Th-AM-MinII-1 THE BIOPHYSICAL BASIS OF EYE STRUCTURE AND TRANSPARENCY

Vision requires light to traverse the eye and be focused on the retina. In order to reach the retina, light passes through very specialized tissues of the eye, including the cornea, the lens, and the vitreous. Each of these tissues has specific functions: the cornea, at the outer surface, has a focusing, protective, and structural role; the lens focuses light on the retina; and the vitreous maintains an optical path between the lens and the retina which is free of cellular material and low in plasma proteins. All of these tissues must be transparent so that light may pass through them unhindered and reach the retina. Elegant biophysical studies have been carried out to elucidate the structure of the components in each tissue, the organization of the components, and the way in which this organization results in their function and transparency. Light scattering studies on the cornea, x-ray crystallographic and scattering investigations on lens crystallins, and spectroscopic and enzymatic susceptibility studies of vitreous hyaluronic acid will be presented in this symposium. These biophysical studies provide a basis for understanding the structural alterations which result in cataracts and opacity of the cornea, and for clinical applications such as vitreous replacement.

Th-AM-MinII-2 THE SUPRAMOLECULAR ORGANISATION OF THE VERTEBRATE EYE LENS: BIOCHEMICAL, X-RAY AND COMPUTER MODELLING OF CRYSTALLINS AND THEIR INTERMOLECULAR INTERACTIONS, Tom Blundell, Huub Driessen, Peter Lindley, Daruka Mahadevan, Linda Miller, David Moss, Sangari Mylvaganam, Christine Slingsby, Lesley Summers and Helen White, Laboratory of Molecular Biology, Department of Crystallography, Birkbeck College, University of London, Malet Street, London WC1E 7HX, UK

The high resolution X-ray analysis of γ-II crystallin has shown that it is a highly symmetrical, bilobal molecule constructed from four similar polypeptide motifs. The structure forms the basis for computer graphics modelling of the various homologous γ- and β-crystallins, the sequences of which are being obtained from cDNA clones and include some from human sources. These allow suggestions to be made concerning the distribution of groups which may effect solubility in the lens, such as ion pairs and surface hydrophobic regions. They also indicate unusual arrangements of sulphydryl groups, which may lead to disulphide bond formation with various catastrophic consequences, possibly associated with cataract formation.

The sequences of the homologous β-crystallins show that NH_2- and COOH- extensions play important roles in oligomer formation and membrane interactions. The gene structure, with its arrangement of exons, reflects the tertiary structure and suggests that the assembly of these complex molecules in evolution occurred in a modular fashion.

Preliminary biophysical and crystallographic studies of the avian δ-crystallins indicate that this is an α-helical protein with rather different properties from the mammalian γ-crystallins.

Th-AM-MinII-3 TRANSPARENCY OR OPACITY OF THE EYE LENS: ROLE OF THE SPATIAL ORGANISATION OF CRYSTALLIN PROTEINS. Annette Tardieu*, Mireille Delaye+, Dominique Laporte+ and Tadeusz Gulik-Krzywicki*, *Centre de Génétique Moléculaire, CNRS, 91190 Gif sur Yvette, +Laboratoire de Physique des Solides, Bât. 510, Université Paris-Sud, 91405 Orsay, France.

In its normal state, eye lens is transparent despite the presence in the cell cytoplasm of high concentrations of lens-specific proteins, the crystallins. They provide the high refractive index necessary for image formation, but could be expected to scatter an important part of the incident light, leading to lens opacity. It was recognized early on that the explanation for eye lens transparency had to be sought in part in the spatial correlations between individual scatterers.

Scattering techniques and, in particular, small angle X-ray scattering are well suited for the analysis of the spatial organisation of proteins. A study of calf lens cytoplasm by both light and X-ray scattering has shown that a short range, liquid-like or glass-like order of the crystallin proteins accounts for eye lens transparency. A quantitative relationship between protein concentration and transparency was obtained: lens transparency is minimum around 150mg/ml and then improves with increasing concentration, in agreement with simple physical models of short range order. Model refinement is now being attempted with a more homogeneous system of concentrated solutions of α crystallins. We are also currently investigating model systems where perturbations of the spatial organisation of the proteins -aggregate formation or domain segregation, e.g. cold cataract phenomena- lead to opacity. The experimental results suggest possible mechanisms for cataract formation.

Th-AM-MinII-4 SPECTROSCOPIC INVESTIGATIONS OF CALF NUCLEAR γ-CRYSTALLINS. K. Mandal, S.K. Bose and B. Chakrabarti, Eye Research Institute, Boston, and R. Siezen, Dept. of Physics, MIT, Cambridge, MA

α-, β- and γ-crystallins are water-soluble protein fractions of the vertebrate eye lens. Subfractions of γ-crystallins (γ-II, γ-III and γ-IV) were isolated from the nuclear portion of calf lenses. The amino acid sequence in these subfractions are closely related, and consequently are expected to have similar folding of the peptide backbone. Circular dichroism (CD) and fluorescence studies, however, show some distinct differences in their chiroptical and emission properties. The emission maxima of these proteins (λ_{ex} = 285 nm) due to tryptophan residues appear at 324, 329 and 334 nm for γ-II, γ-III and γ-IV, respectively; upon denaturation with 6M GuHCl, the maxima shift to 344 nm for γ-II and 350 nm for γ-III and γ-IV. The emission band due to tyrosine is less prominent in denatured γ-II than in the other two γ-crystallins. The fluorescence results indicate that the Trp residues in γ-II are buried in hydrophobic environments and are most exposed in γ-IV. The far-UV CD spectra of all these proteins are essentially of the β-conformation type, with some subtle differences. However, the near-UV CD spectra differ in position, magnitude and sign of Trp and Tyr transitions. In addition, a CD maximum at 235 nm, presumably due to interactions of tyrosine residues and peptide backbone, is seen for γ-III and γ-IV but is insignificant in γ-II. This study shows that the environment and interactions of the aromatic residues of these subfractions are different. These differences may be significant, in terms of interactions of these γ-crystallins with each other and with α- and β-crystallins, for understanding lens transparency and opacity.

Th-AM-MinII-5 MICROARCHITECTURE OF THE VITREOUS: STRUCTURE AND FUNCTION OF HYALURONATE. Mary K. Cowman. Dept. Chemistry, Polytechnic Institute of New York, Brooklyn, NY 11201.

The adult human vitreous is a nearly acellular transparent solution phase, with two morphologically defined regions. The central portion of the vitreous, termed the liquid vitreous, is a collagen-free matrix of hyaluronate (HA). The vitreous outer portion, termed the gel vitreous, is a network of collagen fibers with interspersed HA. The microarchitecture of the collagen - HA matrix provides a barrior to the movement of cells and protein into the vitreous, and may play a role in protection of the retina from mechanical shock. Within this matrix, HA is proposed to function as a molecular filter and collagen network stabilizer.

The solution conformation and interactions of purified and liquid vitreous HA have been investigated by several biophysical and biochemical approaches. Purified oligosaccharide fragments of HA have proven useful in the development of structural correlations for CD and NMR data, and in the analysis of self-association phenomena. Small oligosaccharide fragments match polymeric HA in details of the substituent acetamido group orientation and environment in aqueous solution. Larger oligosaccharides are required to adequately model properties related to the polyelectrolyte character of HA. Oligosaccharides as well as hydrolytic enzymes have also been employed as biochemical probes of HA structure, in order to compare native vitreous HA conformation and organization with preparations of purified HA to be used in surgical applications.

(Supported by NIH Grant EY 04804)

Th-AM-MinII-6 LIGHT SCATTERING AND CORNEAL STRUCTURE

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The cornea is the clear front covering of the eye through which we see and is composed of collagen fibrils embedded in an optically homogeneous ground substance. It has long been recognized that these fibrils scatter light and that transparency results from interference effects due to an ordering in the spatial arrangement of the fibrils about one another. This presentation discusses measurements of the angular and wavelength dependence of corneal light scattering that support the underlying assumption that the collagen fibrils are indeed the primary scatterers in the normal cornea. Moreover, in agreement with electron micrographs, measurements of the wavelength dependence of total scattering suggest that the fibrils are disposed about one another with short-ranged order. The second part of this presentation discusses how small angle light scattering (SALS) and polarization effects can be used to deduce large-scale structural information, particularly about the cornea's lamellar structure. We used SALS and electron microscopy to show that the waviness in the collagen fibrils, which occurs when their tension is relaxed by reduced intraocular pressure, is the morphological feature responsible for the cross-polarized (I+) SALS patterns. Measurements of the cornea's birefringent properties, together with SALS measurements, provide further insight into fibril optical properties and fibril arrangement. Such experiments suggest that the fibrils are optically anisotropic with their polarizability axis at 45° to their geometric axis and that the fibrils have preferential orientations in the cornea.

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Th-AM-A1 MUTATIONS IN THE β-SUBUNIT OF E. COLI F_1-ATPase WHICH AFFECT THE CATALYTIC SITES. T.M. Duncan and A.E. Senior, Box 607 Biochemistry, University of Rochester Medical Center, Rochester, NY 14642.

The three β subunits of the F_1-sector of the E. coli membrane H^+-ATPase are encoded by the uncD gene and are thought to contain the catalytic sites. The uncD412 and uncD484 mutant strains each produce F_1 which is of normal molecular size and subunit composition, but which is catalytically defective (ATPase S.A. = 20% and 0.4% of normal, respectively). The mutant F_1-ATPases were studied first under conditions which allow only one catalytic site to bind and hydrolyse ATP ("unisite hydrolysis", 0.3μM MgATP plus 1μM F_1). Each had a slower ATP association rate and faster dissociation rate than normal (K_D ATP was: unc$^+$, 5 x 10^{-10}M; uncD412, 10^{-8}M; uncD484, 9 x 10^{-8}M). The uncD412 F_1 behaved normally in that it showed a rapid equilibrium between unisite-bound ATP and Pi product, and unisite turnover rate was limited by product dissociation; however uncD484 F_1 showed bound ATP but no bound Pi during net unisite hydrolysis. To study catalytic cooperativity, excess ATP was added so that binding to two or more catalytic sites per F_1 occurred. In normal and uncD412 F_1, this promoted rapid hydrolysis at, and product release from the first catalytic site, with cooperative multisite turnover being 5 x 10^4-fold (unc$^+$) or 9 x 10^3-fold (uncD412) faster than unisite turnover. In marked contrast, in uncD484 F_1, excess ATP induced rapid release of ATP bound at the first catalytic site, and multisite turnover was <400-fold faster than unisite turnover. Therefore both of the uncD mutations affect intrinsic properties of the catalytic site; additionally the uncD484 mutation causes a severe defect in cooperative interactions between catalytic sites.

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Th-AM-A2 FUNCTIONALLY DISTINCT β SUBUNITS IN F_1-ATPASE. Jui H. Wang, Bioenergetics Laboratory, Acheson Hall, State University of New York, Buffalo, NY 14214.

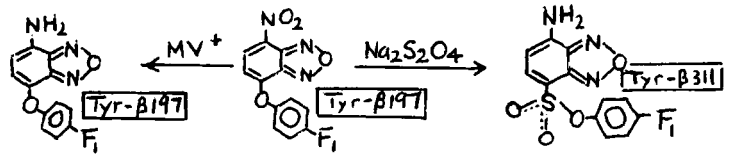
Kinetic data on the catalytic hydrolysis of ATP by F_1-ATPase have often been interpreted by the well-known alternating sites model based on the presence of 3 or 2 strongly interacting but functionally equivalent β subunits in the enzyme molecule. Although there is convincing evidence for strong interaction between the β subunits, direct evidence is still needed to show that the active sites on the three β subunits indeed catalyze alternately during the steady state hydrolysis of ATP. One way to test the assumed functional equivalence of the three β subunits is to inhibit F_1 almost completely by labeling with NBD-Cl until the label to F_1 molar ratio is nearly equal to 1, subsequently making the subunits rearrange through partial dissociation and reassociation, and then measuring the ATPase activity of the reassociated enzyme relative to that of the control F_1. These experiments have been performed. It was found that ATPase activity of covalently labeled F_1 with approximately 1 NBD-label per F_1 could be increased from 1.48 to 18.0 μmol ATP $min^{-1}mg^{-1}$ without losing the covalent label by treatment with buffer containing 3 M LiCl at 0°C and subsequent removal of the salt by centrifugal gel-filtration at 25°C in the presence of 5 mM ATP. The experimental results seem incompatible with models for F_1-ATPase with either 3 or 2 equivalent alternating catalytic sites, but are consistent with the model with one active catalytic site and 2 interacting regulatory sites. Similar partial dissociation-reassociation experiments with unlabeled F_1 in the presence of β or NBD-β show that NBD-β can replace β in F_1 and cause a significant decrease in the specific activity of the ATPase.

Th-AM-A3 EVIDENCE FOR CONFORMATION CHANGE AT THE CATALYTIC SITE OF F_1-ATPASE INDUCED BY ADP BOUND TO REGULATORY SITE(S). Lyn M. Celenza and Jui H. Wang, Bioenergetics Laboratory, Acheson Hall, State University of New York, Buffalo, NY 14214.

The fluorescence emission spectra of bovine heart F_1-ATPase (MF_1) labeled with NBD-Cl have been examined as functions of the concentration of nucleotides. ADP causes a large decrease in the fluorescence of (N-NBD)$_{0.75}$-MF_1, which is presumably not due to quenching by the adenine group but due to ADP-induced protein conformation change (Ferguson et al.(1975) Eur. J. Biochem. 54, 127-133) at the labeled active site, because at the same ligand concentration ATP causes a slight increase in the fluorescence of the labeled enzyme. This observation also shows that inhibition of ATPase activity by the N-NBD-label is probably not due to the prevention of necessary protein conformation change by the label, but due to the labeling of an essential amino group that is required for the catalysis. Since the 0.75 N-NBD-label per MF_1 is almost entirely on the active catalyic subunit β', the stoichiometric formula of this particular N-NBD-labeled enzyme may be written as $\alpha_3\beta''_2[(\text{N-NBD})_{0.75}\beta']\gamma\delta\varepsilon$, where β''_2 represents the two regulatory β subunits. Most of the fluorescence decrease occurs in the range from [ADP] = 0.05 to 1 mM, which corresponds to a dissociation constant of $K_{ADP} \approx 0.2$ mM. Since this value is 2 to 3 orders of magnitude higher than that for the first ADP-binding site of MF (Grubmeyer et al.(1982) J.Biol.Chem. 257, 12092-12100), it may be concluded that the ADP which is responsible for the major portion of the observed fluorescence decrease in $\alpha_3\beta''_2[(\text{N-NBD})_{0.75}\beta']\gamma\delta\varepsilon$ is not bound to the active catalytic site on β' which carries the fluorescent probe, but to the second and/or third site(s) on the two β" subunits which do not carry the probe. These results show for the first time that the exchange of ADP for ATP or vice versa at the regulatory site(s) in β" can cause protein conformation change at the active catalytic site in β', & in this way regulate the catalysis.

Th-AM-A4 EVIDENCE FOR THE TRANSFER OF RADIOACTIVE LABEL FROM TYR-β197 TO TYR-β311 DURING THE REDUCTION OF [^{14}C]NBD-LABELED F_1-ATPASE WITH HYDROSULFITE. Vijay Joshi and Jui H. Wang, Bioenergetics Laboratory, Acheson Hall, State University of New York, Buffalo, NY 14214.

Identification of the single essential Tyr residue in F_1-ATPase labeled by [^{14}C]NBD-Cl (4-chloro-7-nitro-[^{14}C]2,1,3-benzoxadiazole) was hampered by the lability of the radioactive label. This label could be stabilized by reducing its nitro group to amino group, but unfortunately powerful reducing agents are often strong nucleophiles which could also displace the label from the initial Tyr residue before it is stabilized by reduction. By reducing O-[^{14}C]NBD-F_1 with the strongly reducing but almost non-nucleophilic methyl viologen radical (MV^+), subsequently cleaving and sequencing the product, it was found that the radioactive label was initially on Tyr-β197(Ho and Wang,(1983) BBRC 116, 599-604). However, reduction of O-[^{14}C]NBD-F_1 with $Na_2S_2O_4$ and subsequent sequencing showed that the reduced label was on Tyr-β311 (Andrews et al. (1984) JBC 259,8219-8225). The difference in chemical stabilities and spectra of the two reduction products suggest that the radioactive label might have been transferred from Tyr-β197 to the nearby Tyr-β311 during hydrosulfite reduction as shown below:



Accordingly, we have synthesized [^{35}S] hydrosulfite, used it to reduce non-radioactive NBD-F_1 and subsequently isolated ^{35}S-labeled F_1-ATPase with a label/F_1 molar ratio of 0.8 (supported by Grant GM31463 from NIGMS).

Th-AM-A5 FLUORESCENT ANALOGUES OF DCCD AS STRUCTURAL PROBES OF THE MITOCHONDRIAL PROTON CHANNEL IN H^+-ATPASE: A PARAMAGNETIC QUENCHING STUDY. M.J.Pringle and M.Taber, Department of Cell Physiology, Boston Biomedical Research Institute and Department of Biological Chemistry, Harvard Medical School, Boston, Mass., 02114.

N-Cyclohexyl-N'-(4-dimethylamino-α-naphthyl) carbodiimide (NCD-4) and N-cyclohexyl-N'-pyrenyl carbodiimide (NCP) are two novel fluorescent analogues of the mitochondrial inhibitor, dicyclohexylcarbodiimide (DCCD). Although non-fluorescent in aqueous media, both compounds form fluorescent conjugates with mitochondrial electron transport particles (ETP_H) or the purified, lysolecithin-extracted H^+-ATPase. DCCD prevents the reaction of ETP_H with both NCD-4 and NCP. The fluorescent probes are effective inhibitors of ATPase activity and ATP-driven membrane potential, though their reaction rates are considerably slower than that of DCCD. Energisation of ETP_H by NADH is unaffected by any of the diimides.

The fluorescence of NCD-4- or NCP-treated H^+-ATPase is quenched by paramagnetic stearic acid nitroxide derivatives (xNS) in the order 16NS >12NS >7NS ≅5NS, but not by I^- ions. The carboxyl group reactive to the diimides would therefore seem to be accessible to the membrane lipids. Furthermore, the quenching efficacy of the spin labels suggests that this group is located about 12 A from the membrane surface. The quenching behaviour of 16NS is consistent with a mixed population of accessible and inaccessible fluorophores where the latter fraction comprises ~ 18 percent (one out of the six proteolipids) of the total. While these are preliminary results, they do establish that the above fluorescent probes can provide useful structural information on the proton channel of the mitochondrial H^+-ATPase. [Supported by NIH GM 31416]

Th-AM-A6 DIFFERENTIAL SCANNING CALORIMETRY AND KINETICS OF LOCAL ANESTHETIC EFFECTS ON F_1ATPase. G. Vanderkooi, A. B. Adade, and G. C. Kresheck, Department of Chemistry, Northern Illinois University, DeKalb, Illinois 60115.

F_1ATPase, prepared from beef heart submitochondrial particles (SMP) by the chloroform extraction method, has a single sharp endothermic peak as observed by DSC at 80.5°. The half height peak width is 2.0° and the calorimetric transition enthalpy is 19.2 cal/g protein or 6800 kcal/mole, but the van't Hoff enthalpy determined by peak shape analysis is only 470 kcal/mole. The transition is irreversible and does not follow a two state transition model, as evidenced by the large difference between the calorimetric and van't Hoff enthalpies. Tetracaine and dibucaine shift the DSC peak to lower temperatures; 1.0 mM tetracaine gives a peak at 70°, and 0.3 mM dibucaine gives a 72° peak, without peak broadening. These same anesthetic concentrations cause reversible inhibition of enzyme activity at 25° (Chazotte et al. (1982) Biochim. Biophys. Acta 680, 310), and protect the enzyme from irreversible cold inactivation at 0°. Kinetic analysis of ATPase activity as a function of ATP and anesthetic concentration shows that the inhibition is of the partial noncompetitive type. We interpret these DSC and kinetic results to mean that local anesthetics act as protein conformational perturbants of F_1ATPase, on the tertiary or quaternary organizational level. Differential scanning calorimetry of SMP also revealed a sharp endothermic peak at 75°, with a half height peak width of 1.8°. Since this peak is also shifted to lower temperatures by dibucaine and tetracaine in a similar manner as F_1ATPase, we provisionally conclude that the 75° peak is due to membrane-bound ATPase.

Th-AM-A7 STRUCTURAL STUDIES OF F_1-PART OF PROTON TRANSLOCATING ATPASE AT 3.5 Å RESOLUTION. L.M. Amzel, P. Narayanan, T.B. O'Toole, P.L. Pedersen and X. Ysern, Department of Biophysics, Johns Hopkins University School of Medicine, 725 N. Wolfe Street, Baltimore, Md. 21205, USA.

ATP synthase is a large complex membrane bound enzyme that catalyzes the synthesis of ATP coupled to an electrochemical proton gradient. A soluble portion (F_1-ATPase, MW 380,000) can be purified. It contains five different subunits $\alpha,\beta,\gamma,\delta$ and ϵ in the ratio 3:3:1:1:1. We obtained crystals of the F_1-portion of proton translocating ATPase (Space group = R32) from rat liver mitochondria, and collected photographic data to 3.5 Å resolution using synchroton radiation (λ = 1.57 Å) for the native and three heavy atom derivatives. The oscillation films were measured with the optronic scanner and the help of an Evans and Sutherland vector graphics system. The scanned data sets were processed and reduced to the final data set using the post refinement procedures. A 9 Å resolution map had been already presented and a 3.5 Å resolution map based on one derivative is currently being analyzed. (Supported by N.I.H. Grant GM25432).

Th-AM-A8 QUANTITATIVE APPLICATION OF THE LIGHT SCATTERING (L.S.) TECHNIQUE TO PROBLEMS OF SOLUTE TRANSPORT IN MITOCHONDRIA. Andrew D. Beavis and Keith D. Garlid, Dept. of Pharmacology, Medical College of Ohio, Toledo, Ohio 43699

Changes in L.S. as a measure of swelling and contraction of the mitochondrial matrix have been widely used to study transport in mitochondria. Quantitative application of L.S. has been limited by incomplete characterization of the relationships between absorbance, matrix volume and the concentration of mitochondria. We present a simple method for normalization of data for mitochondrial concentration, resulting in an intrinsic L.S. parameter which depends solely on particle volume. Matrix volume is linear with, and is a single-valued function of, inverse osmolality. In contrast, the L.S. osmotic curve is segmentally linear, exhibiting discontinuities at specific matrix volumes. This results from the fact that the optical technique samples total particle volume, including contributions from folded membranes and trapped medium. In the native state, these contributions are minimized by structural components, such as intermembrane connections and the outer membrane. When these structures are broken, transition to a random packing state causes an irreversible increase in total volume. Thus, the observed nonlinearities are found to disappear irreversibly following preswelling. For the interpretation and analysis of kinetic data, it is essential that these complexities be taken into account and we present methods for doing so. Thus, an acceleration in the L.S. trace obtained during erythritol transport, previously attributed to a volume-dependent increase in erythritol permeability, is shown to reflect, instead, a volume-dependent change in structure. When this is taken into account, erythritol permeability is found to be constant over a wide range of matrix volumes. (Supported by USPHS grant GM 31086).

Th-AM-A9 CHARACTERIZATION OF IONOPHORE-MEDIATED TRANSPORT KINETICS IN MITOCHONDRIA USING THE LIGHT SCATTERING (L.S.) TECHNIQUE. Daniel J. DiResta, Andrew D. Beavis and Keith D. Garlid, (Intr. by Amir Askari), Medical College of Ohio, Toledo, Ohio 43699

One of the difficulties inherent in the study of ionophore kinetics in mitochondria is the rapidity of transport mediated by these agents. In this report we show that the L.S. technique is readily adaptable to such studies and that the parameters obtained are well-defined. We have developed a suite of inherently simple phenomenological equations with a minimum of extrathermodynamic assumptions to describe net salt transport. We have applied these equations in a study of ionophore-mediated mitochondrial swelling to obtain ion-specific conductances.

Hydrophobic amines behave as anionophores catalyzing ion pair formation and net transport of anions under some experimental conditions (Garlid, K.D. and Nakashima, R.N. [1983] J. Biol. Chem. 258, 7974-7980). Kinetic studies of quinine-mediated transport of thiocyanate provide independent verification for the ion pair mechanism. In particular, the data confirm that quinine-mediated thiocyanate transport bypasses the intrinsic pathway for transport of thiocyanate anion.

Using the L.S. technique, we have measured the Vmax (nmol/mg.min) for transport of thiocyanate (350) perchlorate (260), nitrate (14), chloride (<10) and acetic acid (1700) at the gradient obtaining under the conditions of study. These values reflect the permeability of the inner mitochondrial membrane to these anions. We have also obtained turnover numbers for CCCP (3/sec), nigericin (21/sec) and valinomycin (467/sec) in the mitochondrial membrane. Nigericin is found to catalyze potassium uniport at high doses and the kinetics indicate that potassium uniport is mediated by a nigericin dimer. (Supported by USPHS grant GM 31086).

Th-AM-A10 INHIBITION BY Ba^{++} OF K^+ FLUX INTO RAT LIVER MITOCHONDRIA. Joyce Johnson Diwan, Biology Department, Rensselaer Polytechnic Institute, Troy, NY 12180-3590.

Ba^{++}, which has an ionic radius similar to that of K^+, inhibits unidirectional flux of K^+ into respiring rat liver mitochondria, measured by means of ^{42}K. Ba^{++} is itself accumulated by the mitochondria, as evidenced by uptake of ^{133}Ba. Treatment with relatively large amounts of Ba^{++} (e.g. 120 nmoles/mg protein) results in reduced rates of respiration. However the inhibition of K^+ influx by Ba^{++} is observable at levels (23-37 nmoles/mg protein) which cause no significant change in state 4 or uncoupler stimulated respiration. The inhibition of K^+ influx by Ba^{++} is seen both in the presence and absence of mersalyl, an activator of K^+ influx. La^{+++}, which inhibits accumulation of ^{133}Ba, decreases the inhibition of K^+ influx by Ba^{++}, when added with the Ba^{++}. Addition of La^{+++} following accumulation of Ba^{++} has little effect. Kinetic plots indicate an essentially non-competitive pattern of inhibition by Ba^{++}. The results suggest that Ba^{++} gains access to inhibitory sites via the internal matrix compartment. In contrast to its effect on K^+ influx, Ba^{++} has no apparent effect on the rate of unidirectional K^+ efflux from the mitochondria. These observations are consistent with the view that K^+ enters and leaves the mitochondria by separate transport mechanisms. (This work was supported by USPHS Grant GM-20726.)

Th-AM-A11 INTRACELLULAR CALCIUM IN LIVER: ELECTRON PROBE ANALYSIS OF MITOCHONDRIA (mito), ENDOPLASMIC RETICULUM (ER) AND NUCLEUS. A.P. Somlyo, Meredith Bond and Avril V. Somlyo, Penn. Muscle Inst., U. of Penn. Sch. of Med., Philadelphia, PA 19104

We wished to determine the subcellular distribution of Ca in liver and, specifically, whether the mito matrix free $[Ca^{2+}]$ in situ is in the range that could regulate mito enzymes (1). Livers of Wistar rats (250-350g) were rapidly frozen in situ under pentobarbital anesthesia. Dry cryosections showing minimal ice crystal damage were used for electron probe analysis (2). The Ca contents in mmol/kg dry wt. +SEM were: mito 0.8 ± 0.1 (97); nucleus 0.8 ± 0.4 (21); whole cell, (analyzed with large defocussed probes) including ER, mitochondria and nucleus: 3.4±0.4 (n=20); ER: 5.0±0.4 SEM (n=24). The ER value is possibly an underestimate, as the probe sizes used were larger than the lumen of the ER. The fractional volumes in liver cells are: nucleus 6%, mito 20%, ER 15% (3). Therefore, not more than ~5% of the total cellular Ca is in the mito. The highest concentration of Ca is in the ER, with some possibly bound to other, high affinity cytoplasmic Ca-binding sites not visualized in unstained cryosections. If the free Ca^{2+}/total Ca is about $7x10^{-4}$ in liver mitos (4), then matrix free $[Ca^{2+}]$ in situ is about 0.2μM; this is consistent with regulation of mito enzymes by matrix free Ca^{2+} (1,5), but argues against the role of mitos in the physiological regulation of cytoplasmic Ca^{2+}. REFS: 1) Denton, R.M. & McCormack, J.G., FEBS LETT. 119, 1, 1980; 2) Kitazawa, et al., Ultramicroscopy 11, 251, 1983; 3) Weibel, E.R. et al., J. Cell Biol. 42, 68, 1969; 4) Coll, K.E. et al., J. Biol. Chem. 257, 8696, 1982; 5) Hansford, R.M. & Castro, F. J. Bioenerg. Biomembr. 14, 361-376, 1982. Supported by HL15835 to the Penn. Muscle Inst.

Th-AM-A12 MAGNESIUM BINDING TO THE PURIFIED F_1-ATPASE OF RAT LIVER MITOCHONDRIA - IMPLICATIONS FOR FUNCTION - Noreen Williams and Peter L. Pedersen, Department of Biological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

The mitochondrial F_1-ATPase has been examined in some detail for its metal binding properties. As isolated, the ATPase contains approximately 2 mols of "tightly" bound Mg^{++} per mol F_1. One mol of this Mg^{++} can be removed by incubation of the enzyme (1 hr.) in 50 mM Tris HCl, 50 mM EDTA, pH 7.5. The other mol is only removed by overnight incubation with this EDTA - containing buffer, with concomitant loss of structural integrity. When the F_1 is cold treated and fractionated the $\alpha\gamma$ portion of the enzyme retains ≅ 75% of this tightly bound Mg^{++}. Employing radioactive Co^{++} (which most closely mimics the Mg^{++} interaction with the enzyme) F_1 as isolated binds less than 0.25 mols metal per mol F_1. Addition of ATP or ADP increases this value to 0.6-0.7 mol Co^{++}/mol F_1. GTP and ITP by contrast do not affect metal binding to F_1. The non-hydrolyzable ATP analog, AMP·PNP increases divalent cation binding to 2-2.5 mols/mol F_1. Significantly, the enzyme containing 2-2.5 mol radioactive Co^{++}/mol F_1 still contains 1 mol tightly bound Mg^{++}. These results are consistent with the view that F_1 contains 1 metal binding site which plays a structural role, a second site which plays an allosteric role, and two additional sites involved in catalysis. [Supported by NIH grant CA 10951]

Th-AM-B1 REGULATION OF CYTOSOLIC CALCIUM IN SYNAPTOSOMES. Daniel A Nachshen, Department of Physiology, Cornell University Medical College, New York, NY 10021, U.S.A

Cytosolic Ca (Ca_i), measured in synaptosomes that were loaded with the fluorescent Ca indicator quin 2, had a control value (Ca_o = 1 mM) of 100 - 200 nM. The mitochondrial inhibitors valinomycin (5 μM) and FCCP (1 μM) increased Ca_i by 100 - 200 nM in the presence, but not in the absence, of external Ca. This increase in Ca_i did not correlate with the level of Ca_i prior to the addition of inhibitors. It therefore seems likely that these inhibitors increase Ca_i as a consequence of metabolic inhibition and not of Ca release from the mitochondria. Caffeine (1 mM), an agent that releases Ca from sarcoplasmic reticulum in muscle, also increased Ca_i in synaptosomes. This increase did, however, correlate with the level of Ca_i prior to the addition of caffeine. The increase observed at resting (200 nM) levels of Ca_i, 50 nM, is consistent with the size of the Ca pool that is sequestered in the smooth endoplasmic reticulum (S.E.R., 1-3 nmoles/mg protein) when the Ca buffering capacity of the nerve terminals (~15,000 nmoles/(nM x liter)) is taken into account. These results suggest that the S.E.R., but not the mitochondria, is important in regulating cytosolic Ca at levels close to the resting value.

Th-AM-B2 Ca ENTRY IN SQUID AXONS DURING VOLTAGE CLAMP PULSES IS Na/Ca EXCHANGE. J. Requena, J. Whittembury, L. J. Mullins. Centro de Biosciencias, Instituto Internacional de Estudios Avanzados, Caracas, Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, Philadelphia, and Department of Biophysics, University of Maryland School of Medicine, Baltimore.

Intact squid giant axons were injected with aequorin and bathed in 3 mM Ca seawater (a concentration close to that of squid blood). Sodium and potassium currents were pharmacologically blocked and repetitive voltage clamp pulses of a duration of 1.5 msec were applied (to simulate the duration of an action potential) with amplitudes of from +30 to +90 mV and at a frequency of 100/s. In a very fresh axon with a low Na_i, no detectable change in aequorin glow resulted from this treatment whether the axons were in Na-containing or in Na-free seawater. In axons subjected to a modest Na loading, repetitive voltage clamp pulsing did not result in an increased aequorin glow if the pulses were delivered while the axon was in Na seawater, while in Na-free seawater there was an easily measurable increase in aequorin light emission during repetitive pulsing. The increase in the number of aequorin photons emitted per voltage clamp pulse was e-fold for 22 mV of depolarization and the process showed no signs of saturating at pulse amplitudes of +180 mV (i.e. at a membrane potential close to that of E_{Ca}). The aequorin light emission per voltage clamp pulse increased linearly with pulse duration (at constant pulse amplitude).

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Th-AM-B3 LYMPHOCYTE MAGNESIUM DETERMINATION, Gregory R. Hook, Jeanette Hosseini and Ronald J. Elin, Clinical Pathology Department, Bioengineering and Instrumentation Branch, National Institutes of Health, Bethesda, MD 20205

Clinically, whole body magnesium (Mg) status has been inversely correlated with cardiac arrhythmias. However, less than 1% of the total body Mg is in extracellular fluid and serum Mg has been shown to be a poor indicator of total body Mg. Peripheral blood lymphocyte Mg, on the other hand, may be useful for assessing skeletal and cardiac muscle Mg status. Methods are needed to determine the mean cell Mg content and the cell Mg content variation among individual cells. Using a three day old Burkitt's lymphoma cell culture as a test sample, methods were developed for determining the mean lymphocyte Mg content and the variation in Mg content among individual lymphocytes. Atomic absorption analysis and cell counting methods were used to measure a mean cell Mg content of 170 $\pm$ 10 fg/cell in three cultures and had an analytical coefficient of variation of 10%. Electron probe X-ray microanalysis of 100 cells gave a mean cell Mg content of 150 $\pm$ 60 fg/cell. Electron probe X-ray microanalysis had a 10% analytical coefficient of variation and showed the cell Mg content among individual cells had a 30% coefficient of variation. Correlative viability, cytology and cell volume data showed cells were alive, intact and had a normal volume after preparation for Mg analysis. The mean cell Mg contents obtained from two very different analytical methods agree and indicate an accurate estimate of the mean lymphocyte Mg content can be obtained. These methods will be useful for determining the mean and variation in peripheral blood lymphocyte Mg content in various patient groups.

Th-AM-B4 IMAGING FLUORESCENCE CHANGES OF THE Ca-INDICATOR QUIN II IN MAMMALIAN CNS CELLS, John A. Connor, AT&T Bell Laboratories, Murray Hill, New Jersey 07974

Cells grown in culture from rat embryo diencephalon (E-18) were loaded with Quin 2/AM (Calbiochem) following the method of Tsien et al. (J. Cell. Biol. 94, 325-334). After loading and a 2 hr. post-load incubation, cells grown on #1 thickness coverslips were mounted on the stage of an inverted microscope and viewed through a 40X objective (1.3 N.A., glycerine). U.V. illumination was provided by a 100 w, short-arc Hg lamp. A charge coupled device (CCD) photometer was mounted at the 35 mm camera port of the microscope in the image focal plane. The CCD had 320 x 512 pixels, though generally, pixel binning was employed to reduce thermal noise variance. Experimental procedure was to obtain sequential images using 340 and 360 nm excitation (.15 to .25 S exposures) and then to form the ratio image (340/360) from these data. In vitro, Quin II fluorescence is maximally dependent on Ca^{2+} at 340 nm excitation, but relatively independent at 360 nm excitation. In normal density cultures 5 to 10 cells could be imaged in a given exposure. There were in most cases large differences in the ratio images of the various cells in a given field (>100%). Also, cells in new cultures (growing) showed brighter ratio images than cells of more mature cultures. It is not completely clear at this time that these findings represent differences in $[Ca^{2+}]$ or dye activation. It has been possible to increase the ratio image intensity by up to 80% in some cells (but not others in the same field) by 6 to 10 min. exposure to high K, Ca saline. In cells of older cultures (>4 days) it has been possible to reduce the ratio image intensity of individual cells by exposure to saline containing Cd (2mM) and TTX. Examples of growth cones with extended filapodia have been observed. In these cases the ratio image of the cone is brighter than the soma of the same cell. Where filapodia are not evident the ratio image is nearly uniform.

Th-AM-B5 THEORY OF THE EFFECT OF EXTRACELLULAR POTASSIUM ON OSCILLATIONS IN THE PANCREATIC β-CELL. Teresa Ree Chay, Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260 and Joel Keizer, Department of Chemistry, University of California, Davis CA 95616.

Based on the observation that potassium ions are compartmentalized near the surface of pancreatic β-cells in mouse islets, we present a theoretical treatment of the effect of external potassium on oscillations in the pancreatic β-cell. Our model includes the effects of ionic diffusion, the Ca^{2+}-activated K^+ channel and is described by four ordinary differential equations. Numerical integration of these equations allows us to examine the effect of glucose, external K^+, quinine, and TEA on the oscillations in membrane potential, intracellular Ca^{2+}, and compartmentalized K^+. The results are in good agreement with experiments by Perez, Atwater, and Rojas.

Th-AM-B6 EXTERNAL DIVALENT CATIONS INHIBIT Na/H EXCHANGE IN FROG SKELETAL MUSCLE. Robert W. Putnam and Albert Roos. Department of Physiology and Biophysics, Washington University School of Medicine, St. Louis, MO. 63110.

Intracellular pH (pH_i) recovery from an acid load was studied using glass pH-sensitive microelectrodes in frog semitendinosus muscle fibers, depolarized in 50 mM K solution to -20 mV. When 5% CO_2/24 mM HCO_3 was substituted for HEPES in the superfusate (keeping pH_o=7.35, Ca=4 mM, Mg=2 mM), pH_i recovered at a rate of 0.31±0.04 ΔpH/h (mean ± SEM, n=10). Amiloride (1 mM) inhibited this recovery by 50%, as did 0.1 mM SITS. Thus, pH_i recovery in frog muscle is due to two membrane transport systems: a Na/H exchanger, and a SITS-sensitive transporter. When external Ca was increased from 4 mM to 10 mM, recovery was reduced to 0.15±0.01 ΔpH/h (n=13), and was not further reduced by raising Ca to 14 mM (0.15±0.04 ΔpH/h, n=4). Reducing Ca from 4 mM to 1 mM did not increase recovery (0.32±0.04 ΔpH/h, n=8). In the presence of 10 mM Ca, 1 mM amiloride failed to inhibit recovery (0.15±0.01 ΔpH/h, n=8), but 0.1 mM SITS did inhibit it (0.05±0.02 ΔpH/h, n=11). Thus, elevated Ca inhibits the Na/H exchanger but not the SITS-sensitive component. Elevating Mg to 8 mM or adding 6 mM Sr did not affect recovery (0.31±0.04 ΔpH/h, n=4 and 0.30±0.04 ΔpH/h, n=6, respectively). However, as little as 1 mM Ni or Cd reduced recovery (0.21±0.01 ΔpH/h, n=6 and 0.12±0.02 ΔpH/h, n=7, respectively). Recovery from acidification induced by NH_4Cl-prepulse (in nominally CO_2-free solutions), which was found to be entirely due to Na/H exchange, was abolished by 10 mM Ca or 1 mM Cd, but unaffected by 8 mM Mg. We propose an inhibitory external divalent cation-binding site on the frog muscle Na/H exchanger with binding order Cd>Ni>Ca, while Mg and Sr were ineffective. (Supported by NIH Grant HL-00082 to A.R.).

Th-AM-B7 **INTRACELLULAR-pH REGULATION BY THE SQUID AXON: COMPETITION OF DNDS, A REVERSIBLE STILBENE DERIVATIVE, WITH EXTERNAL Na^+ AND HCO_3^-.** Walter F. Boron and Roger C. Knakal (Intr. by Gerhard Giebisch). Dept. of Physiology, Yale University School of Medicine, New Haven, CT.

Intracellular pH (pH_i) in squid axons is regulated by a mechanism that mediates the influx of one Na^+ and two HCO_3^- (or an equivalent species), and the efflux of one Cl^-. The results of a previous study suggested that the extracellular substrate actually may be the ion-pair $NaCO_3^-$. If this is true, then any inhibitor that appears to be competitive with HCO_3^- should also appear to be competitive with Na^+. Axons were internally dialyzed with a pH-6.5 fluid containing no Na^+ and 400 mM Cl^- until pH_i fell to ~6.6. The acid-extrusion rate (J, equivalent H^+ efflux) was calculated from the rate of pH_i recovery, measured with a microelectrode. At a fixed pH_o of 8.00 and $[Na^+]_o$ of 425 mM, the apparent J_{max} and K_m when varying $[HCO_3^-]_o$ are 20.5±2.2 pmol·cm^{-2}·s^{-1} (pcs) and 2.6±0.3 mM HCO_3^-, respectively (± std. dev., n=31). In the presence of 0.1 or 0.25 mM 4,4'-dinitrostilbene-2,2'-disulfonate (DNDS), J_{max} is unchanged, whereas K_m increases to 5.7±1.3 (n=40) or 12.7 ±4.3 mM HCO_3^- (n=35), respectively. Thus, DNDS is competitive with HCO_3^-. At a fixed pH_o of 8.00 and $[HCO_3^-]_o$ of 12 mM, varying $[Na^+]_o$ in the absence of DNDS produces a J_{max} of 19.7±3.4 pcs, and a K_m of 70.5±12.1 mM Na^+ (n=35). With [DNDS]=0.1 mM, J_{max} is unchanged, whereas K_m is 179±54 mM Na^+ (n=24). If the fixed $[HCO_3^-]_o$ is raised to 48 mM at pH_o=8.00, then in the absence of DNDS, J_{max} is unchanged and K_m falls to 22.1±5.6 mM Na^+ (n=42). In the presence of 0.1 or 0.25 mM DNDS, the comparable J_{max} values are unchanged, whereas K_m increases to 45.2±12.2 (n=43) or 90.4±31.4 mM Na^+ (n=33), respectively. Thus, DNDS (an anion) is comparably competitive with both Na^+ and HCO_3^-, consistent with the $NaCO_3^-$ ion-pair model. (Supported by NIH grant NS18400).

Th-AM-B8 DECREASED CELL SODIUM DURING DMSO INDUCED DIFFERENTIATION OF MURINE ERYTHROLEUKEMIC (MEL) CELLS; EVIDENCE AGAINST THE Na-Ca HYPOTHESIS. Deborah A. Lannigan and Philip A. Knauf, Dept. of Rad. Biol. and Biophysics, Univ. of Rochester Med. Ctr., Rochester, NY 14642.

MEL (Friend) cells are a virally-transformed cell line that can be induced to differentiate along the erythroid pathway *in vitro*. It has been proposed that an essential early event in MEL cell differentiation is a decrease in Na,K-ATPase activity which causes an increase in cell Na^+ concentration (Na_c) and then, by means of Na^+-Ca^{++} exchange, an increase in intracellular Ca^{++}. A critical prediction of this Na-Ca hypothesis is the rise in Na_c. We have used a triple isotope method involving ^3H_2O, ^{14}C-sucrose and ^{22}Na to measure total water, extracellular fluid, and Na^+, respectively. ^{22}Na equilibration occurred in <10 min. In uninduced cells, Na_c was 15.2 ± 2.2 mM (SD, n = 22); after induction for 14 to 16 h with DMSO Na_c decreased significantly ($P<0.0001$) to 8.4 ± 1.4 mM (n = 21), in direct contradiction to the prediction of the Na-Ca hypothesis. Assuming that the Na,K-ATPase in MEL cells has similar kinetics to that in erythrocytes, the observed decrease in Na_c would cause about a 40 percent decrease in Na,K-ATPase activity. This is very similar to the reported decrease in ouabain-sensitive Rb^+ uptake under the same conditions and, in addition, the time course of the decrease in Na_c parallels that of the decrease in Na,K pump activity. The observed changes in Na,K pump activity therefore can probably be explained solely on the basis of changes in Na_c caused by a decrease in net Na^+ influx. Thus, a direct modulation of Na,K-ATPase activity does not seem to be a primary event in MEL cell differentiation. (Supported by NIH grants AM27495 and HL18208.)

Th-AM-B9 Na/H EXCHANGE IN HUMAN RED BLOOD CELLS: A COUPLED TRANSPORT PROCESS M.A. Milanick, S.D. Dissing, and J.F. Hoffman, Yale Univ. Sch. of Med., New Haven, CT. 06510

In studying the effects of protons on cation fluxes in human red blood cells we found it helpful to clamp the internal and external pH at separate values by using DIDS (4,4'-diisothiocyano-2,2'-stilbene disulfonate) treated red cells. Previously (J.G.P. 80:15a, 1982) we reported that external protons stimulated a net downhill ouabain-insensitive Na efflux of 1 mmole/ liter packed cells (l.p.c.) x hr and that a downhill proton influx occurred in concert with the Na efflux (pHi=7.4, pHo=5.8, Nai=30 mM, Nao=0). Further kinetic evidence supporting the notion that a portion of these fluxes represent Na/H exchange is: 1) Nao inhibits the proton flux. In fact the Nao-sensitive H influx is about the same size as the H stimulated Na efflux. 2) Internal protons stimulate unidirectional Na influx. While these results indicate that the red cell can exchange Na for H, the question becomes is there direct coupling between the Na and H fluxes per se? To test whether a proton gradient can drive a net uphill movement of Na we measured unidirectional Na efflux and influx that occurred when DIDS treated, sulfate loaded red cells containing 5 mmoles Na/l cell water were placed in a medium that contained 7.5 mM Na. In the absence of a pH gradient the unidirectional fluxes were approximately equal (0.103 ± 0.005 and 0.096 ± 0.002 mmoles Na/ l.p.c. x hr, respectively) but when the pHo was lowered to 6.0 (pHi clamped at 7.4), Na efflux (0.081 ± 0.003 mmoles/ l.p.c. x hr) significantly exceeded Na influx (0.051 ± 0.001) ($p<0.01$). Thus there was a net Na efflux against a concentration gradient, suggesting that a portion of Na and H fluxes are coupled under the appropriate conditions in human red blood cells. (Supported by NIH grants HL-09906 and AM-06896)

Th-AM-B10 RADIOSODIUM FLUXES IN MULTICELLULAR TISSUE: SKELETAL MUSCLE AND VASCULAR SMOOTH MUSCLE. M.R. Menard, Dept. of Anatomy, University of British Columbia, Vancouver, Canada. V6T1W5.

The most straightforward interpretation of the radiosodium (Na*) efflux measured in single frog skeletal muscle cells is that there is only one intracellular compartment of readily-exchangeable sodium, but the most straightforward interpretation of the efflux measured in whole muscle is that there is more than one intracellular compartment of readily-exchangeable sodium. A different hypothesis which is consistent with observations in single cells and in whole muscles is that each cell in the muscle has only one compartment, but that different cells have different Na* exchange rates. The rate constant for Na* exchange is proportional to the surface-to-volume ratio of the cell, so diversity of cell size and/or shape in the muscle could produce the diversity in sodium exchange characteristics required by this model. This hypothesis was tested by measuring the surface-to-volume ratio at the light microscopic level for all cells in a muscle, and predicting the efflux and influx under the assumption that each unit area of cell membrane had the same sodium exchange characteristics and each cell had the same internal sodium concentration. This prediction agreed with the major feature of the efflux measured in similar muscles: continuous decline of the fractional loss of Na*. The measured Na* influx showed the appropriate corresponding variation in rate. Similar measurements were done in mixed multicellular preparations of rat vascular smooth muscle (artery strips), and again a consistent interpretation of the influx, efflux, and morphometry was possible. Thus a simple model which takes into account the dimensional characteristics of the tissue provides a consistent interpretation of the measured Na* fluxes in different multicellular preparations. (Supported by the MRC).

Th-AM-B11 CALCIUM AT THE SURFACE OF CARDIAC PLASMA MEMBRANES - CATION BINDING, SURFACE CHARGE SCREENING AND Na-Ca EXCHANGE. Donald M. Bers, Kenneth D. Philipson and Arthur Peskoff. Division of Biomedical Sciences, University of California, Riverside, CA 92521-0121 and Departments of Physiology, Medicine and Biomathematics , University of California, Los Angeles, CA 90024

Ca binding and Na-Ca exchange activity was measured in isolated cardiac plasma membrane vesicles under various ionic conditions. A model was developed to describe the Ca binding characteristics of cardiac sarcolemmal vesicles using the Gouy-Chapman theory of the diffuse double layer with specific cation binding to phospholipid carboxyl and phosphate groups. The surface association constants for Ca, Na, K and H binding to both of these groups were 7, 0.63, 0.3 and 3800 M^{-1} respectively. This model can readily describe Ca and Na binding characteristics of sarcolemmal vesicles and also predicts surface charge, surface potential and surface concentrations of Ca, Na and H under various ionic conditions. The effects of the divalent screening cation, dimethonium on Ca binding and Na-Ca exchange were studied. Dimethonium had no significant effect on Ca binding at high ionic strength (150 mM KCl), but strongly depressed Ca binding at low ionic strength (5 mM KCl). This is consistent with predictions of the model. Dimethonium had no significant effect on Na_i-dependent Ca influx (Na-Ca exchange) at either high or low ionic strength. These latter results suggest that the Ca sites of the Na-Ca exchanger are in a physical environment where they are either not exposed to or not sensitive to surface [Ca] (but rather to bulk [Ca]).

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Th-AM-B12 ACTIVATION OF Na^+/H^+ EXCHANGE BY INSULIN IN L6 MUSCLE CELLS. Amira Klip, Toolsie Ramlal and Liliana Attisano. Neurology, The Hospital for Sick Children, Toronto M5G 1X8 Canada.

The Na^+/H^+ antiport at the plasma membrane of several cell types is virtually quiescent but becomes activated by growth factors. In intact skeletal muscle, a cytoplasmic alkalinization occurs in response to insulin (Moore RD, BBA 737, 1-49, 1983). Whether such alkalinization is mediated by the amiloride-sensitive Na^+/H^+ antiport remains to be determined. In this study we explore whether skeletal muscle cells of the L6 line grown in culture have a Na^+/H^+ antiport, whether insulin activates it and whether such activation is related to stimulation of glucose transport.

Operation of the Na^+/H^+ antiport was detected by measurements of ^{22}Na uptake and of intracellular pH (pH_i). Na^+ uptake measurements were done in monolayer cultures in the presence of ouabain. In the basal state amiloride-sensitive Na^+ influx was negligible, whereas in the presence of insulin it represented over 30% of the total Na^+ uptake. pH_i, determined at 37°C in suspended cells loaded with the fluorescent indicator bis-carboxyethyl-carboxyfluorescein, changed from 7.00 to 7.10 within 10 min of addition of insulin. A 4 min lag period preceded the rise in pH_i. The cytoplasmic alkalinization was prevented by amiloride and was substantially reduced in Na-free medium. This suggests that insulin activated an amiloride-sensitive Na^+/H^+ exchange in L6 muscle cells.

On the other hand, amiloride (300 uM) did not prevent insulin-stimulation of sugar uptake, although it lowered both basal and insulin-stimulated rates of transport. Replacement of extracellular Na by N-methylglucamine or choline did not affect basal glucose transport nor did it prevent its stimulation by insulin. This suggests that stimulation of glucose uptake and activation of Na^+/H^+ exchange by insulin are not causally related. Supported by the MRC and the MDAC, Canada.

Th-AM-C1 ^{31}P NMR SPECTRA OF ROD OUTER SEGMENT AND SARCOPLASMIC RETICULUM MEMBRANES SHOW NO EVIDENCE OF IMMOBILIZED COMPONENTS DUE TO LIPID-PROTEIN INTERACTIONS. Robert D. Pates, Jeffrey F. Ellena, and Michael F. Brown. Department of Chemistry and Biophysics Program, University of Virginia, Charlottesville, VA 22901.

The effects of protein perturbation of the ^{31}P NMR resonance of the phospholipid components in biological membrane specimens are under investigation in this laboratory. Phase-cycled Hahn echo NMR spectroscopy (1) was employed in an effort to eliminate spectral distortions resulting from inability to record the initial part of the free induction decay. Careful attention was paid to pulse widths, pulse spacing and the location of the top of the echo (1). Spectra were taken as a function of temperature, salt concentration, and pulse spacing. Variation of these parameters did not have a large effect on lineshapes or relative intensities. Samples were biochemically characterized before and after spectroscopy and showed no signs of degradation. Spectra have been obtained for bovine rod outer segment membranes, rabbit sarcoplasmic reticulum membranes, and bilayers of the total extracted lipids from each of these systems. We observe no broad spectral component attributable to strong lipid-protein interactions on the ^{31}P NMR timescale in either case. These results are in agreement with some previous studies (2,3), but not others (4,5).

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Th-AM-C2 INTERACTION OF DURAMYCIN WITH MEMBRANES CONTAINING LIPIDS TENDING TO ADOPT HEXAGONAL II STRUCTURES. J. Navarro, J. Chabot, K. Sherrill, R. Aneja, S.A. Zahler and E. Racker. Section of Biochemistry, Molecular and Cell Biology and Section of Genetics and Development, Division of Biological Sciences, Cornell University, Ithaca, NY 14853.

Duramycin is a polypeptide antibiotic obtained from culture filtrates of Streptomyces cinnamomeus forma azacoluta. In this work we show that low concentrations of duramycin induced aggregation of lipid vesicles containing lipids tending to adopt hexagonal II structures (e.g. unsaturated phosphatidylethanolamine and unsaturated monogalactosyldiglyceride), and of sarcoplasmic reticulum vesicles from rabbit skeletal muscle. Furthermore, duramycin inhibited the ATP-dependent Ca^{2+} uptake in sarcoplasmic reticulum vesicles without affecting the hydrolysis of ATP. Also, duramycin only inhibited the bacteriorhodopsin proton pump reconstituted into phospholipid vesicles containing unsaturated phosphatidylethanolamine. We have also found a relationship between the presence of phosphatidylethanolamine in the membrane of Bacillus subtilis and duramycin-induced inhibition of the secretion of protons and influx of calcium in the bacteria. Our findings are consistent with the idea that duramycin recognizes a particular membrane conformation determined by the presence of lipids tending to adopt hexagonal II structures.

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Th-AM-C3 CORRELATION BETWEEN TORPEDO CALIFORNICA ACETYLCHOLINE RECEPTOR FUNCTION AND STRUCTURAL PROPERTIES OF MEMBRANES, Tung Ming Fong and Mark G. McNamee, Department of Biochemistry & Biophysics, University of California, Davis, CA 95616

Lipid-protein interactions were studied using Torpedo californica acetylcholine receptor (AChR) as a model system by reconstituing purified AChR with synthetic lipids. Previous results from studies of spin-labeled lipids have demonstrated that AChR interacts preferentially with steroid and phosphatidic acid (PA) compared to other kinds of phospholipids [J.F.Ellena et.al., (1983) Biochemistry 22, 5523]. In the present studies, membrane fluidity was measured by the order parameter of 5-doxyl-hexadecanoic acid in the membranes. AChR function was determined by two activities: (1) ion-gating activity of vesicles containing AChR in response to agonist; (2) low to high agonist affinity transition of AChR in either vesicles or membrane fragments in the presence of agonist. It was found that both aspects of AChR function depend on the lipid environments. Membrane fluidity appears to play a role in modulating protein conformational changes, because AChR can only manifest the affinity state transition activity in membranes within a narrow range of fluidity, although the average fluidity is not a sufficient condition for conformational changes. In addition, the conformational change is only a necessary, but not a sufficient condition for the ion channel opening. Among membranes that manifest the state transition activity, only those containing both cholesterol and PA retain ion-gating activity. (Supported by USPHS Grant No. NS13050)

Th-AM-C4 A MINIMUM NUMBER OF LIPIDS IS REQUIRED TO PROTECT ACETYLCHOLINE RECEPTOR ION CHANNEL FUNCTION O. Jones, J. Eubanks and M.G. McNamee. Dept. Bioch. & Biophys. U. Cal. Davis. CA 95616

The nicotinic acetylcholine receptor (AchR) of Torpedo californica is an integral membrane protein whose functional properties are highly dependent on the nature of the lipid environment. We have investigated the role of lipids on one aspect of AchR function - stabilization of the ion channel. Membranes containing AchR were incubated with increasing concentrations of the detergent cholate. AchR vesicles were reconstituted by adjusting the cholate and lipid (asolectin) concentrations and subsequent dialysis. Agonist induced ion-flux measurements revealed a critical cholate concentration (C_c), above which irreversible inhibition of ion channel function occurred. Increasing the lipid:protein ratio of the AchR membranes increased C_c. Centrifugation of the cholate-AchR-lipid mixtures into detergent-free sucrose gradients, and analysis of AchR-containing bands, showed a decrease in the lipid:protein ratio with increasing cholate concentration. Flux measurements for AchR complexes reconstituted after centrifugation showed channel inactivation profiles similar to those found prior to centrifugation. When ion channel activity is expressed as a function of the lipid:protein ratio, a critical value representing the minimum lipid content to support maximal activity, is apparent. Cholate concentrations which cause the lipid content to fall below the critical lipid:protein ratio irreversibly inactivate channel function. The critical lipid:protein ratio is around 45 moles of lipid/mole protein and occurs at cholate concentrations corresponding to C_c. The minimum number of lipids needed to protect ion channel function is similar to the number predicted to surround the intramembranous portion of the AchR and to the value determined using EPR spectroscopy. (Ellena et al (1983), Biochemistry 22 5523). Supported by Grant NS13050 and an MDA fellowship to O.T.J.

Th-AM-C5 FLUORESCENCE POLARIZATION STUDIES ON FLUORESCEIN-LABELED COBRA VENOM PHOSPHOLIPASE A_2: THE EFFECT OF METAL IONS AND LIPIDS. Theodore L. Hazlett and Edward A. Dennis, Department of Chemistry, University of California at San Diego, La Jolla, CA 92093 (USA).

Phospholipase A_2 from the venom of the Indian cobra (Naja naja naja) has been shown to undergo a concentration dependent and substrate dependent aggregation and this plays an important role in its mechanism of action [Review: E.A. Dennis, The Enzymes 16, 307-353 (1983)]. Its aggregation has now been studied using fluorescence polarization. The extrinsic probe fluorescein isothiocyanate (FITC) was coupled to the enzyme to serve as the fluorescence probe. Static polarization measurements were made to determine changes in the nature of the coupled probe and enzyme. It was found that the divalent metal ions Ca^{++} and Ba^{++} cause an increase in the polarization, while Mn^{++}, Mg^{++}, and Co^{++} did not. The concentration of enzyme present also greatly affected the polarization. The water-soluble phospholipid diheptanoyl glycerophorylcholine and the lipid analog dodecylphosphorylcholine when present increase the polarization of the phospholipase below their respective critical micelle concentrations. The phospholipase A_2 from rattlesnake (C. adamanteus) and the phospholipase A_2 from pancreas were also coupled to FITC and compared to the cobra venom conjugate. Neither of these latter phospholipases show changes in their fluorescence under the conditions tested. Analysis of the results in terms of premicellar lipid-protein aggregation and probe environment changes are discussed. (NSF PCM 82-16963)

Th-AM-C6 INTERFACIAL PROPERTIES OF MODEL MEMBRANES AND PLASMA LIPOPROTEINS CONTAINING ETHER LIPIDS. John B. Massey and Henry J. Pownall, Baylor College of Medicine, Houston,TX

The interfacial properties of synthetic ester and ether phosphatidylcholines (PCs) were investigated using the polarity sensitive fluorescent probes, Prodan and pyrene. Single bilayer phospholipid vesicles (SBV) formed by sonication and apolipoprotein A-I:phospholipid complexes (R-HDL) were studied. On the basis of a number of spectroscopic and thermodynamic criteria, the interfacial regions of PCs and their ether analogs are similar. The fluorescence properties of Prodan in R-HDL or SBV were independent of the phospholipid fatty acyl chain length, polar head group, and cholesterol content as well as the substitution of ether linkage for ester bonds in the phospholipid. The spectral shifts correlated mainly with the physical state of the phospholipid. The emission spectrum of Prodan appeared at shorter wavelengths upon transfer from water to liquid crystalline phospholipid and blue-shifted further when the lipid was cooled to its gel phase. The quantum yield of Prodan fluorescence in an ether-PC matrix was similar to that observed in water whereas in an ester PC matrix it was enhanced by a factor of about 5. Water-phospholipid partition coefficients of Prodan were independent of the physical state of 1,2-dimyristoyl-sn-glycero-3-phosphorylcholine or 1,2-tetradecyl-sn-glycero-3-phorphorylcholine. The enhancement ratio of the vibronic bands of pyrene indicated similar changes in the environment of the probe during the gel to liquid crystalline phase transition of ether and ester PCs. Spectral differences of the probes in gel or liquid crystalline phase are consistent with differences in entry or exclusion of water from the interfacial region. By analogy, the decreased quantum yield of Prodan in ether PCs suggests that greater water penetration creates a hydrophobic-hydrophilic balance of the interface for ether PCs that is comparable to that of ester PCs.

Th-AM-C7 MISCIBILITY, FUSION AND BINDING OF APOLIPOPROTEIN A-I TO DISPERSIONS OF ETHER AND ESTER PHOSPHATIDYLCHOLINES. B.J. McKeone, H.J. Pownall, and J.B. Massey, Baylor College of Medicine, Houston, TX 77030.

Ether phosphatidylcholines (PC) in model high density lipoprotein (R-HDL) provide a stable environment which allows the differentiation of macromolecular properties such as fluidity and structural variation such as unsaturation for studies of lipolytic enzymes. Studies on the physical properties of ether versus ester PCs were performed to ascertain the compatability of ether and ester PCs in the R-HDL. Miscibility of C-14 and C-16 saturated ester and ether PCs was examined by differential scanning calorimetry. 1,2-Dihexadecyl-sn-glycero-3-phosphorylcholine (C-16 ether) was completely miscible with 1,2-dipalmitoyl-sn-glycero-3-phosphorylcholine, the C-16 and C-14 ester PCs and the C-14 ether (1,2-ditetradecyl-sn-glycero-3-phosphorylcholine). The C-14 ether was completely miscible with the C-16 ether but not with the C-16 ester. Association of apoA-I with multilamellar vesicles of ether or ester PCs followed by changes in turbidity indicates that the binding of apoprotein A-I to the C-16 PCs was negligible while apoA-I binding to the C-14 PCs was fastest at their gel-liquid crystalline transition temperatures and diminished rapidly above and below it. Fusion of single bilayer vesicles of the C-14 and C-16 ether and ester PCs indicates that the ester PC vesicles were more stable. These results indicate that ether PCs are suitable structural analogs for investigating the mechanism of lipolytic enzymes.

Th-AM-C8 TRANSFER OF OLEIC ACID BETWEEN PHOSPHOLIPID VESICLES AND ALBUMIN. James A. Hamilton, David P. Cistola and Donald M. Small, Biophysics Institute, Boston University School of Medicine, Boston MA 02118

The net transfer of oleic acid (OA) between egg phosphatidylcholine (PC) vesicles and bovine serum albumin (BSA) has been monitored using ^{13}C NMR spectroscopy and carboxyl ^{13}C-enriched OA. PC vesicles with 6 mole% OA were incubated with fatty acid-free BSA at 37°C and at initial pH values of 7.0-7.4 in 0.56% KCl. The stoichiometry of the incubation mixture was 5 moles OA/mole BSA. ^{13}C NMR spectra were obtained for this mixture at 37° to determine the distribution of OA between BSA and PC. In the pH range investigated (5.4-8.0), the chemical shifts of OA bound to BSA are different from those of OA in PC vesicles: the former gives multiple peaks at 180.8-184.0 ppm (J.S. Parks, D.P. Cistola, D.M. Small & J.A. Hamilton (1983) J. Biol. Chem. 258:9262) and the latter gives a single pH-dependent peak at 176.3-179.6 ppm. The equilibrium distribution of OA was determined by intensity ratios of the different peaks without separation of PC and BSA. Peak area ratios (uncorrected for possible T_1 and NOE differences) showed that at pH=7.4, $\geq$80%, and at pH= 8.0, $\geq$90% of the OA was bound to BSA. Decreasing the pH decreased the proportion of OA associated with BSA. At pH=5.4, $\leq$10% of the OA was bound to BSA and >90% was vesicle-associated. The distribution was reversible on changing the pH. These results show that (1) the rate of exchange of OA between BSA and PC is slow on the NMR time scale; (2) the partitioning of OA between BSA and PC is strongly pH-dependent between pH 5.4 and 8.0. At physiological pH (7.4) most of the OA is bound to BSA; decreasing the pH by only one unit results in a marked redistribution of the OA, with most of the OA binding to PC vesicles.

Th-AM-C9 PHASE DIAGRAMS FOR LIPID BILAYERS CONTAINING INTRINSIC MEMBRANE PROTEINS: THEORY. James R. Abney and John C. Owicki, Dept. of Biophysics & Medical Physics, Univ. of CA, and Division of Biology & Medicine, Lawrence Berkeley Laboratory, Univ. of CA, Berkeley, CA 94720.

The phase behavior of membranes that are binary mixtures of phospholipids and intrinsic membrane proteins can be analyzed in temperature-composition phase diagrams. Such diagrams summarize, among other things, the gross lateral distribution of proteins in the membrane and the effects of the proteins on the phospholipid phase transition. We have investigated theoretically the influence of lipid-protein interactions and protein electrostatics on these phase diagrams. Lipid-protein interactions were modeled by Landau theory (J.C. Owicki and H.M. McConnell (1979) Proc. Natl. Acad. Sci. U.S.A. 76:4570-4574) using an accurate variational trial function for the decay of the lipid order parameter moving radially outward from the protein. Electrostatic interactions between like-charged proteins were modeled simply by the Gouy-Chapman approximation for uniformly charging the aqueous/nonpolar interface (S. McLaughlin (1977) Current Topics Membr. Transport 9:71-144); the lipids bore no net charge in this analysis. For some ranges of temperature, composition, and molecular properties, the theory predicted lateral phase separation. The coexisting phases differed not only in composition, but also in the amount of conformational order in the lipids. Electrostatic repulsions between proteins, which act (in a relative sense) to destabilize protein-rich phases, can have large effects on the phase behavior of lipid-protein systems. We gratefully acknowledge support from the U.C. Cancer Research Coordinating Committee.

Th-AM-C10 INTERACTIONS OF HAPTEN SENTIZIED LIPOSOMES WITH IMMOBILIZED ANTIBODY: A HOMOGENOUS SOLID-PHASE IMMUNOLIPOSOME ASSAY. R. J. Y. Ho and L. Huang, University of Tennessee, Knoxville, TN 37996-0840.

Dioleoyl phosphatidylethanolamine (DOPE) does not form stable bilayer liposomes at room temperature and neutral pH. However, stable unilamellar liposomes could be prepared by mixing DOPE with a minimum of 12% of a haptenated lipid, N-(dinitrophenylaminocaproyl)-phosphatidyl-ethanolamine (DNP-cap-PE). When the liposomes bound to rabbit anti-DNP IgG which had been adsorbed on a glass surface, lysis of the liposomes occurred with the release of the contents into the medium as judged by the fluorescence enhancement of an entrapped self-quenching dye, calcein. On the other hand, incubation of the same liposomes with glass surfaces coated with normal rabbits IgG had little effect. In addition, free anti-DNP IgG induced aggregation of the liposomes but did not cause any dye release. Liposomes composed of dioleoyl phosphatidylcholine and DNP-cap-PE did not lyse when added to the glass surfaces coated with either anti-DNP IgG or normal IgG. A likely mechanism for liposome lysis is that the DNP-cap-PE laterally diffuse to the contact area between the liposome and the glass. Binding of the haptenated lipid with the immobilized and multivalent antibody trap the haptenated lipids in the contact area. As a result of lateral phase separation, lipids may undergo the bilayer to hexagonal phase transition, leading to the leakage of the entrapped dye. Since both the free hapten and the free antibody inhibited the liposome leakage, this process could be used to assay for the free hapten or antibody. We have shown that inhibition assays using this principle can easily detect 10 p mole of free DNP-glycine in 40 microliters. (Supported by NIH Grant CA 24553).

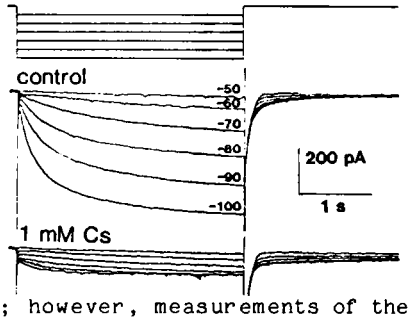
Th-AM-D1 OPTICAL MONITORING OF MYOCARDIAL CONDUCTION: OBSERVATIONS OF REENTRY AND EXAMPLES OF LIDOCAINE ACTION. Bruce C. Hill and Kenneth R. Courtney. Research Institute, Palo Alto Medical Foundation, Palo Alto, California 94301

We have constructed a laser scanning system to optically monitor myocardial conduction. Conduction maps are made by monitoring voltage-dependent fluorescence changes in tissue stained with dye WW781 and illuminated by a 633 nm He-Ne laser. Sixty four discrete points can be selected on the tissue and simultaneously monitored. A custom-built control and data acquisition system sequentially moves the laser beam from point to point and can digitize data at a 128 kHz rate, resulting in a maximum sample rate of 2 kHz for each point on the tissue.

Reentry was induced in isolated Guinea pig left atrium exposed to 2-6 uM carbamylcholine by occasionally applying a single extra stimulus just at the refractory period. Conduction maps were constructed to allow comparison of normal and reentrant propagation pathways. We also observed a mode of antiarrhythmic action of lidocaine in right ventricular flaps. At a fast (4/second) drive rate, application of 25 uM lidocaine resulted in excitation of alternate halves of the tissue on alternate stimuli.

Supported in part by NIH grants HL 25489, HL 24156, and RR 05513.

Th-AM-D2 I_f: IN ISOLATED CELLS FROM THE RABBIT S-A NODE. A. van Ginneken and W. Giles, University of Calgary, Calgary, Alberta T2N 4N1.



Suspensions of individual cardiac pacemaker cells were prepared by enzymatic dispersion from the rabbit sino-atrial node. Whole-cell and patch clamp techniques were used to record i_f (cf. Brown 1982). In normal Tyrodes (33 to 37 C) individual cells showed sustained spontaneous activity which closely resembled that observed in the intact S-A node. Furthermore, (see Fig.) the time-course, steady-state voltage dependence and Cs^+ sensitivity were very similar to thosepreviously recorded in small strips of rabbit S-A node.

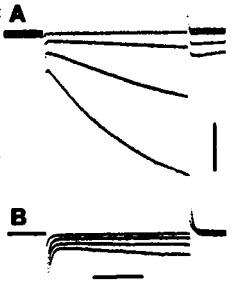
In patch clamp experiments attempts were made to identify the single channel activity which underlies I_f. In cell-attached patches no such single channel transitions could be recorded; however, measurements of the amplitude of the action currents indicate that upon hyperpolarization the membrane resistance of the patch decreases in a voltage- and time-dependent fashion. In outside-out torn-off patches, an I_f-like current could sometimes be recorded, but no discrete single channel transitions were observed. These data suggest either that the single channel conductance of I_f is exceedingly small; or that I_f is generated by an ion transfer process different from a voltage-gated channel.

Brown, H.F. (1982). Physiol. Rev., 62, 505-530.

Supported by DHHS HL-27454 and by the Canadian MRC and CHF.)

Th-AM-D3 VOLTAGE-GATED CURRENTS IN SINGLE CULTURED PACEMAKER CELLS FROM THE SINOATRIAL NODE. Richard D. Nathan and Lou A. Roberts, Departments of Physiology and Anatomy, Texas Tech University Health Sciences Center, Lubbock, Texas 79430.

The sinoatrial node was excised from 1.5-2.5 kg rabbits and dissociated into single cells by multiple exposures to collagenase. After one or two days in culture, voltage-clamp recordings were obtained with the single microelectrode technique. We studied two morphologically and functionally distinct types of cells. One type was spheroidal or spindle shaped (15.8 x 17.4 µm; mean, n=12). It exhibited slow rising action potentials (3-8 V/sec) and a TTX-resistant inward current that was activated at -30.2 ± 2.4 mV (SEM, n=8) and blocked by 0.5 mM $CdCl_2$. Another type was spheroidal (19.4 x 22.4 µm; n=8). It exhibited fast rising action potentials (50 ± 8 V/sec, n=4) and a much larger and faster inward current that was activated at -41.2 ± 1.9 mV (n=6) and blocked by TTX. Both types beat at comparable rates (106 ± 17 and 96 ± 6 beats/min, respectively), displayed a delayed outward current as well as a transient outward current that was markedly reduced by 2 mM 4-aminopyridine or a holding potential of -40 mV, and exhibited an inward current that was activated by hyperpolarization to -54.3 ± 2.2 mV (n=7) (panel A, Fig.) and blocked (at < -100 mV) by 2 mM CsCl (panel B, Fig.). The reversal potential for this current (tails) was -20.8 ± 1.7 mV (n=6). Because our results are similar to those obtained from small multicellular preparations, we believe that these cultured cells can be used in more extensive investigations. Supported by NIH grants HL 20708 (RDN) and HL 21145 (LAR). Fig: (A) Control current at -50, -70, -90 and -110 mV; (B) 7 min in 2 mM CsCl; 37°C; scales, 100 pA and 100 msec.

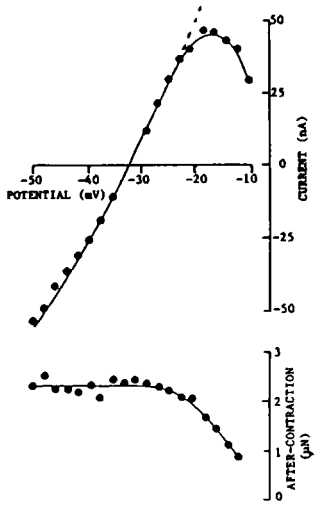


Th-AM-D4 EFFECTS OF ACETYLCHOLINE ON POTASSSIUM CURRENTS IN CHICK EMBRYONIC HEART CELL AGGREGATES. Alvin Shrier[1], Richard M. Brochu[1] and John R. Clay[2]. [1]Dept. of Physiology, McGill Univ., Montreal, Quebec, Canada H3G 1Y6 and [2]Lab. of Biophysics, NINCDS, NIH at the MBL, Woods Hole, MA. 02543 (Intr. by Guy Roy).

We have investigated the effect of ACh on the electrical activity and ionic currents of aggregates of cells derived from the atria of 7-11 day old chick embryos. A dose dependent hyperpolarization was found in the concentration range between 10^{-8} and 10^{-5} M. In spontaneously beating aggregates or in aggregates rendered quiescent by TTX, which rested at -55mV, the application of 10^{-6} M ACh produced a hyperpolarization to -95mV. At concentrations of ACh up to 10^{-6} M there was no change in the action potential duration. The underlying ionic current change induced by ACh was determined with the two microelectrode voltage clamp. At holding potentials in the pacemaker range 10^{-6} M ACh caused a marked outward current. Voltage steps in this range of potentials lacked a time dependent current component before and after application of ACh. At more positive potentials we have recorded two repolarization currents, Ix_1 and Ix_2, neither of which are influenced by ACh. The ACh induced current, obtained by subtracting the control steady state IV from that recorded in the presence of ACh, was maximal at about -50 mV and showed marked inward rectification at more positive potentials. In fact, due to the marked negative slope character of the IV virtually no ACh induced current was found positive to 0mV. The steady state IV's from control and in the presence of ACh cross at approximately the potassium equilibrium potential. When incorporated into a computer model of atrial aggregate electrical activity the ACH induced current produces a marked hyperpolarization but no change in the action potential duration. Supported by the MRC, Canada.

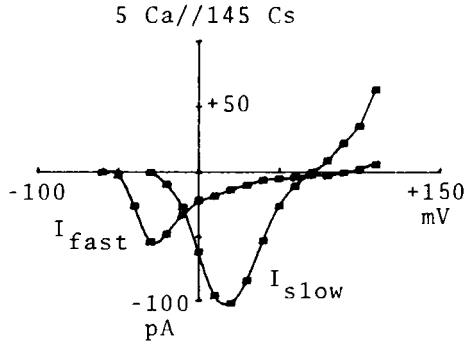
Th-AM-D5 **TRANSIENT INWARD CURRENT (I_{TI}) IN A SODIUM-FREE SOLUTION: IMPLICATIONS FOR THE ROLE OF NA-CA EXCHANGE AS THE CHARGE CARRIER FOR THIS CURRENT.** **M.B. Cannell and W.J. Lederer, Dept. Physiology, Univ. Maryland, Baltimore, MD**

The role of Na-Ca exchange in generating the oscillatory membrane current I_{TI} was examined in experiments using sheep cardiac Purkinje fibers. Tension was recorded while membrane voltage was controlled by a two micro-electrode voltage clamp technique. Fig. 1 shows the current-voltage relationship for I_{TI} and the aftercontraction-voltage relationship in a preparation exposed to K-free, Na-free modified Tyrode's solution containing isotonic $CaCl_2$ (105 mM, 293 mOsm). The oscillatory current observed under these conditions was identified as I_{TI} because of its timing relative to the aftercontraction. I_{TI} reversed at -32 mV indicating that the current was carried by Ca^{2+} as well as K^+. Although Na-Ca exchange may contribute some current to I_{TI} when Na^+ is present, this study shows that I_{TI} can exist independently of Na-Ca exchange current.



Th-AM-D6 TWO KINDS OF CALCIUM CHANNELS IN ATRIAL CELLS FROM DOG AND FROG HEARTS. Bruce P. Bean, Dept. Physiol. and Biophys., University of Iowa, Iowa City, IA 52242

Ca channel currents were studied in single cells isolated from dog or frog atria. Whole-cell currents were recorded with an internal solution of (in mM) 120 CsCl, 10 Cs_2EGTA, 5 $MgCl_2$, 10 HEPES, pH 7.45 and external solutions of 110 $BaCl_2$, 10 mM HEPES or 5-40 $CaCl_2$, 135 TEACl, 2 $MgCl_2$, 10 HEPES, pH 7.4. In cells from both species, there were two components of Ca channel current: one (Ifast) was present only if cells were held negative to -50 mV, became activated at ~-40 mV, and inactivated quickly ($t_{\frac{1}{2}}$ 10-40 msec); the other (Islow) was inactivated only by holding potentials positive to -40 mV, became activated at ~-20 mV, and inactivated much more slowly. Neither current was affected by 20 μM TTX; 2 mM Co blocked both to ~35%. Estimated by fluctuation analysis (dog cells in 110 Ba), the single channel current was smaller for Ifast (0.28 ± .04 pA at -20 mV) than for Islow (0.96 ± .18).



Isoproterenol (4 μM) applied to dog cells in 110 Ba increased the current at +30 mV (mainly Islow) by 116 ± 3% (N=4) but increased the current at -20 or -10 mV (mainly Ifast) by only 6 ± 6%, suggesting selective β-adrenergic regulation of Islow channels. Supported by NIH grant HL 32663.

Th-AM-D7 THE EFFECTS OF CARDIOTONIC STEROIDS ON Ca CURRENT IN FROG VENTRICULAR MYOCYTES. R. Fischmeister, J.A. Argibay* and G. Vassort. Laboratoire de Physiologie Cellulaire Cardiaque U-241 INSERM, Université Paris Sud, 91405 Orsay France.

Ca currents (I_{Ca}) were recorded with the whole-cell patch clamp technique from single, enzymatically dispersed adult frog ventricular cells, bathed in 20 mM Cs (K-free) Ringer solution (pH=7.4) containing TTX. Patch-electrodes (2-5 MΩ) were filled with buffer containing (mM) CsCl, 120; EGTA, 5; $MgCl_2$, 2.5; Na_2CP, 5; Na_2ATP, 3; Hepes, 10; pH 7.1. Cells were polarized every 8 sec, using 400 msec command pulses to various potentials (-120 to +80 mV) from a holding potential of - 60 mV. Few seconds after the membrane patch was disrupted, the cell ceased to contract. Zero current potential was ≅ -46 mV and steady state membrane resistance was ≅ 83 K .cm^2 and showed no significant voltage dependence. When no appreciable rundown of I_{Ca} was observed during a control period of ≅ 10 min, the cell was exposed to a Ringer solution containing either 10^{-6}M ouabain or $5x10^{-5}$M dihydroouabain (DHO). Both ouabain and DHO depolarized the cells by 10 to 25 mV and induced an inward shift in the background current of ≅-14 and -12 pA at -60 mV and -23 and -17 pA at +60 mV, respectively. Maximal effects were observed within 3-5 min. I_{Ca} was also reduced by both glucosides. The reduction occurred simultaneously to the decrease in Na/K pump current. I_{Ca} was depressed by ≅40% with ouabain and ≅30% with DHO. The effects of glucosides on I_{Ca} were identical at each potential and the U-shaped inactivation curve (Mentrard et al., 1984, J. Gen. Physiol. 83, 105-131) was not affected by the steroids. These observations would not be expected if an increase in Ca_i, consecutive to the Na/K pump inhibition, was the primary reason to I_{Ca} reduction. The effect of DHO on I_{Ca} was partially or totally reversible. (* *Supported by France-Venezuela exchange program).*

Th-AM-D8 EFFECTS OF EXTRACELLULAR CALCIUM DEPLETION ON TRANSMEMBRANE CALCIUM INFLUX IN FROG VENTRICULAR MYOCARDIUM K.P.Dresdner & R.P.Kline, Pharmacology Department, College of Physicians and Surgeons, Columbia University, N.Y.,N.Y. 10032

Significant depletions in extracellular calcium ion concentration (Cao) during beating have now been shown in frog ventricular myocardium using calcium sensistive microelectrodes (Ca-ISE; Dresdner et al. Biophys.Soc. abstr. 1982,1983) and optical techniques (Hilgemann & Langer, Fed. Proc.abstr.1984). We examined the effects of ongoing depletion on subsequent transmembrane Ca++ flux by examining: 1)rate dependence of the time course of slow Cao depletion; 2)effect of Cao buffer Nitriloacetic acid (NTA) on Ca-ISE signal;and 3)effects of NTA on the developement of tension. Our results are consistent with Cao depletion induced reduction in the rate of Ca++ influx. Time constant of Cao depletion induced by an abrupt increase in stimulation rate (but not recovery of Cao levels following reduction in rate) is reduced below that for diffusion alone.This effect is more pronounced at higher rates, where magnitude of depletion saturates. NTA (2-10 mM plus 1.2-5.2 mM total calcium;free calcium concentration ~ 0.2 mM at pH 7.1) reduces the magnitude and time constant of Cao depletion by countering the lowering of Cao due to net transmembrane Ca++ influx. NTA also reduces the negative component of the biphasic tension staircase at low free Cao, while potentiating peak twitch tension by ~ 30%. Action potential plateau and duration were not significantly changed by Cao depletion with or without NTA. In the presence of isoproterenol (2 μM), which greatly increases twitch tension and Cao depletion, the potentiating effects of NTA on tension developement are more pronounced. (Funded by PPG #HL30557, & HL 07271.)

Th-AM-D9 RYANODINE AND AMINOPYRIDINE SENSITIVE CURRENTS OF CARDIAC PURKINJE FIBERS. J.L.Kenyon and J.L.Sutko. Departments of Pharmacology, Physiology, and Internal Medicine, University of Texas Health Science Center, Dallas, TX 75235

We used the two microelectrode voltage clamp technique to record membrane currents from shortened calf cardiac Purkinje fibers and we measured isometric force. We examined the effects of 1 μM ryanodine, 0.5 mM 4-aminopyridine (4-AP), and 50 μM 3,4-diaminopyridine (3,4-DAP) on currents and twitches evoked by 250 msec depolarizations from about -60 mV. 3,4-DAP or 4-AP reduced the transient outward current. 3,4-DAP caused either a slight reduction or no change in the twitch. Ryanodine had complex effects on the current as it abolished the twitch. At early times (0-20 msec) the transient outward current was reduced while at later times (100-250 msec) the net outward current was increased. In combination, ryanodine plus either of the aminopyridines apparently abolished the transient outward current and revealed the slow inward current. We conclude that there are two current components that contribute to the transient outward current of calf Purkinje fibers, one is sensitive to aminopyridines and one is sensitive to ryanodine. The correlation between the outward current inhibited by ryanodine and the twitch is similar to that observed by Siegelbaum and Tsien (J. Physiol. 299:485 1980) and we believe that this is a calcium-activated current that is abolished as a consequence of the ryanodine inhibition of sarcoplasmic reticulum calcium release (Sutko and Kenyon, J.Gen.Physiol. 82:385 1983). The mechanism of the enhancement of outward current by ryanodine is unclear; it may be the result of a direct effect on sarcolemmal channels or an indirect effect of altered intracellular calcium activity. Supported by NIH 26528, AHA 81-815, NSF PCM-8402100.

Th-AM-D10 IMPEDANCE STUDIES OF VENTRICULAR TRABECULAE AND PAPILLARY MUSCLE FROM GUINEA-PIG HEART. R.T. Mathias, J. Bloom and D. Saar; Department of Physiology, Rush Medical College, Chicago.

The impedance of small (< 0.25 mm^3) sections of trabeculae and papillary muscle was recorded in solutions containing varying concentrations of potassium and in the presence/absence of barium, cadmium and cesium. The results from 6 studies in 4 mM K-Tyrode are: membrane potential, V_m = -80 ± 1 mV; membrane conductance, g_m = 0.67 ± .09 mS/cm^2; effective intercellular resistivity, R_e = 1800 ± 250 Ω cm; series resistance, R_s = 21 ± 6 Ω cm^2; surface of cleft membrane per unit volume of tissue (assuming a capacitance of 1 μF/cm^2), S_c/V_T = 2600 ± 300 cm^{-1}; surface of restricted access membrane per surface of myocyte = 52 ± 9%. These parameters can be related to structural properties of the tissue (see the abstract by Eisenberg et al, 1985) as follows: R_e implies loosely packed cells; R_s is due to the epithelial layer surrounding the tissue; S_c/V_T implies a cell diameter of about 10 μm; the restricted access membrane is T-system and intercalated disk.

The above impedance data showed evidence that K accumulation/depletion was occurring with a time constant of about 0.27 ± .03 sec. Further studies in solutions of varying K concentrations strengthened this conclusion and showed that g_m changed approximately 2 to 3 fold for a 10 fold change in bath potassium. Moreover V_m varied in accordance with E_K, suggesting a large resting K-conductance in these preparations. The addition of Ba to block the K-conductance induced spontaneous activity, which could be stopped by adding Cd to block the slow inward current. In this situation g_m decreases about 5 fold and the evidence of K-accumulation/depletion disappears; however, the impedance develops a resonance at 1 to 2 Hz (V_m = -69 mV). This resonance is partially but not totally eliminated by the addition of Cs. (Supported by NIH grant HL29205.)

Th-AM-D11 STEADY STATE PROPERTIES OF ISOLATED FROG ATRIAL AND SINUS VENOUS CELLS. L.E. Moore, E. F. Shibata, R. B. Clark and W. Giles. Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550

Isolated frog atrial cells have been used to investigate the ionic currents underlying the electrical activity of the atrial myocardium. A two microelectrode cable analysis of these isolated cells has shown that the resting space constant is many times the length of these cells (Hume and Giles, 1981) suggesting that this preparation can be adequately voltage clamped. Frequency domain measurements were made in order to substantiate these observations, as well as establish a passive equivalent circuit for the isolated cell. The admittance measurements reported here show that frog atrial cells have a passive resistance-capacitance equivalent circuit with an inconsequential internal membrane system. These results support the ultrastructural observations that the sarcotubular membrane structures in frog atrial cells are quite sparse.

In normal Ringer's solution the admittance function is markedly affected by the inward rectifier. The barium blocked atrial cell shows marked similarity to the behavior of sinus venosus cells which show no signs of inward rectification. Both cell types show a TTX insensitive voltage dependent component in the admittance which can be provisionally identified as a calcium conductance system. In addition the atrial cells show a cadmium insensitive component that may reflect a steady state sodium conductance. Supported in part by DHHS 1R01-HL32281.

Th-AM-D12 IMPEDANCE AND MORPHOLOGY OF ISOLATED CANINE CARDIAC PURKINJE MYOCYTES: COMPARISON WITH INTACT STRAND PREPARATIONS. R.T. Mathias, B.R. Eisenberg, N.B. Datyner, G.A. Gintant, and I.S. Cohen. Depts. of Physiology, Rush Medical College, Chicago, IL and S.U.N.Y., Stony Brook, NY

Acutely dissociated single Purkinje myocytes were obtained from canine cardiac Purkinje strands by gentle trituration and collagenase treatment. Morphological studies confirmed excellent myocyte ultrastructure in physiologic Tyrode's solution. Morphometric techniques indicate that the cell membrane area is increased by a factor of 2.1 above the area of an unfolded right circular cylinder. Impedance properties of myocytes were studied using a single microelectrode impedance technique with compensation for microelectode series resistance. Impedance data (9 cells, 13 μm average radius, 155 μm average length, -74 mV average membrane potential) were fit using a parallel RC circuit membrane model with a specific membrane conductance (Gm) of 47 μS/μF (calculated using the above factor of 2.1) and a specific membrane capacitance (Cm) of 1.1 μF/cm^2. No evidence for extracellular accumulation and depletion of ions was found from the impedance studies, nor was there evidence of accumulation spaces from electron micrographs.

Similar impedance studies of canine Purkinje strands provide evidence for extracellular accumulation and depletion of ions. Consistent with this finding, morphometric studies show the existence of narrow extracellular spaces. The specific membrane conductance from strand preparations (38 μS/μF) was similar to that obtained from isolated myocytes. Isolated Purkinje myocytes should (1) prove invaluable for studies of potassium currents in the absence of extracellular K^+ accumulation and depletion, and (2) provide information regarding the effect of tissue geometry on membrane currents of multicellular preparations. Supported by NIH HL-29205, -20558, -28958 and the A.H.A.

Th-AM-E1 EQUILIBRIUM AND KINETIC STUDIES OF THE NATURE OF METAL-ION BINDING IN PARVALBUMIN, W. DeW. Horrocks, Jr., P. J. Breen, E. K. Hild and K. A. Johnson, Departments of Chemistry and BMMCB, Pennsylvania State University, University Park, PA 16802.

Parvalbumin is a small (MW 12000), structurally well-characterized protein prototypical of calcium-modulated proteins. It undergoes conformational changes upon binding of metal ions to two primary calcium-binding sites. Changes in the protein fluorescence or absorption spectra (from the single trp residue) as Ca(II) or Ln(III) ions are titrated into apo codfish parvalbumin reveal a break after one equivalent of added metal ion, indicating that the two binding sites are filled sequentially by these ions. The kinetics of metal ion removal or substitution in this protein were studied using a stopped-flow apparatus. The metal bound protein was mixed with (a) an excess of the metal ion chelator 1,2-diaminocyclohexanetetraacetic acid (DCTA) or (b) an excess of a competing metal ion. The reactions were monitored by (a) changes in trp fluorescence in going from metal-bound to apo, (b) quenching of trp fluorescence upon the binding of Eu(III) or Yb(III), or (c) changes in the sensitized Tb(III) luminescence upon addition or removal of this ion. The double exponential traces were fitted to obtain k_{off} values for the two sites of parvalbumin. For the DCTA experiments these (0.50 and 0.05 sec^{-1}) vary little across the Ln(III) series. The corresponding k_{off} values for Ca(II) are 1.0 and 5.9 sec^{-1}. For the metal substitution experiments, k_{off} varies directly with the concentration of the substituting metal ion, M', indicating an additional mechanism involving direct attack on the metal-bound protein by M'. The k_{off} values extrapolated to [M'] = 0 agree well with the values for k_{off} from the DCTA experiments. Together these results provide strong evidence for the lack of cooperative binding to the two sites.

Th-AM-E2 DETERMINATION OF THE TRIPLET QUENCHING CONSTANT FOR MODEL INDOLES IN VARIOUS SOLVENTS AND SINGLE TRYPTOPHAN-CONTAINING PROTEINS USING ACRYLAMIDE. by C.A. Ghiron*, M. Bazin and R. Santus. *Department of Biochemistry, University of Missouri, Columbia, MO 65211, U.S.A. and Laboratoire de Physico-Chimie de L'adaption Biologique du Museum National d'Histoire Naturelle, 43 rue Cuvier, 75231, Paris, France.

The decay of the indole triplet of single tryptophan-containing proteins and model compounds can be readily measured in aqueous solution by monitoring triplet-triplet absorption and/or phosphorescence emission at room temperature following a 30ns, 265nm exciting Laser pulse. The acrylamide triplet quenching constant (k_q^t) ranged from a high of $7.8 \times 10^8 M^{-1}s^{-1}$ for the exposed indole of ACTH to a low of $2 \times 10^5 M^{-1}s^{-1}$ for the buried indole of RNase T_1. The ratio (γ) of these values with their respective acrylamide singlet quenching constants (k_q^s) ($\gamma = k_q^t/k_q^s$) ranged from a high of 0.22 for ACTH to a low of 0.001 for RNase T_1. Acrylamide is also an inefficient quencher of model indoles in various solvents. The magnitude of γ varied from a high of 0.3 in H_2O to a low of 0.02 in acetonitrile, but did not correlate with viscosity, dielectric constant or polarity. The much greater inefficiency observed for "buried" indole groups can not be explained by that class of models which predict the presence of "static" quenching at the triplet level, since none was observed within experimental error. For example: The acrylamide quencher might be partitioned into an internal binding site adjacent to, but not in contact with the buried indole side chain of RNase T_1. The present results confirm the observation of Calhoun et al. Biochemistry 22, 1533 (1983) and suggest that it may be largely explained by acrylamide's inefficient triplet quenching. The magnitude of this inefficiency is probably determined by specific microenvironmental factors.

Th-AM-E3 RESOLUTION OF THREE-COMPONENT MIXTURES OF FLUOROPHORES BY PHASE SENSITIVE DETECTION OF FLUORESCENCE, by S. Keating-Nakamoto and J.R. Lakowicz, University of Maryland, School of Medicine, Department of Biological Chemistry, Baltimore, Maryland 21201.

We describe a new application of phase sensitive detection of fluorescence which permits the determination of the lifetimes and fractional intensities of each component in multi-component mixtures of fluorophores. Resolution of three components is possible using data obtained at a single modulation frequency. In this method phase sensitive spectra are recorded at about ten detector phase angles, without concern of supressing the emission of any individual component. These phase sensitive spectra are then fit using the method of non-linear least squares to yield the lifetimes and fractional intensities of the individual component. It is necesary to know the steady state spectra of the individual components. We resolved the lifetimes and intensities of a two-component mixture of 9-methylanthracene (4.5 nsec) and 9,10-diphenylanthracence (5.9 nsec), and a three-component mixture of POPOP (1.3 nsec), 9-methylanthracene (4.5 nsec) and 9,10-diphenylanthracene (5.9 nsec). To illustrate the potential usefulness of this method for studies of protein fluorescence we resolved a mixture of N-acetyl-L-tyrosinamide (1.5 nsec), N-acetyl-L-tryptophanamide (2.9 nsec) and indole (4.5 nsec). These results indicate that phase sensitive detection fluorescence, even at a single modulation frequency, can provide useful resolution of multi-component emissions.

Th-AM-E4 TIME-RESOLVED FLUORESCENCE ANISOTROPIES OF FLUOROPHORES AND MEMBRANE-BOUND MELITTIN BY FREQUENCY-DOMAIN FLUOROMETRY. By B.P. Maliwal and J.R. Lakowicz, Department of Biological Chemistry, University of Maryland, School of Medicine, Baltimore, Maryland 21201.

We report the measurement of the time-resolved anisotropy decays and the determination of rotational correlation times using a newly constructed variable frequency phase fluorometer (Lakowicz and Maliwal, Biophysical Chemistry, in press). This instrument provides impressive resolution of complex anisotropy decays. For instance, we resolved two rotational correlation times for perylene in glycerol, and three rotational correlation times for this anisotropic molecule in DMPC vesicles. Remarkably, we resolved two correlation times for 9-aminoacridine which differ only 1.4 fold. The resolution of anisotropy decays now appears adequate to reveal the shapes of proteins in solution. Additionally, we descirbe the anisotropy decays of N-methylanthraniloyl melittin as the monomer, tetramer and bound to DMPC vesicles. These decays are sensitive to the chemical composition and phase state of the lipids. In summary, frequency-domain fluorometer provides rapid and reliable resolution of complex anisotropy decays.

Th-AM-E5 RESOLUTION OF COMPLEX FLUORESCENCE DECAY KINETICS USING THE METHOD OF MOMENTS. Enoch W. Small and Louis J. Libertini, Department of Biochemistry and Biophysics, Oregon State University, Corvallis, Oregon 97331.

In the past year we have made a number of improvements on our fluorescence lifetime instrument. Our dye laser is now synchronously pumped by a new Nd:YAG laser and we have added a proximity type triple microchannel plate photomultiplier. When the dye laser is operated with rhodamine 575, the cavity-dumped and frequency-doubled output supplies more than an order of magnitude more intensity at 280 nm than our previous argon laser pumped system. This has enhanced our ability to work with the intrinsic tyrosine fluorescence of chromatin. The new photomultiplier is capable of differentiating between single and multiple photon events, has a response half width of less than 100 ps and no wavelength dependence that we can detect. Because of improvements in our instrument and advances in our data analysis methods we can now routinely perform difficult three and four component analyses of measured data. We can even analyze some five component decays if we use tricks such as F/F deconvolution or Cheng-Eisenfeld moment filtering. We are now testing new methods of analyzing fluorescence anisotropies, including the use of the total fluorescence intensity to analyze the difference between the parallel and perpendicular intensity components. This F/F deconvolution approach is insensitive to errors in the sensitivity correction. By using this method we have been able to detect anisotropic rotational diffusion of a relatively short lifetime (2.47 ns) dye, rhodamine B in methanol solution. We have also written and tested a new program for the analysis of nonconvoluted multiexponential decays.

Th-AM-E6 INTERNAL SITE DIRECTED FLUORESCENCE LABELING OF A MONOCLONAL ANTIBODY. Beverly Packard, Akira Komoriya, and Michael Edidin. Biology Department, The Johns Hopkins University, Baltimore, MD. 21218 and Revlon Biotechnology Research Center, Rockville, MD. 20850.

Fluorescently labeled antibodies are used both to identify surface antigens of diverse cell types and to probe the dynamics of these molecules. The most common targets of direct labeling of antibodies as well as proteins in general are the nucleophilic sites, i.e., α-amino termini and the ε-amino group of lysine. Drawbacks of this approach include nonspecifity of labeling due to a multitude of reactive sites and difficulty in the purification of monospecifically labeled proteins. Additionally, with the commonly used chemical linkages between proteins and fluorophores, the latter often retain autonomous motion to a significant degree. We have chemically modified 5-carboxyfluorescein by the addition of two sulfhydryls so that it may insert into the disulfide bonds of immunoglobulins; thus, the design includes minimization of possible autonomous motion of the fluorophore as well as exploitation of the limited number of disulfide environments of immunoglobulins. Using this probe we have specifically labeled a monoclonal antibody with retention of biologic activity. Crabescein, a fluorescent molecule with a claw, may serve as a prototype for specific labeling of disulfide containing peptides with the fluorescence signal coming from a relatively rigid environment.

Th-AM-E7 DIRECT MEASUREMENT OF THE INTERACTION OF eIF-4A WITH mRNA AND NUCLEOTIDES
Dixie J. Goss, Chemistry Dept., Hunter College of CUNY, 695 Park Ave., New York, NY 10021 and Charles L. Woodley, and Albert J. Wahba, Dept. of Biochemistry, U. of Mississippi Medical Center, Jackson, MS 39216-4505.

We have previously used fluorescence techniques to monitor directly protein-protein and protein-nucleic acid interactions. Fluorescent labeling of eucaryotic initiation factor 2 (eIF-2) allowed us to describe a mechanism for the interaction of eIF-2 with guanosine nucleotide exchange factor (GEF), GDP, and GTP (J. Biol. Chem. (1984) 259, 7374). To further explore the roles of various initiation factors in polypeptide chain initiation, we have fluorescently labeled eucaryotic initiation factor 4A. By monitoring changes in fluorescence intensity and fluorescence anisotropy, direct interaction of eIF-4A with ATP, ADP, eIF-4B and various mRNA have been detected. Analysis of fluorescence quenching data allowed us to determine the K_D for ATP and ADP binding to eIF-4A. The equilibrium constants (K_D) obtained were approximately the same (80μM) for both nucleotides. The presense of Mg^{2+} decreased K_D more than two-fold. Messenger RNA with and without the 5' cap and having different secondary structure will be assayed for binding to eIF-4A and other initiation factors.

Th-AM-E8 ULTRAVIOLET RESONANCE RAMAN SPECTROSCOPY OF AROMATIC AMINO ACIDS AND CYTOCHROME C
Robert A. Copeland and Thomas G. Spiro, Department of Chemistry, Princeton University, Princeton, New Jersey 08544 USA

Ultraviolet resonance Raman (UVRR) spectra are reported for aromatic amino acids and the heme protein cytochrome c using 200 and 218 nm excitation. With 200 nm excitation tyrosine and amide modes are most strongly enhanced while at 218 nm excitation tryptophan, tyrosine, and phenylalanine modes are seen.

For heme proteins, UV excitation allows selective enhancement of aromatic amino acid residues and amide modes without interference from the heme chromophore. Spectra are presented for cytochrome c from horse and tuna heart in several pH dependent conformational states in both H_2O and D_2O. Changes seen in these spectra are interpreted by study of the individual aromatic amino acid UV RR spectra in H_2O and D_2O.

Th-AM-E9 ULTRAVIOLTET RESONANCE RAMAN SPECTROSCOPY OF NUCLEOTIDES AND NUCLEIC ACID POLYMERS
Stephen P.A. Fodor and Thomas G. Spiro, Department of Chemistry, Princeton University, Princeton, New Jersey 08544 USA

UV resonance Raman spectra of mononucleotides, synthetic and naturally occurring DNA duplexes are presented. Resonance excitation into the strong ultraviolet absorption bands of purine and pyrimidine bases yield spectra rich in vibrational detail.

Selective enhancement of bases and exocyclic components is achieved via appropriate choice of excitation wavelength, allowing discrimination of individual base components in the polynucleotide spectra. Vibrational features obscured in the non-resonance Raman spectra are clearly resolved using UV excitation, leading to a more detailed analysis of structural variations in these biopolymers.

Th-AM-E10 GAMMA-GAMMA PERTURBED ANGULAR CORRELATION SPECTRA FROM STOCHASTIC MOLECULAR DYNAMICS MODELS. Christopher Haydock and Lynda McDowell, Department of Pharmacology, Mayo Foundation, Rochester, MN 55905.

Perturbed angular correlation (PAC) spectra are computed from a Langevin dynamics model for the labeled group trajectory. These model spectra are qualitatively compared with experimental data for 111-Indium chelate complexes in water-solvent mixtures at temperatures from 200 to 300° K. In the model, a rigid probe-ligand complex interacts with the solvent and the remainder of the chelating molecule. This interaction is parameterized by three orientational friction coefficients and an angular constraint parameter (d). The parameter d defines the allowed orientations of an approximate symmetry axis of the rigid complex by the condition that $-1 < d < \cos(\theta) < +1$, where θ is the polar angle of this axis relative to the remainder of the chelator. Additional model parameters include the magnitude and asymmetry of the hyperfine interaction electric field gradient, the Euler angles of the electric field gradient with respect to the probe-ligand complex, and an isotropically fluctuating electric field gradient component. The magnitude and the correlation time of this fluctuating component are adjusted to model the effects of internal vibrations and solvent collisions on the ligand-probe bonding geometry. Experimental data are presented for 111-Indium complexes with ethylenediaminetetraacetate (EDTA), 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetate (BAPTA), citrate, nitrilotriacetate (NTA), N-benzyliminodiacetate (NBIDA), and 4-isothiocyanatobenzylethylenediaminetetraacetate (ITC-Bz-EDTA). This research was supported by NIH grants GM 31241 and BRP 1 R03 RR02219-01.

Th-AM-F1 PERMEABILITY AND ELECTROPHYSIOLOGICAL PROPERTIES OF ELECTROTONIC SYNAPSES BETWEEN APLYSIA NEURONS IN SITU AND IN CULTURE. R. Bodmer and D.C. Spray
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Coupling between identified abdominal motoneurons in situ and that between unidentified neurons in culture have similar properties. Junctional conductance (g_j) is insensitive to transjunctional or inside-outside potentials of at least 50 mV of both polarities, although coupling coefficients can be voltage dependent, due to rectification by nonjunctional membranes. The relation between g_j and intracellular pH (pH_i) measured with liquid ion selective electrodes during exposure to CO_2 is approximately linear over the pH range 7.3 to 5.8; at higher pH_i, g_j levels off and at the lowest pH_i a residual 10-30% conductance remains. Exposure to 1mM octanol, which decreases g_j in other tissues, has little effect on coupling between Aplysia neurons. Permeability (P_j) of Aplysia junctions to tetramethyl and tetraethyl ammonium ions was measured with K sensitive microelectrodes; P_j/g_j is similar to

Th-AM-F4 BIOPHYSICAL PROPERTIES OF RAT LIVER GAP JUNCTION CHANNELS. D.C. Spray and E.L. Hertzberg, Dept. Neuroscience, A. Einstein College Of Medicine, Bronx, N.Y. 10461 and Dept. Biochem. Baylor Coll. of Medicine, Houston Tx. 77030

We have studied the electrical properties of gap junctions between pairs of freshly dissociated rat hepatocytes and compared them to those of membranes in which isolated gap junctions are presumably incorporated. Under dual voltage clamp using patch electrodes, gap junctional conductance (g_j) between hepatocyte pairs is insensitive to either transjunctional or inside-outside voltage. When intracellular pH (pH_i) is simultaneously measured, acidification with CO_2 reveals a steep pH dependence of g_j in the range 6.5 to 6.0. Injection of affinity purified antibodies to the 27kD rat liver gap junction polypeptide irreversibly blocks g_j and dye transfer between cells; injection of preimmune serum and bathing cells in the potent antibodies are ineffective. When patch pipettes are dipped several times through a layer of mixed brain phospholipid into a solution containing 1 ug/ml gap junction membrane, pipette conductance decreases, possibly due to membrane formation but usually remains high (10 nS). This macroscopic conductance shows no dependence on pipette voltage, is dramatically but reversibly decreased between pH 6.5 and 6.0, and is irreversibly decreased, often to very low conductances, by addition of antibody. Channel like events are sometimes seen which are also voltage independent and blocked by antibody. We appear to have a membrane preparation resembling in situ liver gap junctions in several important respects. Supported by NIH NS 16524 (D.C.S) and GM 30667 (E.L.H.) and McKnight Foundation Award to D.C.S.

Th-AM-F5 INCREASED CELL-TO-CELL DIFFUSION OF LUCIFER YELLOW CH PRODUCED BY dBcAMP IN HEART FIBERS. W. C. De Mello, P. van Loon and N. Vizcarra. Department of Pharmacology, Medical Sciences Campus, GPO Box 5067, San Juan, Puerto Rico 00936.

The longitudinal movement of Lucifer Yellow CH along dog trabeculae was studied with the cut-end method. The results indicated that in muscles immersed in normal Tyrode solution the longitudinal redistribution of the dye occurred over 3.5 mm (about 29 cells). The average effective diffusity (D) was found to be $4.3 \pm 1.3 \times 10^{-7}$ cm^2/s. From non-steady state diffusion measurements (diffusion time - 45 min.) the permeability of the nexus (P_{nexus}) to Lucifer Yellow CH was found to be 3.5×10^{-4} cm/s. In fibers exposed to dBcAMP (5×10^{-4} M), which increases the intracellular concentration of cAMP, the average diffusity was enhanced to $1.6 \pm 1.05 \times 10^{-6}$ cm^2/s. The permeability of the gap junctions to Lucifer Yellow was also appreciably increased (1.2×10^{-3} cm/s) by dBcAMP. No change in D or P_{nexus} was found with 8-Br-cGMP (5×10^{-9} M). The results support the idea that cAMP modulates the junctional permeability in cardiac muscle (De Mello, 1983). (Supported by Grant RR08102-13, and in part by Grant HL30614-01 from NIH.)

Th-AM-F6 VOLTAGE-GATED AND TRANSMITTER-GATED MEMBRANE CURRENTS RECORDED FROM ISOLATED BIPOLAR CELLS OF THE AXOLOTL RETINA, Martin Wilson,* David Attwell, Peter Mobbs, and Marc Tessier-Lavigne, Department of Physiology, University College London and *Department of Zoology, University of California at Davis.

Bipolar cells constitute the pathway of information flow from the outer to the inner retina. Examination of the membrane currents of these cells in the retina is made difficult by their inaccessibility and the complexity of their connections with other cells. We have avoided these problems by examining bipolar cells isolated from enzymatically-dissociated retinae. Bipolar cells were recognized by the presence of a Landolt club and an axon at opposite poles of the cell body.

Whole-cell patch-clamp recordings were made with patch pipettes filled with a variety of solutions. At a holding potential of -55 mV, cells had an input resistance of about 1 GΩ and showed strong outward rectification. Following a voltage step to potentials more positive than -35 mV, large outward currents could be seen at short times but these decreased substantially over a few hundred millseconds. This time-dependent current may contribute to the shaping of the bipolar cell light response.

Pressure ejection of aspartate [230 μM] failed to elicit a response, but glutamate, another candidate photoreceptor transmitter, did produce a conductance increase in a small fraction of cells. Both GABA, a possible horizontal cell transmitter, and glycine, a possible transmitter of interplexiform cells, produced a conductance increase, probably to Cl^-, when applied by pressure ejection [100 μm]. Supported by the MRC, the Nuffield Foundation, the Central Research Fund of London University and NIH [EY04112]. MT-L is a Commonwealth scholar.

Th-AM-F7 MODULATION BY GLYCOLIPIDS OF MEMBRANE ADHESION AND CONDUCTANCE CHANGES IN LARGE SPHERICAL MODEL MEMBRANES. G.J. Brewer and P.D. Thomas, Department of Medical Microbiology and Immunology, Southern Illinois University, School of Medicine, Springfield, IL. 62708

The formation of two spherical model membranes at the tips of two syringes has allowed us to study the role of gangliosides in membrane adhesion and look for changes in conductance between two such membranes during the process of adhesion. Membranes were formed in aqueous 100 mM NaCl, 10 mM KCl, 1 mM $CaCl_2$ from 1% (w/v) egg phosphatidylcholine in n-decane, with or without mixed beef brain gangliosides. After thinning to the "black" bilayer state, the apposition of two such membranes resulted in adhesion, not fusion. The rate of formation of the adhesion area was greatest at 0 to 0.8 mole % gangliosides; it then declined 25% and remained constant up to 10 mole %. Adhesion was frequently blocked at 11 to 15 mole % gangliosides. With gangliosides, the contact area and conductance increased colinearly with time over a 5 to 20 min period of adhesion. Electrostatic bridging by calcium contributed to the rate of adhesion, but calcium was not required to observe adhesion. As the ganglioside concentration was increased from 0 to 15 mole %, the electrical conductance of individual membranes decreased three-fold from 48±30 nS/cm^2 to 17±13 nS/cm^2. The conductance was pH dependent with a minimum at neutral values. At neutral pH, when two membranes containing 4.1 mole % gangliosides adhered, the region of adhesion had a specific conductance three times that of the nonadhering regions of membranes. Without gangliosides, the specific conductance of the contact region was the same as that of nonadhering regions of the membrane. A polarity-sensitive fluorescent probe indicated an environment in the junctional region less polar than that in nonadhering regions. These data suggest that mixed gangliosides can mediate an adhesion dependent increase in conductance, a signal for contact sensation.

Th-AM-F8 C-TERMINAL ARM AND CELL-TO-CELL CHANNEL GATING Camillo Peracchia and Stephen J. Girsch, Dept. of Physiology, Univ. of Rochester Medical Center, Rochester, New York 14642.

There is evidence that cell-to-cell channels close when $[Ca^{++}]_i$ or $[H^+]_i$ increase and that calmodulin (CaM) may be involved, either directly or indirectly, in channel gating. To study in vitro channel permeability and gating we have incorporated into liposomes the lens junction protein (MIP26), a 28.2 kDa component, and its trypsin-cleaved product (MIP21), a 21-22 kDa component which has lost the 5-7 kDa C-terminal arm, with and without CaM (equimolar). The function of the resulting channels (hemichannels) was studied by means of an osmotic swelling assay (Lukey and Nikaido, 1980). This assay consists in loading the liposomes with a channel impermeant, Dextran T-10 (MW=10,000) and suspending them into iso- or hypertonic solutions of channel permeants: KCl, sucrose (SUC) or polyethyleneglycol (PEG, MW=1500). The presence of open channels is determined by the decrease in optical density ($OD_{500\ nm}$), caused by liposome swelling, as the permeant molecules diffuse through the channels while T-10 does not. MIP26 and MIP21 incorporation into liposomes was monitored with freeze-fracture EM. MIP26 and MIP21 liposomes swelled in KCl, SUC or PEG both with or without Ca^{++} (100 µM) indicating the presence of channels larger than 1 nm in diameter. In the absence of Ca^{++}, MIP26-CaM and MIP21-CaM liposomes swelled in all permeants. Upon addition of Ca^{++} (100 µM), MIP26-CaM liposomes did not swell in any of the permeants, indicating complete channel closure, while MIP21-CaM liposomes swelled in KCl and sucrose but not in PEG, indicating that these channels close only partially. These data indicate that the C-terminal arm of the lens channel protein may represent the major component of the channel gate. Since in the lens MIP26 is naturally cleaved to MIP21 with age, the presence of ungatable channels in old lenses may be a safety device for maintaining transparency. Supported by NIH GM20113.

Th-AM-F9 ARE GAP JUNCTIONAL PITS AND PARTICLES COMPLEMENTARY STRUCTURES? Leszek Kordylewski and Ernest Page, University of Chicago.

Gap junctional (GJ) pits and particles are thought to be complementary structures; although it is well known that, in replicas of freeze fractured tissues, pits are more closely spaced and more highly ordered than particles, these discrepancies have been attributed to "plastic deformation" affecting the P-face particles more than the E-face pits. We have reexamined this explanation by studying complementary replicas (CR) of GJ in sheep cardiac Purkinje strands fixed with glutaraldehyde, cryoprotected with glycerol, freeze fractured with unidirectional shadowing using the Balzers apparatus, and photographed at original magnifications of 50,000 or 200,000 X with tilting on the goniometer stage of a Hitachi 600 electron microscope. Using several techniques for superimposing stereoimages of the complementary E- and P-faces, we found that pits fall between particles, not on them. In CR in which the numbers of pits and particles could be compared directly for defined corresponding (complementary) areas of E- and P-face in the same GJ, the ratio (number of pits/number of particles) ranged from 1.3 - 1.5. Similar ratios were found by measuring the number of particles or pits per unit area in rotary shadowed E- and P-faces of the same cardiac GJ prepared (without CR) using rotary shadowing, in which underestimation of P-face particle number due to apparent "fusion" of particles could be ruled out. We conclude that, whatever the role of plastic deformation, GJ pits and particles are not complementary structures and the pits do not lie on the same transmembrane axis as the particles. Supported by USPHS NHLBI Grants HL 10503 and 20592.

Th-AM-F10 LIPOSOME-INCORPORATED LIVER GAP JUNCTION CHANNELS ARE LESS PERMEABLE THAN LENS CHANNELS
Stephen J. Girsch and Camillo Peracchia (Intr. by Bruce C. Spalding), Department of Physiology, University of Rochester, Medical Center, Rochester, New York 14642

Most cells communicate directly with each other by means of channels 1-1.5 nm in size located in gap junctions. These cell-to-cell channels are regulated by $[Ca^{++}]_i$ and $[H^+]_i$, with the possible involvement of calmodulin (CaM). Previous studies have shown that lens cell-to-cell channel protein (MIP26, 28.2 kDa) can be incorporated into liposomes and the permeability and calmodulin-mediated gating of the resulting channels elucidated by a spectrophotometric osmotic swelling assay (Girsch and Peracchia, J. Membr. Biol. in press). Liver gap junctions were isolated using the recent NaOH-wash method of Hertzberg (JBC, 259, 9936-9943, 1984). Junctional protein was extracted with 2% SDS (overnight, 4° C), clarified by centrifugation at 48K x g and checked for purity by SDS-PAGE without preboiling. Liposomes were prepared by resuspension and sonication using brain phospholipids. After dehydration, liposomes were loaded with Dextran T-10 (a 10 kDa channel impermeant) and suspended into hyperosmotic channel-probe solutions (KCl, sucrose, and 1.5 kDa polyethylene glycol, PEG) both with and without Ca^{++}. Like lens channels, liver gap junction channels readily admit KCl and sucrose with first-order kinetics. Unlike lens channels, the liver channels are impermeable to PEG, indicating an apparently smaller bore diameter. The different permeability properties of lens and liver channels may reflect tissue-specific functional needs. Experiments on the gating properties of the incorporated channels are in progress. Supported by NIH GM20113

Th-AM-F11 SUPPRESSION OF PACEMAKER ACTIVITY BY PASSIVE INTERACTIONS BETWEEN ELECTRICALLY COUPLED HEART CELL AGGREGATES.
R.D. Veenstra AND R.L. DeHaan, Dept. of Anatomy, Emory University School of Medicine, Atlanta, Georgia 30322.

Pairs of spontaneously active heart cell aggregates establish a synchronized beat rate shortly after they are brought into contact which may differ substantially from the intrinsic beat rate of either member. The synchronous rate is determined by pacemaker phase-resetting and other action potential interactions, as well as by passive subthreshold electrical interactions (Ypey et al., Dev. in Cardiovasc. Med. 17, 1982). To study passive electrical interactions further we have used 150 um diameter aggregates prepared from cells of 4-day, 7-day and 14-day embryonic chick ventricle as models of pacemaker and non-pacemaker tissue. In medium containing 1.3 mM K^+, the synchronized spontaneous beat rates of pairs of 4+1 (4-day ventricle + 1 day in culture), and 7+1 aggregates were 89±6 b/m (n=69) and 59±14 b/m (n=72), respectively. 14+1 aggregates do not beat spontaneously. Mixed pairs consisting of a 4+1 and a 7+1 aggregate established an intermediate rate of 65±6 b/m (n=54). Elevation of bath K^+ to 2.8 mM reduced the beat rate of 4+1 pairs only slightly (83±13 b/m) but slowed 7+1 pairs by half (31±7 b/m). Mixed pairs were also slowed significantly, to 48±9 b/m. As the degree of electrical coupling increased after a 4+1 and a quiescent 14+1 aggregate were brought into contact, the beat rate of the 4+1 member gradually declined to a minimum value (16±13 b/m; n=16) just before the 14+1 aggregate began to beat in synchrony. The rate then slowly recovered to a stable value of 40±12 b/m. We conclude that the spontaneous beat rate of a primary pacemaker can be slowed or suppressed by passive interactions with latent pacemaker or non-pacemaker tissue.(Supported by NIH HL06909 to RDV and HL27385 to RLD)

Th-AM-G1 A REGION OF TROPONIN C CONTAINING RESIDUES 89-100 UNDERGOES FAST CONFORMATIONAL CHANGES UPON Ca^{2+}-BINDING TO SITES I AND II. Z. Grabarek, P.C. Leavis and J. Gergely., Dept. Muscle Res., Boston Biomed. Res. Inst. and Dept. Neurology, Mass General Hosp., Boston MA 02114.

Residues 89-100 of TnC (C_{89-100}) and 96-116 of TnI (I_{96-116}) interact with each other in the troponin complex (Dalgarno et al., 1982, FEBS Letters, 150;54) and are necessary for the Ca^{2+}-sensitive inhibition of actomyosin ATPase activity (Syska et al., 1976, Biochem. J. 153;375., Grabarek et al., 1981, J.Biol. Chem. 256; 13121). In order to determine whether regulatory Ca^{2+}-binding sites (I,II) or Ca^{2+}-Mg^{2+} sites (III,IV), induce changes in C_{89-100} we have monitored the fluorescence of TnC labelled at Cys-98 with 1,5-IAEDANS. Titration of the labeled TnC with Ca^{2+} has shown that the probe is sensitive to binding to site III and IV with or without TnI present. We have performed stopped flow experiments on labeled TnC in the presence of Mg^{2+}. There is rapid fluorescence increase related to Ca^{2+}-binding to unoccupied sites I and II followed by a slower phase ($k=11.4\ s^{-1}$) that probably represents Mg^{2+}-Ca^{2+} exchange in sites III and IV. In the presence of TnI only the fast change is detected. Thus the presence of Mg^{2+} at site III and IV permits the detection of rapid changes in the environment of Cys-98 upon Ca^{2+}-binding to the regulatory sites I and II. This rapid change may be instrumental in triggering activation of the thin filament by transferring information to TnI via a change in the contact between C_{89-100} and I_{96-116}. (Supported by NIH grants HL20464 HL5949 and HL05811)

Th-AM-G2 BINDING STUDIES OF TROPONIN T WITH TROPONIN C. Nouman A. Malik and Herbert C. Cheung (Intro. by Nepalli R. Krishna), Dept. of Biochemistry, University of Alabama in Birmingham, Birmingham, AL 35294

The interaction between skeletal troponin T (TNT) and tropoinin C (TNC) was monitored by changes in intrinsic and extrinsic fluorescence intensity at 20°C in 0.3 M KCl, 2 mM EGTA and 50 mM Tris at pH 7.5. The binding constants derived from the Trp. fluorescence of TNT were $2.5 \times 10^4\ M^{-1}$ in the absence of cation and $4.5 \times 10^4\ M^{-1}$ in the presence of Mg^{2+}. No binding constant could be determined in the presence of Ca^{2+} since the fluorescence signal was little changed as increasing TNC was added. Two sets of binding studies were performed with labeled proteins. TNT labeled with dansyl chloride yielded the following constants: $6.2 \times 10^5\ M^{-1}$ in the absence of cations, $9.2 \times 10^5\ M^{-1}$ in the presence of Mg^{2+} and $6.5 \times 10^5\ M^{-1}$ in the presence of Ca^{2+}. When TNC was modified with 5-(iodoacetamido)eosin, the binding constants determined in the absence of cation and in the presence of Mg^{2+} were $1.1 \times 10^6\ M^{-1}$ and $1.5 \times 10^6\ M^{-1}$, respectively. Because of insufficient fluorescence change, a good estimate of the binding constant could not be obtained in the presence of saturating Ca^{2+}. The binding constants derived from intrinsic fluorescence were considerably smaller than those from labeled proteins. In spite of this difference, the results indicate that the TNT·TNC complex is only marginally stabilized by Mg^{2+} binding to the Ca^{2+}-Mg^{2+} sites of TNC. The results from labeled TNC suggest that the stability of the binary complex is not affected by saturation of all four Ca^{2+} sites. Mg^{2+} or Ca^{2+} binding to TNC has little or no effect on the strength of the TNT-TNC linkage. This lack of an effect is in contrast to the role of metal binding in the stabilization of the TNI·TNC linkage previously reported. (Supported in part by AM25193).

Th-AM-G3 A FAMILY OF FAST TROPONIN T SPECIES IN RABBIT SKELETAL MUSCLE THAT DIFFER IN THE N-TERMINAL REGION. Margaret M. Briggs and Frederick H. Schachat. Department of Anatomy, Duke University Medical Center, Durham, NC 27710. (Introduced by H.C. Beall).

Four species of fast troponin T (TnT) have been identified by immunoblot analysis of myofibrils from the intrinsic musculature of rabbit tongue. Two have been characterized previously. TnT_{2f} (M_r 37,000) is the major TnT in fast muscles of the rabbit back sequenced by Pearlstone et al. (J. Biol. Chem. (1977) 252, 983-989), and TnT_{1f} (M_r 37,500) has been purified and shown to differ from TnT_{2f} in the N-terminal CNBr fragment (Briggs et al., J. Biol. Chem. (1984) 259, 10369-10375). The two new species are designated TnT_{Cf} (M_r 39,000) and TnT_{3f} (M_r 35,000). Time course degradation experiments indicated that they do not arise from proteolysis, and the molecular bases of the heterogeneity were further investigated.

The N-terminal region of each TnT was [^{32}P]-phosphorylated with TnT kinase. By comparison with CNBr peptides purified from TnT_{1f} and TnT_{2f}, stained and autoradiographed SDS-PAGE peptide maps of the four labeled TnT species showed that each one has a different N-terminal CNBr peptide (CB3). No difference in the CB2 peptides is apparent at this level of resolution. While TnT_{3f} is present in the trunk, hindlimb, and diaphragm muscles as well as in the tongue, TnT_{Cf} has not yet been detected elsewhere and may be restricted to craniofacial muscles. The four fast TnT species appear to be members of a family of homologous proteins that differ in the N-terminal region.

This work was supported by a grant from the NIH(NS18228) to F.H.S.

Th-AM-G4 FLUORESCENCE ENERGY TRANSFER BETWEEN Tb^{3+} AT THE HIGH AFFINITY SITES AND LABELS IN THE REGION OF THE LOW AFFINITY SITES OF TROPONIN-C. C.-L.A. Wang, T. Tao and J. Gergely, Dept. of Muscle Res., Boston Biomed. Res. Inst., Dept. of Biol. Chem. and Neurology, Harvard Med. School, and Dept. of Neurology, Mass. Gen. Hosp., Boston, MA 02114

Tb^{3+} bound at the two high affinity sites of troponin-C (TnC) can be excited either indirectly through Tyr-109 with a pulsed UV lamp, or directly with a N_2-pumped dye-laser. The Tb^{3+} luminescence resulting from either method of excitation decays with a single lifetime of 1.3 ms, suggesting that the immediate environments of both bound Tb^{3+} ions are identical. Upon direct excitation of Tb_2-TnC labeled at Met-25 with 4-nitrobenz-2-oxa-1,3-diazole (NBD) or 4-dimethylamino-phenylazobenzene (DAB) as Forster type energy transfer acceptor the luminescence decays bi-exponentially, the lifetimes being 0.76 ms and 1.40 ms for TnC^{NBD}, and 1.0 ms and 1.38 ms for TnC^{DAB}. These two lifetimes indicate that (i) only one of the two bound Tb^{3+} ions transfers energy to the label, the transfer efficiencies yielding a distance of 3.7 nm between them; and (ii) Tb^{3+} at the other site, corresponding to the component associated with the unquenched emission, is at a greater distance from the label. Indirect excitation of Tb^{3+} bound to labeled TnC results in emission with a single--unquenched--lifetime, suggesting that Tyr-109 transfers energy to only that Tb^{3+} which is bound at the site more distant from the label. This Tb^{3+} luminescence is quenched when Ca^{2+} is added, suggesting that binding of Ca^{2+} to the low affinity sites accompanies a conformational change which either alters the position of Tyr-109 or shortens the separation between the label and one of the two high affinity sites. (Supported by NIH Grants AM32727, HL5949, and AM21673 and grants from NSF and MDA)

Th-AM-G5 EXCITATION ENERGY TRANSFER MEASUREMENTS OF THE DISTANCE BETWEEN CYS-98 OF TNC AND CYS-133 OF TNI IN RECONSTITUTED RABBIT SKELETAL TROPONIN. T. Tao, G. Strasburg, E. Gowell & P.C. Leavis. Dept. of Muscle Research, Boston Biomedical Research Institute and Dept. of Neurology, Harvard Medical School, Boston MA.

We have used excitation energy transfer to measure the distance between specific sites in the troponin complex and to study how this distance changes with respect to bound metal ions. The fluorescent donor 1,5-IAEDANS was used to label Cys-98 of TnC. The chromophoric acceptor DAB-Mal (4-dimethylaminophenylazophenyl-4'-maleimide) was used to label Cys-133 of TnI. These labeled subunits were combined with unlabeled TnT to form the ternary troponin complex. Using fluorescence lifetime measurements, energy transfer efficiencies of 72, 59, and 52% were obtained for the complex in the presence of 0.1 mM $CaCl_2$, 2 mM EGTA, and 2 mM $MgCl_2$, respectively. Based on a critical transfer distance of 40 Å, and the assumption that the orientation factor $\kappa^2=2/3$, these efficiencies correspond to separation distances of 34, 37, and 39.5 Å, respectively. Similar results were obtained when the locations of the donor-acceptor pair were reversed. Ca^{2+}-titration studies showed that there was a decrease in the transfer efficiency when the high affinity metal binding sites were occupied followed by an increase when the low affinity sites were occupied, indicating an increase in the donor-acceptor separation distance in the former case and a decrease in the latter. Since the low affinity sites have been implicated as the triggering sites for Ca^{2+}-regulation, our findings suggest that one of the first events in the Ca^{2+}-regulation process is a movement of the Cys-98 region of TnC toward the Cys-133 region of TnI by ~5 Å. (Supported by NIH AM21673 and HL20464).

Th-AM-G6 DISULFIDE CROSSLINKING STUDIES OF RABBIT SKELETAL TROPOMYOSIN: DIFFERENCES IN LOCAL CONFORMATION AROUND CYS IN αα, αβ AND ββ TROPOMYOSIN. S.S.Lehrer and D.Joseph, Boston Biomedical Research Institute, Boston, MA 02114. (Intr. by P.Graceffa)

Rabbit skeletal tropomyosin (RSTm), consists of α and β chains in relative abundance 3-4 α/β in register in a 2-chain coiled-coil structure. αTm contains one Cys at 190; βTm contains 2 Cys at 190 and 36. The Cys 190 of RSTm can be quantitatively crosslinked by 5,5'-dithiobis(2-nitrobenzoate (Nbs_2) to give 2 bands on SDS-gels identified as α-α and α-β. In this study the ability of Nbs_2 (1mM) to disulfide crosslink reduced RSTm and ββTm was investigated as a function of [urea] in 0.5M NaCl, 20mM Hepes buffer, pH 7.4, at 15°. The reaction with Nbs_2 was monitored at 412nm; after completion, SDS was added to quench the crosslinking reaction and the samples were run on SDS-PAGE. In the absence of urea, RSTm gave 2 crosslinked bands, α-α and α-β in a 60/40 ratio. As the [urea] increased, the fraction of crosslinked species decreased and the fraction of Nbs-blocked monomer chains increased. The α-α and α-β bands were lost between 3-4M and 2-3M urea, respectively, showing that the region around Cys 190 was much less stable for αβTm. In the absence of urea, ββTm gave 2 crosslinked bands; a higher(H) and lower(L) mobility band in a 70/30 ratio. H decreased to 0% and L increased to 50% of the total species between 1-2M urea before decreasing to 0% between 2-3M urea. These data indicate that H is crosslinked at both pairs of Cys, 36 and 190; L is only crosslinked at one Cys pair. When the [urea] was increased, the region around the partially crosslinkable pair unfolds further, resulting only in L. Thus, ββTm contains two regions differing in stability; compared to the Cys 190 region of RSTm, one region has about the same stability and the other has less stability than αβTm. (Supported by NIH HL22461 and the MDA)

Th-AM-G7 FLUORESCENCE OF PYRENE-TROPOMYOSIN (PTM): EFFECTS OF MYOSIN SUBFRAGMENT 1 (S1) BINDING TO PTM-F-ACTIN. Y.Ishii and S.S.Lehrer, Department of Muscle Research, Boston Biomedical Research Institute, Boston MA 02114.

The fluorescence of rabbit striated tropomyosin labeled with pyrene-maleimide consists of monomer(M) and excimer(E) bands. E arises from pyrenes at Cys190 of each chain which interact only if the chains locally separate; increases of E with temperature are due to a shift in equilibrium from a chain-closed state(N) to a localized chain-open state(X). Perturbation of the N-state by pyrene-pyrene interaction was reflected by a 10% loss in helix and an appreciable value of initial E(E_o) at temperatures below the N=X pretransition. When bound to F-actin, M_o did not change appreciably and E remained at E_o over the pretransition indicating little change in pyrene environment and a suppression of the N=X equilibrium without overcoming the pyrene perturbation. S1 binding to PTm-F-actin at 25° caused changes in M and E in two kinetic phases. In a fast phase (< a few seconds), M increased ~20% without affecting E due to an environmental change of pyrene associated with S1 binding to the Tm-F-actin complex. The fast fluorescence increase saturated at low levels of S1 binding (1-2 S1/7 actin) as compared to a linear light scattering increase which saturated at 1 S1/1 actin. In a slow phase (hours), E_o decreased and M_o increased at the same rate due to further stabilization of the N state by bound S1 overcoming pyrene perturbation. The M fluorescence of singly labeled-PTm-F-actin only changed in a fast phase and gave a similar titration curve showing that the change does not involve E forming species and that S1 does not preferentially bind to pyrene-containing regions of Tm. Thus, low levels of S1 binding appear to be sufficient for the slow and fast fluorescence changes indicating long range effects of myosin binding on Tm conformation on the thin filament. (Supported by NIH HL22461 and the MDA)

Th-AM-G8 SERUM ALBUMIN BINDS TO TROPOMYOSIN. S. E. Hitchcock-DeGregori, Z. Yunossi, and W. E. Brown, Department of Biological Sciences, Carnegie-Mellon University, Pittsburgh, PA 15213

We have isolated a tropomyosin binding protein (TMBP) from human platelets that binds to muscle and non-muscle tropomyosins (Gerhard et al., submitted). During study of this protein we noted its similarity in molecular weight (67,000) and immunological crossreactivity to human serum albumin (HSA). The sequences of the first 33 amino acids are identical, and we cannot distinguish between the two proteins using a variety of analytical procedures (peptide maps, immunological crossreactivity, isoelectric focussing). When HSA is purified from plasma on hydroxylapatite, it will bind to skTM-Affi-Gel 15. Although HSA (or TMBP) does bind to tropomyosin, it does not affect end-to-end association since it does not increase the viscosity of sk tropomyosin, as does troponin. HSA (or TMBP) does not cosediment with actin, actin-sk tropomyosin, or actin-tropomyosin-troponin, and does not prevent binding of sk tropomyosin to actin in a variety of experimental conditions we have tried. It does not depolymerize actin. Albumin is present in muscle and antibody stains myofibrils periodically, as has been reported elsewhere (Heizmann and Hauptle, 1981). When myofibrils are washed extensively, the albumin is greatly reduced in amount, if not completely removed. Immunofluorescence studies have shown localization of albumin at the A-I junction; it does not co-localize with tropomyosin implicating, as do the results from the binding experiments, that component(s) in addition to tropomyosin are involved in localization of albumin in myofibrils.

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Th-AM-G9 EFFECT OF HETEROBIFUNCTIONAL CROSSLINKERS ON TROPOMYOSIN FUNCTION. T. WILLIAM HOUK and SONDRA KARIPIDES, Department of Physics, Miami University, Oxford, Ohio 45056. Tropomyosin and actin were crosslinked with a number of heterobifunctional photoreactive reagents including N-hydroxysuccinimidyl 4-azidosalicylic acid, p-azidophenacyl bromide, sulfosuccinimidyl (4-azidophenyldithio) propionate, and benzophenone isothiocyanate. These reagents are directed at specific reactive groups on tropomyosin or actin. Reconstituted thin filaments were formed and the actin and tropomyosin were photoreactively crosslinked. The effect of the cross-linking reagents on the ability of the system to react to calcium and activate myosin atp'ase both before and after the photoreactive crosslinking are reported.

Th-AM-G10 DISTANCE MEASUREMENTS BETWEEN METAL ION-BINDING SITES OF CALMODULIN AND FROM THESE SITES TO CYS-133 OF TROPONIN-I IN THE BINARY COMPLEX. C.-L.A. Wang, Dept. of Muscle Research, Boston Biomedical Research Institute, Boston, MA 02114

Distances among the four Ca^{2+}-binding sites of calmodulin (CaM) have been measured by fluorescence energy transfer experiments using trivalent lanthanide ions as substitutes for Ca^{2+}. Eu^{3+} and Tb^{3+} were used as energy donors, and a number of lanthanide ions as acceptors. It has been shown previously that, for lanthanide ions, sites I and II are the high affinity sites, whereas sites III and IV are the low affinity sites (Wang et al., Eur. J. Biochem. (1982) 124, 7-12). Thus upon direct excitation with a dye-laser the luminescence lifetimes of Eu-Ln-CaM and Tb-Ln-CaM (Ln: lanthanide ions acting as energy acceptors) afford distance information between sites I and II. Since Tb^{3+} ions bound to sites III and IV are sensitizable through tyrosine residues, upon excitation with a UV light source, lifetime measurements of Tb_3Ln-CaM yield the distance between sites III and IV. Both pairs of sites are found to be separated by a distance of 1.0-1.2 nm. Binding of Ca^{2+} to sites III and IV apparently does not alter the distance between sites I and II. We have also attached a chromophoric label, dimethylaminophenylazobenzene (DAB), to Cys-133 of troponin-I (TnI), and carried out distance measurements in the CaM-TnI complex by both direct and indirect excitation. The averaged distance from sites I and II in the N-terminal half and that from sites III and IV in the C-terminal half of the CaM molecule to the label were calculated to be 2.7 nm and 2.5 nm, respectively. (Supported by NIH grant AM32727)

Th-AM-G11 COOPERATIVITY AMONG DRUG BINDING SITES ON CALMODULIN
John S. Mills and J. David Johnson, Dept. of Physiological Chemistry, The Ohio State University Medical Center, Columbus, Ohio 43210.

Previously, we have reported allosteric interactions among the calcium dependent drug binding sites on calmodulin (J.D. Johnson, BBRC 112, 787-793, 1983). This was evidenced by the ability of the calmodulin antagonists prenylamine (P) and R24571 (R), to potentiate the binding (fluorescence enhancement) of felodipine (a dihydropyridine calcium antagonist) to calmodulin. Using 3 H-felodipine binding to calibrate the fluorescence changes occuring with felodipine binding, we have performed Hill plots and Scatchard analysis of felodipine binding with and without its allosteric effectors. Felodipine binds to two sites on calmodulin at $pCa^{++}=3$ in a highly cooperative (Hill coefficient=1.9) and calcium dependent fashion with a Kapparent of 23μM. In the presence of 2μM R or 10μM P, the Hill coefficient is shifted from 1.9 to 1.0 or 0.7 with K_{app} of 15μM and 58μM and Bmax of 1.8 and 2.0 moles/mole respectively. These allosteric effectors, R and P are thus able to abolish cooperativity between the two felodipine (F) binding sites presumably by occupying one of these F sites to potentiate F binding to the other F site. In the absence of 4% ethanol, used in these studies to enhance F solubility, similar phenomena were observed with ~ 2 fold higher affinity. Scatchard analysis of felodipine binding in the presence of 2 moles/mole of R show a reduction in B_{max} at lower Ca^{++} concentrations. These studies are discussed in terms of allosteric control mechanisms regulating the cellular action and specificity of calmodulin.

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Th-AM-H1 SURFACE CHARGE MODIFICATION INFLUENCES BLOCKING ACTIVITY OF DIHYDROPYRIDINE CA CHANNEL ANTAGONISTS. D. Krafte, B. Coplin, P. Bennett, and R.S. Kass. University of Rochester, Department of Physiology, 601 Elmwood Avenue, Rochester, NY 14642.

The observation that elevation of Ca and other divalent cations could counter drug induced inhibition of Ca-dependent responses in cardiac and smooth muscle contributed to the classification of these compounds as Ca antagonists despite evidence that Ca does not antagonize drug binding. The present experiments were designed to test the hypothesis that the antagonistic actions of divalent cations occur in part by voltage-dependent shifts in inactivation caused by screening of negative surface charges. These experiments were carried out using a two-microelectrode voltage clamp in calf cardiac Purkinje fibers and a whole-cell patch clamp of isolated guinea pig myocytes. In the absence of drug, raising the extracellular divalent cation concentration (Ca^{+2}, Sr^{+2}, Ba^{+2}, or Mg^{+2}) causes depolarizing shifts in the 500 ms and 20 sec inactivation curves in both single cells and in multicellular Purkinje fibers. The shift occurs in a concentration-dependent manner and is relatively insensitive to the divalent cation species. Its magnitude is similar in the enzymatically-dissociated cells and the Purkinje fiber, suggesting that enzyme treatment does not disrupt the negative surface charge layer of the single cells. The steady-state inactivation curve is also shifted by elevated divalents in the presence of nisoldipine. Thus block appears to be relieved after negative prepulses in solutions containing elevated divalents. Since the magnitude of this depolarizing shift is the same order as that measured under drug-free conditions, we conclude that the "antagonistic" actions of divalent cations on this drug are due, in part, to a modification of negative surface charge.

Th-AM-H2 THE INTERACTION OF A Ca CHANNEL AGONIST, BAY-K-8644, AND A UNIQUE Ca-CHANNEL INHIBITOR, DIACETYL MONOXIME (DAM), ON YOUNG AND OLD EMBRYONIC CHICK HEARTS AND RABBIT PAPILLARY MUSCLES. **Hideaki Sada and Nick Sperelakis, Dept. of Physiology & Biophysics, Univ. of Cincinnati, Cincinnati, OH 45267-0576**

The effects of a novel Ca channel agonist, Bay-K-8644 (BAY), and a negative inotropic agent, diacetyl monoxime (DAM), and their interaction were assessed on the action potentials (APs) and the time derivative ($\dot{V}_{max}$) of several cardiac muscle preparations. On Na^+-dependent spontaneous slow APs of young (3-day-old) chick embryos, BAY (10^{-6}M) increased the amplitude, $\dot{V}_{max}$, and AP duration, whereas DAM (10 mM) decreased these parameters. On Ca^{++}-dependent slow APs of K^+-depolarized (22 mM) old embryonic chick hearts, BAY and DAM also increased and decreased, respectively, the amplitude, $\dot{V}_{max}$, and AP duration. On Ca^{++}-dependent slow APs of K^+ -depolarized (22 mM) rabbit papillary muscles, BAY increased the amplitude, $\dot{V}_{max}$, and AP duration, whereas DAM did not change these AP parameters except for the shortening of the AP duration. The actions of DAM were quickly produced and reversed after drug addition and washout, respectively. In contrast, the onset and washout of the Bay effects were slower. The changes in AP parameters by DAM on slow APs of young and old embryonic chick hearts were nullified by simultaneous addition of BAY. The results suggest: 1) BAY stimulates, and DAM depresses, the slow Na^+ channels present in young (3-day) chick embryos; 2) in addition, BAY stimulates, and DAM depresses, the Ca channels present in old chick embryonic hearts; 3) on the slow APs of rabbit hearts, BAY had a stimulating action, but DAM did not have a depressing action; 4) antagonism of these two drugs are probably produced by changes in the slow Na^+ current (in young) or in the slow Ca^{++} current (in old embryonic chick hearts). Since the TTX-insensitive slow Na^+ channel found in young embryonic chick heart is stimulated by BAY-K-8644, this compound is more appropriately called a slow channel agonist. Supported by NIH Grant HL-31942.

Th-AM-H3 SPECIFIC BINDING OF DIHYDROPYRIDINES TO INTACT FROG SARTORIUS MUSCLE: BINDING IS VOLTAGE-DEPENDENT, BUT MOST BINDING SITES ARE NOT FUNCTIONAL CA CHANNELS. L.M.Schwartz,E.W. McClesky & W. Almers, Dept. Physiology and Biophysics, Univ. of Washington, Seattle, WA 98195

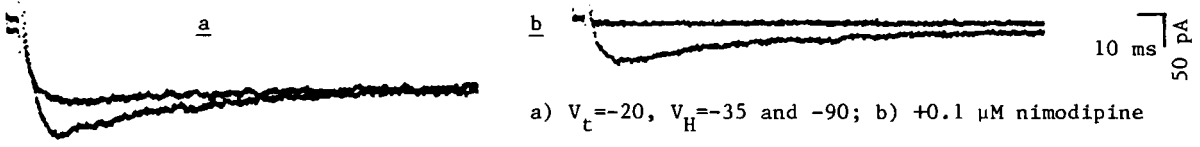
Dihydropyridines are thought to bind specifically to Ca channels, and are used for their extraction and purification. The richest source of dihydropyridine (DHP) binding sites is skeletal muscle. We have incubated intact muscles in small volumes of Ringer's fluid containing the antagonist DHP, ^{3}H-PN-200/110, and determined drug uptake from the disappearance of label from the incubate. After 4 hrs, uptake had reached equilibrium, and an excess of unlabelled nitrendipine or PN-200/110 was added. This caused release of label from the muscle and indicated displacement from a finite number of sites. The sites bound label with a dissociation constant of K_D = 0.9-1.0 nM. Their number correspond to U = 26 pmoles/g wet. Depolarization to -15 mV by high-K^+ Ringer did not change K_D but increased U to 79 pmoles/g, which represents $5 \cdot 10^{13}$ sites/ml fibre volume, or 230 sites/μm^2 of tubule membrane. We suggest that the additional sites are recruited by conversion from a pool of sites with an affinity too low to be detected by our method. Ca currents recorded from intact sartorii with the three-electrode voltage clamp were, on average, 102 mA/ml fibre volume at -20 mV and 10 mM Ca^{++}. With $5 \cdot 10^{13}$ DHP sites/ml, this corresponds to 2 fA/site. Ca currents through single channels under similar conditions are 30 to 50 times higher (e.g. Hess & Tsien, Nature 309, 453). PN-200/110 blocks Ca channels one-to-one with K_D = 430 nM, but virtually no block is observed at 10 nM, where nearly all high-affinity sites are loaded. Evidently there are many more DHP sites than Ca channels, and PN-200/110 can bind without causing block. Probably, most DHP sites are not functional Ca channels. Supported by DHHS grant AM17803.

Th-AM-H4 VOLTAGE SELECTS ACTIVITY OF THE CA CHANNEL MODULATOR BAY K 8644. M.C. Sanguinetti and R.S. Kass. University of Rochester, Department of Physiology, 601 Elmwood Avenue, Rochester, NY 14642.

Bay K 8644 is a dihydropyridine derivative that has been shown to alter Ca channel gating in cardiac cells and is capable of enhancing or decreasing Ca channel currents depending on membrane potential. We have investigated the of this compound on Purkinje fiber Ca channel currents and compared it to other, more specific, dihydropyridine Ca channel blockers. When currents are measured from holding potentials more negative than -50 mV, the drug produces a concentration-dependent increase in peak inward currents and causes a hyperpolarizing shift in the peak inward current/voltage relationship. The drug causes a similar shift in the conductance/voltage relation, suggesting that activation of the channels occurs over a more negative voltage range. Bay K 8644 causes hyperpolarizing shifts of the relationship between Ca channel availability and membrane potential, but in contrast to nisoldipine and other more specific blockers, these shifts occur after brief (500 ms) prepulses. From the maximal shift (-20 mV), we estimate that Bay K 8644 is a 10x less potent blocker than nisoldipine. Bay K 8644 causes use-dependent reduction in Ca channel current, and the pattern of development of block suggests that open channel block may be significant, in contrast to the results of similar experiments with nisoldipine and nifedipine. We conclude that Bay K 8644 is a partial agonist that can produce blocking activity if preparations are depolarized, stimulated at high rates, or exposed to very high concentrations of the compound.

Th-AM-H5 DIFFERENTIAL EFFECTS OF DIHYDROPYRIDINES ON TWO POPULATIONS OF Ca CHANNELS IN ANTERIOR PITUITARY CELLS. C.J. Cohen and R.T. McCarthy, Miles Inst. Preclin. Pharmacol., New Haven, CT.

High K induced hormone secretion from GH4C1 pituitary cells is increased by BAY k 8644 and blocked by nimodipine (Enyeart and Hinkle). Whole cell patch voltage clamp measurements on GH4C1 cells tested for effects of these drugs on Ca channels. Na and K channels were blocked by replacing Na_o and K_o with TEA, adding 0.2 μM TTX and perfusing the cell with (in mM):130 CsCl, 10 TBA Cl, 11 EGTA, 0.9 $CaCl_2$, 2 $MgCl_2$, 1 ATP. Like GH3 cells and some neurons, GH4C1 cells have 2 populations of Ca channels. One population of channels is maximally available for activation at $V_h \cong -40$ mV and little or no inactivation occurs during test pulses (non-inactivating channels). The other population of channels is only available for opening for $V_h < -40$, they activate at more negative voltages and the channels inactivate rapidly for $-20 < V < 20$. 0.1 μM nimodipine blocks almost all current for $V_h = -35$, but has little effect on the extra current elicited when $V_h = -90$ (see fig). 0.2μM BAY k 8644 produces a larger % increase in current when $V_h > -45$ than when $V_h = -90$. BAY k 8644 also increases the time constants for Ca channel activation, deactivation and inactivation. These experiments indicate that nimodipine and BAY k 8644 preferentially act on the non-inactivating Ca channels.



a) $V_t = -20$, $V_H = -35$ and -90; b) +0.1 μM nimodipine

Th-AM-H6 AFFINITY PURIFICATION OF HIGH-AFFINITY ANTIBODIES SPECIFIC FOR THE DIHYDROPYRIDINE Ca^{2+} CHANNEL BLOCKERS. Alan Sharp, Steven D. Kahl and Kevin P. Campbell. Dept. of Physiology and Biophysics, The University of Iowa, Iowa City, Iowa, 52242.

High-affinity antibodies to the dihydropyridine Ca^{2+} channel blocker nifedipine have been produced and affinity purified in preparation for production of anti-idiotypic antibodies directed against the dihydropyridine receptor of the Ca^{2+} channel. Anti-dihydropyridine antibodies were raised using covalent conjugates of nifedipine with BSA and KLH. A sheep was initially injected with a BSA-nifedipine conjugate followed by booster injections at 2, 4 and 8 weeks. Booster injections were switched to KLH-nifedipine for 12, 16 and 20 week injections. Antisera contained high titer anti-dihydropyridine antibodies after 16 weeks. Anti-dihydropyridine antibodies exhibited average K_A's of 2-6 x 10^9 M^{-1} for nitrendipine and bound various dihydropyridine Ca^{2+} channel blockers with the same order of specificity as the membrane dihydropyridine receptor. Structurally unrelated Ca^{2+} channel blockers, verapamil and diltiazem, did not modify the binding of nitrendipine to the anti-dihydropyridine antibodies. A nifedipine-Sepharose affinity matrix was used for affinity purification of the anti-dihydropyridine antibodies. The nifedipine-Sepharose was first incubated with 100 ml anti-dihydropyridine specific antiserum, separated from antiserum by centrifugation, and washed. Anti-dihydropyridine specific antibodies were then eluted by incubation with a high pH solution. Eluted antibodies were quickly passed through a Sephadex G25 column and returned to neutral pH. This procedure yielded a total of 6.5 mg purified antibody with a peak affinity constant of 1.6 x 10^{10} M^{-1}. This preparation is now being injected into other sheep to produce anti-idiotypic antibodies directed to the dihydropyridine receptor. (Supported by Miles Lab.)

Th-AM-H7 DEVELOPMENT OF AN AFFINITY COLUMN FOR THE PURIFICATION OF THE DIHYDROPYRIDINE RECEPTOR OF THE Ca2+ CHANNEL. Kevin P. Campbell, Molly Strom and Alan Sharp, (Intr. by Earle Stellwagen) Dept. of Physiology and Biophysics, The University of Iowa, Iowa City, IA 52242.

Cardiac and smooth muscle contraction are initiated and modulated by Ca^{2+} influx across the cell membrane via voltage-dependent Ca^{2+} channels. Although the properties of the cardiac and smooth muscle Ca^{2+} channels have been examined with electrophysiological techniques, little is known about the molecular structure or subunit composition of these Ca^{2+} channels. Dihydropyridines are the most potent class of Ca^{2+} channel blockers and [^{3}H]nitrendipine has been shown to bind with high affinity to membranes isolated from skeletal, cardiac and smooth muscle. We have used dihydropyridine-specific antibodies to develop an affinity column, nifedipine-Sepharose, for the affinity purification of the dihydropyridine receptor of the cardiac Ca^{2+} channel. Nifedipine-isothiocyanate was coupled (approximately 100-200 nmoles ligand/ml of gel) to several different amino-containing Sepharose beads in sodium bicarbonate (pH 9.0). Anti-dihydropyridine specific antibodies were used to determine the specificity and binding capacity of the affinity column. Nifedipine-Sepharose was able to specifically bind the membrane bound receptor or the CHAPS-solubilized receptor from either cardiac or skeletal muscle. Specific elution of the receptor bound to the affinity column has been accomplished using various treatments which lower the affinity of the dihydropyridine binding to the solubilized receptor in order to dissociate the receptor complex from the affinity column. The purity of the eluted purified receptor is currently being assessed using binding analysis and SDS gel electrophoresis. (Supported by AHA and NIH (HL14388)

Th-AM-H8 CHARACTERIZATION OF LOW AND HIGH AFFINITY NITRENDIPINE BINDING SITES IN CANINE CARDIAC SARCOLEMMA. Pal L. Vaghy, Judith S. Williams and Arnold Schwartz, Department of Pharmacology and Cell Biophysics, University of Cincinnati, Cincinnati, Ohio 45267-0575

A membrane fraction enriched in sarcolemmal markers was isolated from canine heart and nitrendipine (NTD) binding sites were studied using [^{3}H]NTD as a labeled ligand. In competition binding experiments, the [^{3}H]NTD concentration was kept constant at 0.3, 7, 25 or 100 nM and the nonlabeled NTD concentration was varied between 0.05 and 1500 nM. In saturation binding experiments, the [^{3}H]NTD concentration was varied between 0.05 and 650 nM and the nonspecific binding was measured in the presence of either 100 nM or 1200 nM nonlabeled NTD. The specific NTD binding was linear with the protein concentration ranging from 5 to 100 µg/ml both at low and at high NTD concentrations. The binding data were analyzed with a nonlinear least squares LIGAND program. Saturable and reversible high (K_D = 0.3 nM) and low (K_D = 140-220 nM) affinity NTD binding sites were identified. The effect of heat treatment, proteases, lipases, concanavalin A, bivalent cations, La^{3+}, ionic strength, pH, nimodipine, nifedipine, and Bay k 8644 were studied on both high and low affinity NTD binding sites. The data suggest the existence of two distinct but interacting, high and low affinity NTD binding sites in canine cardiac sarcolemma. A model will be presented which suggests that the tested dihydropyridine Ca^{2+} channel modulators are bivalent ligands, and, depending on the concentrations used, they can either activate or inactivate Ca^{2+} channels.

Th-AM-H9 INJECTION OF cGMP, PGF2α, NITROPROSIDE AND THE SUBUNIT OF cGMP - DEPENDENT PROTEIN KINASE BLOCKED THE MYOCARDIAL SLOW CHANNELS. G. Bkaily, J.D. Carbin[1], and N. Sperelakis. Department of Biophysics, Faculty of Medicine, University of Sherbrooke, Sherbrooke, Quebec, Canada. Department of Physiology and Biophysics, University of Cincinnati, Cincinnati, Ohio, USA and [1]Department of Physiology, Howard Hughes Medical Institute, Vanderbilt University, Nashville, Tennessee.

The role played by cyclic AMP in heart muscle is well documented, but the role of cyclic GMP in cellular function is almost as obscure today as it was 10 years ago and controversial. In order to elucidate the role played by cGMP at the cellular level, intracellular injection of cyclic nucleotides into cultured heart cells (reaggregates) was done using the liposome method. We report here that injection of cyclic GMP blocked the on-going naturally-occurring slow APs. Addition of isoproterenol did not substantially reverse the effects of the injected cGMP, but prior addition of isoproterenol or simultaneous injection of cAMP prevented the effects of cGMP injection. Also, PGF2α, and nitroproside that increase intracellular cGMP blocked the slow APs. Injection of the subunit of cGMP - dependent protein kinase rapidly blocked the inward slow current (Isi). These data show that cAMP and cGMP have antagonistic roles in the regulation of slow channel function.

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Th-AM-H10 INDUCTION OR ENHANCEMENT OF CARDIAC SLOW ACTION POTENTIALS (APs) BY FORSKOLIN. Gordon M. Wahler and Nicholas Sperelakis. Department of Physiology and Biophysics, University of Cincinnati, College of Medicine, Cincinnati, OH 45267.

Our laboratory has shown that isoproterenol, which enhances cAMP levels by stimulation of adenylate cyclase through the β-adrenergic receptors, induces or enhances cardiac slow APs. Dibutyryl cAMP, or intracellular injection of cAMP, has similar effects, as does intracellular injection of the catalytic subunit of the cAMP-dependent protein kinase. Intracellular injection of cGMP has opposite effects. It is thought that isoproterenol induces slow APs by enhancing the slow inward current through a cAMP-dependent phosphorylation of the slow channel or an associated regulatory protein. We hypothesized that an agent, such as forskolin, which directly activates the adenylate cyclase, should also induce or enhance slow APs in myocardial cells. Thus, cells of guinea pig papillary muscles (stimulated at 0.5 Hz at 37° C) were depolarized to about -40 mV with 25 mM K^+-Tyrode's solution to voltage-inactivate the fast Na^+ channels. Slow APs were elicited by electrical stimulation within 10 min. following the addition of forskolin (10^{-6} M). Slow APs are dependent on the slow inward current (I_{si}) carried through the voltage- and time-dependent slow channels. Addition of 10^{-8} to 10^{-6} M forskolin enhanced the ongoing slow APs, induced by 10 mM TEA plus doubled bath [Ca] (which increases the net inward current without affecting the slow channels) within 10 min (increased V_{max} to 115-314% of control). These results indicate that direct activation of adenylate cyclase can result in induction or enhancement of slow APs, consistent with the phosphorylation hypothesis. Thus, direct activation of the adenylate cyclase by forskolin induces slow APs which are not dependent on membrane receptors, providing a useful tool for studying the role of cAMP in slow channel function. (Supported by grant HL-31942 and fellowship 1F32 HL-06736-02.)

Th-AM-H11 NOREPINEPHRINE AFFECTS CA CHANNEL CURRENTS THROUGH TIGHT RECEPTOR-CHANNEL COUPLING IN DRG CELLS. Paul Forscher and G.S. Oxford. The Neurobiology Program, University of North Carolina, Chapel Hill, NC 27514.

Whole-cell and on-cell patch configurations of the patch voltage clamp technique have been used to study NE modulation of Ca channel currents in tissue cultured DRG cells. 10uM NE rapidly (2 s) and reversibly decreases whole-cell Ca currents recorded at E_{Na} from neurons dialyzed with Na/NMG mixtures containing 5mM MgATP/2mM EGTA. Expression of the NE effect is not inhibited by cumulative sequential addition of 5mM cAMP, 5mM cGMP, 10mM EGTA, or 50uM trifluoperazine, indicating that acute changes in intracellular cAMP, cGMP, or Ca are probably not involved in mediating this NE effect.

Receptor-channel coupling was investigated in on-cell patch experiments by recording ensemble-averaged Ba currents from the patch and exposing the extra-patch cell membrane to NE. 1-2 min. exposures to 10-100uM NE never reversibly decreased Ba currents from Ca channels in the patch. Occasionally patch currents increased after NE exposure, but usually remained stable. These results support the hypothesis that NE directly effects Ca channels or a molecule tightly coupled to the channel in DRG cells.

DRG cells possess both inactivating and non-inactivating calcium channels. Examination of 200 ms whole-cell Ca or Ba current records reveals that while NE affects both inactivating and non-inactivating components, the inactivating channel fraction is more sensitive. This preferential sensitivity of the inactivating component results in marked changes in apparent activation kinetics of Ca channel currents by NE. (Supported by NIH grant NS18788 to G.S.O.).