as diffusible intermediaries, is the existence of an adequate supply of precursor polyphosphoinositides, mainly PtdIns(4,5)P_2 (PIP_2), at the T-tubules. Hidalgo et al. (FEBS Letters,202:69,1986) showed that T-tubule membranes isolated from frog skeletal muscle have endogenous phosphoinositide kinases capable of synthesizing PtdIns4P (PIP) and PIP_2. We have studied the lipid phosphorylation process in resting frog skeletal muscles from Rana catesbeiana using a fast freezing hammer smasher technique followed by extractions with 1N HCl and chloroform:methanol (1:1). We found that, in addition to PtdIns (PI) and PIP, skeletal muscles contain about 25 nmole/g wet weight of PIP_2 and 9 nmole/g wet weight of a novel phospholipid, lysoPtdIns(4,5)P_2 (lysoPIP$_2$), whose presence in tissues is now reported for the first time. The identity of this latter lipid was obtained by coelution with standards, prepared by incubation of commercial polyphosphoinositides with bee venom phospholipase A_2 (PH A_2), and separated in HPTLC plates. Incubation of endogenous ^{32}P labelled lipid extracts from muscle with PH A_2 showed a significant reduction in PIP_2 and a proportional increase in lysoPIP$_2$ in the HPTLC autoradiogram. Experiments using heavy SR isolated membranes, incubated with [γ-^{32}P]ATP, showed label incorporations into lysoPIP$_2$, preceding any other lipid phosphorylation process. The endogenous occurrence of this polyphosphoinositide and its rapid synthesis suggest that it may play a crucial role as an intermediary in the metabolic biochemical steps at the triadic junctions in skeletal muscle.

Supported by MDA,NSF and NIH grants. N.L. and K.A. supported by AHA GLAA postdoctoral fellowships.

W-Pos366 CALCIUM RELEASE BY INOSITOL TRISPHOSPHATE IN AMPHIBIAN AND MAMMALIAN SKELETAL MUSCLE IS AN ARTIFACT OF CELL DISRUPTION, AND PROBABLY RESULTS FROM DEPOLARIZATION OF SEALED-OFF T-TUBULES. J. D. Hannon, N. K. M. Lee, and J. R. Blinks. Pharmacology Dept., Mayo Fdn., Rochester, MN 55905.

It has been reported by several investigators that inositol (1,4,5)-trisphosphate ($InsP_3$) causes the release of Ca^{++} in skeletal muscle fibers skinned in various ways, and in suspensions of vesicles derived from junctional sarcoplasmic reticulum (SR). This and other evidence led to speculation that $InsP_3$ might serve as a chemical transmitter linking transverse tubular (TT) depolarization to the release of Ca^{++} by the SR. However, there have also been several reports (and many unpublished observations) indicating no such effect of $InsP_3$. We now have evidence that may reconcile these conflicting observations and also indicates that $InsP_3$ does not play an essential role in E-C coupling. Microinjection of $InsP_3$ (1 μM - 1 mM) into intact frog, mouse, and guinea-pig twitch fibers produces no local activation. After TT disruption by glycerol shock, the same fibers contract locally in response to injection of 1 μM $InsP_3$. The contraction can then be abolished by applying the membrane-permeant cardiac glycoside digitoxin (100 μM for 30 min) to the detubulated fiber (or prevented by soaking the fibers in the non-permeant glycoside ouabain (1 mM for 2 hr) before detubulation). The injection of caffeine (25 mM) or Ca^{++} (100 μM) causes local contraction under all these circumstances. Our observations suggest that $InsP_3$ triggers Ca^{++} release only when T-tubules have been disrupted, and that it probably does so by depolarizing sealed-off T-tubules. Vesicles of junctional SR sometimes have sealed T-tubules attached; a current capable of depolarizing sealed-off tubules need not significantly depolarize the T-tubules of normal fibers because they are under the voltage control of the surface membrane. (Support: USPHS grant HL12186.)

W-Pos367 INORGANIC PHOSPHATE (Pi) HAS A BIPHASIC EFFECT ON CALCIUM LOADING BY THE SARCOPLASMIC RETICULUM (SR) OF SKINNED CARDIAC FIBERS. Thomas M. Nosek and Ashish Jain (Intro. by Keith Green) Dept.of Physiology & Endocrinology, Medical College of Georgia, Augusta, Georgia 30912.

With hypoxia, ischemia, or increased work load, intracellular [Pi] of cardiac muscle increases markedly and might well affect excitation-contraction coupling. The purpose of this study was to examine this possibility by measuring the effect of [Pi] on Ca^{2+} uptake by the SR. All experiments were carried out on rabbit papillary muscle bundles treated with saponin to make the sarcolemma hyperpermeable but to leave the SR membrane intact. The amount of calcium loaded by the SR under test conditions was estimated from the peak isometric force generated when Ca^{2+} was subsequently released from the SR by 25 mM caffeine (for details see Nosek et al., Am. J. Physiol. 250:C807, 1986) We found that Ca^{2+} loading was depressed for 1 mM $\leq$ [Pi] $\leq$15 mM but was enhanced when [Pi] > 20 mM. In contrast, inhibitors of the SR Ca-ATPase (vanadate (0.1 mM) & arsenate (30 mM)) only decrease SR calcium loading while oxalate (30 mM), a well known calcium chelator, significantly increased calcium loading. These data suggest that the biphasic response with Pi is due to: i) inhibition of SR Ca-ATPase activity concurrent with ii) precipitation of calcium by Pi within the SR. (Supported by NIH HL/AR 37022).

W-Pos368 A COMPARISON OF INTRACELLULAR SODIUM ION ACTIVITIES IN RAT AND RABBIT VENTRICULAR MUSCLE MEASURED WITH ION-SELECTIVE MICROELECTRODES. Michael J. Shattock and Donald M. Bers. Division of Biomedical Sciences, University of California, Riverside, CA 92521.

The adult rat heart demonstrates a number of anomolous physiological characteristics such as a short action potential, a negative force-frequency staircase, an insensitivity to glycosides and E-C coupling that is relatively dependent on SR Ca release. Furthermore, a number of reports have indicated that the intracellular sodium ion activity (aNa_i) of adult rat ventricle may be unusually high, 28-30 mM, (Szabo and Armstrong, Fed.Proc. 43, 1022, 1984; Wasserstrom, Fed.Proc. 42, 1113, 1983). If resting aNa_i is indeed regulated at such a high level in rat ventricle, this may be expected to have profound effects on cellular Ca regulation and E-C coupling. However, a lower measurement of aNa_i in rat ventricle of 8.5 mM has also been reported (Grupp et al. J.Physiol. 360, 149-160, 1985). We have therefore measured aNa_i in isolated rat and rabbit ventricular preparations maintained at 30°C using Na-selective microelectrodes filled with the liquid ion exchanger ETH 227 and simultaneous measurement of membrane potential via a conventional 3 M KCl-filled microelectrode. Only impalements which did not show a rapid change in aNa_i upon depolarization with 30 mM KCl were considered acceptable (thus excluding unsatisfactory electrode subtractions). In rat, aNa_i in quiescent preparations was found to be 12.7 ± 0.5 (n=13) mM and in rabbit 7.2 ± 0.5 (n=8) mM. Thus, our observations would indicate that aNa_i in the rat appears to be regulated at a level between the high and low values previously reported. The relatively high value of resting aNa_i in the rat heart is likely to contribute to diastolic Ca-loading of this tissue (due to a shift in Na/Ca exchange). Results from rapid cooling contracture experiments have indicated that, in rat ventricle, SR Ca content increases during rest and decreases upon resumption of stimulation (in contrast to rabbit ventricle). This could be explained by a combination of a large Ca release (due to this large Ca load) and the short action potential of the rat which may both lead to enhanced Ca extrusion (via Na/Ca exchange) during contraction.

W-Pos369 **VOLTAGE AND SODIUM DEPENDENCE OF CONTRACTION OF FELINE VENTRICULAR MYOCYTES** R.B. Kleiman and S.R. Houser, Dept of Physiology, Temple University School of Medicine, Philadelphia PA 19140

The voltage and sodium (Na) dependence of contraction of isolated feline ventricular myocytes were evaluated using a discontinuous single microelectrode voltage clamp and a video edge detector to measure myocyte contraction. Membrane potential (Vm) was held at -80 mV to allow calcium (Ca) influx via both low and high threshold channels. In solutions containing 150 mM NaCl, 2 mM Ca, depolarizations to -30 mV elicited small, slow contractions (low threshold) whose magnitude was dependent on voltage step duration, without eliciting slow inward Ca current (Isi). Stepping Vm to +10 mV elicited peak Isi, and large, rapidly rising contractions (high threshold), whose magnitude was also dependent on voltage step duration. In solutions containing 0 Na 135 TrisCl 2 mM Ca, low threshold contractions elicited by steps to -30 mV were similar to those in normal Na. The magnitude of high threshold contractions (steps to +10 mV), however, was not dependent on voltage step duration. The inactivation of Isi was also markedly hastened. We conclude that 1) low threshold contractions result from Ca influx through a non or slowly inactivating channel, and 2) high threshold contractions result from Ca induced Ca release from the sarcoplasmic reticulum (SR) with SR Ca loading in part dependent on extracellular Na, possibly via Na-Ca exchange. (Supported by NIH grants 33921, HL 33648 to SRH and an AHA SEPA grant to RBK.)

W-Pos370 PERCHLORATE CONTRACTURES AND THE 3Na/Ca EXCHANGE MODEL OF t-SR COUPLING. Brian A. Curtis, University of Illinois College of Medicine at Peoria, Peoria, IL 61656.

Perchlorate (8mM) prolongs 20 mM K contractures to upwards of 100 sec at 12 C in comparison to 10 sec duration for 120 mM K contractures with or without ClO_4. Many authors suggest that perchlorate allows the recycling of the t-SR coupling mechanism. I have proposed that depolarization activates the single rotation of a 3Na/Ca exchanger in the t wall to bring a fixed amount of Ca into the t-SR junction to trigger Ca activated Ca release from the SR. Recycling the 3Na/Ca exchanger in perchlorate contractures would bring extracellular Ca into the t-SR space. After a 20 mM K, 8 mM ClO_4, 5 mM Mg contracture had reached plateau tension, flushing with the same solution plus 10 mM EDTA terminated the contracture prematurely in 8 bundles of 2-5 frog twitch fibers by removing the continuing source of trigger Ca. Adding 10 mM EDTA to the contracture inducing solution further shortened the contracture, indeed the time-tension index is the same as in a 120 mM K contracture. In 7 bundles, the ratio of time-tension indexes was 0.86 ± 0.12 which is not significantly ($P<.05$) different than 1; the only Ca available for t-SR transmission is that originally bound to the 3Na/Ca exchanger. These shortened contractures often give 2-4 oscillations in tension. Diltiazem (100 μM) does not alter tension or duration of 20 mM K, 8 mM ClO_4 contractures; voltage gated Ca channels are unlikely to be the source of the continued Ca influx. Supported by American Heart Association, Illinois Affiliate.

W-Pos371 [K^+] and [Na^+] IN MALIGNANT HYPERTHERMIA-SUSCEPTIBLE SWINE. J.R. López[1], M. Dershwitz[2], V. Sanchez[1], and F.A. Sréter[2], [1]Centro de Biofísica y Bioquímica, Instituto Venezolano de Investigaciones Científicas, Apartado 1827, Caracas, Venezuela; [2]Department of Anesthesia, Massachusetts General Hospital and Harvard Medical School, and Department of Muscle Research, Boston Biomedical Research Institute, Boston, MA 02114.

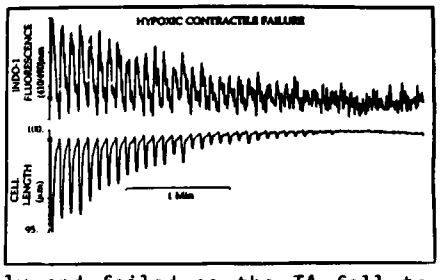
Malignant hyperthermia (MH) is a genetically determined syndrome characterized by hypermetabolic reactions of skeletal muscle. The intracellular free K^+ and Na^+ concentrations were measured in vivo in the superficial fibers of the peroneus longus muscle in anesthetized MH-susceptible and non-susceptible (control) swine. The glass micropipettes were cleaned, pulled, and silanized as described previously (López, et al., *Biophys. J.*, **43**:1, 1983). The K^+-selective microelectrodes were prepared with K^+ resin (valinomycin) from Fluka, and were calibrated before and after each series of measurements in mixed solutions containing varying [K^+] from 50 to 150 mM. The Na^+-selective microelectrodes were prepared using neutral carrier ETH (Fluka), and were calibrated in mixed solutions containing varying [Na^+] from 0 to 116.5 mM. The values of $[K^+]_i$ and $[Na^+]_i$ in control fibers were 108 ± 1.3 mM (mean ± SEM) and 7.5 ± 0.6 mM, respectively, while in MH-susceptible fibers they were 110 ± 1.6 mM and 11.3 ± 1.0 mM, respectively. Thus, no significant differences were found for $[K^+]_i$ in the two populations of swine studied. In contrast, $[Na^+]_i$ was significantly higher in the MH fibers as compared to controls. The increase observed in $[Na^+]_i$ might be related to the elevation in $[Ca^{2+}]_i$ found in MH-susceptible swine (López, et al., *Muscle Nerve*, **9**:85, 1986) through a possible malfunction of Na^+/Ca^{2+} exchange. [Supported by grants from MDA and CONICIT of Venezuela S1-1277 to JRL, NIH Grant GM11656 to MD, and NIH Center Grant GM15904 to the Department of Anesthesia].

W-Pos372 TWO TYPES OF CALCIUM CHANNELS IN FROG SARCOPLASMIC RETICULUM (SR) MEMBRANES. Verónica Irribarra, R. Bull, A. Oberhauser, J. J. Marengo and B. A. Suárez-Isla. (Intr. by M. Núñez). Centro de Estudios Científicos de Santiago, P.O. Box 16443, Santiago 9 and Dept. Physiol. & Biophysics, Fac. of Medicine, University of Chile, P.O. Box 70055, Santiago 7, Chile.

Highly purified SR membranes vesicles isolated from frog skeletal muscle (*C. caudiverbera*) contain two types of Ca and Ba selective channels with distinct pharmacological profiles. SR vesicles were fused into neutral POPE/PC (4:1) or charged POPE/PS (1:1) planar bilayers. The high conductance channel ($InsP_3$-sensitive; Suárez-Isla et al., this meeting) was observed at 15 uM cis Ca. The I/V relationship, linear between -25 to +25 mV in neutral or charged bilayers, was sublinear at V more positive than +25 mV. The direction of the current could not be reversed. Single channel conductance was 100 ± 4 pS with 37 mM trans Ba (mean ± S.E.M.; n = 6) and 103 pS with 37 mM trans Ca and 225 mM HEPES Tris in the cis compartment. Extrapolation of the linear part of the I/V curve gave a pseudo E_{rev} potential of +30.0 ± 1.2 mV (n = 6) (minimum P_{Ba}/P_{Tris} of 9.2). Fractional open time (P_o), was voltage-independent (-40 to +25 mV), but was steeply dependent on the free cis [Ca] (P_o = 0.02 at 10 uM cis Ca and 0.77 at 150 uM Ca; estimated Hill coefficient: 1.6). ATP (1 mM; cis) further increased P_o to 0.94. Calcium activation was reverted by micromolar EGTA. Mg (2 mM) increased the frequency of rapid closures and 8 mM Mg decreased I from 3.4 to 1.2 pA at 0 mV but did not completely block the channel, suggesting a reversible fast blockade. The channel was blocked by 0.5 mM cis lanthanum or ruthenium red (0.5 uM). In addition, ryanodine (50 nM), which binds to the SR vesicles with high affinity (Kd~5 nM), blocked this channel. The low conductance channel (60 pS with 37 mM trans Ca) was insensitive to cis Ca, Mg and ATP and was not activated by $InsP_3$. However, P_o increased significantly upon addition of caffeine (9 mM) to the cis compartment. These findings suggest the existence of two pharmacologically distinct pathways for Ca release from the SR. Supported by NIH Grants GM35981 and HL23007,FONDECYT 598, MDA, DIB 2123 and Tinker Foundation.

W-Pos373 CONTRACTILE FAILURE IN HYPOXIC RAT VENTRICULAR MYOCYTES RESULTS FROM FAILURE OF INTRACELLULAR CALCIUM TRANSIENTS. Howard S. Silverman, Maurizio C. Capogrossi, Harold A. Spurgeon, Edward G. Lakatta, and Michael D. Stern (Intr. by G. Gerstenblith). Gerontology Research Center, NIA, NIH and Johns Hopkins Medical Institutions, Baltimore Maryland

It has not previously been possible to study contractile function in cardiac myocytes during hypoxia. We devised a new instrument, the Laminar Counterflow Barrier Well, which allows the simultaneous measurement of contractility, membrane potential, and intracellular calcium transients in single adult rat ventricular myocytes under conditions of extreme hypoxia. Five cells, loaded with the calcium fluorescent probe Indo-1 AM, and superfused with anoxic glucose-free HEPES buffer were field stimulated at 0.2 Hz. After a lag period of 19±2 min twitch amplitude (TA) fell rapidly to zero. The fall in twitch amplitude was paralleled by the fall in intracellular calcium transients (ratio of fluorescence 410/490 nm). After the failure of stimulated contraction, rapid ejection of caffeine (10 mM) from a micropipette adjacent to the cell, which causes calcium release from sarcoplasmic reticulum (SR), caused vigorous contraction and relaxation. In 6 cells membrane potential was monitored by the "whole cell" patch pipette technique. The AP duration shortened rapidly and failed as the TA fell to zero. TA failure is principally the result of a failure in SR calcium release and not SR calcium depletion or altered myofibrillar calcium sensitivity.



W-Pos374 EFFECT OF REDUCING ACID EFFLUX RATE OF FATIGUED MUSCLES ON THE RATE OF FORCE RECOVERY. J.M. Renaud, Department of Biological Sciences, University of Calgary, Alta. Canada. T2N 1N4

When frog sartorius muscles are stimulated at a rate of one contraction per second tetanic force decreases by about 80-90% in 3 min. Following fatigue development, tetanic force returns toward pre-fatigue levels but the rate of force recovery is a function of pHo (extracellular pH). Muscles exposed to pHo 8.0 and 7.2 recover their tetanic force completely in about 40 and 70 min, respectively. After 90 min of recovery at pHo 6.4, tetanic force is only 50-60% of pre-fatigue level. This inhibition of force recovery in acidic pHo was believed to be an effect on acid efflux rate, so the rate of pHi (intracellular pH) recovery is slower in acidic pHo than in alkaline pHo. Measurements of pHi with pH microelectrodes indeed show reduced rates of pHi recovery at pHo 6.4 when compared to rates at pHo 8.0 and 7.2. Addition of 40 mM L-lactate to the medium bathing muscles, while maintaining pHo at 7.2, causes a 5-fold reduction in the rate of pHi recovery when compared to the rate observed at pHo 7.2 in absence of L-lactate. The rate of tetanic force recovery, on the other hand, is unaffected by the presence of L-lactate. In other words, rates of force recovery is independent of the rate of pHi recovery. It is therefore suggested that the inhibition of force recovery in acidic pHo is due to a direct effect of the extracellular H^+ on the sarcolemma and not an effect of the intracellular H^+ on a step occurring in the myoplasm.

W-Pos375

WITHDRAWN

W-Pos376 End-to-End Joining of Taxol-Stabilized GDP-Containing Microtubules. R.C. Williams, Jr. and L.A. Rone, Vanderbilt University, Nashville, TN 37235.

Incubation with GDP under Mg^{2+}-free conditions, followed by replacement of Mg^{2+}, yields tubulin (without MAPs) with >95% of its E-sites occupied by GDP. In 10 - 50 µM taxol, this tubulin forms long microtubules (MTs). Short pieces of these MTs, produced by shearing, increase in length upon incubation. Observation, at intervals, of samples centrifugally deposited on EM grids and negatively stained showed that mean length increased about 2-fold (e.g., from 7.0 to 14.1 µm) per hour. The rate constant for dissociation of tubulin dimers from the ends of MTs, measured from the rate of interchange of labelled nucleotide between MTs and solvent, was found to be < 4 subunits MT^{-1} s^{-1}. This value is far too small to allow the observed rapid increase in microtubular lengths to be explained by processes involving exchange of tubulin dimers. "Treadmilling" and dynamic instability are ruled out in this system because GTP is absent. The observed length redistribution must therefore occur by an end-to-end joining mechanism such as the annealing described by Rothwell et al. [J. Cell Biol. 102, 619 (1986)]. Our results show that such annealing can happen without the involvement of GTP hydrolysis. On the assumption that MTs diffuse freely, the apparent rate at which joining events occur was calculated at three MT concentrations. The concentration-dependence of this rate was found to be less than second order. Supported by NIH Grant GM25638.

W-Pos377 TOWARD A COMPLETE EQUILIBRIUM DESCRIPTION OF DIVALENT CATION BINDING TO TUBULIN. J.J. Correia and R.C. Williams, Jr., Department of Molecular Biology, Vanderbilt University, Nashville, TN 37235.

The tubulin heterodimer is known to bind two molecules of guanine nucleotide, GTP at the nonexchangeable or N-site and either GTP or GDP at the exchangeable or E-site. GTP binding at the E-site is strongly coupled to Mg^{2+} binding, while GDP binding is Mg^{2+}-independent (Correia, J.J., Baty, L.T. and Williams, R.C., Jr., 1987, J. Biol. Chem., in press). Association constants for Mg^{2+} were determined by competitive binding studies ([^{3}H]-GDP vs. GTP) over a wide range of [Mg^{2+}] to be as follows (assuming $K_{GDP} = 1.6 \times 10^7\ M^{-1}$): $K_{GDPMg} = 2.5\text{-}2.7 \times 10^7\ M^{-1}$, $K_{GTP} < 1.4 \times 10^4\ M^{-1}$, $K_{GTPMg} = 6.4\text{-}9.0 \times 10^7\ M^{-1}$. Other divalent cations are known to substitute for Mg^{2+}. The exchange of Mn^{2+} for bound Mg^{2+} (monitored by atomic absorption and EPR) demonstrates that divalent cations bind to both the E-site and the N-site of tubulin (Correia, J.J., Beth, A.H. and Williams, R.C., Jr., 1987, J. Cell Biol., abstract). GDP•TB (GDP at the E-site) possesses one tight metal binding site and GTP•TB (GTP at the E-site) possesses two tight metal binding sites (K_{Mg}'s = $1.3 \times 10^7\ M^{-1}$; K_{Mn}'s $\cong 1 \times 10^8\ M^{-1}$). Thus ,divalent cations bind to GTP at both the N-site and E-site of tubulin. [Divalent cations bind weakly to GDP at the E-site ($K_{Mg} = 1.1 \times 10^3\ M^{-1}$; $K_{Mn} \cong 9.8 \times 10^3\ M^{-1}$) and to at least 7 nonspecific sites ($K_{Mg} = 106\ M^{-1}$; $K_{Mn} \cong 1 \times 10^3\ M^{-1}$).] The N-site metal exchanges very slowly (hours), suggesting a mechanism involving protein "breathing". The binding and exchange data have been modeled and all the relevant equilibrium constants (pertaining to 0.1 M Pipes, 1 mM DTE, pH 6.9 at 0-4°C) for Mg^{2+} and Mn^{2+} have been determined. (Supported by grant GM 25638 of the NIH.)

W-Pos378 BIREFRINGENCE IN SOLUTIONS OF MICROTUBULES. Anne Hitt, Alan R. Cross and Robley C. Williams, Jr., Dept. of Molecular Biology, Vanderbilt University, Nashville, TN 37235 (Intr. by Michael P. Stone).

Microtubules (MTs), assembled at 37° in vitro from mixtures of tubulin and MT-associated proteins at concentrations >1.3 mg/ml, exhibit macroscopic regions of intense form birefringence, most likely due to their mutual alignment. When MT assembly is initiated by warming the solution, birefringence appears within 5 min of the onset of turbidity and continues to increase for more than 30 min after polymerization has reached a steady state. It decreases, reversibly, by 35% when the temperature is changed from 37° to 25°, although the turbidity changes only 3%. Densitometry of photographs taken with light of 280 nm, corrected for scattering by the use of photographs of the same solution taken at 340 nm, indicates that the birefringent regions are higher in concentration than their surroundings by several tenths of a mg/ml. The solutions yield a highly birefringent pellet and an isotropic supernatant after centrifugation for 10 min at 10,000 g, indicating that the anisotropic domains may sediment intact. Oscillations in birefringence accompany the rapid oscillations in turbidity [Carlier et al., P.N.A.S. 84, 5257-5261 (1987)] seen at concentrations >6 mg/ml in solutions of pure tubulin assembled in 12 mM Mg^{2+}. These observations, taken together, indicate that spontaneous alignment of MTs occurs rapidly under conditions ordinarily employed for their study in vitro. Supported by NIH grants GM29834 and GM25638.

W-Pos379 CHARACTERIZATION OF THE MICROTUBULE-ACTIVATED ATPASE OF MAP 1C, A CYTOSOLIC FORM OF DYNEIN FROM BRAIN. H. S. Shpetner, B. M. Paschal, R. B. Vallee, Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

We have found that the microtubule-associated protein MAP 1C is a microtubule-activated ATPase that can translocate microtubules in an in vitro assay (Paschal et al., J. Cell Biol. 105: 1273, 1987). The direction of movement indicated a likely role in retrograde organelle motility in the cell (Paschal and Vallee, Nature, in press). A number of biochemical properties as well as the morphology and mass of the molecule as determined by scanning transmission electron microscopy (Vallee et al., J. Cell. Biol. abst., in press) indicated that MAP 1C is a cytosolic form of the axonemal enzyme dynein. We have now characterized the MAP 1C ATPase activity in detail. Half-inhibition of the microtubule stimulated Mg^{++} ATPase occurred at 5-10 μM vanadate; the activity was also inhibited 66% by 1 mM NEM and 24% by 1 mM EHNA. Mg^{++} ATP was hydrolyzed 2.4 times faster than Ca^{++} ATP and 11.5 times faster than ATP alone. Microtubule activation was sensitive to ionic strength and sulfhydryl oxidation state. The K_m for microtubules was 0.16 mg/ml with a V_{max} of 186 nmol/min/mg (6.4-fold activation) in the standard assay buffer (20 mM Tris/HCl, pH 7.6, 32 mM KCl) and standard purification conditions (no added DTT). A 30 mM increase in [KCl] caused a 37-fold increase in the K_m for tubulin; inclusion of DTT during purification increased the K_m by 28-fold. The V_{max} was unaffected by either treatment. Although MAP 1C resembled axonemal dynein in its pharmacological properties and substrate specificity, half-saturation of the MAP 1C Mg^{++} ATPase by microtubules occurred at concentrations at least 150-fold lower than those reported for axonemal dynein (Omoto and Johnson, Biochem. 25:419, 1986). We are currently investigating if this is due to intrinsic differences between the two enzymes. Supported by GM 26701 to R.B.V.

W-Pos380 THE NORMAL COORDINATES OF BACTERIOCHLOROPHYLL. Robert J. Donohoe and David F. Bocian, Department of Chemistry, Carnegie Mellon University, Pittsburgh, PA 15213

The normal coordinates of bacteriochlorophyll (BChl) have been calculated via the quantum chemistry force field method of Warshel and Karplus. The prominent features of the resonance Raman spectrum of BChl have been assigned via systematic comparison with the calculated and observed vibrational frequencies of a number of related systems (chlorins, chlorophyll a). Although the observed vibrational energies of the tetrahydro and dihydro pigments are similar, the forms of the normal coordinates are in general quite different.

W-Pos381 INTERMEDIATE STATES IN PHOTOSYNTHETIC ELECTRON TRANSFER Julian Joseph and William Bialek, *Departments of Physics and Biophysics, University of California, Berkeley, CA 94720*

There has been considerable interest of late in the possible role of intermediate states in photosynthetic electron transfer. Essentially all of the discussion has been based on a classical picture in which the role of the intermediate electronic state is to generate some effective matrix element between initial and final electronic states, where this matrix element is to be evaluated at the classical 'transition state' for the reaction; in this picture the activation energy for the reaction is unaffected by the details of the intermediate state. We have begun to explore fully quantum mechanical calculations of the transfer rate in three state systems, hoping to understand in particular whether the commonly used classical expressions for the rate constant can be derived as a systematic approximation to the correct quantum mechanical transfer rate. Classically one can adjust the energetics of the intermediate state to generate an effective matrix element which exactly cancels any small direct hopping from initial to final state; in this way one could in principle generate infinitely long lived charge separation even though one has allowed for electron tunneling. We find that this is not possible quantum mechanically — quantum fluctuations spoil the infinite stability of the classical system. These arguments suggest that there is a quantum limit to the stability of charge separation, and we will explore the possibility that bacterial reaction centers actually reach this limit.

This work is supported in part by the National Science Foundation.

W-Pos382 **DYNAMIC INTERACTIONS BETWEEN THE PRIMARY REACTANTS IN PHOTOSYNTHESIS.** **L. L. Feezel, P. Gast*, U. H. Smith, D. E. Budil, A. L. Morris*, J. R. Norris and M. C. Thurnauer. Chemistry Division, Argonne National Laboratory, Argonne, Illinois 60439 and *Department of Chemistry, The University of Chicago, Chicago, Illinois 60637.** Initial charge separation in photosynthetic bacteria involves the donor bacteriochlorophyll dimer (P_{870}), an intermediate acceptor bacteriopheophytin (I), and the more stable acceptor quinone-iron (Q-Fe) complex. When the iron is decoupled from Q, electron spin polarized (ESP) spectra of $P^+_{870}Q^-$ are observed. Similar ESP spectra have been observed in studies of green plant photosystem I (PSI) and are believed to be due to the primary donor, P^+_{700}, and the reduced acceptor, A^-_1. Interpretation of these ESP spectra through simulations should provide information on the dynamic interactions of the primary reactants and the structure of the photoexcited reaction center. Yet, several interpretations of the published 9.5 GHz ESP EPR spectrum of $P^+_{870}Q^-$ differ on whether $P^+_{870}I^-$ or $P^+_{870}Q^-$ radical pair interactions are the primary source of ESP. Resolution of this question has implications for determining the chemical identities and primary reaction sequence of PSI. We have obtained 35 GHz ESP EPR spectra of iron decoupled Rhodobacter sphaeroides R26 bacterial reaction centers of the following isotopic compositions: ^{1}H-R26, ^{2}H-R26, ^{1}H-R26(^{2}H-Q), ^{2}H-R26(^{1}H-Q). These experiments yield the individual P^+_{870} and Q^- polarization contributions. This information is required to determine the correct mechanism of ESP production. Simulations which include both $P^+_{870}I^-$ and $P^+_{870}Q^-$ radical pair interactions will be presented in comparison with the experimental spectra.

This work was supported by the Office of Basic Energy Sciences, Department of Chemical Sciences, U.S. Department of Energy, under contract W-31-109-Eng-38.

W-Pos383 EFFECTS OF DEUTERATION ON PHOTOSYNTHETIC REACTION CENTERS: RADICAL PAIR RECOMBINATION RATE, TRIPLET ENERGY TRANSFER, AND CAROTENOID TRIPLET EPR SPECTRA. S.V. Kolaczkowski, D.B. Budil, M. K. Bowman and J.R. Norris. Chemistry Division, Argonne National Laboratory, Argonne, Illinois, 60439. We have measured, by optical techniques, the rate of P^+I^-recombination in reaction centers from wild type and R-26 Rhodobacter sphaeroides as a function of small static magnetic fields and temperature. The radical pair ISC rate is decreased by approximately a factor of 2 when both P and I are fully deuterated. Similarly triplet energy transfer from ^3P to spheroidene, mediated by spin-orbit coupling effects, is slowed by about factor of 2 when both P and spheroidene are fully ^2H. The triplet EPR spectrum of fully ^2H spheroidene in RCs at 60^0K shows shoulders on the respective low and high field sides of the low and high field Z peaks (figure 1). These shoulders disappear and the Z peak linewidth decreases as the temperature of the sample is increased (figure 1). Also as the temperature is increased the zero field D value associated with the inner Z peaks increases towards a maximum high temperature value 3.3% larger than the low temperature value (figure 1). This data indicates the possible equilibrium of two spheroidene triplet states in the RC. (EPR conditions: Microwave Freq. 9.16 GHz, Power 5mW; Gain $2.5x10^3$, Mod. Amplitude 8 Gauss, Scan width 1000 Gauss, Scan time 2min, 8 total scans; Lockin Detection: Light Mod. 1000Hz, Sensitivity 100mV, time constant 0.3s)

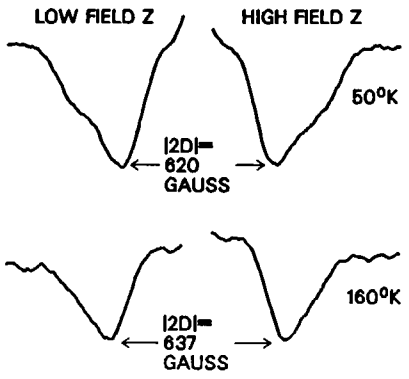


FIGURE 1

Work Performed under USDOE CONTRACT W-31-109-Eng-38

W-Pos384 EFFECT OF EXOGENOUS DY(III) ON THE MICROWAVE POWER SATURATION OF EPR SIGNAL II_S Jennifer B. Innes and Gary W. Brudvig, Yale University, New Haven, CT 06511

The role of EPR Signal II_S ($SigII_S$) in photosystem II (PSII) is still unknown, although $SigII_S$ has been shown to be oxidized by the S_2 and S_3 states and reduced by the S_0 state of the oxygen-evolving complex. Recently $SigII_S$ has been found to arise from Tyr-160 of the D2 polypeptide of PSII (Debus et al., PNAS, in press), but the location of this residue in the membrane has not been established. To obtain information on the location of the site, we have studied the microwave power saturation of $SigII_S$ in the presence and absence of added Dy(III)-EDTA. $SigII_S$ has an unusual temperature dependence of the microwave power at half-saturation ($P_{\frac{1}{2}}$); $P_{\frac{1}{2}}$ is nearly temperature independent between 10 and 70K and then increases as $T^{2.5}$ at higher temperatures. The presence of up to 20mM Dy-EDTA caused only a small increase in $P_{\frac{1}{2}}$, indicating that the Dy(III)-EDTA and the $SigII_S$ site are separated by a fairly large distance. Estimates for the distance from the $SigII_S$ site to the exposed membrane surface were obtained by using myoglobin-NO to calibrate the distance. In thylakoids, where only the outer membrane surface is exposed, the estimate was 24Å. In PSII membranes, with both surfaces exposed, the estimated distance was not significantly different at 23.4Å. In Tris-washed PSII membranes, where polypeptides and Mn have been removed from the inner membrane surface, the distance obtained was 21.4Å. These results support a deeply buried $SigII_S$ site, with access from the inner membrane surface blocked when the extrinsic polypeptides are present. Our results also fit with the expected location of Tyr-160, nearer the inner membrane surface, based on analogy of the D1 and D2 subunits of PSII to the bacterial L and M proteins. This work was supported by NIH (GM32175) and a Heyl Fellowship to JBI.

W-Pos385 PARAMAGNETIC INTERMEDIATES IN THE CATALYTIC CYCLE OF PHOTOSYNTHETIC OXYGEN EVOLUTION R.R. Sharp, A.N. Srinivasan, University of Michigan, Ann Arbor, MI 48109-1055 USA

NMR proton relaxation has been used to monitor in situ the manganese redox chemistry which occurs during the catalytic cycle of photosynthetic oxygen evolution. The acitve site of water oxidation is a cluster of four manganese ions which passes through a sequence of five oxidation states, denoted S_0-S_4, during the catalytic cycle; an ordered passage through these states can be achieved photochemically by illumination with trains of brief (10 USEC) saturating flashes form a xenon source. Flash-induced enhancements in the solvent proton relaxation rate R_1 in suspensions of photosynthetic membranes have been observed and linked to manganese redox chemistry in the S-State cycle. The relaxation transients exhibit kinetic behavior which mirrors the properties of S-State decay. They also respond positively to a variety of chemical treatments known to perturb the manganese center; such treatments include (1) chemical extraction of manganese, (2) agents which accelerate the decay of the higher S-States, (3) agents which alter the physiological S-State cycle. Two manganese redox changes have been detected and linked to the S-State cycle. One S-State transition $S_0 \rightarrow S_1$, is accompanied by the destruction of a strongly relaxing paramagnetic center. Another, $S_1 \rightarrow S_2$ is accompanied by the production of a strongly relaxing center. The $S_2 \rightarrow S_3$ transition shows no evidence of associated manganese redox chemistry. Across a 5-flash cycle, the flash-induced relaxation transients exhibit a period-4 oscillation, characteristic of phenomena arising in the S-States. Efficient relaxation traps are provided by paramagnetic species with large magnetic moments and long electronic relaxation times. Among the common manganese oxidation states, those that are orbital singlets, Mn(II) and Mn(IV), typically have long paramagnetic relaxation times and provide relaxation traps that are much more highly efficient than Mn(III).

W-Pos386 RESONANCE RAMAN SPECTRA OF THE RHODOSPIRILLUM RUBRUM LIGHT HARVESTING PROTEIN AND ITS DETERGENT MODIFIED FORMS Patricia M. Callahan, Paul A. Loach* and Therese M. Cotton, Department of Chemistry, University of Nebraska-Lincoln, Lincoln, NE 68588-0304 and *Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston IL 60201

The 873-nm absorbing antenna protein of Rhodospirillum rubrum can be reversibly modified to two different spectral forms by addition of the detergent octyl glucoside. The modified antenna species display Qy absorption maxima in the near-infrared at 818 and 777 nm. Dilution of octyl glucoside results in the reformation of the antenna complex with a Qy absorption maximum of 870 nm. Soret region (350-450 nm) resonance Raman (RR) spectra have been obtained of the 870-, 818- and 777-nm forms of the antenna protein. The RR spectra of all three antenna forms are indicative of five-coordinate ligation of the central Mg^{2+} ion. The major changes in the vibrational spectra are observed in the carbonyl stretching region and in the 1300-1400 cm^{-1} region where contributions from (Ca-N) stretching vibrations are expected. Because of possible overlap of the acetyl stretching frequencies and ring vibrations in the B818 spectrum, the carbonyl stretching vibrations were identified by varying the excitation wavelength throughout the Soret region. Detergent-solubilized BChl a in both monomeric and aggregate forms were prepared in an effort to duplicate the spectra of the modified LH protein. The optical and vibrational spectra of monomeric BChl a in 5% octyl glucoside reproduce the B777 form well and the carbonyl stretching region of B818 can be mimicked by aggregates of BChl a in 1% octyl glucoside although the absorption maxima do not coincide.

W-Pos387 Determination of the Order of the Four Hemes in the Cytochrome C Subunit of the Rhodopseudomonas viridis Reaction Center. Guillermo Alegria and P. Leslie Dutton. Dept. Biochem./Biophys. Univ. of Pennsylvania, Phila. PA 19104

The X-ray crystal structure of the Rps. viridis RC shows that the four hemes in the cyt. c subunit are arranged linearly (1). Establishing the order of the two low potential and two high potential hemes relative to the RC special pair $(BChl)_2$ is of relevance to the understanding of the mechanism of electron transfer in this and other species. Spectrophotometric and redox potentiometric studies on Langmuir-Blodgett films of oriented monolayers of Rps. viridis RCs has revealed with this system that the four hemes become resolved into four different electrochemical species each with distinct absorption spectra. Redox titrations of the heme -absorption bands, as well as flash-induced spectra obtained at different potentials, yield the following E_m values (mV) at pH8 and α-band max (nm) for the four hemes: a) 340,558, b) 225,556, c) 90,553, and d) -90,551. Differential, linearly-polarized spectroscopy revealed that the hemes are oriented in two angular populations. Each population comprises a high and a low potential heme, paired as (a,d) and (b,c); of the two pairs the (a,d) pair appears more tilted out of the membrane plane. This together with the recognition that the second heme has low potential ligands (1) and may be presumed to be a low potential heme, yields the following ordering: RC-high,low,high,low. This can accomodate two possibilities: RC-a,c,b,d or RC-d,c,b,a. However, kinetic and electrochemical arguments support the former arrangement (2). Refs. 1) Nature 318 19. 2) In Cyt Syst.:Molec.Biol.&Bioen. (Papa ed. 1987). DOE

W-Pos388 USE OF A RESPIRATORY OSCILLATION TO MEASURE THE OPTICAL CROSS SECTIONS OF PSII AND PSI IN ALGAE. N.L. Greenbaum and D. Mauzerall, The Rockefeller Univ., New York, N.Y. 10021.

The optical cross section, σ, of a photosynthetic unit, the equivalent "area" available for photon capture by a reaction center and its associated pigments at a given wavelength, can be determined by the pulsed light-saturation behavior of a signal specific to the photosystem (PS) in question. Plots of the light-saturation curves of PSII activity in Chlorella, Scenedesmus, and isolated spinach chloroplasts, monitored by O_2 production following single-turnover flashes, are the complement of an exponential (Poissonian). PSI was measured by the magnitude of a flash-induced oscillation in the rate of O_2 uptake (Greenbaum et al. (1987) Plant Physiol. 84:879-882). As this oscillation is not exhibited by isolated chloroplasts, it is hypothesized that the signal reflects a transient ATP-mediated inhibition of mitochondrial respiration following initiation of photophosphorylation. The light-saturation curve of this signal in Chlorella is broader than that of O_2 production. Measured at 723 nm, the best fit is achieved by a curve which is the sum of equal contributions of Poissonians with σ = 25 and 3 A^2. In the presence of DCMU, however, the resulting curve is Poissonian with σ = 20 A^2. The plot of O_2 production remains a Poissonian with σ = 2.5 A^2. These data suggest that the observed composite curve in the absence of DCMU represents contributions by both photosystems. The amplitude of the respiratory signal in the presence of the inhibitor is not, however, half that of the control, as would be expected, but remains at its full value. A possible explanation for this behavior is that, in the absence of significant electron transfer from PSII, PSI-dependent ATP production is increased by cyclic photophosphorylation (Arnon and Chain (1975) PNAS 72:4961-4965). This research was supported by NSF DMB 83-16373.

W-Pos389 Na^+ AND LIGHT-DEPENDENT REGULATION OF CYTOPLASMIC pH IN CYANOBACTERIUM SYNECHOCOCCUS 6311. Margaret E. Huflejt, Paul A. Negulescu, Terry E. Machen, Lester Packer. Department of Physiology-Anatomy, University of California, Berkeley 94720

The membrane-permeable acetoxymethyl ester of the pH-sensitive, fluorescent dye 2', 7'-bis (carboxyethyl)-5(6)-carboxyfluorescein (BCECF/AM) was used to measure internal pH in fresh water unicellular cyanobacterium Synechococcus 6311. Fluorescence intensity ratio (excitation 490/439, emission $<$600nm) was converted to intracellular pH by equilibrating pH_o and pH_i using 40μM nigericin/100mM KCl; the pH calibration curve was obtained by titration of pH_o. In light aerobic conditions pH was 7.65±0.1. Removal of 20mM Na^+ caused a transient decrease in pH to 7.30. After acidification with 30mM NH_4Cl/2 min., pH decreased to 6.95 with or without 20mM Na^+. Restoration of pH occurred within 100 sec. with 20mM Na^+ or 300 sec. without 20mM Na^+ in light. In dark, full recovery of pH was absolutely Na^+-dependent. Without Na^+ cells remained acidic; but after illumination, even at low light intensity, complete realkalinization occurred in 100 sec. KCN did not inhibit realkalinization, but inhibitors of PS II (15μM DCMU) and PS I (2μM DBMIB) did inhibit pH recovery by 75%. We conclude that photosynthetic electron transport is mainly responsible for maintaining the internal pH in Synechococcus 6311.
Supported by Office of Biological Energy Research, DOE

W-Pos390 EXOGENOUS REDUCTANTS ACCELERATE THE DECAY OF Z^+ IN PHOTOSYSTEM II REGARDLESS OF THE PRESENCE OF THE 33 KDA POLYPEPTIDE. Curtis W. Hoganson, Gerald T. Babcock and Charles F. Yocum*. Chemistry Department, Michigan State University, East Lansing, MI and *Division of Biological Sciences, The University of Michigan, Ann Arbor, MI.

Among the polypeptides of the oxygen-evolving complex of Photosystem II is a 33 kDa watersoluble polypeptide. It appears to be essential for oxygen evolution activity and for binding the 17 and 23 kDa polypeptides which protect the manganese ensemble from attack by exogenous reductants. The 33 kDa polypeptide appears not to contribute ligands to manganese in the S_1 and S_2 states, as judged by the appearance of the S_2 multiline EPR spectrum and by analysis of EXAFS data. The arrangement of these three polypeptides and the manganese is unknown. One possibility is that the 33 kDa polypeptide might provide a steric hindrance to the reduction of Z^+ by exogenous reductants. To test this possibility we examined the lifetime of Z^+ by EPR as a function of reductant concentration in PSII membrane samples in which the 33 kDa polypeptide was present or absent. Benzidine and manganese chloride were used as reductants. The decay of the Z^+ EPR signal is pseudo first order (within experimental error) and allows calculation of a second order rate constant for the $R + Z^+ \rightarrow R^+ + Z$ reaction. The second order rate constants are not affected by the presence of the 33 kDa polypeptide for either reductant. We conclude that this polypeptide does not shield Z^+ from exogenous reductants. This work was supported by the McKnight Foundation, NIH, the Competitive Research Grants Program of the USDA and the NSF Metabolic Biology Program.

W-Pos391 CHARACTERIZATION OF THE TYROSINE RADICALS THAT ARE REDOX COMPONENTS IN THE OXYGENIC PHOTOSYNTHETIC SYSTEM B.A. Barry, J.J. Byrne, S. Ferguson-Miller†, L. McIntosh†, C.F. Yocum* and G.T. Babcock, Departments of Chemistry and Biochemistry†, Michigan State University, East Lansing, MI 44824, Division of Biology Sciences*, The University of Michigan, Ann Arbor, MI 48109.

Through amino acid labeling of Photosystem II in the cyanobacterium,Synechocystis 6803, we have recently shown that $D^{\bullet}$, the cofactor giving rise to a dark stable EPR spectrum (Signal II), is a tyrosine radical. (B.A. Barry and G.T. Babcock, PNAS, 84, in press.) Here, we present experiments in which the $D^{\bullet}$ tyrosine radical has been specifically labeled with deuterium. These data were obtained with the aim of determining hyperfine couplings constants to specific tyrosine protons. We find that deuteration at the 2,6 position produces little change in the $D^{\bullet}$ EPR spectrum, in agreement with the low spin density found at this position in tyrosine radical model compounds. Deuteration at both the methylene position and at the 3,5 position results in a substantial change in the spectrum. We will also report on the development of a procedure to isolate active Photosystem II preparations from Synechocystis 6803. Such a preparation is a necessary requirement for the definitive identification of another Photosystem II redox component, $Z^{\bullet}$, which is an electron transfer intermediate and which may also be a tyrosine radical. Supported by NIH (GM37300), the Photosynthesis Program of the USDA (CRGO) and the McKnight Foundation.

W-Pos392 INHIBITION OF Q_A^- OXIDATION IN BACTERIAL RCs BY COVALENT MODIFIERS. J. Gao, D.R. Paterson and C.A. Wraight, University of Illinois, Urbana IL 61801

We have examined a number of protein-modifying agents for affects on the activity of the acceptor quinone complex (two-electron gate) in reaction centers from the photosynthetic bacterium Rb. sphaeroides. We observed that high concentrations of DCCD (~1mM) simultaneously inhibit Q_A^- reoxidation and release of Q_BH_2. However, the inhibition by DCCD was reversible upon dilution and was not related to the covalent attachment of DCCD, which occurs at much lower concentrations (<10μM) and specifically labels the L subunit. This labelling pattern is in contrast to earlier observations on R. rubrum chromatophores (1). Various sulfhydryl reagents were also tested and many were found to effectively block Q_A^- reoxidation. The charged, water soluble mercurial, PCMBS, was as effective as neutral reagents. The inhibition was not reversed by dialysis unless a thiol reagent was present, indicating the inhibition to arise from covalent attachment. This is a little surprising considering the known lack of cysteine residues in the immediate vicinity of the quinone binding sites (2). The structural/functional implications of this inhibition will be discussed.

1) Zürrer, H., Snozzi, M. and Bachofen, R. (1983) FEBS Lett 153, 151-155.
2) Allen, J.P., Feher, G., Yeates, T.O., Komiya, H. and Rees, D.C. (1987) Proc. Natl. Acad. Sci. US 84, 6162-66.

Supported by NSF PCM 83-16487 and USDA/CRGO AG 84-1-CRCR-1-1518.

W-Pos393 STRUCTURAL IDENTIFICATION AND ESTIMATION OF ENERGY TRANSFER RATES AND SPECIES-ASSOCIATED EMISSION AND ABSORPTION SPECTRA FROM TIME-RESOLVED FLUOROMETRIC DATA. Ralph Weidner and Solon Georghiou (Intr. by P. P. Constantinides), Biophysics Laboratory, Department of Physics, University of Tennessee, Knoxville, TN 37996.

Compartmental modeling is commonly used to simulate total and polarized fluorescence decay in the presence of excited-state interactions. However, the reverse process of extracting estimates for all model parameters from data (experimental or simulated) has only been accomplished in isolated instances because of the largely unsolved problem of structural identification. Using global principles we construct a general approach to structural identification and parameter estimation for fluorescence decay models. Special attention is paid to energy transfer in macromolecules, which does not depend on an independent (global) variable in the simple way that processes such as proton transfer and excimer formation depend on pH, temperature or concentration. As an example, the three decay rates, six energy transfer rates and species-associated spectra for C-phycocyanin from Synechococcus 6301 are extracted from total decay data simulated from recently reported results. This approach can also be applied to fluorescence decay in the absence of excited-state interactions to resolve emission and absorption spectra that may completely overlap and to obtain more accurate parameter estimates than those possible by previously introduced global methods.

W-Pos394 QUINONE RECONSTITUTION IN PHOTOSYSTEM 2 MEMBRANES. Gerard P. Palace and Joseph T. Warden, Department of Chemistry, Rensselaer Polytechnic Institute, Troy, NY 12180-3590.

We have documented recently that the principal loci of fatty acid inhibition in photosystem 2 are quinone binding sites associated with the reaction center. Spectroscopic data are consistent with the hypothesis that long-chain, unsaturated fatty acids displace competitively endogenous quinone from quinone-binding peptides (J.T. Warden and K. Csatorday, *Biochim. Biophys. Acta* 890 (1987) 215-223). In support of this hypothesis, we have been able to restore stable photochemistry in digitonin photosystem 2 preparations, as evidenced by $P_{680}^+Q_a^-$ formation, through reconstitution with trimethylbenzoquinone or duroquinone. Restoration of reaction-center photochemistry has been achieved in varying degrees utilizing other substituted benzoquinones as well.

The reconstitution yields for exogenous quinone have been found to be highly dependent on quinone uptake into the photosynthetic membranes, with the degree of incorporation into the membrane dependent on the structure of the quinone. At pH 6.5, the relative extents of incorporation of selected quinones are observed to be 2,5-dichloro- >1,4-anthra- >1,4-naphtho- >ubi- >plasto- >phyllo- >cumo- >duro- >2,5-dimethyl- >benzoquinone. Partitioning of substituted benzoquinones as well as prenylquinones between the aqueous medium and the membrane has been studied with a variety of photosystem 2 preparations; the extent of uptake is highly influenced by the experimental conditions used. For example, an increase in uptake has been shown to result from use of small or highly charged buffers at high molarity and pH, high quinone concentrations and minimal amounts of glycerol or detergent in the medium. At optimal conditions, maximal levels of incorporation are reached after only short incubation periods (circa 30 minutes). The free energies of partition have been obtained for trimethylbenzoquinol (-4.75 kcal/mol) and trimethylbenzoquinone (-4.82 kcal/mol); these values are comparable to that determined for ubiquinone-0 (-4.5 kcal/mol) partitioning into a phospholipid bilayer (Fato *et al.*, *Biochemistry* 25 (1986) 3378-3390).

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W-Pos395 Requirements for Function and Binding at the Q_B Site in Rb. Sphaeroides. Kathleen M. Giangiacomo, M.R. Gunner and P.Leslie Dutton. Intr. by John R. Williamson, Dept. Biochem./Biophys. Univ. of Pennsylvania, Phila. PA 19104

Failure to reconstitute activity in the Q_B site of RCs with anything other than tailed, ubiquinone-like quinones has led to the view that the Q_B site is specific. However, the apparent failure of other quinones to bind and function at the Q_B site can largely be ascribed to a small-$\Delta G°$ between Q_A and Q_B($\ln K_D' = \ln K_D + \Delta G°/RT$ where K_D' and K_D are the apparent and true dissociation constants). We have overcome this problem by providing a more favorable-$\Delta G°$ by a) replacing the native UQ_{10} of Q_A with lower potential 9,10-AQ or b) testing higher potential Q_B candidates. For most of the compounds tested, we have measured Q_A to Q_B electron transfer kinetics ($t_{1/2}$=50-500μsec) and rates of binding ($k_{on} \sim 10^7 M^{-1}s^{-1}$). The results reveal: 1) a hydrophobic tail is not required. 2) The site functions with quinones containing up to three rings (e.g. 1,4-BQ=3mM; 1,4-NQ=50μM; 9,10-phenanthraQ=100μM). 3) The site will accept a variety of substituents on the quinone rings (e.g. 2-methyl-BQ=50μM; 2-methoxy-BQ=110μM; 2,3-dichloro-1,4-NQ=2μM). 4) The para-carbonyl is not obligatory (e.g. 1,2-NQ=50μM; 3,5-di-t-but-1,2-BQ=10μM). 5) The dependence on hydrophobicity for the methyl substituted benzoquinones is weak: $-\log K_D'/\log P = 0.3$; cf. the Q_A site where $-\log K_D/\log P = 2.0$. By recognizing restrictions caused by a small $-\Delta G°$ we have demonstrated that the Q_B site has a broad specificity for quinone binding. This provides the opportunity for detailed studies into factors that govern binding, electrochemistry and catalysis at the Q_B site. NSF DMB.85-18433, NIH GM.27309

W-Pos396 AFFINITY OF VARIOUS QUINONES FOR THE Q_A AND Q_B SITES IN REACTION CENTERS OF RHODOBACTER SPHAEROIDES R26 IN SOLVENT HEXANE. K.WARNCKE, B.S.BRAUN, P.L.DUTTON (INTRO. BY J.M.VANDERKOOI) DEPT. OF BIOCHEM. AND BIOPHYS., UNIV. OF PENN., PHILA., PA 19104

Dissociation constants (K_D^X, where "X" refers to the mole fraction standard state) of various quinones at the Q_A and Q_B sites have been determined in hexane with reaction centers (RC) solubilized in inverted phospholipid/detergent micelles. Protein-ligand affinities ($-\log K_D^X$) are thus determined in the absence of large, non-specific hydrophobic bonding contributions. In this work, we: (A) assesss the validity of ligand logP(hexane/water) values as estimates of these contributions, and (B) apply these concepts to identify specific quinone-RC interactions. Aqueous and hydrocarbon phase binding affinities are ideally related as: $-\log K_D^X(hc) = -\log K_D^X(w) - \log P(hc/w)$. Only BQ ($-\log K_D^X(hc) = 2.6$), which exhibits no preference for either phase (logP=-0.1), obeys the relation. All other quinones tested (logP>1) bind about 10x weaker than predicted, indicating that logP does represent the differential hydrophobic bonding contribution when affinities are compared. For example, in the aromatic quinone series, NQ and AQ exhibit identical hexane phase affinities ($-\log K_D^X$=3.8; predicted=4.8). The affinity increment of 1.2 for AQ over NQ in water thus arises totally from hydrophobic bonding because $\Delta \log P(AQ-NQ) = 1.2$. Hexane binding affinities of the ten-member isoprenoid-UQ0 series reveal the tail's role in the physiological quinone-protein interaction. At the Q_A site, only the first two units contribute (UQ_0=7.0; UQ_1=7.5; UQ_2=8.4). Affinity for UQ_{10} (6.7) is lower than for UQ_0. These results reveal that in vivo: (1) the tail makes no net affinity contribution; (2) head group binding energy is extremely important; (3) specific interactions occur in the binding domains of the first two isoprenoid units. Supported by NSF grant DMB 85-18433.

W-Pos397 **DIRECTED MUTAGENESIS OF QUINONE BINDING SITES IN *Rhodobacter capsulatus*.** William J. Coleman, Edward J. Bylina and Douglas C. Youvan, Department of Applied Biological Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139 U.S.A.

Analysis of the *Rhodobacter viridis* photosynthetic reaction center complex by X-ray crystallography has indicated that histidine 217 (H217) of the M subunit is situated between the Q_A and Fe binding sites. In order to examine the role of this histidyl residue in electron transfer, we have introduced mutations at this position using oligonucleotide-mediated site-directed mutagenesis in a related bacterium, *Rhodobacter capsulatus*. We have generated a number of mutants at H217 by this technique, including aspartic acid, glutamic acid, asparagine, glutamine, cysteine, methionine, alanine and leucine. Preliminary biophysical characterizations of these mutants will be presented.

W-Pos398 MECHANISM OF BICARBONATE ACTIVATION OF PLASTOQUINONE REDUCTION IN PHOTOSYNTHESIS
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Our current model for the role, in vivo, of HCO_3^- in the intersystem electron transport is:(1)HCO_3^- is the active species and is an essential requirement;(2)there are, at least, 2 high-affinity sites of HCO_3^- binding: one, presumed to be a ligand to Fe^{2+} (in Q_A-Fe-Q_B complex) and to H-bond with an aminoacid residue (possibly HIS) on the D_2 protein, may create a salt bridge necessary for the functional configuration of the reaction center; and a second, possibly bound to ARG in D_1, is involved in protonating a HIS near the Q_B site to stabilize the negative charge on Q_B^- -- rapid exchange of resulting CO_3^{2-} with another HCO_3^- ensures irreversibility of the protonation reaction;(3)a third, low affinity HCO_3^- binding site may exist as an intramembrane pool of HCO_3^- to create a H^+ buffering domain; (4)low pH and high salt, both necessary for effective HCO_3^- depletion, are suggested to disrupt the HCO_3^- salt bridge between Fe^{2+} and D_2; the resulting conformational change exposes the intramembrane HCO_3^- pool to bulk phase, and impairs plastoquinone (PQ) binding. The above model is supported by: (a) restoration of electron transport in HCO_3^- - depleted thylakoids is dependent on equilibrium [HCO_3^-]; CO_2, H_2CO_3 and CO_3^{2-} have no direct involvement;(b) in thylakoids, well depleted of HCO_3^- (residual activity<7% of the fully restored rate), the activity vs. [Chl] was non-linear after addition of 1/2 saturating [HCO_3^-] indicating the presence of some endogenously bound HCO_3^- in the depleted membrane;(c) with a conservative correction for the endogenous [HCO_3^-], the double reciprocal plot of activity vs. [HCO_3^-] is non-linear suggesting 2 cooperating sites (>1.4);(d) an azido analog of PQ appears to bind less after removal of HCO_3^- (data obtained in collaboration with C.A. Yu). We thank NSF PCM 83-06061 for support.

W-Pos399 SEQUENTIAL HETERO-STIMULATION OF DELAYED LUMINESCENCE: FEATURES AND SIGNIFICANCE.
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The recombination following light-induced transmembranal charge separation in photosynthetic systems results in the emission of delayed luminescence (DL) containing structural and functional information by virtue of its dependence on photosynthetic and membrane-related variables. In chloroplasts, imposition of a transmembrane electrochemical potential (e.g., by salt + ionophore or acid injection to the suspension) as well as a number of other treatments (organic solvent addition, temperature-jump) can significantly increase DL, giving rise to stimulated delayed luminescences (SDL). Their quantitative study is hampered by (a) slow perturbation kinetics, (b) lasting physicochemical modification of the system by the stimulus and (c) complicated relationships between the light-induced precursors of DL and SDL. The use of external electric fields to stimulate DL answers problems (a) and (b), and the resulting emission (termed electrophotoluminescence or EPL) has already yielded valuable information. To investigate the relationship between various precursor pools, we stimulated a preilluminated suspension of chloroplasts by one of the SDL-inducing methods listed above, preceded (or not, for comparison) by an electric field stimulation which - upon removal - leaves the sample unchanged except for precursor depletion. The main findings for the time-integrated emission intensities were as follows: (1) diffusion-potential (KCl + valinomycin) - induced SDL was decreased by a preceding EPL by the very amount of EPL, strongly suggesting common precursors; (2) the methanol-induced and temperature-jump induced SDL were also decreased by a preceding EPL, but their quantitative relations were less clear.