M-PM-SYM1 ATOMIC SOLVATION PARAMETERS AND HYDROPHOBIC MOMENTS FOR ASSESSING PROTEIN AND LIGAND STABILITY David Eisenberg, Mason Yamashita, William Wilcox, Joe Talafous, Morgan Wesson
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Atomic solvation parameters (ASPs) and hydrophobic moments are semiempirical quantities for interpretation of protein structure and function, which offer some advantages over conventional energetics. ASPs are essentially atomic hydrophobicities, which give the free energy change for increasing the aqueous-accessible surface area of each protein atom. ASPs can be used to estimate the free energy of protein folding or binding, including the effect of the entropy of the solvent. ASPs are based on measurements of free energy of transfer of amino acid analogs into aqueous solution. This means that they give an estimate of the free energy change which is based, at least indirectly, on experiment. Also ASPs, like hydrophobic moments which describe asymmetry of hydrophobicity, can provide a pictorial representation of the hydrophobic forces at work within a protein. These descriptions of forces are at a more coarse-grained level than conventional energetics, and provide alternative, and in some cases, more tangible insights into the biophysics. Several applications of ASPs and hydrophobic moments to proteins and DNA will be given.

One example is of metal binding sites within proteins. Many divalent cations, particularly Ca^{2+}, bind at sites characterized by a spherical hydrophilic region around the ion, surrounded in turn by a hydrophobic region. This situation is described by a function that gives the contrast in the local hydrophobicity. Within many proteins, this function has its maximum values at metal binding sites.

M-PM-SYM2 ELECTROSTATIC INTERACTIONS IN PROTEINS. M.K. Gilson[1]. K. Sharp[1], B. Honig[1], R. Fine[2], R. Hagstrom[3]. [1]Dept. of Biochem. & Molecular Biophysics, Columbia Univ., N.Y., N.Y. 10032. [2]Dept. of Biological Sciences, Columbia Univ., N.Y., N.Y. 10027 & Dept. of Structural Biology, Brookhaven National Lab., Upton, N.Y. 11973. [3]High Energy Div., Argonne National Laboratory, Argonne, IL. 60439.

We have recently developed a general approach to the treatment of electrostatic interactions in proteins. The protein is treated as a low dielectric medium containing real and partial charges whose coordinates are known from x-ray data. The solvent is treated as a high dielectric medium which contains a simple electrolyte. Electric fields inside and outside the protein are obtained by solving the Poisson-Boltzmann equation. The theoretical basis for this model, including its range of validity and the appropriate choice for the dielectric constant of a protein will be discussed. A finite difference algorithm which solves the Poisson-Boltzmann equation for molecules of arbitrary shape will be introduced. The method has been applied to problems of solvent screening of interatomic electrostatic interactions and the role of helix dipoles in protein stability and function. The electrostatic potential around the enzyme Cu,Zn superoxide dimutase will also be described. The role of this potential in determining the rate of substrate diffusion is considered in light of stochastic dynamics simulations that include an accurate description of the shape of the protein.

M-PM-SYM3 WEAKLY-POLAR INTERACTIONS IN PROTEINS by S.K. Burley*$ and G.A. Petsko*
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Recent geometric and energetic analyses of the atomic environments of aromatic side chains in protein and oligopeptide crystal structures have identified a group of weakly-polar, enthalpically-favorable interactions that exploit the characteristic segregation of positive and negative partial electronic charges in aromatic moities. Members of this group include (1) edge-to-face interactions between aromatic side chains, which bring a $\delta(+)$ hydrogen atom of one aromatic ring near to the $\delta(-)$ π-electron cloud of the other aromatic ring, (2) amino-aromatic interactions, which bring a positively-charged or $\delta(+)$ side chain amino group near to the $\delta(-)$ π-electron cloud of an aromatic ring, (3) sulfur-aromatic interactions, which bring the $\delta(-)$ sulfur atoms of cysteine and methionine near to the $\delta(+)$ hydrogen atoms of an aromatic ring, and (4) oxygen-aromatic interactions, which bring a $\delta(-)$ oxygen atom near to the $\delta(+)$ hydrogen atoms of an aromatic side chain. These four interactions are both ubiquitous and numerous, and the distribution of observed geometries for each type of weakly-polar interactions differs substantially from random and does not arise solely as a function of packing constraints. In addition, the results of *ab initio* quantum mechanical calculations suggest that these interactions make enthalpic contributions of between -1 and -2kcal/mol per interaction to the three-dimensional structural stability of a protein. Examples of the biological importance of these weakly-polar interactions involving proteins, oligopeptides and protein-ligand binding will be presented.

M-PM-SYM4 **TOWARDS QUANTITATIVE STRUCTURE-FUNCTION CORRELATION IN PROTEINS** : A. Warshel, Department of Chemistry, University of Southern California, Los Angeles, California 90089-0482

Different approaches for simulation of the function of solvated proteins are described[1]. Emphasis is placed on our current progress using free energy perturbation methods[1-3] for simulating electrostatic free energies of charged groups, ligand binding, hydrogen exchange and enzymatic reactions. The results presented indicate that we are approaching the stage of semi-quantitative modeling of protein functions.

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M-PM-A1 NEUTRON SCATTERING RESULTS INDICATE THAT S1 DOES NOT CHANGE SHAPE DURING FORCE GENERATION. Robert Mendelson and Paul M. G. Curmi. C.V.R.I. and Dept. of Biochem./ Biophys., Univ. of Calif., San Francisco CA 94143

Previously we have reported that results of neutron scattering by myosin subfragment-one bound to actin made invisible by deuteration were not significantly different (resolution 2.5 nm) from those produced by free S1. We now discuss the results of modeling studies aimed at exploring the sensitivity of these measurements to distortions of S1 structure that could be responsible for force generation. We have considered four general types of distortion which we applied numerically to several S1 shapes from 3D EM reconstructions. Deformations of S1 structures were achieved by cutting the molecule and rotating one part with respect to the other or by deforming part of the molecule by shearing. Several hundred scattering curves were computed and each was convolved with the beam geometry and neutron wavelength spread. Results indicate the data are sensitive to longitudinal translations of the distal end of S1 of about 4.0 nm or larger. If we assume that free S1 and rigor (+MgADP) S1 structure represent the beginning and end, respectively, of the force generating step and the power stroke is 4.0 nm or larger, we conclude that force can only be produced with most (or all) of the S1 retaining its overall shape.

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M-PM-A2 **SINGLE TURNOVER EXPERIMENTS IN SKINNED RABBIT PSOAS MUSCLE FIBERS USING LASER FLASH PHOTOLYSIS OF CAGED-ATP.** C. Ian Spencer and Michael A. Ferenczi National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K.

The mechanism of ATP hydrolysis in chemically skinned rabbit psoas muscle fibers has been investigated by determining the fate of sub-stoichiometric concentrations of [^{3}H]ATP generated by laser-flash photolysis of caged-[^{3}H]ATP. The reaction may be summarised as follows:

$$E + ATP \overset{K_1}{\rightleftharpoons} E{\cdot}ATP \overset{K_2}{\rightleftharpoons} E{\cdot}ADP{\cdot}Pi \overset{K_3}{\longrightarrow} E + Products$$

where E represents myosin or actomyosin ATPase sites. The hydrolysis equlibrium constant K_2 given by [^{3}H]ADP/[^{3}H]ATP has been measured in freeze-clamping experiments at a sarcomere of 2.5-2.8μm. The fibers were frozen at 1 and 4.5 seconds after the laser-flash and a value of 6 ± 1 (SEM, n=11) was obtained for K_2 with ATP generated in the presence or absence of 32 μM Ca^{++} at 13°C, pH 7.1, and I=200mM. The time at which the fibers were frozen had no effect on K_2. In cold chase experiments, the ratio of [^{3}H]ADP to [^{3}H]ATP released after bathing the fibers in solutions of 5 mM unlabelled ATP 2 to 7s after caged-[^{3}H]ATP photolysis gives the value for $K_2.k_{+3}/k_{-1}$, and was found to be approximately 1 in the presence and absence of Ca^{++}. Again the ratio was insensitive to the time of the cold chase. These results give a quantitative description of early steps in the mechanism of ATP hydrolysis by muscle fibers at full myofilament overlap.

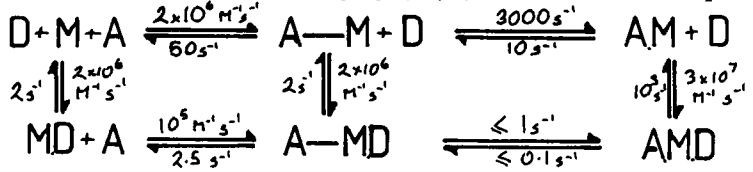
M-PM-A3 **RAPID BURST OF ATP HYDROLYSIS IN SKINNED CARDIAC MUSCLE FOLLOWING LASER FLASH PHOTOLYSIS OF CAGED-ATP.** R.J. BARSOTTI and M.A. FERENCZI. National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K.

We studied the kinetics of activation, relaxation and MgATP hydrolysis in contractions initiated by caged-ATP photolysis in guinea-pig ventricular trabeculae. Triton-skinned trabeculae (1-3mm long, 100-250μm ϕ) were incubated in a rigor solution containing 2mM caged-ATP, 200mM ionic strength, pH 7.1, 12°C. Approximately 1mM ATP was liberated by a 347nm laser pulse in the presence and absence of Ca^{2+}. In the presence of 32μM free Ca^{2+}, liberation of ATP caused a rapid exponential rise in isometric tension with a rate constant of $2.3s^{-1}$. The average force per cross-sectional area was 22 kN/m^2. Following the release of ATP in the absence of Ca^{2+}, tension either increased or in some cases remained constant for ~0.3s before declining with an approximately exponential time course to the relaxed level with a rate constant of $1.4s^{-1}$. The time course of ATP hydrolysis in the absence of Ca^{2+} was measured using tritiated caged-ATP. The fibers were frozen between copper blocks precooled in liquid N_2 at 1s and 5s following the laser pulse. The nucleotides were extracted as described by Ferenczi, Homsher and Trentham (1984, J. Physiol. **352**;575). The amount of ADP formed at 1s was 170μM (n=7) and not significantly different from the value obtained at 5s (147μM, n=6). These values are similar to the active site concentration of 164μM. Further aspects of the hydrolysis mechanism are being investigated.
R.J.B. is a fellow of the American Heart Association and the British Heart Foundation.

M-PM-A4 THE DYNAMICS OF THE INTERACTION BETWEEN ACTIN AND MYOSIN IN THE PRESENCE OF ADP. Michael A.GEEVES. Intr.by H.GUTFREUND. Dept of Biochemistry, University of Bristol,Bristol, UK.

The dynamics of actin-myosin interactions were investigated using the method of pressure induced relaxations. Coates Criddle & Geeves (1985 Biochem.J.282,351) have previously shown that pressure jumps of 100 atmospheres induce two relaxations in solutions of acto-S1 when the reaction is monitored by following changes in the fluorescence of a pyrene group attached to cys 374 of actin. The first relaxation is complete within the pressure release time (200μsec). The rate of the second relaxation is dependent upon the protein concentration and is compatible with a second order rate constant of $2x10^6$ M^{-1} s^{-1}.

In the presence of 2mM ADP only one relaxation is observed, the rate of the relaxation is compatible with a second order rate constant of $1x10^5$ M^{-1} s^{-1}. Varying the ADP concentration in the range 0-2mM showed that the fast relaxation remained fast, the amplitude being reduced by ADP and totally eliminated at 500μM ADP. The rate of the slow relaxation was reduced by ADP concentrations such that the 20 fold reduction in the 2nd order rate constant was complete when the [ADP] was stoichiometric with the [S1] (40μM). The data are compatible with the model shown below:



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M-PM-A5 RIGOR AND ACTIVE CROSS-BRIDGES ENHANCE THE FLUORESCENCE OF FIBER-INCORPORATED LABELED TnC IN THE PRESENCE AND ABSENCE OF CALCIUM. B. Schultie, L.D. Yates, T. Allen And A.M. Gordon. Dept. of Physiology and Biophysics, Univ. of Washington, Seattle, Wa 98195.

We showed previously that calcium and rigor cross-bridges affect fluorescently labeled TnC incorporated into a skinned skeletal rabbit muscle fiber (Yates et al., 1985, Biophys. J. 47:468a). Yet, little is known about the effect of active or force producing cross-bridges on TnC, particularly in the absence of calcium. To this end, the Met-25 of TnC was labeled with 5-dimethylaminonaphthalene-1-sulfonyl aziridine (DANZ), further purified using a TnI affinity column, and incorporated into partially TnC extracted skinned skeletal-muscle fibers. The fibers were then activated in the absence of calcium by decreasing the [MgATP]. With decreasing MgATP, both force and fluorescence increased with force peaking just below 1 μM MgATP before declining slightly to the elevated values in rigor. The addition of calcium to fibers in rigor only increased fluorescence from 55% to 65% of the fluorescence observed during activation in the presence of 3 mM MgATP. When the levels of MgATP were then increased in the presence of calcium, both force and fluorescence increased above their values in rigor peaking near 10 μM before declining slightly at higher levels of MgATP. At this peak, maximum fluorescence was 14% greater than that observed with 3 mM MgATP. These results show that the probe is more sensitive to cross-bridges, both rigor and active, than calcium binding, and that greater fluorescence enhancement is associated with the active-cycling cross-bridge. Analyzing the results using a simple three state model consisting of rigor, detached and active cross-bridges (Goldman et al., 1984, J. Physiol. 354:605) indicated that the fluorescent transitions correlated with the formation of active and rigor cross-bridges. Furthermore, if the fluorescence contribution by calcium binding in rigor (10%) is taken to be the same at all levels of MgATP, then the force producing cross-bridge enhanced fluorescence two times as much as that observed with rigor cross-bridges. This is most consistent with a model in which the thin filament is activated by either calcium or rigor cross-bridges. The calcium activated thin filament is further activated by cross-bridges with the degree of activation dependent upon the state of the bound cross-bridge. This was supported by grants from NIH (NS 08384 and HL 31962), MDA and the MSRTP at UW (B. Schultie).

M-PM-A6 VANADATE PROMOTED PHOTOCHEMICAL MODIFICATION OF MYOSIN SUBFRAGMENT-1 (SF-1). Jean C. Grammer and Ralph G. Yount, Biochemistry/Biophysics Program, Washington State University, Pullman, WA 99164-4660.

Irradiation with long wavelength uv light of SF-1 containing MgADP trapped at the active site by vanadate (Vi), results in covalent modification of the SF-1 and in the release of most of the trapped MgADP and Vi. After 4 minutes of irradiation, the CaATPase and MgATPase activities were increased 4-5 fold over non-irradiated controls. The K+EDTA-ATPase activity remained below 10% of controls. There was a linear correlation between the increase in CaATPase activity and the release of both ADP and Vi. The number of SH groups in the MgADP·Vi·SF-1 complex remained unchanged after irradiation. In addition, the photo-modified SF-1 trapped MgADP by action of p-phenylene dimaleimide (pPDM) 70 to 80% as well as unmodified SF-1. Analysis by SDS-PAGE and fluorography of [^{14}C]pPDM cross-linked SF_1 showed essentially all the radioactivity was located in the 20 kDa heavy chain tryptic peptide. These results indicate that the site of photomodification does not involve either SH_1 or SH_2. UV spectral changes were observed upon irradiation of the MgADP·Vi·SF-1 complex which were correlated linearly with the release of Vi. These results indicate that Vi promotes photomodification of a specific aromatic amino acid which presumably is at or near the γ-phosphate binding site of ATP.

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M-PM-A7 MECHANISM OF ACTIN ACTIVATION OF BOVINE CARDIAC MYOSIN-S1 ATP HYDROLYSIS, Howard D. White and Betty Belknap, Dept. of Biochemistry, Eastern Virginia Medical School, Norfolk Va. 23501 and David D. Hackney, Dept. of Biological Science, Carnegie Mellon University, Pittsburg Pa. 15213

We have studied the mechanism of actin activation of bovine cardiac actomyosin-S1 ATP hydrolysis by comparing the dependence upon actin concentration of the rate of ATP hydrolysis and the fraction of myosin-S1 bound to actin during steady state ATP hydrolysis. ATP hydrolysis was measured by colorimetric determination of phosphate. The amount of myosin-S1 bound to actin was measured by the airfuge method, adapted for use in a refrigerated centrifuge and by measuring the amount of light scattered at 90° in a stopped-flow cell during steady state ATP hydrolysis. Low ionic strength conditions (5 mM mops, 2 mM $MgCl_2$, 1 mM ATP, 0.1 mM DTT pH 7, 20° C) were used to simplify the measurements by making the actin affinity as high as possible. The apparent binding constants of bovine cardiac myosin-S1 for actin: 4 +/- 1 x 10^{-6} M (K_m actin activation of the steady state rate of ATP hydrolysis), 5 +/- 2 x 10^{-6} M (K_c measured by the centrifuge method), and 7 +/- 2 x 10^{-6} M (K_L measured by light scattering) were the same within experimental error. Increasing the ionic strength by the addition of 10 mM KCl increased the K_m and K_c to 22 +/- 3 and 23 +/- 6 x 10^{-6} M, respectively. We have, therefore been unable to observe a significant difference between the amount of bovine cardiac myosin-S1 bound to actin and the extent of actin activation of myosin-S1 ATP hydrolysis. These results indicate that the four state model of Taylor is sufficient to explain the actin activated ATP hydrolyis of bovine cardiac myosin-S1 and would not require (nor disprove) the six state model proposed by Eisenberg for rabbit skeletal actomyosin-S1.

M-PM-A8 ACTIN DEPENDENCE OF Pi = HOH EXCHANGE OF BOVINE CARDIAC S1. David D. Hackney and Howard D. White. Dept. Biol. Sci., Carnegie-Mellon Univ., Pittsburgh, PA 15213 and Dept. Biochem., Eastern Virginia Medical School, Norfork, VA 23501.

The dependence on the actin concentration of the oxygen exchange accompanying ATP hydrolysis was determined for bovine cardiac S1. The buffer was 5 mM MOPS pH 7, 2 mM $MgCl_2$, 0.1 mM DTT, 0.04 mM diadenosine pentaphosphate with 1 mM [^{18}O]ATP and KCl as indicated. In the absence of actin, extensive incorporation of water-derived oxygens into the product Pi is observed indicating many reversals of the hydrolysis of bound ATP before product release. Increasing actin decreases the incorporation of water-derived oxygens and increases the net ATPase rate. The distributions of the Pi species containing 0 to 3 ^{18}O-oxygens per Pi are approximately that predicted for a single pathway, but a limited degree of heterogeneity is observed. Plots of the average number of reversals of the hydrolysis step, R, versus the inverse of the actin concentration are linear over a wide range, although some upward curvature is observed at 0 mM KCl and very high actin. The extrapolated R values are 1.3 and 1.9 at infinite actin for 0 and 10 mM KCl respectively. The K_R values for half saturation with actin are 18 and 10 micromolar at 0 and 10 mM KCl which are approximately equal to the values observed for saturation of the ATPase rate and steady state actin binding (see preceeding abstract).

Cross-linked actoS1 also exhibits an R value of 1.5 under similar conditions. At KCl levels above 50 mM, the R value increases and the distributions become markedly heterogeneous. Changes in pH in the range of 6.4 to 8.1 in contrast have little effect on the net rate or R value. The effect of temperature is more pronounced at 150 versus 10 mM KCl for both the ATPase rates and R values.

M-PM-A9 BIOCHEMICAL KINETICS OF PORCINE SUBFRAGMENT-1: COMPARISON OF $K_{BINDING}$ AND K_{ATPase} AT LOW IONIC STRENGTH. Stein, L.A. and White, M.O. Cardiology Division, SUNY at Stony Brook, Stony Brook, New York.

Previous kinetic studies on rabbit skeletal subfragment-1 (S-1) have revealed two important features of the actomyosin ATPase activity. (Stein, et al. 1979, Biochem 18, 3897, Stein, et al. 1985, Biochem 24, 1357). First, hydrolysis of ATP by myosin subfragment-1 proceeds both when S-1 is bound to actin and when it is dissociated from actin. Second, the actin concentration required to reach half the maximum ATPase activity, K_a (ATPase), is considerably lower than the actin concentration required to bind half the subfragment-1 during steady state hydrolysis of ATP, K_a (binding). These kinetic facts require that skeletal myosin can hydrolyze ATP without dissociating from actin, i.e., there exists a "non-dissociating" pathway for ATP hydrolysis.

The studies reported here show that porcine cardiac S-1 is very similar to rabbit skeletal S-1. Under identical conditions to prior work on skeletal S-1, the K_a (ATPase) of procine cardiac S-1, is approximately equal to that reported for skeletal S-1. This is also true for K_a (binding). Comparison of K_a (APTase) and K_a (binding) show that for cardiac proteins K_a(ATPase) is four to six-fold stronger that K_a (binding) (i.e., half maximal ATPase activity is achieved at about one fifth the actin necessary to reach 50% binding). The extrapolated maximum ATPase activity at saturating actin concentration for cardiac S-1 is consistently slower than skeletal S-1 by about a factor of 2.5. Furthermore, studies of the actoS-1 ATPase activity at high actin concentrations as well as with crosslinked actoS-1 show no significant inhibition, implying the requirement of a "non-dissociating" pathway for ATP hydrolysis by cardiac myosin subfragment-1.

M-PM-A10 THE EXTENT OF ^{18}O-EXCHANGE INDUCED BY THE ACTIN-ACTIVATED ATPase ACTIVITY OF PORCINE CARDIAC MYOSIN SUBFRAGMENT-1 (S-1). John A. Evans, Evan Eisenberg, and Leonard A. Stein, Laboratory of Cell Biology, NHLBI, NIH, Bethesda, MD and SUNY at Stony Brook, Stony Brook, NY.

While there is substantial agreement on the kinetic mechanism of the acto·S-1 ATPase activity, the nature of the rate-limiting step in the ATPase cycle is still controversial. This rate-limiting step is important because it may govern the rate of force development *in vivo*. We previously suggested that the rate-limiting step is a separate step following ATP hydrolysis, while Rosenfeld and Taylor suggested that ATP hydrolysis, itself, is rate-limiting. Both the cardiac and skeletal acto·S-1 ATPase cycles appear to have similar properties, in particular, both show a large difference in K_{ATPase} and $K_{binding}$ and, therefore, presumably have the same rate-limiting step. If ATP hydrolysis were rate-limiting, then the P_i release step would have to be rapid in both sytems to explain the difference between K_{ATPase} and $K_{binding}$. This, in turn, predicts that very little ^{18}O-exchange should occur at high actin concentration since P_i would be released before ^{18}O exchange could occur. In fact,with skeletal S-1, we and others have shown that very little ^{18}O exchange occurs at high [actin] which is consistent with ATP hydrolysis being rate-limiting. However, we now find that, with cardiac S-1, considerable ^{18}O exchange occurs both at high [actin] and with the S-1 crosslinked to actin. These data strongly suggest that ATP hydrolysis is not rate-limiting in the cardiac acto·S-1 ATPase cycle. Rather a separate step, following the ATP hydrolysis step may be rate-limiting. If a similar mechanism occurs with skeletal S-1, as seems likely, then the low level of ^{18}O exchange observed in the skeletal system may be due to phosphate rotation limiting the amount of exchange which can occur.

M-PM-A11 EFFECT OF MILD HEAT TREATMENT ON BINDING OF ACTIN AND NUCLEOTIDE BY MYOSIN SUBFRAGMENT-1. Adrianne Setton, Mary Dan-Gur and Andras Muhlrad. Dept.Oral Biology, Hebrew University-Hadassah School of Dental Medicine, Jerusalem 91010, Israel.

We have shown recently that chymotryptic subfragment-1 (S1) lost its ATPase activity upon incubation at 35°C and this loss was accompanied by unfolding of the 50K domain of S1 (Arch.Biochem.Biophys.235,411(1984). Here it is shown by sedimentation that S1 which lost its ATPase activity following 3 hours incubation at 35°C still retained its capacity to bind to F-actin under rigor conditions, however, with decreased affinity. Heat-treated S1 dissociated from F-actin upon addition of MgATP or $MgPP_i$, and under these conditions its affinity to actin was identical with that of native S1. No increase in tryptophan fluorescence was observed upon addition of MgATP to heat treated S1 in contrast to native S1. Heat treated S1 lost its ability to bind the purine ring of the nucleotide since the fluorescence of ε-ADP added to the heat treated S1 was quenched by acrylamide contrary to which was observed with native S1. Limited tryptic digestion of heat treated S1 resulted in two stable heavy chain fragments (27K and 20K) instead of the usual three (27K, 50K and 20K). A 30K fragment has also appeared in the SDS-PAGE following short tryptic digestion of heat treated S1. The 30K fragment was derived from the N-terminal part of the missing 50K domain as it was shown by a specific monoclonal antibody. It seems that the mild heat treatment of S1 leads to unfolding of the C-terminal part of 50K which unstabilizes this domain and results in the loss of the ATPase activity and the binding of the purine ring of ATP but hardly affects the binding of actin and the pyrophosphate moiety of the nucleotide. Supported by grants from U.S.-Israel Binational Science Foundation and Muscular Distrophy Association.

M-PM-A12 TROPONIN-I AS A MOLECULAR SWITCH IN CALCIUM REGULATION OF SKELETAL MUSCLE CONTRACTION: RESONANCE ENERGY TRANSFER AND SITE-SPECIFIC PHOTOCROSSLINKING STUDIES. T. Tao, B.-J. Gong, & P.C. Leavis, Dept. of Muscle Research, Boston Biomedical Research Institute, & Dept. of Neurology, Harvard Medical School, Boston, MA 02114.

Troponin-I (TnI), the inhibitory component of skeletal mucle troponin, is capable of inhibiting acto-myosin ATPase activity by itself. Its role in the regulation of skeletal muscle contraction by Ca^{2+} is still poorly understood. We have sought answers to this question by examining the interactions between troponin-C (TnC), TnI and actin in reconstituted rabbit skeletal thin filaments using a combination of resonance energy transfer and site-specific photocrosslinking studies. When TnI was labeled at Cys-133 with the fluorescent donor 1,5-IAEDANS, and TnC at Cys-98 with the acceptor DAB-Mal, apparent distances of >7.5, >7.5 and 3.9 nm were obtained in the presence of EGTA, Mg^{2+} and Ca^{2+}, respectively. With the donor again attached at Cys-133 of TnI, but with the acceptor on Cys-374 of actin, distances of 3.9, 4.0 and 5.5 nm were obtained in EGTA, Mg^{2+}, and Ca^{2+}, respectively. When TnI was labeled at Cys-133 with the photocrosslinker BP-Mal, crosslinking to actin was obtained in the presence of EGTA or Mg^{2+}, but not in the presence of Ca^{2+}. Taken together, these results suggest that upon binding of Ca^{2+} to the low affinity Ca^{2+}-specific sites of TnC, TnI "swings" towards TnC and away from actin by a distance of ~1.5 nm. These observations are consistent with models for thin filament regulation in which binding of Ca^{2+} to TnC induces the detachment of TnI from actin, which in turn triggers a movement of tropomyosin that results in deinhibition of acto-myosin ATPase activity. Supported by grants from NIH (AM 21673 and HL20464) and MDA.

M-PM-A13 COOPERATIVE INTERACTIONS BETWEEN TROPONIN-TROPOMYOSIN UNITS EXTEND THE LENGTH OF THE THIN FILAMENT IN SKELETAL MUSCLE. PW Brandt[+], MS Diamond[+], JS Rutchik[+], FH Schachat[#]. [+]Columbia University, [#]Duke University.

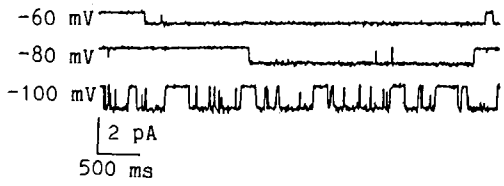
Ca^{2+} binding to troponin C (TnC), a subunit of the thin filament regulatory strand, activates vertebrate skeletal muscle contraction. Tension, however, increases with Ca^{2+} too abruptly to be the result of binding to sites on a single TnC. Because extraction of one TnC on average per regulatory strand dramatically reduces the slope of the tension/Ca^{2+} relationship, we proposed that all 26 troponin-tropomyosin complexes of the regulatory strand form a cooperative system (Brandt et al, JMB, 1984). This study of permeabilized (chemically skinned) rabbit psoas fibers analyzes the extraction time-course, the distribution of extraction sites on regulatory strands and the effects of extraction on the cooperativity of the tension/Ca^{2+} relationship. Two components of TnC are resolved in the time-course of extraction: a "rapidly extracting" component which can be selectively removed without affecting tension or cooperativity, and a "slowly extracting" component whose loss reduces tension and cooperativity. Extraction of ^{14}C-TnC shows that the slowly extracting component is lost randomly, so that, after removal of 5% of the TnC, most extracted strands have lost one TnC. Extraction interrupts the transmission of cooperativity by dividing the regulatory strand into smaller, independent cooperative systems; it reduces tension by preventing Ca^{2+} activation of TnC depleted regulatory units. Cooperativity of the tension/Ca^{2+} relationship is modeled by the concerted-transition formalism (Monod et al, JMB, 1965) for intact cooperative systems of 26 regulatory units, and for the smaller systems in extracted fibers. The effects of different combinations of troponin-tropomyosin isoforms on cooperativity are also analyzed with this model.

M-PM-A14 THE EFFECT OF IASL MODIFICATION ON THE CALCIUM CONTROL OF THE ACTOMYOSIN ATPase. M.A. Titus, G. Ashiba & A.G. Szent-Györgyi, Department of Biology, Brandeis University

Iodoacetamide-based spin label (IASL) modification of the fast reacting thiol group of myosin impaired calcium regulation in both rabbit and scallop. The parent compound, iodoacetamide (IAAm), is known to specifically modify rabbit SH-1. Reaction of the thiol group was indicated by a 90% loss of K/EDTA ATPase following overnight incubation with stoichiometric amounts of IASL, at low ionic strength at 4° C. The actin-activated ATPase of rabbit myosin was measured in the presence of regulated actin, and that of scallop myosin in the presence of pure actin. IASL modification of both rabbit and scallop myosin resulted in a 2-fold reduction of the actomyosin ATPase activity in the presence of calcium, and an increased activity in the absence of calcium. The calcium activation was decreased to 2- to 3- fold which contrasted with the 80-fold activation of unmodified myosin. The increased MgATPase of myosin, which typically occurred following SH-1 modification, was subtracted from each assay point and did not account for the loss of calcium sensitivity. The modification of rabbit SH-1 by either IAEDANS or IAAm also significantly reduced the calcium sensitivity of the actomyosin ATPase. In contrast, IAAm modification did not affect the calcium sensitivity of scallop myosin. This may be due to a differential susceptibility of two reactive thiol groups, or to the effect of each reagent on the surrounding environment. These results indicate that thiol-directed probes may not be suitable for following cross-bridge behavior during rest. Supported by NIH AR-15963 and MDA grants.

M-PM-B1 CALCIUM-PERMEABLE CHANNELS FROM CARDIAC SARCOLEMMA OPEN AT RESTING MEMBRANE POTENTIALS. R.L. Rosenberg & R.W. Tsien. Department of Physiology, Yale Univ. New Haven, CT 06510.

When cardiac sarcolemmal membranes are incorporated into planar lipid bilayers (50% PE/50% PS), we observe activity of two different types of Ca channels: L-type Ca channels that are very similar to those in intact cells (Rosenberg et al., Science 231, 1564), and another type of Ca channel ("B-type") with very different properties. Unlike L-type channels, B-type Ca channels open even at steady negative potentials; channel open probability is maximum at -80 to -60 mV and falls to very low levels at depolarized potentials. With 100 Ba, 50 Na (out)//50 Na (in), or with 100 Ca, 50 Na//50 Na, B-type channels have a slope conductance of ~10 pS at -60 mV, whereas the L-type channels have a conductance of 23 pS with external Ba and 7 pS with external Ca. E_{rev} with external Ba is 50 mV so P_{Ba}/P_{Na} is ~18, in contrast to the >1000-fold selectivity for L-type channels. In the presence of Bay K 8644, B-type channel open time averages 50-100 msec at -100 mV but increases to >500 msec at -60 to 0 mV, compared to 20-40 msec for L-type channels. B-type channel openings were seen even in the absence of Bay K 8644. B-type and L-type Ca channels are both found in light but not heavy sarcolemmal fractions, and can appear together in bilayer recordings. If present in intact heart cells, B-type channels could contribute to tonic influx of Ca. We have seen unitary Ba currents at negative potentials in heart and smooth muscle cells and in hippocampal neurons, but the openings were briefer or smaller than those in the bilayers.



M-PM-B2 RECONSTITUTION OF A PURIFIED VOLTAGE-DEPENDENT CALCIUM CHANNEL (VDCC) INTO PLANAR LIPID BILAYERS. Edward J. McKenna, Jeffrey S. Smith, Janjie Ma, Janeen Vilven, Pal Vaghy, Arnold Schwartz, and Roberto Coronado, Department of Pharmacology and Cell Biophysics, University of Cincinnati, Cincinnati, Ohio 45267 and Department of Physiology and Molecular Biophysics, Baylor College of Medicine, Houston, Texas 77030.

A purified VDCC from rabbit skeletal muscle t-tubules has calcium channel activity when incorporated into planar bilayers. The purified VDCC consists of one major polypeptide of M_r 150,000 under reducing conditions, but several minor polypeptides are also present. The VDCC exhibits high (+)[^{3}H]PN200-110 binding and retains allosteric regulation by d-cis-diltiazem and (-)D888. VDCC spontaneously induced a multiplicity of channels with conductances ranging from 4 pS to 50 pS; some are statistically coupled in time suggestive of substates of the VDCC. The predominant calcium channel activity is indistinguishable from that of native t-tubule membranes. This channel exhibits: 1) an amplitude of 0.5 pA and a slope conductance of 10 pS in 100 mM Ba^{2+}, 2) voltage-dependent kinetics, unlike the purified protein used by Flockerzi et al., 1986 (NATURE 323:66-68), 3) selectivity for divalent cations (but not Mg^{2+}), 4) a minimum permeability ratio for monovalents (P(Ba)/P(Na) = 20), and 5) sensitivity to µM Bay k 8644 which prolongs mean open time and increases frequency of channel opening. However, a second type of channel activity with a unitary conductance of 22 pS is also apparent and this channel has a high divalent over monovalent ion selectivity. (Supported by NIH Grants PO1 HL22619, GM36852 and HL37044 (RC), and MDA Postdoctoral Fellowship (JSS)).

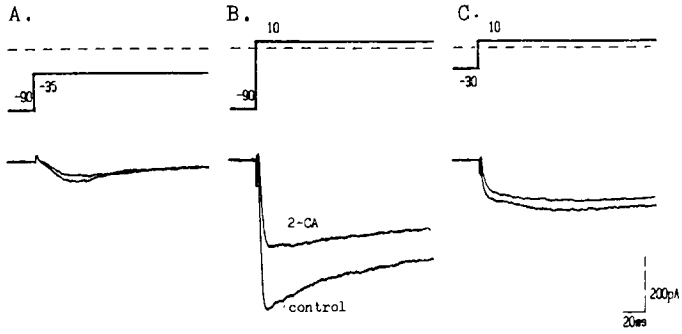
M-PM-B3 MODIFICATIONS OF CARDIAC CALCIUM CURRENT IN CELLS WITH INHERENT DIFFERENCES IN CURRENT DENSITY. Rodolphe Fischmeister, Jorge A. Argibay*, and H. Criss Hartzell. Unité INSERM U-241, Université de Paris-Sud, Bâtiment 443, F91405 Orsay, France.

Whole-cell calcium currents were recorded in single ventricular myocytes from adult frogs with low resistance patch pipettes. Other ionic currents were blocked by TTX and caesium. I-V relationships obtained from different holding potentials (V_h = -100 to -40 mV) showed the presence of a single type of calcium current (I_{Ca}). In 79 cells studied under identical experimental conditions, cell membrane capacitance (C_m) ranged from 34 to 92 pF (62 ± 12 pF) and I_{Ca} density (i.e. I_{Ca}/C_m), measured at 0 mV from V_h = -80 mV, varied significantly between 0.8 and 20 pA/pF (4.5 ± 3.7 pA/pF). Inactivation of I_{Ca} at positive potentials was less pronounced in cells with lower I_{Ca} density, while reactivation of I_{Ca} was faster. The degree of inactivation induced by a 200 ms pulse at +100 mV increased from 0 to ≈50 % in cells with I_{Ca} density increasing from 1 to 5 pA/pF, while the time for 50% reactivation at -80 mV ($t_{1/2}$) increased from ≈50 to 90 ms. In cells with I_{Ca} density over 6 pA/pF, the degree of inactivation and $t_{1/2}$ asymptoted around 45 % and 100 ms, respectively. A facilitation of I_{Ca} was observed in cells with I_{Ca} density below 7 pA/pF. A positive I_{Ca} staircase could be elicited in few of these cells at high stimulation frequency (2 Hz), while I_{Ca} staircase was always negative with I_{Ca} density above 4 pA/pF. Enhancement of I_{Ca} by superfusion of a cell with isoprenaline or perfusion of the patch pipette with cAMP induced changes in both inactivation and reactivation that were predictable from the increase in I_{Ca} density. These results suggest that the properties of a single Ca channel may vary from cell to cell or/and may be strongly dependent on the density of functional Ca channels per membrane surface area.

*Supported by France-Venezuela exchange program.

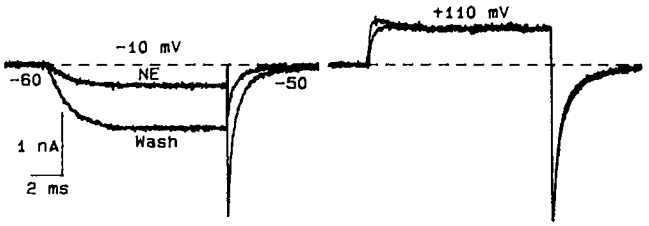
M-PM-B4 ADENOSINE REDUCES AN INACTIVATING COMPONENT OF CALCIUM CURRENT IN HIPPOCAMPAL CA3 NEURONS. D.V. Madison, A.P. Fox and R.W. Tsien. Dept of Physiology, Yale Univ., New Haven, CT 06510.(Intr. by W.N. Green)

We have studied Ca currents in cultured rat hippocampal CA3 neurons with patch clamp recording. Preliminary evidence suggests the possible existence of three components of Ca current (cf. Gray and Johnston, 1986) corresponding to T, N and L-type Ca channels in DRG neurons. We find that 2-chloroadenosine (2-CA) reduces whole cell Ca current in CA3 neurons, as in DRG neurons (Macdonald et. al., 1986; Dolphin et. al., 1986). 2-CA has little effect on the T-like current (A) or on the L-type current (C). However, an inactivating N-like component seen with strong depolarizations from negative holding potentials is reversibly reduced by 2-CA (B). N-type calcium channels of sympathetic neurons have been implicated in controlling neurotransmitter release (Hirning et. al., 1986). Because adenosine blocks synaptic transmission in the hippocampus, we are invesigating the possibility that modulation of N-type calcium currents by adenosine can account for this action.



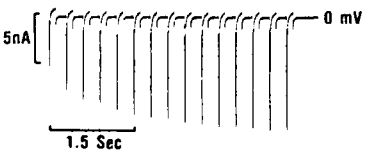
M-PM-B5 NOREPINEPHRINE CHANGES THE VOLTAGE-DEPENDENCE OF CALCIUM CHANNELS IN SENSORY NEURONS
Bruce P. Bean, Department of Neurobiology, Harvard Medical School, Boston, MA

Previous studies have concluded that norepinephrine (NE) decreases neuronal Ca currents without changing their voltage-dependence. I have re-examined this point using freshly dispersed cell bodies from dorsal root ganglia of adult bullfrogs, a process-free preparation allowing rapid, high-quality space clamps. Cells (20-35 μ) were isolated by collagenase treatment. Whole-cell currents were recorded using external solutions of 1-10 mM Ca or Ba in a TEA Cl solution (with 2 μM TTX) and internal solutions of Cs Glutamate that also contained an ATP-regenerating solution (Forscher and Oxford, J Gen Physiol 85,743). Depolarizing pulses from holding potentials positive to -70 mV elicited a single type of Ca current. Application of 30 μM NE produced rapid, reversible decreases in the current. In agreement with earlier studies, the decrease was about the same for voltages from -30 to about +60 mV. However, the outward current activated by large depolarizations - identified as Ca channel current by its sensitivity to La (30-100 μM) - was little affected by NE. Similarly, tail currents following moderate depolarizations were greatly reduced, but those following large depolarizations were little changed. I conclude that NE decreases Ca current by changing the voltage dependence with which channels open.



M-PM-B6 VOLTAGE DEPENDENT POTENTIATION OF Ca CHANNEL CURRENTS OF INTERNALLY PERFUSED SINGLE MAMMALIAN HEART CELLS.
Kai S. Lee. Cardiovascular Diseases Research, The Upjohn Co., Kalamazoo, MI

The influx of Ca^{2+} through voltage dependent Ca channels is controlled by channel gatings. Repeated depolarization promotes channel inactivation leading to reduction of channel conductance. Recently, Noble and Shimoni provided contrasting evidences that repeated depolarization increases Ca channel currents in frog atrial cells. Their results suggest that the open Ca channel not only can be down-regulated, as channel inactivates, but can be up-regulated intrinsically by a voltage or Ca dependent process that has yet to be identified. This study reveals that the Ca currents of internally perfused single ventricular cells of guinea-pigs can be markedly increased by repeated depolarization. The increase or potentiation is maximal at peak current potentials of 0 to 10 mV. Time courses of potentiation development and decline are very slow, occurring in seconds. Concomitant with the marked increase of peak current amplitude, rate of current decline or inactivation during the pulse is strongly reduced, thus resulting significant amount of current flow at the end of a 200 msec pulse. Microelectrode studies of the same preparation in the presence or absence of 60 μM TTx revealed that the developmental time course of muscle twitch intensity (positive staircase) in response to repeated depolarization matches closely with the developmental time course of Ca current potentiation. It is proposed that the Ca channel current potentiation is mainly responsible for the generation of positive staircase activity of the myocardial cells.



M-PM-B7 MULTIDISCIPLINARY APPROACH TO THE STRUCTURE OF THE PURIFIED VOLTAGE-DEPENDENT CALCIUM CHANNEL (VDCC). Arnold Schwartz, Edward McKenna, Pal Vaghy, Terence Kirley, Jeffrey Robbins, Natsuki Nakayama, Kevin Campbell, Alan Sharp, Albert Leung, Toshiaki Imagawa, Mark Williams, Steven Ellis, Michael Harpold, Department of Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267-0575; SIBIA Inc., P.O. Box 85200, San Diego, California, 92138-9216; Department of Physiology and Biophysics, University of Iowa, Iowa City, Iowa 52242.

Partially purified VDCC from rabbit skeletal muscle t-tubules contains one major (150 kDa) and several minor peptides that can be demonstrated on silver-stained SDS-Polyacrylamide gels. This preparation is enriched in dihydropyridine (DHP) receptors B_{max} ~2 nmol (+)[^{3}H]PN200-110/mg with 50 µM d-cis diltiazem. Non-DHP Ca channel modulators, d-cis-diltiazem and (-)D888 allosterically regulate (+)[^{3}H]PN200-110 binding. Reconstitution revealed VDCC activity. An expression cDNA library from rabbit skeletal muscle was prepared in λgt11 and is being screened by two methods: 1) oligonucleotide hybridization probes designed from N-terminal amino acid sequence and 2) antibodies which are capable of immunoprecipitating (+)[^{3}H]PN200-110 labeled VDCC. This two-pronged approach is necessary to confirm the identity of the putative clones of the VDCC. (Supported by NIH Grants PO1 HL22619 (A.S.) and RO1 HL37187 (K.C.).)

M-PM-B8 BLOCK OF PARAMECIUM CALCIUM CHANNELS BY THE CALMODULIN ANTAGONIST W-7 AND ITS ANALOGUES. B.E. Ehrlich, *M. Forte, +A.R. Jacobson, +L.M. Sayre. Division of Cardiology, University of Connecticut Medical Center, Farmington, CT and Department of *Biology and +Chemistry, Case Western Reserve University, Cleveland, OH.

The only compounds known to block Paramecium calcium channels are the napthalene sulfonamide calmodulin antagonists (eg., W-7). We tested the effectiveness of analogues of W-7 on a behavioral response in intact Paramecium and on single calcium channels that had been incorporated into planar lipid bilayers. Since the behavioral assay is simple and since it correlates well with direct measurements of calcium currents in intact voltage-clamped Paramecium, all compounds were tested behaviorally. Only the most potent analogues were tested on the single channel currents. To date we have focused on one compound that is more effective than W-7: W(12)Br. Complete block with W-7 occurs with 100 uM; the K1/2 is 20 uM. With W(12)Br, 1 uM leads to complete block; the K1/2 is 0.1 uM. When we measured the effect of 0.1 uM W(12)Br on single channel currents, open times decreased to 30% of control and very long silent periods began to appear. The single channel conductance was unchanged. Since these compounds are effective in blocking channels in the cell-free system, it seems unlikely that they are working via calmodulin despite the fact that they are potent antagonists of Paramecium calmodulin. However, the mechanism of action is likely to be complex since more than one channel parameter is altered by these agents.

Supported by NSF grant DCB83-09110. BEE is a PEW Scholar in the Biomedical Sciences.

M-PM-B9 PRESYNAPTIC Ca-ANTAGONIST W-CONOTOXIN IRREVERSIBLY BLOCKS N-TYPE Ca-CHANNEL IN CHICK SENSORY NEURONS. Haruo Kasai and Tosihiko Aosaki, (Intr. by Christopher Follmer) Department of Physiology, Faculty of Medicine, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan.

Three types of Ca-currents and their sensitivities to w-conotoxin (wCTX) and dihydropyridines were studied using the whole-cell voltage-clamp method in cultured dorsal root ganglion cells of chick embrios. The T-type current could purely be recorded by small membrane depolarization (-60 mV) while holding the membrane potential to hyperpolarized level (-100 mV). The two high-threshold currents, L- and N-type currents, were separated using the pharmacological criteria: the L-type current is augmented by Bay K 8644 and reduced by nifedipine, while the N-type current is reduced by Bay K but insensitive to nifedipine. According to this criteria, the high-threshold currents recorded from 28 cells out of 56 were considered to be composed purely of the N-type current, and the remaining cells contained both the L- and N-type currents. We found that wCTX (5 µM) was a complete and irreversible blocker of the N-type Ca-current, while it was a partial and reversible blocker of both the L- and T-type Ca-currents. The N-type Ca-current is a dominant type of Ca-channel in the neurons and that the content of L-type channels, on which DHPs act as specific inhibitors, is small (mean=6 %). The L- and N-type currents separated using wCTX showed different voltage-dependencies and ionic selectivities.

These findings suggest that three types of Ca-currents are carried through different types of Ca-channel molecules, and that the N-type channel is a dominant Ca-channel in the neurons, which explains well the small DHP-sensitivity of Ca-currents in various nerve cells. Furthermore, the action of wCTX on synaptic transmission suggests that the major Ca-channel which regulates the release of transmitters from neurons is the N-type.

M-PM-B10 SINGLE UNIT ANALYSIS OF THE PROTON-INDUCED TRANSFORMATION OF CA^{2+} CHANNEL IN SENSORY NEURON. M. Morad and H.D. Lux. Max-Planck-Institute for Psychiatry, 8033 Planegg, FRG. Recently we described a Na^+ current ($I_{Na(H)}$) in whole cell DRG neurons which was activated by step changes in proton concentration (Morad et al., J.Physiol. P113, 1986). Activation of $I_{Na(H)}$ is accompanied by transformation of Ca^{2+} channel such that Ca^{2+} channel loses transiently (1-2s) its voltage-gating and became selective to Na^+. Activation of $I_{Na(H)}$ depends on the $(H^+)_o$ but not on its gradient across the membrane, thus outside-out but not inside-out patches generated $I_{Na(H)}$. The single channel associated with $I_{Na(H)}$ opened in bursts with mean open times of 0.3 to 1.0 ms and a conductance of 17-25 ps (-80 to +60 mV). Activation ($t_{1/2} \simeq$ 20 ms) and inactivation ($t_{1/2} \simeq$ 150 ms) of the channel were weakly voltage-dependent, consistent with an external site of action. Inactivation was characterized by an increase in long shut times. $I_{Na(H)}$ deactivated by a step reduction of $(H)_o$ or increase in $(Ca)_o$. 30 mM $(Ca)_o$ fully blocked $I_{Na(H)}$. Displacement of Ca^{2+} from the channel binding sites by H^+ appeared to be responsible for transformation of the Ca^{2+} channel since reduction of $(Ca)_o$, also induced a Na^+ current with similar properties. Kinetics and the conductance of the proton-induced unitary current were similar to those of Na^+ conducting Ca^{2+} channel. However, the concentration of Ca^{2+} needed to block $I_{Na(H)}$ were much higher than those needed to block Na^+ permeation through the voltage-gated form of the Ca^{2+} channel. Our studies suggest that Ca^{2+} channel may exist in 2 possible states. In the voltage-gated state it transports preferentially bivalents and in proton-gated state it prefers monovalents and is blocked by divalents.

M-PM-B11 Ca CURRENTS IN BC_3H1 MYOCYTES CORRESPOND TO THOSE OF SKELETAL MUSCLE T-TUBULES. John M. Caffrey*, A.M. Brown* and Michael D. Schneider*†. Departments of *Physiology and Molecular Biophysics, †Medicine, and †Cell Biology, Baylor College of Medicine, Houston, TX.

The non-fusing myogenic cell line, BC_3H1, has been extensively studied as a model of skeletal or, alternatively, smooth muscle differentiation. We found that BC_3H1 myocytes express two independent voltage-gated Ca currents, which could be resolved on the basis of voltage-dependence, kinetics and pharmacology. These components were designated "fast" and "slow" Ica, analogous with but distinct from the "low-" and "high-threshold" Ca currents expressed in cardiac muscle and neurones:

	Threshold V	Peak V	Activ. (τ)	½ Inactiv.	Inactiv. (τ)	DHP Sensitiv.
Fast	-40 to -30 mV	-10 to 0 mV	1-15 msec	-10 mV	0.5-5 sec	none
Slow	-10 to 0 mV	+10 to +20 mV	60-200 msec	-70 mV	1-10 sec	strong

Two classes of elementary Ca channels were detected in single-channel records. The fast and slow channels exhibited 8 and 12 pS conductance, respectively, in 50 mM Ba. The fast channel was equally selective for Ba and Ca, whereas the slow channel was more permeant to Ba than Ca. Both currents were blocked by cadmium. The slow current could also be modulated by β-adrenergic agonists. The biophysical properties of Ca channels formed in BC_3H1 cells correspond to those observed previously for the "fast" and "slow" Ca currents in T-tubules of skeletal muscle (Cota and Stefani, J. Physiol. 370: 155, 1986; Almers and Palade, J. Physiol. 312: 139, 1981).

M-PM-B12 TRANSFECTED ONCOGENES CAN BLOCK THE EXPRESSION OF SPECIFIC VOLTAGE-GATED ION CHANNELS. Michael D. Schneider*†, John M. Caffrey† and Arthur M. Brown†. Depts. of *Medicine, *Cell Biology, and †Physiology & Molecular Biophysics, Baylor Coll. of Med., Houston, Texas.

The molecular mechanisms underlying developmental regulation of voltage-gated ion channels are unknown. We have used the BC_3H1 muscle cell line to determine whether expression of voltage-gated ion channels is contingent on mitogen withdrawal and growth arrest, as appears to be generally true for muscle-specific gene induction. Because cellular oncogenes have been postulated to function in a cascade for transduction of growth factor signals, we also have examined BC_3H1 cells that had been modified by gene transfer with oncogene expression vectors. BC_3H1 myocytes first expressed functional Na and Ca channels after ~ 5 days of mitogen withdrawal. We identified both a "fast", low-threshold Ca current and a "slow", higher-threshold Ca current, whose properties corresponded to those of skeletal muscle Ca channels. Ca current density doubled every two days through days 15-20 ($\sim 10^4$ functional "slow" Ca channels per cell). The c-*myc* and v-*erbB* expression vectors (pSVc-*myc*-1 and pAEV11-d1-3) delayed but did not prevent the induction of Na and Ca channels. Down-regulation of c-*myc* expression thus was not a prerequisite for biophysical differentiation. In contrast, BC_3H1 cells that had been modified with a valine-12 c-Ha-*ras* vector (pEJ6.6) by itself, or by co-transfection of pSVc-*myc*-1 together with pAEV11-d1-3, failed to produce functional Na and Ca channels at up to 4 weeks. Functional Ca channels could not be unmasked in *ras*-transfectants by BAY k8644 (5 μM) or isoproterenol (1 μM). Delayed rectifier K currents, in contrast, were affected neither by mitogenic medium nor by the transfected oncogenes. These results comprise the first biophysical studies of the selective effects of oncogenes on voltage-gated ion channels following gene transfer.

M-PM-B13 ASSOCIATION OF PURIFIED T-TUBULE CALCIUM CHANNELS RECONSTITUTED IN PLANAR LIPID BILAYERS. Lin Hymel[1], Jörg Striessnig[2], Hartmut Glossmann[2], and Hansgeorg Schindler[1]. [1]Institut für Biophysik, Universität Linz, A-4040 Linz, Austria and [2]Institut für biochemische Pharmakologie, Universität Innsbruck, A-6020 Innsbruck, Austria.

Purified Ca^{2+} channels from skeletal muscle T-tubule have been reconstituted using a rapid dilution method to obtain vesicles containing at most one protein molecule. Vesicle-derived planar bilayers were formed and singly-distributed Ca^{2+} channels were observed. Channels showed a high selectivity for Ba^{2+} over Na^{+}, were activated by BAY-K 8644 and inhibited by 10 μM Cd^{2+}. The open channel current-voltage curve was linear in symmetrical solutions. The initially randomly-distributed channel proteins were observed to associate in a time- and concentration-dependent manner. This association leads to strong functional coupling of channels which results in: (i) positive channel cooperativity, (ii) voltage dependence of channel opening, and (iii) altered gating kinetics. Such effects, epescially that of voltage, were not observed if channels were singly distributed. From this we conclude that association of channel proteins is essential in establishing the properties of Ca^{2+} channels as observed in native T-tubule membranes.

M-PM-B14 MODULATION OF [^{3}H] DIHYDROPYRIDINE RECEPTORS BY ACTIVATION OF PROTEIN KINASE C IN CHICK MUSCLE CELLS. Javier Navarro, Department of Physiology, Boston University School of Medicine, Boston, MA 02118.

Modulation of ion channels is an essential step for understanding the regulation of cellular functions. 1,4 - dihydropyridines (nitrendepine, nifedipine, PN 200-110, etc.) are potent inhibitors of voltage dependent calcium channels and are important therapeutic agents in the treatment of various cardiovascular disorders such as angina and cardiac arrhythmias. In this work a new procedure is employed to determine the density of surface dihydropyridine receptors in contracting muscle cells in culture. Activation of endogenous protein kinase C by the tumor promotor phorbol-12-myristate (PMA) or 1-oleoyl-2-acetylglycerol (OAG) enhanced the number of dihydropyridine receptors without significant change in the receptor affinity. The increase in the number of receptors was associated with changes of the dihydropyridine sensitive ^{45}Ca uptake as well as activation of protein kinase C in myotubes treated with phorbol esters. These data strongly suggest that activation of protein kinase C promotes the appearance of dihydropyridine receptors in the plasma membrane. Supported by National Science Foundation Grant #DCB-8511671 and Grant #1N-97 from the American Cancer Society.

M-PM-B15 TRIFLUOPERAZINE BLOCKS Ca^{2+} INWARD CURRENTS IN HELIX NEURONS IN A REVERSIBLE AND DOSE DEPENDENT MANNER. Bernal, M.J., Fernández, J.R. and Alvarez-Leefmans, F.J. Department of Pharmacology. CINVESTAV del IPN, Ap. Postal 14-740, Mexico 07000, D.F. and Instituto Mexicano de Psiquiatría, Calz. Xochimilco 101, Mexico 14370, D.F.

Identified neurons from subaesophageal ganglia of Helix aspersa were voltage clamped with two microelectrodes. Outward currents were suppressed by filling the current and voltage microelectrodes with 2M Cs-Acetate and superfusing the cells with a solution containing (mM): TEA-Cl, 70; 4AP-Cl, 5; KCl, 5; $CaCl_2$, 20; glucose, 5; K-HEPES, 5; pH, 7.5. Command pulses (40 to 100 ms, 10 to 200 mV) were applied from a holding potential of -50 mV. $I_{Ca^{2+}}$ max was attained between +30 and +50 mV. The currents were stationary over periods of more than 2 hs. After 30 min superfusion with trifluoperazine hydrochloride (TFP), a phenothiazine known to interfere with calmodulin (CaM) and Protein Kinase C, $I_{Ca^{2+}}$ max was reversely reduced as follows: for 20 μM by 26.6±2.9%, n=5; for 50 μM, by 43.9±4.7%, n=5 and for 100 μM by 65%, n=2. TFP sulfoxide, a TFP analogue without CaM blocking action at 50 μM had little or no effect on $I_{Ca^{2+}}$ max, suggesting that TFP action is not simply due to a surface membrane hydrophobic effect (Landry et al. Biochem. Pharmacol 30: 2031, 1981) or to rundown of $I_{Ca^{2+}}$ (Clapham & Neher, J. Physiol. 353: 541, 1984). The results might be explained by an specific effect of TFP on some CaM or C Kinase process modulating Ca^{2+} channels.

Sponsored by CONACYT PCEXCNA-030834 Grant to F.J.A.L. and Fellowship No. 30673 to J.B.M.

We are grateful to Dr. T.J. Rink from SK&F Research Limited for supplying a sample of TFP Sulfoxide.

M-PM-C1 THE EFFECT OF RECEPTOR DENSITY ON THE DIFFUSION LIMITED FORWARD RATE CONSTANT FOR BINDING OF LIGANDS TO CELL SURFACE RECEPTORS Jon W. Erickson, *Byron Goldstein, David Holowka, and Barbara Baird. Department of Chemistry, Cornell University, Ithaca, N. Y. 14853 and *Theoretical Division, Los Alamos National Laboratory, Los Alamos, N. M. 87545.

In the course of investigating the dynamic aspects of ligand-receptor interactions we have directly tested the functional dependence of the diffusion-limited forward rate constant (k_{on}) on the number of receptors per cell (N) predicted by Berg and Purcell (1977). The observation that bound 2,4-dinitrophenyl (DNP) ligands quench ~20% of the available fluorescence emission from fluorescein isothiocyanate-modified monoclonal anti-DNP IgE provides a sensitive monitor for F_{ab} site occupation (Erickson et al., 1986). Using the quenching method one can observe directly the binding of monovalent DNP-aminocaproyl-L-tyrosine (DCT) to FITC-IgE when the antibody is bound to its high affinity receptor on rat basophilic leukemia (RBL) cells. Surface density of F_{ab} is easily varied by partial IgE saturation of available receptors. Kinetic constants (k_{on}) can be extracted for different extents of saturation (N) by computer fitting the quenching that results after injection of ligand. In these experiments k_{on} ranged from ~$12\text{-}2\times10^{7}$ $(M\cdot sec)^{-1}$ when F_{ab}s/cell was varied from ~$0.5\text{-}7\times10^{5}$. The results are well fitted by the equation $k_{on}(cm^3/cell\cdot sec)=4\pi aN\chi_{on}/(4\pi Da+N\chi_{on})$ where D is the diffusion constant of the ligand in cm^2/sec, a the radius of the cell, and χ_{on} is the intrinsic forward rate constant for a single F_{ab}-DNP interaction. With D fixed at $10^{-5}cm^2/sec$ the best fit predicts an average cell radius of ~4μm and a χ_{on} of ~1.8×10^{-13} $cm^3/F_{ab}\cdot sec$ (=$1.1\times10^{8}(M\cdot sec)^{-1}$). Interestingly, k_{on} for IgE in solution at all measured concentrations approaches the predicted value of χ_{on}. These studies demonstrate the primary importance of N in determining the rate of ligand binding to cells. Supported by the NIH.

M-PM-C2 **A CYCLIC NUCLEOTIDE-GATED CONDUCTANCE IN OLFACTORY RECEPTOR CILIA.** Tadashi Nakamura and Geoffrey H. Gold, Dept. of Physiol., Yale U. Sch. of Med., New Haven, CT 06510

An odorant-sensitive adenylate cyclase has recently been reported in olfactory receptor cilia (Pace et al., Nature 316, 255). If cAMP is an intracellular messenger for olfactory transduction, there must be a conductance in the cilia plasma membrane that is regulated by cAMP. We studied the conductive properties of olfactory receptor cilia by use of the patch clamp technique. With patch pipettes that were fire-polished to resistances of 30-50 Mohm, we obtained gigaohm seals on cilia of enzymatically dissociated receptor cells from the olfactory mucosa of the toad, Bufo Marinus. In excised, inside-out patches, we observed a conductance which was reversibly induced by the bath application of cAMP. This conductance was observed in the absence of added NTP's, indicating that it is gated directly by cAMP, rather than resulting from protein phosphorylation. The timecourse of the ciliary conductance changes followed step changes in bath cAMP concentration with a lag of <~200 msec. With Ringer's solution in the patch pipette and a pseudo-intracellular solution in the bath, the reversal potential for this conductance was -5±3 mV. The value of the reversal potential is similar to reported values for the reversal potential of the odorant response, suggesting that this conductance mediates olfactory transduction in vivo. The steady-state concentration dependence of the ciliary conductance was measured for cAMP, cGMP and cCMP; the $K_{1/2}$ values were 3.4±1.0, 1.5±0.6, and 57±10 uM, respectively. The slightly higher affinity for cGMP than for cAMP raises the possibility that the intracellular messenger may be cGMP, or a mixture of cyclic nucleotides, rather than cAMP alone. The similarities between the ciliary and rod and cone outer segment conductances, suggest considerable similarity between the mechanisms of olfactory and visual transduction.

M-PM-C3 CELL SURFACE DYNAMICS OF THE IgE RECEPTOR IN SURFACE ELECTROPHORESIS AND POST-FIELD RELAXATION USING DIGITAL VIDEO FLUORESCENCE MICROSCOPY. Timothy A. Ryan, Jeffrey Myers, David Holowka, Barbara Baird and Watt W. Webb, Department of Physics (T.A.R.), Applied Physics (W.W.W.), and Chemistry (J.M., D.H. and B.B.), Cornell University, Ithaca, New York 14853.

We have performed a detailed investigation of electrophoresis and post-field relaxation of both monomeric and cross-linked fluorescein-labeled IgE-receptor complexes on the surface of rat basophilic leukemia cells. A comparison of measurements of the lateral diffusion coefficients obtained from post-field relaxation with those from fluorescence photobleaching recovery show that the immobilizing constraints induced by external cross-linking of the IgE-receptor complex can be released by surface electrophoresis. However, in the kinetics of post-field relaxation of both monomeric and cross-linked IgE-receptor complexes, we have found that very long initial exposures to the externally applied electric field induces interactions resulting in an effectively time dependent diffusion coefficient during the surface redistribution with apparent immobilization of the complex. Using the high spatial resolution of video microscopy we have obtained detailed measurements of the equilibrium surface distribution of the IgE-receptor complex during electrophoresis and relaxation. A comparison of these data with the expected equilibrium distributions in the presence of the applied electric field indicates that only regions of low surface concentration can be described accurately by application of the ideal Boltzmann distribution.

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M-PM-C4 ACID-INDUCED RESPONSES FROM NECTURUS OLFACTORY RECEPTOR NEURONS. V.E. Dionne. Division of Pharmacology, University of California, San Diego, CA 92093

Olfactory receptor neurons were enzymatically dissociated from the sensory epithelium of adult Necturus maculosus and studied using whole-cell patch-clamp methods. As reported earlier, the whole-cell currents from these neurons consisted of at least five components: inward Na^+ and Ca^{++} currents, outward Ca^{++}-dependent and voltage-dependent K^+ currents, and an outward (possibly nonspecific) cation current [Soc. Neuroscience Abstr. 12(1986)1179]. The outward currents were especially sensitive to run-down during whole-cell recording, in spite of the inclusion of ATP and GTP in the electrode. Typical outward currents stabilized within 30s after beginning whole-cell recording at about 50% of their initial magnitude. Amino acids are potent olfactory stimuli in salamanders [Artz, et al., J. Comp. Physiol. A 158(1986)479-487] and were tested for activity here. l-Amino acids (Arg, Cys, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Tyr, Val) dissolved in saline at 1-250 uM were locally perfused onto neurons. Since amino acids are zwitterions, they were applied at neutral and acid pH to control the predominant charged form. Exposure to amino acids at pH 7 failed to induce any response; there was no change in whole-cell current nor was there any change in resting potential during current-clamp. In contrast, at pH 3.5 amino acids always induced a substantial change in the whole-cell current; however, this was not specific to the compounds and could be duplicated by salines made acid with HCl. The acid-induced response consisted of a substantial or complete reduction in I_{Na} and I_{Ca} as well as a small reduction in I_K. The substantial run-down of outward currents that was noted suggests that the conductances as well as the lack of chemical responsiveness may not fully reflect the membrane properties of olfactory neurons in vivo. Supported by NIH grant NS20962.

M-PM-C5 SOUR TASTE STIMULI DECREASE A VOLTAGE-DEPENDENT POTASSIUM CURRENT IN MUDPUPPY TASTE RECEPTOR CELLS. Sue C. Kinnamon and Stephen D. Roper, Department of Anatomy, Colorado State University, Fort Collins, CO 80523, and Rocky Mountain Taste and Smell Center, Denver, CO 80263.

In previous studies we have shown that weak acids (citric or HCl) elicit graded depolarizations when applied focally to the apical membrane of taste cells, and that these receptor potentials are blocked by bath perfusion with tetraethylammonium bromide (TEA). To determine if voltage-dependent K^+ currents are involved in the transduction of sour taste stimuli, we have applied the whole-cell patch-clamp to isolated mudpuppy taste cells so that individual ionic currents could be studied under voltage-clamp. Depolarizing voltage steps to +40 mV from a holding potential of -100 mV elicited transient inward, followed by sustained outward currents in taste cells. The transient inward current was completely blocked by 100 nM tetrodotoxin (TTX), indicating that it is a Na^+ current. Bath-applied TEA (8 mM) completely blocked the outward current and revealed the presence of a sustained inward current that had the pharmacological and kinetic properties of a slow Ca^{++} current. Outward K^+ currents were isolated by holding the cell at -50 mV to inactivate inward currents. Under these conditions the outward current activated at -40 to -10 mV and did not inactivate over the duration of the voltage pulse (10 sec). 1 mM citric acid (focally-applied to the membrane from a puffer pipette) reversibly decreased the peak outward current approximately 60%; 10 mM citric acid nearly abolished the outward current. These data suggest that sour taste stimuli depolarize taste cells by modulating this voltage-sensitive K^+ conductance. Experiments are underway to determine if weak acids modulate other ionic currents in taste cells.

M-PM-C6 MECHANISMS MODULATING THE SENSITIVITY AND INTENSITY OF Ca^{2+}- ACTIVATED [^{3}H]RYANODINE BINDING TO SITES IN Ca^{2+} RELEASE VESICLES FROM SKELETAL MUSCLE SARCOPLASMIC RETICULUM. Isaac N. Pessah, Roxanne A. Stambuk, Alexander O. Francini and John E. Casida (Intr. Kenneth W. Anderson), Pesticide Chemistry and Toxicology Laboratory, Department of Entomological Sciences, University of California, Berkeley, CA 94720

Ca^{2+} is essential for activating the [^{3}H]ryanodine binding domain to a state which will recognize the alkaloid and permit its binding to highly specific sites localized at the terminal cisternae of the sarcoplasmic reticulum of skeletal muscle. The apparent affinity of the activator site for Ca^{2+} ($K_{d/Ca}$) is ~30 μM and contains critical sulfhydryl moieties which upon derivatization with Ag^+ or aryldisulfides rapidly dissociates the Ca^{2+}-ryanodine receptor equilibrium complex. The alkaloid binding domain does not remain accessible but instead undergoes rapid occlusion upon complex formation suggesting close proximity to the channel. Mg^{2+} (1mM) decreases the affinity (K_d) and the number of binding sites (B_m) for the alkaloid, and retards the association kinetics (k_{+1}) without altering $K_{d/Ca}$. Caffeine (20mM) lowers the threshold for Ca^{2+} activation below 1 μM and increases $K_{d/Ca}$ ~10-fold which in the presence of Mg^{2+} restores K_d and B_m to the level observed in the absence of Mg^{2+} while only partially restoring k_{+1}. In contrast, AMP-PCP (1mM) causes a dramatic increase in K_d and B_m to a level above that observed in the absence of Mg^{2+} and completely restores k_{+1} without affecting $K_{d/Ca}$ suggesting distinct modulatory domains for caffeine and adenine nucleotides. We propose a model by which Ca^{2+}, Mg^{2+}, caffeine, and adenine nucleotides modulate the [^{3}H]ryanodine binding site and recognize four functional domains of the Ca^{2+}-ryanodine receptor complex. Supported by National Institutes of Health Grant ES00049.

M-PM-C7 **A MODEL FOR THE EGF RECEPTOR/KINASE CONTAINING MULTIPLE TRANSMEMBRANE α-HELICES.** Mark W. Russo, Cheryl A. Guyer, Adrian Goldman*, and James V. Staros, Vanderbilt University, Dept. of Biochemistry, School of Medicine, Nashville, TN 37232, and *Yale University, Dept. of Molecular Biophysics and Biochemistry, New Haven, CT 06511

When epidermal growth factor (EGF) binds to its cell surface receptor, a Tyr specific protein kinase is stimulated, and this kinase catalyzes the phosphorylation of substrates in the cytoplasm. Affinity labeling studies in our laboratory have demonstrated that the EGF receptor and the EGF-stimulable protein kinase are two domains of the same polypeptide chain. We have suggested that the EGF receptor/kinase can be viewed as a transmembrane allosteric enzyme, in which the binding of EGF induces a conformational change that is transmitted to the kinase domain, resulting in the stimulation of kinase activity. Other workers have cloned and sequenced cDNA's to the mRNA for the EGF receptor/kinase, resulting in a derived primary sequence. These workers have noted a strikingly hydrophobic sequence, Ile622-Met644, and they have suggested that the receptor/kinase might be oriented in the membrane with the amino terminal 621 residues forming the receptor domain on the cell surface, the 23 residue hydrophobic sequence forming a transmembrane α-helix, and the carboxyl terminal 542 residues forming the kinase domain on the cytoplasmic surface of the membrane. We propose a testable alternative model in which the polypeptide chain of the EGF receptor/kinase traverses the membrane multiple times. Our model and the single transmembrane segment model are equally consistent with known structural features of the receptor. However, the more substantial transmembrane domain of our model is more easily rationalized with the concept of allosteric coupling between receptor and kinase domains. Supported by the NIH: DK25489, GM07347, HD07043, GM22778.

M-PM-C8 PERIPHERAL-TYPE BENZODIAZEPINE RECEPTOR EXISTS IN VASCULAR SMOOTH MUSCLE. John F. French and Mohammed A. Matlib, Department of Pharmacology and Cell Biophysics, University of Cincinnati Medical Center, Cincinnati, OH 45267

Recently, a novel class of benzodiazepine receptor, distinct from the well known central-type receptor, has been identified in peripheral tissues including heart, adrenal gland, kidney, testis, salivary gland and even in selected areas of the brain. The physiological role of this receptor is unknown. The benzodiazepine Ro5-4864 binds with high affinity to the peripheral sites which are insensitive to clonazepam. With the use of ^{3}H-Ro5-4864, we attempted to demonstrate the existence of the peripheral-type benzodiazepine receptor in smooth muscle membranes of the rat aortae isolated by homogenization and centrifugation. Radioligand binding experiments revealed the existence of highly specific, saturable and reversible binding of ^{3}H-Ro5-4864 in the isolated membranes. Scatchard analysis of the binding data demonstrated a large number of high affinity (K_d = 5.32 ± .84 nM) binding sites (Bmax = 27.74 ± 5.43 pmoles/mg protein). Ro5-4864, PK-11195 (putative antagonist of peripheral-type benzodiazepine binding sites) and diazepam inhibited the binding with calculated K_i being 6.45 nM, 9.41 nM and 94.09 nM, respectively. However, clonazepam, a specific ligand for the central-type benzodiazepine receptor, had no effect. The data indicate that the Ro5-4864 binding in vascular smooth muscle is of the peripheral type. In conclusion, we have identified a large number of highly specific binding sites for Ro5-4864 in vascular smooth muscle characteristic of the peripheral-type benzodiazepine receptor. (Supported in part by a grant from the Southwestern Ohio Chapter of the American Heart Association).

M-PM-C9 KINETICS OF MEMBRANE TO SURFACE ADHESION AND DETACHMENT. Micah Dembo, David C. Torney, Karl Saxman, and Dan Hammer. Theoretical Biophysics, Theoretical Division, Los Alamos National Laboratory, Los Alamos, New Mexico 87545.

We consider a one-dimensional plate (or membrane) that is bonded to a surface as an arc length variable "S," approaches +infinity. As S approaches -infinity, the plate bends so as to asmptotically approach a certain "contact angle" with the surface. At the unattached extremity, the plate is subject to an arbitrary tension tending to detach it from the surface. Bonding between the surface and the plate occurs by a first order chemical rate process, there being a certain number of "receptors" per unit length of plate. The rate constants for forma tion and rupture of cell to surface bonds are variables that depend on the gap separating the plate from the surface. For this simple model, a system of coupled nonlinear PDE's describe the bending of the plate and the formation and breakage of bonds in the boundary layer or contact zone that separates the region of complete bonding from the nonbonded or unattached region.

We have developed a numberical method that efficiently solves the initial value problem corresponding the the aformentioned PDE's in a coordinate system that is fixed with respect to the moving contact zone. For arbitrary input parameters (tension, contact angle, bending modulus, receptor density, etc.), we are able to predict the geometry of the plate, the bond distribution over the surface of the plate, and the velocity of the contact zone, as a function of time. We will describe the results of our numerical studies and compare these results with relevant experimental observations.

M-PM-C10 HUMAN NEUTROPHILS EXHIBIT A DOUBLE BIPHASIC CALCIUM RESPONSE TO FMLP. D.L. Mazorow, C.O. Simpkins, C.H. June, D.B. Millar, Naval Medical Research Institute, Bethesda, Maryland 20814-5055.

Human neutrophils, which are pretreated with the calcium binding chromophore Fura-2, exhibit a double biphasic time-dependent increase in cytosolic calcium after treatment with N-formyl-methionyl-leucyl-phenylalanine (FMLP). The first peak occurs very rapidly after addition of FMLP, with the maximum fluorescence occurring within seconds. At 25°C, the second peak which is smaller in magnitude than the first, occurs approximately 7 to 10 minutes after the maximum of the first peak. The initial response to FMLP does not appear to be highly temperature sensitive, at least between 15°C and 42°C. However, the second peak is temperature sensitive. It is not observed at 15°C, but at temperatures above 25°C, the second peak increases in magnitude relative to the first and the time of appearance decreases. At 37°C, the time is half that at 25°C, and at 42°C, there is a further measurable decrease in time. Flow cytometry experiments, using neutrophils, which have been labelled with Indo-1, exhibit the same calcium double biphasic temperature dependence as seen with Fura-2. Furthermore, we were able to determine that the cells belonged to one population; that is, all cells underwent the double biphasic time-dependent response to FMLP. Studies attempting to determine the significance of the second peak in the functional pathways of neutrophils are underway.

M-PM-C11 THE EFFECT OF MEMBRANE FLOW ON THE CAPTURE OF RECEPTORS BY COATED PITS: THEORETICAL RESULTS. B. Goldstein,[1] H. Echavarría-Heras,[2] and C. Wofsy.[3] [1]Theoretical Division, Los Alamos National Laboratory, Los Alamos, NM 87545, [2]Centro de Investigación Cientifica y de Educación Superior de Ensenada (CICESE), Mexico, and [3]Department of Mathematics and Statistics, University of New Mexico, Albuquerque, NM 87131.

Coated pits trap cell surface receptors and internalize them, as well as plasma membrane, by rounding up and forming coated vesicles. Once internalized, many receptors recycle back to the cell surface. When recycled receptors are inserted into the plasma membrane, they move until they are again trapped by coated pits. The mechanisms for moving receptors from their insertion sites to coated pits are unknown. Unaided diffusion as the transport mechanism is consistent with the observed kinetics of receptor recycling. Another candidate for the transport mechanism is convection. For receptors that recycle to random positions on the cell surface, we assess the importance of convective flow in the transport of receptors to coated pits. First we consider local flows set up by the formation of coated pits. As coated pits round up, surrounding membrane must be pulled in to form coated vesicles. This will cause membrane to flow toward the center of coated pits. Such flows will occur on all cells undergoing receptor-mediated endocytosis. We also investigate whether this type of flow can, in addition to transporting receptors to coated pits, play a role in trapping them in coated pits. Next we consider bulk membrane flow as, for example, may occur on spreading or moving cells. For receptors diffusing and experiencing such flows, we calculate their rate of transport to coated pits and their mean time to find a coated pit. (Supported by NIH Grant GM35556.)

M-PM-C12 DIRECT ACTIVATION OF A MAMMALIAN ATRIAL K CHANNEL BY A HUMAN ERYTHROCYTE PERTUSSIS TOXIN (PTX)-SENSITIVE G PROTEIN, G_k*.
A. Yatani*, J. Codina†, A.M. Brown* and L. Birnbaumer*†,
*Physiol. & Molec. Biophys. and †Cell Biology, Baylor College of Medicine

Electrophysiological experiments suggest that acetylcholine (ACh)-activated opening of K channels is mediated by G proteins (subunit structure $\alpha\beta\gamma$) that interact with muscarinic ACh receptors. To test the hypothesis that regulation of ACh activated K channels is due to direct interaction between G protein and K channel protein(s), we examined the effects of the purified PTX-sensitive G protein from human erythrocytes on guinea pig atrial K channels recorded in "inside-out" patches under voltage clamp conditions. Without agonist or G protein, we observed no K^+ channel activity. Results: (1) Bath application of G protein preactivated with GTPγS and Mg^{2+} (G_k*, α subunit: 40 kDa) caused opening of K channels. The activation of the K channel was concentration-dependent and channel opening occurred at concentrations as low as 0.2-1pM. (2) G_k*-induced K channels had the same conductance (45pS) and opentime distributions ($\approx$ 1.5 ms) as ACh-activated K channels. (3) The opening of the K channel was G_k* specific. Unactivated G_k, unactivated human erythrocyte G_s as well as preactivated G_s* had no effect on channel openings. (4) At equal concentrations, preactivated bovine brain G_o (G_o*, α subunit: 39 kDa) was only 1-5% as active as G_k*. (5) Addition of the protein kinase C activator, phorbolester (TPA), or substitution of AMP-P(NH)P for ATP in the bath did not mimic or interfere with the effects of G_k*. The results demonstrate that a pure GTP-binding protein G_k* directly activates muscarinic mammalian atrial K channels. Supported by AHA 851159 and HL-31164.

M-PM-D1 HOW LONG DOES IT TAKE CARDIAC GAP JUNCTION CHANNELS TO OPEN? R. L. DeHaan and R. D. Veenstra*, Anatomy and Cell Biology Dept., Emory University, Atlanta, GA 30322.

Single gap junction channel currents were measured using the whole-cell patch-clamp recording technique on pre-formed pairs of ventricular cells, isolated from the heart of 7-day chick embryos and cultured for 1 day in vitro. Two patch pipettes, filled with identical high-K, Ca-buffered solutions were used to voltage clamp both cells independently to -40 and -80 mV. With a constant transjunctional voltage gradient (V_j) of 40 mV, 6-7 pA open-close events were observed as equal and opposite signals in the two cells, indicating current flow through single 160-170 pS gap junction channels (Science 233: 972-974, 1986). The rate of rise of junctional current (I_j) associated with these channel events was near or limited by the maximal bandwidth of our recording equipment (10 KHz bandwidth, 45 μs sampling rate). In 225 channel openings, the transition from the basal current level to the open state level was completed in 285.2 ± 152.7 μsec (range 40-860 μs) at 22°C. A few openings had slower transitions in current level (mean transition time = 1730.7 ± 600.1 μs, range 1000-2880 μs, N=15). In addition, slow drifts in I_j occasionally occurred over a period of several milliseconds. We conclude that gap junction channels in embryonic heart cells usually open rapidly. (Supported by NIH Grant HL27385 to RLD; *Present address: Pharmacology Dept., SUNY/Health Science Center at Syracuse, Syracuse, NY 13210).

M-PM-D2 SINGLE CHANNEL ACTIVITY IN CALCIUM-BLOCKED GAP JUNCTIONS. R. D. Veenstra* and R. L. DeHaan, Dept. of Anatomy and Cell Biology, Emory University, Atlanta, GA 30322.

Pairs of ventricular cells, isolated from the hearts of 7-day chick embryos and cultured for 1 day in vitro, were voltage clamped with two patch electrodes in whole-cell clamp configuration. Both patch pipettes contained high-K, low-Na solution buffered to a calculated [Ca^{2+}] of either 10^{-9} M (low-Ca) or 10^{-6} M (hi-Ca) with 5 mM EGTA. Macroscopic junctional conductance ($G_j = I_j/V_j$) measured immediately after the patch was ruptured under both electrode tips, was 0.15-26.9 nS with pipettes containing low-Ca, and 3.8-32.2 nS with hi-Ca. When the pipettes contained low-Ca, G_j increased in every case (N=6), with a time to half maximal ($T_{1/2}$) of 5.46 ± 3.50 minutes. With hi-Ca in the pipettes, G_j decreased with $T_{1/2}$ = 5.32 ± 3.43 minutes (N=5). At G_j = 0.1-1.5 nS, frequent single-channel open/close events could be observed (Science 233:972-974, 1986). Single-channel conductance was unaffected by $[Ca^{2+}]_i$ (158.78 ± 43.27 pS, low-Ca; 170.27 ± 46.07 pS, hi-Ca). Moreover, in both Ca conditions channel activity, single-channel conductance, and G_j were insensitive to V_m or V_j. At $G_j \geq 1.5$ nS single-channel events could not be extracted from the large junctional current noise. With a weak Ca-buffer (0.1 mM EGTA) in the pipette solution, G_j was reduced to 4% of control levels 2-11 minutes after superfusion with 1-octanol (120 μM, N=4) and single-channel events were suppressed. Octanol reduced G_j by 56% with low-Ca in the pipettes and by only 15% with hi-Ca. (Supported by NIH grant HL27385; *Present address: Pharmacology Dept., SUNY/Health Science Center at Syracuse, Syracuse, NY 13210).

M-PM-D3 ANTIBODY BLOCK OF VOLTAGE-DEPENDENT LENS JUNCTION CHANNELS INCORPORATED INTO PLANAR LIPID BILAYERS. George R. Ehring*, Guido A. Zampighi**, James E. Hall*. *Department of Physiology and Biophysics, University of California, Irvine CA, 92717. **Department of Anatomy and Jerry Lewis Neuromuscular Research Center, University of California, Los Angeles, CA 90024.

Lens junction protein incorporated into planar lipid bilayers forms voltage-dependent channels (PNAS 82:8468-8472 1985). In 1.0 M KCl, the channels have two main conductance states: maximum conductance (3200 pS) and minimum conductance (1200 pS). With transmembrane voltages of less than 20 mV the channels reside mostly in the maximum conductance state. As the transmembrane voltage is increased the channels close to the minimum conductance state. The channels are partly anion-selective (20 mV/decade) and do not appear to distinguish between chloride and nitrate as the anions or sodium and potassium as the cations.

In the presence of rabbit antibody raised to the major intrinsic lens protein (bovine), the latency to first closing at all voltages is markedly reduced. The mean time spent in the maximum conductance state is greatly reduced. Further, additional modes of channel activity with new conductance states appear: a slow burst mode and a fast burst mode. In the slow burst mode, the channel fluctuates between the normal minimum conductance state and a new lower conductance state (500 pS) with a fluctuation rate of 100 transitions per second. In the fast burst mode, the channels fluctuate between the normal minimum conductance state, a new intermediate conductance state (2200 pS), and bare membrane conductance with a fluctuation rate of about 350 transitions per sec. We thank Dr. Joseph Horwitz for the kind gift of the antibody to lens MIP. This work was supported by NIH grants EY05661 and EY04110.

M-PM-D4 CELL-CELL CHANNELS MADE FROM GAP JUNCTION SPECIFIC mRNA ARE GATED. G.P. Dahl, E. Levine, R. Werner, Depts. of Physiology and Biophysics and of Biochemistry, University of Miami, Florida 33101.

Microinjection into Xenopus oocytes of mRNA transcribed in vitro from liver gap junction cDNA (Paul, J. Cell Biol. 1986) results in the induction of cell-cell channels between paired oocytes. Depending on the maturation state of the oocytes, the induction of channels can be observed against a nondetectable background or as a 20-30 fold increase of junctional conductance over conductance provided by endogenous channels.

Junctional conductance was determined by dual voltage clamp in mRNA-injected pairs of oocytes. The effect of Ca^{2+}, H^+ and voltage on channel conductance was studied. Similar gating characteristics of these channels were observed as described previously for channels induced in oocytes by total mRNA preparations from uterus or heart (Dahl and Werner, Bioph. J. 1986). The channels can be closed by Ca^{2+} and H^+ but are insensitive to both transjunctional voltage and membrane potential. These data are consistent with the notion that the liver gap junction cDNA encodes a protein that is essential as well as sufficient to form gated cell-cell channels in paired oocytes.

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M-PM-D5 OXIDANT STRESS BLOCKS HEPATOCYTE GAP JUNCTIONS INDEPENDENTLY OF CHANGES IN INTRACELLULAR pH, Ca^{2+} OR MP27 PHOSPHORYLATION STATE. J.C. Saez, M.V.L. Bennett and D.C. Spray. A. Einstein College of Medicine, Bronx, NY 10461.

It is accepted that the hepatotoxicity of several halomethanes is due to generation of free radicals. Similarly, accumulation of metabolites, such as arachidonic acid, during ischemia leads to massive generation of free radicals upon reperfusion. We studied effects of different halomethanes and arachidonic acid on junctional conductance (g_j) and dye transfer (Lucifer yellow) between pairs of rat hepatocytes. g_j was assayed by 4 electrode current clamp. Perfusion with CCl_4 (650uM) totally blocked g_j and dye coupling within 1 min. The effect was reversed by several minutes perfusion with saline. Uncoupling occurred with or without extracellular Ca^{2+} and was not accompanied by changes in intracellular Ca^{2+} or H^+ as shown by intracellular ion sensitive electrodes. CCl_4 did not change the phosphorylation state of MP27, the principal gap junction polypeptide (measured as in PNAS 83: 2437, 1986). Minimal concentrations of halomethanes to cause 0% incidence of dye coupling $CBrCl_3$ (210uM), CCl_4 (540 uM), $CHCl_3$ (830uM) and CH_2Cl_2 (not determined, 1940uM) are comparable to hepatotoxicity levels in vivo and in the same order as their tendency to form free radicals. CCl_4 uncoupling was largely prevented by a P_{450} monooxidase inhibitor (SK&F 525A) and by a reducing agent (β-mercaptoethanol). Arachidonic acid (50uM) uncoupling, which is also reversible, was totally prevented by an inhibitor of its metabolism (1-phenyl-3-pyrazoline, 10uM). These data, rapid onset, reversibility and block by specific agents, suggest that oxidation of the gap junction protein or of a cytoplasmic regulatory molecule leads to closure of the channels.

M-PM-D6 CAFFEINE INCREASES JUNCTIONAL CONDUCTANCE BETWEEN CULTURED MYOCARDIAL CELLS WHILE SIMULTANEOUSLY ELIMINATING AN INTRACELLULAR COMPARTMENT FOR CALCIUM.
Janis M. Burt, Department of Physiology, University of Arizona, Tucson, AZ 85724.

The electrical properties of the gap junctions between pairs of neonatal rat myocardial cells (in culture for 1 to 5 days) were studied. The effect of caffeine, an inhibitor of sarcoplasmic reticulum calcium pumping capacity and an inhibitor of phosphodiesterases, on junctional conductance and cellular calcium fluxes was examined. Under dual whole cell voltage clamp using patch electrodes (containing either 130mM K glutamate, 10mM KCl, 1mM HEPES and 5mM ATP or 140mM KCl, 1mM HEPS and 5mM ATP), gap junctional conductance (g_j) is reversibly decreased by pH (exposure to bicarbonate buffered solutions at pH 6.0-6.2), as has been shown by Spray and colleagues for other mammalian cell types including adult cardiac cells. Application of 10mM caffeine causes a significant average increase in g_j of 35% within 4 minutes of application. Within 7 minutes, g_j is maximally stimulated, by as much as 100% in some cases. Exposure to zero sodium-caffeine solution following caffeine treatment results in a reversible decrease in junctional conductance of 40-60% within 4-8 minutes. Compartmental analysis of calcium fluxes for these cells reveals two intracellular compartments with half times of exchange of approximately 6 and 50 minutes. The exchange characteristics of both compartments are sensitive to 10mM caffeine. The more rapidly exchanging compartment is virtually eliminated by caffeine while the rate constants for the slowly exchanging compartment are significantly reduced by caffeine. These results support the hypothesis that the conductance of cardiac gap junctions is modulated by cAMP mediated processes and that significant impairment of the calcium pumping capacity of the sarcoplasmic reticulum under normal physiological conditions does not lead to reduced junctional conductance. The low Na-caffeine experiments would be expected to load the cytoplasm with calcium as well as protons. Which of these ions accounts for the observed decrease in junctional conductance remains to be determined. Supported by NIH HL31008, Arizona Disease Control Research Commission and the American Heart Association, Arizona Chapter.

M-PM-D7 EFFECTS OF ISOTONIC SEROSAL (S) CHANGES ON CELL VOLUME IN NECTURUS GALLBLADDER EPITHELIUM. C. E. Parr and Arthur L. Finn. Dept. of Med., UNC Sch. of Med., Chapel Hill, NC 27514.

We studied the effects of lowering S Na^+ and Cl^- and raising S K^+ on cell volume using quantitative light microscopy. Reduction of S Na^+ to 10 mM by isotonic replacement with N-methyl-D-glucamine (NMDG) resulted in shrinkage of cells to 89.9 ± 1.0% of original volume at a rate of 2.0 ± 0.4% of original volume/min. After a new steady state volume was achieved, K^+ (95 mM) was substituted for NMDG in the S bath. The cells then underwent a small (4.0 ± 0.4%) but significant ($P < 0.02$) volume decrease which was followed, approximately 1.5 min. after raising K^+, by rapid (14.7 ± 2.2%/min.) cell swelling to a volume 110.8 ± 2.1% above control. The cell shrinkage which accompanied lowered S Na^+ persisted in the presence of S bumetanide (10^{-4} M). In normal Ringer bumetanide had no effect on cell volume but did prevent K^+-induced cell swelling. When S Cl^- was replaced by the impermeant anion cyclamate, the cells shrank at a rate of 1.5 ± 0.2%/min. to a minimum volume 82.7 ± 1.8% of original volume; subsequent elevation of S K^+ resulted in an immediate swelling (10.0 ± 0.5%/min.), i.e., with no intervening shrinkage phase, to a peak volume 116.0 ± 1.4% of control volume. Complete replacement of mucosal Na^+ by NMDG, which by itself decreases cell volume, had no effect on the pattern of K^+-induced cell swelling in the presence of normal S Cl^-. Spontaneous volume-regulatory behavior did not occur in any of these experiments. These data indicate that: (1) the entry of K^+ and Cl^- induced by high S K^+ is not mediated by a neutral cotransporter; (2) the inhibitory effect of bumetanide is not restricted to a basolateral Cl^- pathway; (3) changes in S Na^+ activity may influence apical membrane permeability; and (4) a decrease in cell volume per se is not sufficient to activate the apical membrane volume-regulatory mechanism. Supported by NIH grant # DK25483.

M-PM-D8 EGTA INDUCES PROLONGED SUMMED DEPOLARIZATIONS IN MYTILUS GILL COUPLED CILIATED EPITHELIAL CELLS. E. W. Stommel and R. E. Stephens, Marine Biological Laboratory, Woods Hole, MA 02543.

Abfrontal ciliated cells of Mytilus gill beat when mechanically stimulated, a consequence of a Ca^{++}-based generator potential and regenerative response (Stommel, J. Comp. Physiol. 155A: 445, 1984). In contrast, lateral ciliated epithelial cells arrest when stimulated, a consequence of a Ca^{++}-based generator potential and $Na+/Ca^{++}$-based regenerative response (Saimi et al, Comp. Biochem. Physiol. 74A: 499, 1983). Iontophoretic injection of EGTA in abfrontal cells, followed by mechanical stimulation, results in a prolonged depolarization that returns to the resting level step-wise (Stommel, loc cit). It was hypothesized that this phenomenon was caused by successive Ca^{++}-dependent repolarizations, first in adjacent cells and then in the injected cell, in accord with relative EGTA loading. We have now demonstrated this same step-wise repolarization phenomenon in the $Na+$-dependent lateral ciliated cells. In this case, each repolarization step is preceded by a small spike. With either cell type, using two-electrode recording techniques, we can detect the step-wise repolarization in distant cells, proportionately decreased when the second (KCl) electrode is some distance from the injection (EGTA) electrode and stimulus. When force is applied between the electrodes and nearest the KCl electrode, a greater initial response is recorded from this electrode, resulting from depolarization of its impaled cell, prolonged by EGTA diffusion through the intervening cell junctions. The subsequent repolarization steps are of approximately the same size, suggesting repolarization of cells between the two electrodes. These observations are consistent with the coupling/loading hypothesis and indicate that both cell types mediate repolarization through Ca^{++} and propagate ciliary beat or arrest through coupling. Support: NIH GM 29,503.

M-PM-D9 EXOCYTOTIC MEMBRANES INCORPORATED INTO APICAL EPITHELIAL MEMBRANES ORIGINATE IN DIFFERENT CYTOPLASMIC POOLS. D. Erlij, W. Van Driessche and I. Aelvoet. Dept. of Physiol. SUNY Downstate Med. Center, Brooklyn, N.Y. 11203 and Labo voor Fysiol. K. U. L. Leuven, Belgium.

We measured the effects of phorbol myristate acetate (PMA) and oxytocin on osmotic water flow and impedance in toad urinary bladders. Addition of PMA (10^{-6} M) to the mucosal side of tissues incubated with isotonic solutions increased apical membrane capacitance by about 25%. Subsequent addition of oxytocin (0.1 U/ml) increased capacitance by an additional 25%. When the apical solution was diluted, mucosal PMA produced an increase in capacitance similar to that observed in tissues incubated with isotonic solutions and water flow was stimulated to about 20% of the maximum response to oxytocin. Addition of oxytocin to these bladders further stimulated water flow to its characteristic high value i.e., about 1 ul/min cm^2. On the other hand, in these bladders, oxytocin caused a small and transient additional increase of capacitance. Such a reduced increase in capacitance has been previously attributed to stimulation of endocytosis. In agreement with this suggestion, we find that after reaching the maximum increase in capacitance caused by the combined action of oxytocin and PMA in isotonic solutions, dilution of the apical medium caused a prompt reduction in capacitance. In conclusion, the additive effects of oxytocin and PMA on membrane capacitance indicate that additional amounts of membrane are delivered to the apical membrane under the influence of each agent. Moreover, the measurements of water flow indicate that each agent induces the incorporation of membranes with different water permeability characteristics. Finally, it appears that when oxytocin is given in the presence of an osmotic gradient mebrane retrieval is stimulated with little effects on water permeability.
Supported by the N.I.H.

M-PM-D10 PROPERTIES OF AN ANION-SELECTIVE CHANNEL FROM RAT COLONIC ENTEROCYTE PLASMA MEMBRANES INCORPORATED INTO PLANAR LIPID BILAYERS

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The model for electrogenic Cl^- secretion across epithelia predicts the existence of a regulated apical membrane channel that can conduct Cl^-. Here we report some properties of an anion-selective channel that may be the apical membrane Cl^- channel.

Vesicles derived from epithelial cells of the colonic mucosa of the rat were fused to planar phospholipid bilayer membranes revealing spontaneously switching anion-conducting channels of 50 pS conductance at -30 mV with 200 mM Cl^- each side. The equilibrium selectivity series was I^- (1.7) / Br^- (1.3) / Cl^- (1.0) / F^- / HCO_3^- (0.4) / Na^+ (<0.1). Only one dominant open-state conductance could be resolved, which responded linearly to Cl^- concentrations up to 600 mM. The single-channel current-voltage curve was weakly rectifying with symmetrical solutions. When 50 mV were exceeded at the high-conductance branch of the curve, switching was arrested in the closed-state. At more moderate voltages (±40 mV) kinetics were dominated by one open-state of about 35 ms lifetime and two closed-states of about 2 and 9 ms lifetime. Of these, the more stable closed-state occurred less often. At these voltages one additional closed-state of significantly longer lifetime (>0.5 s) was observed.

Supported by SFB 246 Projects C1 and C2.

M-PM-D11 PHOTOAFFINITY LABELING OF THE BOVINE LENS CALMODULIN-BINDING COMPONENTS.

C. Louis, P. Hogan, G. Strasburg and K. Hur, Dept. Vet. Biol., Univ. Minn, St. Paul, MN 55108.

Calcium plus calmodulin (CaM) have been proposed to regulate intercellular communication, adenylate cyclase activity and Ca pumping in the mammalian lens. To define the membrane proteins mediating these CaM effects, we have examined the lens membrane CaM receptors using the photoaffinity probe benzophenone-^{125}I-CaM (Bz-^{125}I-CaM). In a urea-washed (UW) lens membrane preparation stripped of extrinsic membrane proteins), the major affinity-labeled complexes had Mr = 36, 46, & 48 kDa; labeling was maximal in 1-2 µM Ca^{2+} plus 10 mM Mg^{2+}, was inhibited by µM R24571 and represented 1:1 complexes between CaM and membrane components. Treatment of Bz-^{125}I-CaM labeled UW lens membranes with reducing agents resulted in the loss of the 48 kDa affinity-labeled complex indicating that it possibly represents the interaction of CaM (17 kDa) with the approx. 28 kDa lens membrane substrate for cAMP-dependent protein kinase [Louis et al. Eur. J. Biochem. (1985), 150, 279]. Affinity labeling of buffer-washed (BW) membranes (which contained extrinsic membrane-bound proteins) revealed a number of Bz-^{125}I-CaM labeled complexes not observed in the UW membranes. While some of these BW membrane complexes were present in both the cortex and nucleus regions of the lens (Mr = 78, 68 & 40 kDa), others were localized exclusively to the nucleus (Mr = 48 & 43 kDa). No Bz-^{125}I-CaM labeled lens membrane complexes were identified with Mr corresponding to CaM-dependent Ca pump or adenylate cyclase subunits previously identified in other tissues. Photoaffinity labeling of lens membranes with a CaM derivative that can react with a variety of amino acid side chains, confirms that CaM does not interact with MP26, the 26 kDa major lens membrane protein that is localized to lens gap junctions. Supported by NIH EY-5684.

M-PM-D12 DIVALENT ION EFFECTS ON LENS NON-SELECTIVE CATION CHANNELS AND LENS IMPEDANCE.

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We have begun to correlate the single channel properties of the "stretch-activated" cation selective channel of frog lens membranes with macroscopic lens properties such as morphology and electrical impedance. The channel is an inward rectifier to current carried by small monovalent cations when Ca^{++} is low on both sides of the channel. We have studied IV relations from single channels in on-cell patches when several divalents are substituted for Ca^{++} in the pipette. Zn^{++}, Ni^{++}, Mn^{++}, Co^{++}, and Cd^{++} in millimolar concentrations linearize the IV at hyperpolarizing potentials to the smaller conductance seen for outward currents. Ba^{++}, Sr^{++}, and Mg^{++} are less effective in this regard. This linearizing ability of the divalents correlates well with their ability to prevent epithelial cell swelling. In addition, the whole lens impedance decrease which follows the removal of Ca^{++} from the bathing medium is largely blocked by adding 2.5 mM Mn^{++} to the lens bath. The effect of other divalents on lens impedance is currently under study.

These results suggest the possibility of a role for these "non-selective" cation channels in the response of cell membranes to low Ca^{++}. Work supported by EY03282, EY06005, and EY06391.

M-PM-D13 STRUCTURE OF CONCENTRATION BOUNDARY LAYERS. R. David Baker. Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550

The resistance to mass transfer imposed by aqueous boundary layers is often important, yet little experimental information is available concerning boundary layer structure. I have used O_2 polarographic microelectrodes to determine the concentration profile for O_2 in the boundary layer overlying an O_2-permeable membrane (dimethyl silicone). The membrane was mounted horizontally on a thin circular chamber (1.2 cm ID) filled with O_2 gas. The chamber was immersed in N_2-equilibrated saline in a circular dish; rotation of the dish produced adjustable free stream velocities over the membrane. The incidence angle was set to zero. Electrodes had recessed tips to eliminate flow sensitivity. Most measurements were made over the trailing edge of the membrane. Steady state O_2 profiles through the boundary layer did not resemble those predicted by classic Blasius-Pohlhausen theory; they were S-shaped and far steeper than predicted. With free stream velocity 9.1 cm/sec, 85% of the total drop in O_2 concentration was reached at about 50 μm from the surface, while theory predicts an 85% distance (i.e. local "unstirred layer" thickness) of 135 μm. Similar discrepancies were noted over a wide range of free stream velocities. The deviations from Blasius-Pohlhausen theory probably result mainly from forward stagnation point effects leading to 1) a "stagnant subphase" extending as far as 20 μm from the membrane surface, and 2) flow velocities in the concentration boundary layer beyond the stagnant subphase which greatly exceed (roughly 20 fold) those predicted by Blasius theory. Velocity profiles measured with flow-sensitive O_2 electrodes (no recess) have confirmed #2. Estimation of boundary layer mass transfer coefficients from boundary layer theory may be hazardous in experimental situations applicable to biological membranes.

M-PM-E1 **THE STABILIZATION OF LIPOSOME-ENCAPSULATED HEMOGLOBIN BY LYOPHILIZATION. Alan S. Rudolph, Martha C. Farmer, Sue Johnson, and Sandra Bayne, Biomolecular Engineering Branch, Code 6190, Naval Research Laboratory, Washington, DC 20375-5000.**

One of the goals of hemoglobin-based blood surrogate research is the long term stabilization of the product. At NRL we have investigated stabilization of liposome-encapsulated hemoglobin (LEH) with antioxidants and lyophilization. Multilamellar vesicles are prepared from lipids (hydrogenated soy phosphatidylcholine, cholesterol, dimyristoyl phosphatidylglycerol, α-tocopherol, 5:4:1:0.2) hydrated in a 15 mM hemoglobin solution. The dispersion is pressure-extruded (Microfluidics Corp.), and the resultant product consists of 0.2μ unilamellar vesicles with an average of 8mM hemoglobin encapsulated, as has been described previously. Experiments to test the efficacy of a series of antioxidants co-encapsulated with the hemoglobin have shown that 10mM glutathione significantly inhibits oxidation to methemoglobin at both 4°C and 37°C, and that the effect is enhanced markedly by addition of glucose. Stabilization of liposomal products in the dry state has previously been accomplished using sugars as a water replacement molecule. The sugars are thought to hydrogen bond to the bilayer surface, maintaining the bilayer in its native liquid-crystalline state even when dehydrated. Our initial findings indicate that we can stabilize the components of the LEH in the dry state. We have measured the lyophilization-induced methemoglobin formation as a function of concentration of trehalose, sucrose, and glucose, and find that at 0.5M these sugars markedly inhibit the oxidation of hemoglobin. Photon correlation spectroscopy results indicate that lyophilizing LEH yields liposomes which, upon rehydration, are large (1-2μ) and multilamellar, but identically treated LEH containing the disacharide trehalose rehydrate as 0.2μ unilamellar liposomes.

M-PM-E2 NOVEL FLUORESCENT LIPIDS FOR ASSAYS OF MEMBRANE FUSION AND LIPID LATERAL DISTRIBUTION. Rania Leventis, Michael Gardam and John R. Silvius. Department of Biochemistry, McGill University, Montréal, Qué., Canada.

A series of novel fluorescent phospholipids has been prepared, in which one acyl chain is labelled either with a bimane or substituted coumarin moiety (λ_{em}= 460-480 nm), or with an N-methyl-N-(nitrobenzoxadiazolyl)amino (NBDMA-) or dimethylaminophenylazophenyl (DAB-) group, with absorption maxima at 440-480 nm. The bimane- and particularly the coumarin-labeled species exhibit strong blue fluorescence that is largely insensitive to perturbations in the physical state of the membrane environment but can be quenched efficiently by low bilayer concentrations (< 1 mole %) of NBDMA- and DAB- labeled lipids. Appropriate donor-acceptor pairs chosen from among these probes can be used in sensitive and reliable assays for lipid mixing between membranes (e.g., during membrane fusion), even in cases where the commonly used NBD-PE/rhodamine-PE energy-transfer pair is difficult to employ because of direct effects of fusogenic agents on the fluorescence of the headgroup-labeled phospholipids. Our acyl chain-labeled fluorescent phospholipids can also be used in a variety of designs to probe the lateral distribution of different types of phospholipids in bilayer membranes of heterogeneous lipid composition, measuring either self-quenching of fluorescence or the quenching of the fluorescence of one type of probe by another. The favorable spectroscopic properties of these probes, and the relative simplicity and flexibility of their preparation, make them highly suitable to monitor processes of membrane coalescence and to study lipid lateral interactions in bilayer membranes. (Supported by the Medical Research Council of Canada).

M-PM-E3 ELECTROFUSION KINETICS OF ERYTHROCYTE MEMBRANES AS MEASURED BY FLUORESCENCE INTENSITY. D.A. Stenger and S.W. Hui, Department of Biophysics, Roswell Park Memorial Institute, Buffalo, New York 14263.

We are currently using the terbium (Tb)/dipicolinic acid (DPA) assay to study the kinetics of electrofusion between human erythrocyte ghost membranes. Ghosts are prepared from neuraminidase-treated erythrocytes and their aqueous compartments are loaded with Tb or DPA as previously described (Hoekstra et al. (1985) Eur. J. Biochem. 146:131). To reduce the inner filter effect of the optically dense sample, fluorescence is measured from the front surface of a 0.5 mm pathlength demountable spectrophotometer cell. The cell has horizontally-mounted electrodes and the front face is positioned at 45 degrees to the excitation beam. The peak fluorescence intensity is obtained within 10 sec following application of a square, 15 usec, 2.5 kV/cm electric pulse to a suspension of dielectrophoretically-aligned ghosts. The additional fluorescence caused by leakage is detected in separate experiments and occurs simultaneously with that attributed to fusion. The subsequent fluorescence decreases by only about 5% over the course of 3 min, suggesting that post-fusion leakage is relatively minor in this case. These results are in agreement with the proposed ultrastructural model of electrofusion (Stenger and Hui (1986) J. Membr. Biol. in press).

M-PM-E4 THEORY AND EXPERIMENTS OF MEMBRANE FUSION. S. Ohki, Department of Biophysical Sciences, State University of New York at Buffalo, Buffalo, New York 14214.

A theory of membrane fusion is given in terms of molecular interaction energy for a simplified model for two interacting membranes; two large flat hydrocarbon plates immersed in aqueous medium, which are separated at a certain distance and possess a thin hydrophilic layer on their surfaces.

It is found that the dehydration energy of the membrane surface, whose magnitude depends on the nature of surface hydrophilic layers, plays a major role in membrane fusion, while the van der Waals interaction energy between the two plates at a given separation distance contributes only a secondary factor to the membrane fusion forces, which depend on the nature of the surface hydrophilic layer and its thickness. When the surface hydrophilic layer becomes sufficiently similar to the bulk hydrocarbon phase in nature, which is related to the increase in interfacial tension of the membrane surface, the dehydration energy becomes small and the attractive interaction energy becomes large, and consequently the two plates in close contact could fuse and become one. The theory is compared with the experimental results obtained with lipid membranes. (supported by a grant from the U.S. National Institutes of Health (GM24840)).

M-PM-E5 NMR STUDIES OF AMPHIPATHIC MOLECULES ACTIVE IN VESICLE FUSION. M.P. O'Brien and J.H. Prestegard, Department of Chemistry, Yale University, New Haven, CT 06511

It is known that exogenous fatty acids and related amphipathic compounds induce vesicle fusion in both model and natural systems. In homogenous phosphatidylcholine vesicles, an increase in fusion rate is particularly apparent around the phase transition temperature of the lipid. To better understand the fusogenic nature of fatty acids, studies of the physical properties of myristic acid and host DMPC bilayers were undertaken using ^{13}C and ^{2}H NMR methods.

For ^{2}H-NMR studies, bilayers oriented on glass plates were employed. The quadrupole doublets which result in oriented media are sufficiently well-resolved to allow monitoring of individual labelled species and species in different phases. Attempts have been made to analyze lineshapes in terms of distribution of fatty acid between phases and the dynamics of exchange between phases. The temperature dependence of fatty acid behavior and lipid behavior has been pursued using double-label experiments in which both fatty acid and lipid are deuterium-labelled. Conditions most directly correlated with enhanced fusion have been identified.

Studies of ^{13}C-labelled myristic acid incorporated into unoriented DMPC multilayers were also performed. Chemical shift anisotropy powder patterns and the time course of polarization transfer from protons have been used to characterize the geometry and motional properties of fatty acids in bilayer lipid phases.

Using the above methodologies, investigations will be carried out on aliphatic aldehydes in oriented vesicles. Aldehydes have been implicated in myelin basic protein induced fusion, and there is substantial interest in comparing the behavior of fatty acids in pure lipid bilayers with that of aldehydes in protein-containing bilayers.

M-PM-E6 VIDEO FLUORESCENCE MICROSCOPY OF VESICLE FUSION WITH PLANAR MEMBRANES. W.D. Niles and F.S. Cohen, Department of Physiology, Rush Medical College, Chicago, IL 60612.

Phospholipid vesicles fuse with a planar phospholipid bilayer membrane when they are osmotically swollen. We have employed a combination of video technology and fluorescence microscopy to study the steps in the fusion process: approach, binding, and fusion. Large unilamellar vesicles (2 to 10 μm in diameter) were made of asolectin and loaded with the membrane-impermeant fluorescent dye calcein at self-quenching concentrations. Vesicles were ejected from a pipette brought near the planar membrane, also made of asolectin, in order to surmount the unstirred layer. The number of vesicles bound to the planar membrane exhibited a steep dependence on calcium concentration between 5 and 20 mM. Vesicles were osmotically swollen by making the cis compartment hyperosmotic with urea. Alternatively, the channel forming antibiotic nystatin was added, which allowed KCl to enter the vesicles with concomitant water flow and vesicle swelling. Osmotically induced vesicle rupture appeared as a bright flash of light which lasted several video fields (each 16.7 ms) in duration. Cobalt, a quencher of calcein fluorescence, was used to determine the side of the planar membrane to which dye was released. When dye was released into 40 mM cobalt citrate, the duration of the flash was shortened. When cobalt was present only on one side of the planar membrane, the duration of the flash was used to determine whether the flash was quenched or unquenched. By this method, 50% of the vesicle ruptures were found to result in fusion with the planar membrane. It is concluded that osmotically swollen vesicles tend to rupture in the region of contact with the planar membrane. Supported by NIH grant GM 31039.

M-PM-E7 THE RELATIONSHIP BETWEEN H_{II}, "ISOTROPIC", OR INVERTED CUBIC PHASE FORMATION AND MEMBRANE FUSION, D.P. Siegel[a], H. Ellens[b], and J. Bentz[c]. [a]Procter & Gamble Co., P.O. Box 39175, Cincinnati, OH 45247; [b]Dept. of Pharmacology & [c]Pharmacy & Pharmaceutical Chem., Univ. Calif., San Francisco. CA 94143.

Recent work [1-6] suggests a general relationship between proximity to bulk L_α/H_{II} phase transitions and occurrence of inter-liposomal interactions (aggregation-induced lipid exchange and contents leakage, membrane fusion). The theoretical basis for this relationship and a summary of some recent results will be presented. The interactions are proposed to occur via structures forming as intermediates in L_α/H_{II} phase transitions. Observations to date are consistent with this. The relative rates of leakage and fusion are determined by a structural parameter of the equilibrium L_α and H_{II} phases, Z, related to the curvature parameter of Kirk, et al. [7]. It appears that qualitative liposome-liposome interaction behavior can be predicted as a function of temperature near T_H (the equilibrium L_α/H_{II} phase transition temperature), liposome radius, and Z. Liposomes composed of lipids that exhibit isotropic ^{31}P-NMR resonances and that form isotropic inverted cubic phases [8] undergo fusion to a much greater extent that those with facile L_α/H_{II} transitions. Liposomal interactions via L_α/H_{II} transition intermediates can occur even at temperatures below T_H, and lateral phase separations in liposomes can produce patches of H_{II}-capable lipids. Thus, even some systems apparently stable in the L_α phase may be susceptible to such interactions. The possible relevance of these findings to biomembrane fusion and its metabolic control and to the action of certain fusogens will be discussed. --(1) Siegel, Biophys. J. 49:1155 (1986); (2) ibid., 1171; (3) Siegel, in: Cell Fusion (A.E. Sowers, ed.; to appear); (4) Ellens et al., Biochem. 25:4141 (1986); (5) ibid., 285; (6) Bentz et al., PNAS 82:5742 (1985); (7) Kirk et al., Biochem. 23:1093 (1984); (8) Siegel, Chem. Phys. Lipids (in press).

M-PM-E8 CALCIUM DEPENDENT INCORPORATION OF SYNEXIN TO ACIDIC PHOSPHOLIPID BILAYERS. Harvey B. Pollard and Eduardo Rojas, Laboratory of Cell Biology and Genetics, NIH, Bethesda, MD.

The mechanism by which synexin mediates Ca^{2+}-dependent aggregation and fusion of chromaffin granules and chromaffin granule ghosts may involve specific interactions with the lipid component of the membrane. To study the details of this interaction, we prepared bilayers of phospholipid at the tip of a patch clamp pipette (10 Mohm) and followed the incorporation of synexin into the bilayers by concomitant changes in the capacitance (C_m) of the membrane. Bilayers were exposed to symmetrical K^+ solutions (mM: 140 KCl, 10 NaHepes at pH 6.8). Stable phosphatidyl serine membranes were formed (10^3 Gohm) and the capacitance, as calculated from fast current transients due to applied rectangular clamp pulses (10 mV), was measured. The changes in capacity caused by the addition of either synexin in the absence of Ca^{2+} or, of Ca^{2+} in the absence of synexin were too small to measure. Addition of synexin to the external medium in the presence of Ca^{2+} (0.1-2.5 mM) induced an increase in capacitance from 20 to 2000fF in less than 20 sec. Control experiments with the pipette attached to a sylgard ball demonstrated that the addition of synexin in the presence of Ca^{2+} causes no measurable changes in capacitance, thus ruling out the possibility of any contributions from the pipette capacitance. The size of the charge displacement can be accounted for by assuming an effective valence of 3 per synexin molecule within the bilayer. Thus, the molar fraction "phosphatidyl serine/synexin" is close to 100. Since synexin is an extremely hydrophobic protein which both polymerizes and inserts into the membranes in the presence of Ca^{2+}, it is possible that for exocytosis, membrane fusion may involve formation of a hydrophobic bridge between fusing membranes.

M-PM-E9 **PORIN CHANNELS GREATLY ENHANCE OSMOTICALLY INDUCED FUSION OF VESICLES WITH PLANAR BILAYERS.** Dixon J. Woodbury and James E. Hall; Dept. of Physiology and Biphysics, University of California, Irvine, CA 92717.

Unilamellar vesicles made with or without the membrane channel porin were filled with the fluorescent dye calcein. Fusion was induced by changing the osmolarity of the solution on the cis (vesicle) side of the planar bilayer. Osmolarity was controlled by perfusing in and washing out different solutions of urea in 400 mM KCl. Fusion of adhering vesicles was detected by the fluorescent flash resulting from the release of trapped dye. Although flashes were detected from both porin-containing and porin-free vesicles, the conditions necessary to induce flashes were much different. Porin-containing vesicles readily flash during perfusion with 400 mM urea, whereas porin-free vesicles rarely flash during perfusion of up to 3 M urea. However, porin-free vesicles readily flash during washout of 3 M urea. A simple model predicts the effects of a steady-state osmotic gradient on fusion. The model assumes that fusion occurs when a critical surface tension in the vesicle membrane is reached (due to an increase in hydrostatic pressure inside the vesicle). This surface-tension-induced-fusion (STIF) model predicts the following conditions for fusion: The vesicle must adhere to the planar membrane; the vesicle membrane must contain a pore; and an osmotic gradient must be applied across the planar membrane with the cis side hyperosmotic. The experimental results support these predictions and suggest that biological fusion may also require channels in the vesicle membrane. This work was supported by NIH grant #HL 30657.

M-PM-E10 EVIDENCE FOR CATION AND ANION PERMEABLE CHANNELS IN SECRETORY VESICLE MEMBRANES. E.F. Stanley*, G. Ehrenstein*, and J.T. Russell+ (Intr. by Robert Taylor). *Biophysics Lab, NINCDS, and +Neurochemistry & Neuroimmunology Lab, NICHD, NIH, Bethesda, MD 20892.

We have recently presented a model of exocytosis based on anion and K(Ca) channels in the secretory vesicle membrane (Life Sci. 37: 1985-1995). In support of this model, we previously reported the incorporation of K(Ca) channels into lipid bilayers from a subcellular fraction enriched for secretory vesicles. Here we present evidence that these channels, and in addition, a newly identified anion channel, are present in the secretory vesicles of the subcellular fraction.

Three subcellular fractions were purified from bovine neurohypophysis enriched for: secretory vesicles (VES), surface membrane (NSS), and mitochondria (MIT). However, each fraction contained some of the other components as contaminants. Lipid bilayers were formed in the presence of each membrane fraction, and cation or anion permeable channels were identified on the basis of the composition and concentration gradients of ions in the bathing solutions. Incorporation of the VES fraction into a painted lipid bilayer confirmed the existence of a potassium-permeable channel of high conductance (>200pS) and in addition, demonstrated an anion channel (conductance 10-20pS) that was blocked with DIDS (K_d=80 uM). Although both of these channel types were also observed in bilayers formed with the NSS or MIT fractions, on the basis of the percentage of bilayers that incorporated these channels, we conclude that the vesicles contain both the large cation channel and the anion channel, consistent with the ion channel model of exocytosis.

M-PM-E11 A MODEL TO EXPLAIN THE INVERSE DEPENDENCE OF PARATHYROID HORMONE SECRETION ON CALCIUM CONCENTRATION. Gerald Ehrenstein, Elis Stanley, and Min Jia, Lab. of Biophysics, NINCDS, NIH, Bethesda, MD 20892.

Parathyroid hormone release is unique in that it decreases with increasing concentrations of calcium. We propose a model to explain this phenomenon based on the following assumptions:

1) The primary role of calcium in causing exocytosis is to open Ca-activated K channels present in membranes of secretory vesicles, leading to an increase in vesicle osmolarity and to fusion (Cf. Stanley and Ehrenstein, Life Sciences 37: 1985-1995, 1985).

2) The open probability for Ca-activated K channels in vesicles containing parathyroid hormone is a biphasic function of calcium concentration, increasing as calcium is increased at very low concentrations and then decreasing as calcium is further increased (Cf. abstract in this volume by Jia et al. for evidence that this is true for Ca-activated K channels in the plasma membrane of parathyroid cells.)

3) The normal calcium concentration in parathyroid cells corresponds to the right-hand limb of the biphasic curve described above.

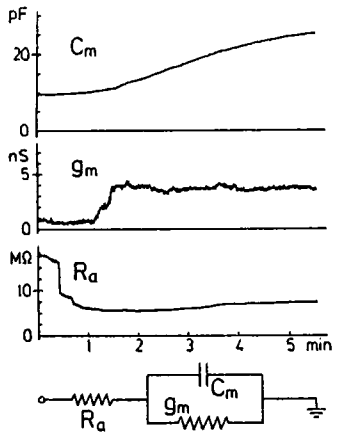
On the basis of these assumptions, an increase in the internal calcium concentration would tend to reduce the probability that the Ca-activated K channels in the vesicles are open, and hence to reduce the liklihood that vesicles fuse with the plasma membrane. This reduction in vesicle fusion results in a reduction in hormone release.

M-PM-E12 pH-DEPENDENT FUSION OF VESICULAR STOMATITIS VIRUS WITH VERO CELLS: MEASUREMENT OF KINETICS AND EXTENT BY FLUORESCENCE DEQUENCHING. Robert Blumenthal, Anu Bali-Puri, Anne Walter, David Covell and Ofer Eidelman Section on Membrane Structure and Function, LMMB, DCBD, NCI

We are developing new ways of studying fusion of intact or reconstituted enveloped viruses with cells and liposomes to gain a better understanding of membrane fusion. The kinetics and extent of fusion between membranes of Vesicular Stomatitis Virus (VSV) and Vero cells were investigated with an assay for lipid mixing based on the relief of fluorescence self-quenching of octadecylrhodamine. VSV was bound to cells at neutral pH and 4°C, and the free virus removed. Addition of the Vero-VSV complexes to a medium with a pH lower than 6.5 at 37°C resulted in rapid fusion, reaching a plateau at 30-50% after 5 min. Fusion was blocked by adding neutralizing antibody to the Vero-VSV complexes. The pH profile of Vero-VSV, fusion as measured by the dequenching method, paralleled that observed for VSV-induced cell-cell fusion. At neutral pH, a slow rate of fusion was observed between the VSV subsequent to viral entry by receptor-mediated endocytosis. This process was inhibited by treating cells with compounds that raise the pH of the endocytic vesicle. The temperature dependence for fusion at pH 7.4 was much steeper than that for fusion at pH 5.9 further implicating endocytosis as the pathway for fusion at pH 7.4. Fusion of VSV with plasma membrane fragments and with intact cells occurred at similar rates. Therefore, pH, voltage, or osmotic gradients are not required for viral fusion. The change induced in the viral spike glycoprotein by lowering the pH to trigger fusion was reversible, in that the rate of fusion was attenuated by raising the pH back to 7.4. With these results we are in a position to test certain hypotheses regarding the role of viral spike glycoproteins in mediating membrane fusion at the molecular level.

M-PM-E13 RECONSTRUCTION OF CAPACITANCE AND CONDUCTANCE CHANGES DURING EXOCYTOSIS FROM TIME DOMAIN MEASUREMENTS. Manfred Lindau, Intr. by M.P.Heyn, Biophysics Group, Dept. Physics, Freie Universität Berlin, D-1000 Berlin 33, West Germany.

The fusion of secretory granules with the plasma membrane during exocytosis leads to an increase of the membrane area which can be measured as a capacitance increase using the whole-cell patch-clamp technique. The time course of capacitance changes associated with secretion has mainly been studied by frequency domain methods using a lock-in amplifier (Neher & Marty,1982,PNAS,79,6712) or a PRBS generator (Fernandez et al.1984,Nature,312,453). I have used a time domain method to reconstruct the time course of capacitance (C_m), membrane conductance (g_m) and access resistance (R_a) during exocytosis of mast cells. In the experiment shown here, the cell was stimulated by including GTP-γ-S in the patch-pipette. A 20mV pulse was given every 56ms and 6,000 current signals evoked by these pulses were fitted with a single exponential. The changes in C_m, g_m and R_a are well separated resulting in a capacitance trace with a noise level of less than 1%. This method was also used to study exocytosis in response to receptor directed stimulation in the slow-whole-cell configuration (Lindau & Fernandez,1986,Nature,319,150). The time domain method presented here requires no additional instrumentation and allows simultaneous tracking of large capacitance and conductance changes at high resolution. Supported by DFG Sfb312.



M-PM-E14 PRESENCE AND LOCALIZATION OF A STIMULUS-SENSITIVE PROTEIN IN PARAMECIUM AND TETRAHYMENA. B. H. Satir, R. Herschenfeld, T. Hamasaki and T. Murtaugh, Department of Anatomy and Structural Biology, Albert Einstein College of Medicine, Bronx, New York.

A stimulus-sensitive phosphoprotein (M_r 63,000) is dephosphorylated when Paramecium tetraurelia cells are triggered to secrete (Gilligan and Satir, J. Biol. Chem. 275, 1982). This protein has been purified from Paramecium and a polyclonal antibody against it produced. The affinity purified antibody has been used to localize the protein via indirect immunofluorescence and biochemically via immunoblotting. Subcellular fractionation followed by SDS-PAGE and immunoblotting demonstrated that the protein was present in both wt and exo$^-$ mutants of Paramecium, predominantly in the cytosolic fraction. A similar, though slightly smaller (M_r 60,000), immunoreactive phosphoprotein was present in the cytosolic fraction of wt Tetrahymena thermophila as well. Similar to Paramecium, the M_r 60,000 phosphoprotein of Tetrahymena is dephosphorylated in response to trinitrophenol. Indirect immunofluorescence of wt Paramecium showed rows of fluorescent rings, 1-2μm in diameter. In places where rings could not be clearly discerned, a pattern of rows of continuous squares were seen, reminiscent of the polygonal network characteristic of Paramecium cortex. The exo$^-$ mutants tested showed similar localization patterns with the exception of the mutant tl (no trichocysts); in this case the rings appeared disrupted or fused, and occasionally replaced by a mixture of regular and irregular rows of fluorescent dots. The fluorescent pattern seen in Tetrahymena was slightly different in that solid rows of fluorescent lines were seen in between rows of double rings; in general the pattern was slightly more diffuse than that in Paramecium. This study suggests that the 63kDa stimulus-sensitive phosphoprotein may be a common feature in ciliate exocytosis.

M-Pos1 SAMPLING, BINNING, FITTING, AND PLOTTING DURATIONS OF OPEN AND SHUT INTERVALS FROM SINGLE CHANNELS. O. B. McManus, A. L. Blatz and K. L. Magleby. Dept. of Physiology & Biophysics, University of Miami School of Medicine, Miami FL 33101.

When single channel current records are sampled by computer at a fixed rate and threshold crossing is used to determine channel opening and closing, errors in measured interval durations can occur. We first extend the methods of Colquhoun & Sigworth (1983) and Sine and Steinbach (1986) to evaluate and correct for these sampling errors. Sampling at a fixed rate effectively combines intervals into bins which have a magnitude greater than that of the exponential (at the midtimes of the bins) describing the distribution of true intervals. When the probabilities that sampled intervals are drawn from the true distribution are taken into account with maximum likelihood fitting, then the true magnitudes and time constants of the underlying exponential components can be determined. Correction for sampling errors should be applied if the sampling period is greater than about 20% of the fastest time constant. We then present a method of binning intervals based on the logarithms of their durations, in which bin width remains a relatively constant fraction of bin midtime. This method allows any number of intervals of any expected duration to be stored in the least number of bins with essentially no loss of resolution. Such log binning of data greatly speeds fitting without adding error and automatically combines intervals for convenient plotting. Potential errors resulting from combining data into bins are avoided since each exponential component decays to insignificant levels before bin width becomes greater than about 20% of the time constant for that component. Supported by grants from the National Institutes of Health and the Muscular Dystrophy Association.

M-Pos2 DIFFUSIVE FLUX THROUGH IONIC CHANNELS. P.Y. Gates, K.E. Cooper & R.S. Eisenberg Physiology Dept., Rush Medical College, 1750 W. Harrison, Chicago, IL 60612

Using the theory of first-passage times, we have analyzed diffusive flux through a channel containing an arbitrary set of barriers and wells. An algorithm has been developed that computes the flux for a channel with a complex profile of potential energy but containing at most one ion. Expressions previously derived by David Levitt for a particular barrier structure acquire clear physical meaning in this formalism.

The simplest situation--a symmetrical channel in symmetrical solutions--has been studied in detail. The dependence of channel current on bath concentration and voltage will be shown. The first-passage time analysis defines an "affinity constant" as a function of transmembrane voltage and potential profile. Other functions of these electrical variables also arise naturally and allow intuitive insight into the channel flux over a wide range of membrane potentials and ionic concentrations.

M-Pos3 RATE CONSTANTS FOR IONIC DIFFUSION OVER BARRIERS. K.E. Cooper, P.Y. Gates and R.S. Eisenberg, Department of Physiology, Rush Medical College, Chicago, IL 60612.

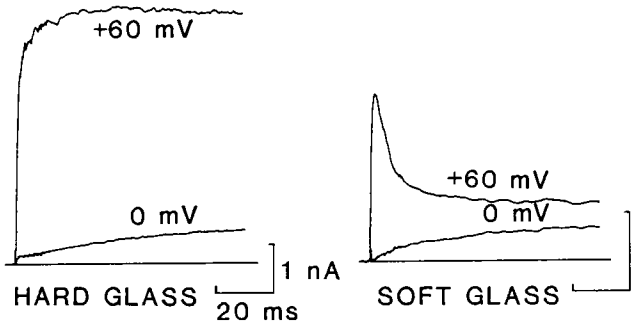
Ions jump picometers as they diffuse nanometers through channels. Ions jump nanometers over barriers in traditional theories of membrane permeation. We describe ionic diffusion over barriers as Brownian motion, a Markov diffusion process, deriving the appropriate Fokker-Planck equation. First-passage times (FPT) are computed to estimate the rate constant of barrier crossing. Unlike the rate constants of Eyring transition state theory (TST), these FPT's depend on the entire form of the barrier, not just its height ΔG^{+} (G = Gibbs *free* energy). The FPT theory thus predicts a richer set of experimental properties for a given profile of potential energy than TST. Like the rate constants of TST, the FPT's completely characterize the channel and can be determined experimentally (in principle) from measurements of the dependence of channel current on ionic concentration and membrane potential. If the barrier is large enough, the FPT simplifies, showing an exponential dependence on barrier height and a linear dependence on the curvature of the well and barrier. Simplified and 'exact' computations of first passage times across barriers will be shown, illustrating the effects of barrier height, well depth, and fine structure, for example, ledges.

M-Pos4 CONDUCTION AND SELECTIVITY IN A K CHANNEL FROM CARDIAC SARCOPLASMIC RETICULUM.
Joseph A. Hill, Jr., Roberto Coronado, Harold C. Strauss. (Intr. by T.J. McManus). Departments of Medicine and Pharmacology, Duke University Medical Center, Durham, NC 27710 and Department of Physiology, Baylor College of Medicine, Houston, TX.

We have studied a K-conducting channel from a cardiac sarcolemmal preparation reconstituted into planar Mueller-Rudin bilayers (PE:PS, 1:1). In the presence of 0.1 M salt, the single channel conductance was 190 pS (O_2); a prominent subconductance state was observed consistently (O_1, 110 pS). Biionic potential measurements (relative to K^+) reveal a selectivity pattern consistent with a low anionic field strength selectivity filter (Eisenman Seq. I). The relative permeabilities of Tl^+ (9.7) and NH_4^+ (1.7) are anomalously high given their ionic radii. The conductance-versus-activity relations for both states are well fit by Michaelis-Menten type equations ($Km(O_2)$=17 mM, $Km(O_1)$=20 mM; $Gmax(O_2)$=230 pS, $Gmax(O_1)$=130 pS). Cs^+ blocks K^+ conduction through the channel and is itself permeant ($P_{Cs}:P_K$ = 1.2). Analysis of the mole-fraction dependence of Cs^+ and K^+ conductance reveals a minimum at X(Cs) = 0.75. Experiments performed using sarcoplasmic reticulum isolated from canine ventricular muscle and rabbit skeletal muscle reveal K^+ channels with similar conductances, gating kinetics, and Cs^+ blockade effects. These data provide insight into the mechanisms of conduction within this channel and suggest that the channel originates in the sarcoplasmic reticulum membrane.

M-Pos5 WHOLE-CELL RECORDED K CURRENTS USING THE PATCH-CLAMP TECHNIQUE ARE INFLUENCED BY THE GLASS OF THE PATCH PIPETTE.
Gabriel Cota and Clay M. Armstrong, Department of Physiology, University of Pennsylvania, School of Medicine, Philadelphia, PA 19104-6085.

We have performed experiments on cultured pituitary cells to characterize their K channels, and have found that the observed kinetic properties of K currents depend on the kind of glass of the recording pipette. Experiments were done at 20-22°C on rat pars intermedia cells bathed in (mM) 135 NaCl, 5 KCl, 10 $CaCl_2$, 10 Hepes and 2 μM TTX. The pipette-filling solution contained (in mM) 110 Kglutamate, 20 KCl, 20 KF, 2 $MgCl_2$, 0.5 EGTA and 10 Hepes. Using hard, borosilicate glass (Kimax-51), depolarization to voltages positive to -20 mV from a HP of -40 mV induces long-lasting outward currents (left panel). In contrast, if the pipette is fabricated from soft, lead-containing glass (Corning #8161) the outward current inactivates (right panel). Soda glass (VWR hematocrit tubing, blue band) was also capable of inducing K current inactivation. Periodic depolarizations at 0.05 Hz progressively decreased the peak amplitude of the current and speeded up its decay. Soft-glass-induced inactivation of the K current was slowed by using a more negative HP (-80 to -100 mV), and was prevented by internal EGTA in high concentrations (10 or 20 mM).



M-Pos6 CALCIUM-ACTIVATED POTASSIUM CHANNEL CURRENTS COMPARED IN THE CELL-ATTACHED AND EXCISED PATCH CONFIGURATION.
B.S. Pallotta, J.R. Hepler, S.A. Oglesby and T.K. Harden, Dept. of Pharmacology and the Curriculum in Neurobiology, Univ. North Carolina School of Medicine, Chapel Hill, NC 27514.

We have tested the assumption that large-conductance (220-250 pS) calcium-activated potassium channels from 1321N1 human astrocytoma cells have similar properties in both the cell-attached and excised patch configuration. Our approach was to K-depolarize the cells (110 mM KCl) to a resting potential near 0 mV, and to load the cells with known [Ca] by using a calcium ionophore (50-100 μM A23187 or ionomycin) in the presence of 0.3 or 1 μM extracellular free Ca. Under these conditions, currents were recorded first in the cell-attached mode, and then after the patch was excised. Equilibration of the intra-and extracellular [Ca] was tested by measuring intracellular [Ca] with the fluorescent Ca chelator Quin 2 during exposure to ionophore.

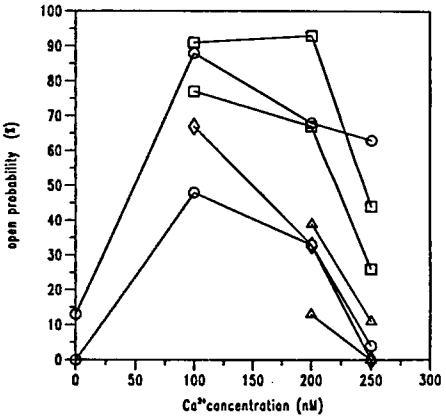
We found few, if any, differences between single channel currents in the cell-attached and excised patch configuration. Measurements included single channel conductance, and the voltage and [Ca] sensitivities of channel % open time. Autocorrelation functions calculated from the currents suggest that no significant changes in channel gating occurred upon excision. After excision, % open time remained relatively constant for several minutes (up to 1.5 h). These results suggest that results obtained from excised patches can be extrapolated to the more physiological cell-attached state. Supported by NIH GM32211 (B.P.) & GM29536 (T.K.H.), and a fellowship from the Lilly Research Laboratories (J.R.H.). T.K.H. is an Established Investigator of the Amer. Heart Association.

M-Pos7 PATCH CLAMP RECORDING OF SINGLE CALCIUM-ACTIVATED K CHANNELS IN TRACHEAL SMOOTH MUSCLE FROM SWINE. Hsiu Ming Huang, Terry M. Dwyer and Jerry M. Farley. Depts. Physiology & Biophysics and Pharmacology & Toxicology, University of Mississippi Med. Ctr., Jackson, MS 39216.

The characteristics of calcium activated K-channels were determined using the patch clamp technique. Tracheal smooth muscle cells were isolated from weanling swine by enzymatic digestion. Cells were used within 4-5 days after isolation. The patch clamp technique was performed to permit the measurement of channel properties in inside-out patches of muscle membrane. The external (pipet) solution contained 140 mM KCl, 2 mM $CaCl_2$, and 5 mM Hepes. The internal (bath) solution contained either 70, 140, or 280 mM KCl, 5 mM Hepes and calcium buffered to various levels with EGTA. Large conductance K-channels were observed in virtually every patch with many patches being unusable due to the large number of channels. The K-channel openings decreased in frequency when the internal solution (containing 1 or 7.7 μM Ca^{++}) was replaced with one containing low calcium levels (10 mM EGTA + 0 added Ca^{++}). The probability of channel opening increased with depolarization. When internal calcium was decreased to low levels by addition of EGTA, the occurrence of single channel events at negative potentials was limited to those events observed immediately after a hyperpolarizing voltage step from positive potentials. The reversal potential was shifted by 31 mV when the internal K was changed from 70 to 280 mM. Thus the channels were very selective for K over Cl. The chord conductance of the channel was 224 pS in the 70 mM K, 290 pS in 140 K, and 260 pS in 280 K internal as measured from E_r to +100 mV. The conductance as measured from E_r to -100 mV was 214 pS with 140//140 K inside and out. We conclude that tracheal smooth muscle has a high density of Ca^{++}-activated K channels which may be very important in the control of the activity of this muscle. This work was supported by Army Contract DAMD-17-C-3248.

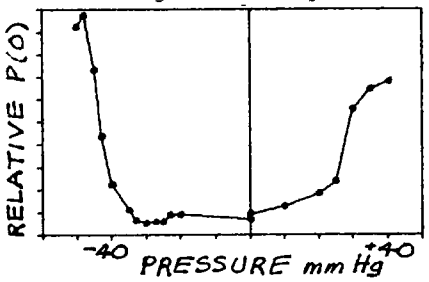
M-Pos8 BIPHASIC CALCIUM DEPENDENCE OF THE OPEN PROBABILITY OF CA-ACTIVATED K CHANNELS IN PARATHYROID CELLS. Min Jia, Kunihiko Iwasa, and Gerald Ehrenstein, Lab. of Biophysics, NINCDS, NIH, Bethesda, MD 20892.

We have performed patch clamp experiments on inside-out patches of isolated bovine parathyroid cells. With 140 mM $[K^+]$ external and 5mM $[K^+]$ internal (bath medium), Ca-activated K channels were observed with a single-channel conductance of 100 pS. The graph at the right shows the open probability for these channels as a function of internal $[Ca^{++}]$ at a membrane potential of -30 mV. The normal internal $[Ca^{++}]$ of these cells is more than 200 nM. Therefore, increasing $[Ca^{++}]$ would tend to close Ca-activated K channels, depolarizing the cells. These results could explain the depolarizing response of rat parathyroid cells to divalent cations reported by Lopez-Barneo and Armstrong [J. Gen. Physiol. 82:269 (1983)].



In another abstract in this volume, a model is proposed to explain the decrease in release of parathyroid hormone caused by an increase in $[Ca^{++}]$. That model is based on the assumption that secretory vesicles of parathyroid cells contain the same channels described here.

M-Pos9 STRETCH-ACTIVATED K CHANNELS IN MOLLUSCAN NEURONS. W.J. Sigurdson, E. Bédard and C.E. Morris. Dept. of Biology, U. of Ottawa, Ottawa, Canada. K1N 6N5. We have previously reported that cardiac cells of the freshwater snail Lymnaea stagnalis possess stretch-activated K^+ (SAK) channels (J.exp.Biol.123:175; Sigurdson et al., in press). Here we report that isolated Lymnaea neurons have similar SAK channels. Patches generally had between 2 and 5 channels. The extrapolated reversal potential is close to the resting potential and increasing the pipette $[K^+]$ from 1.6 mM to 30 mM produces a +35 mV shift in the reversal potential. When Na^+ is the dominant ion on the outside or inside surfaces of the membrane no inward or outward currents are observed. The SAK channel is activated by membrane stretch in cell attached and excised patch conditions. Both negative and positive pressures activate the channel (See Figure). Kinetic analysis shows that the stretch-induced increase in the probability of being open (P(O)) arises mostly from a decrease in the interval between bursts of channel activity, as opposed to increases in the channel open time(s). Quinidine blocks the neuron SAK channel. At 10^{-5} M and higher, it produces a flickery block associated with an increased burst length and a dose-dependent reduction of channel conductance. We also find similar stretch-activated channels in neurons of the terrestrial snail Cepaea nemoralis (family Helicidae). Supported by NSERC and Canadian Muscular Dystrophy Association.



M-Pos10 TRANSIENT VOLTAGE DEPENDENT K CONDUCTANCE IN RAT NEURONS. M. Rizzo and W. Nonner, Dept. of Physiology and Biophysics, Univ. of Miami, Miami, Fl 33101.

A voltage dependent K conductance of the somatic membrane of embryonic rat neurons was studied using a patch-clamp technique. Neurons, from hippocampus and basal ganglia were grown in primary tissue culture for 2-10 weeks. In order to achieve proper space-clamp conditions, we isolated spheroids of membrane ('blebs') by first forming the whole-cell clamp configuration, then slowly pulling the pipette away from the cell. The blebs formed by this method had diameters from 2-10 μm. The preparation was stable for periods up to 1 h and revealed maximal K conductances of 1-20 nS. With external 140 mM NaCl saline plus 1 uM TTX, and 0.5 mM Cd to block current in Na and Ca channels, and with an internal 140 mM KCl saline, a voltage dependent, transient outward current is observed. It activates rapidly (tau >= 0.5 ms, decreasing with depolarization), peaks within 3 to 6 ms, then spontaneously inactivates. Substitution of external KCl for NaCl shifts the reversal potential in the way expected for a K-selective channel; the current is blocked by 5 mM 4-aminopyridine. The channel is activated at depolarizations > -50 mV and reaches 90% of the maximal conductance at 0 mV. Conditioning at potentials (Vc) more negative than -90 mV and maintained for 300 ms reduces inactivation to less than 10%, while 50% inactivation is found at Vc=-55 mV, and 90% at Vc=-40 mV. After complete inactivation, 10% of the current is recovered within 30 ms at -90 mV, and 90% after 200 ms. The inactivation during a depolarizing pulse proceeds with a time course described by one to two exponentials. Time constants group near three values: 20, 40, and 75 ms. Whereas the inactivation time course is little affected by Vc, a large test depolarization enhances the slower components of the decay. Inactivation time courses vary from cell to cell, but are stable for a given preparation. Supported by NIH grant GM 30377.

M-Pos11 TRANSIENT AND STEADY-STATE ANALYSIS OF K CONDUCTANCE RELAXATIONS IN SQUID AXONS. H.M. Fishman and R.J. Lipicky, UTMB, Galveston, TX and FDA, Rockville, MD.

Fits of the equation $I_K=I_o[1-\exp(t/\tau_n)]^n$ to voltage-clamp elicited, K-current responses in series-resistance (R_s) compensated, intact squid axons in ASW + TTX (1μM) were made after removal of linear capacitive and leakage currents. Variable n(1.0 to 2.5) yielded best fits of transient responses for steps of 1 to 80mV from a holding of -65mV. For fixed n, n=2 gave better fits than n=4. Compared to the HH model, which gives a peak τ_n=4.2 msec at -70mV and 9.2°C, τ_n(V) data at 9.2°C peaked at -50mV more sharply with voltage; $(\tau_n)_{max}$=10 and 5.4 msec for n=2 and 4, respectively. τ_n(V) was also determined from fits of the membrane complex admittance, $Y_M(j\omega)$, measured during step clamps in the same axons under the same ionic conditions. The equation $Y(j\omega)=[R_s + (Y_M)^{-1}]^{-1}$ where $Y_M = (j\omega)^{\alpha} C_M + g_o + g_K (1+j\omega\tau_n)^{-1}$ was fitted to the measured admittance in the frequency range 2.5 to 5000Hz with α, C_M, g_K and R_s constant to yield estimates of τ_n. $Y(j\omega)$, determined either at 0.1 or 0.5 sec after step clamps of up to 80 mV from -65 mV and with correction for current "droop", yielded the same τ_n(V), indicative of steady state conditions. τ_n from admittance data corresponded most closely to τ_n(v) from transient data fitted with n=2, but both differ markedly from the HH τ_n(V). The best macroscopic estimates of K conductance relaxations appear to come from admittance determinations because they are not subject to corrections for R_s, I_{CM} and leakage and do not depend on the value of n.
Supported by NIH grant NS11764 and ONR contract.

M-Pos12 POTASSIUM EFFLUX THROUGH THE RESTING CHANNEL OF SQUID AXONS. J.R. Hunt and D.C. Chang, (Intr. by C.L. Seidel), Department of Physiology and Molecular Biophysics, Baylor College of Medicine, Houston, TX 77030.

It has been suggested based on electrophysiological studies that the majority of resting potassium current passes through a membrane pathway different than the delayed rectifier K channel (Chang, Biophys. J., in press.). To further examine this hypothesis, we measured the K efflux from squid giant axons under voltage clamp, with or without blocking the excitable K channel by internal application of TEA (tetraethylammonium). ^{42}K was applied inside the axon by an internal perfusion technique. The axoplasmic concentration of radioisotope thus remained constant throughout the experiment. Labelled K ions passing across the membrane were collected in the external perfusate. This method allows measurement of a steady-state unidirectional flux and avoids complications due to loading and unloading of ^{42}K into the periaxonal space and Schwann cell layer. Our measurements indicate: (1) When the axon was clamped at the normal resting potential (-65 mV), the ^{42}K efflux was not significantly affected by the internal application of 20 mM TEA, suggesting that very little of the resting flux passes through the delayed rectifier. (2) The K efflux increased with depolarization as expected. However, the V-dependence of efflux was less steep than that previously observed using a wash-out method (Hodgkin and Keynes, J. Physiol. 128, 61-88, 1955). (3) Elevation of the external K concentration from 10 mM to 100 mM stimulated ^{42}K efflux when the axon was clamped at constant voltage (-65 mV). This result suggests that an ion exchange mechanism may be involved in the functioning of the resting channel. (Supported by ONR contract N00014-85-K-0424 and NSF grant BNS-84-06932.)

M-Pos13 ION-SELECTIVITY OF THE DELAYED RECTIFIER K CHANNEL IN SQUID AXON. Z.Y. Zhao and D.C. Chang. (Intr. by D. Rampe), Department of Physiology and Molecular Biophysics, Baylor College of Medicine, Houston, Texas 77030

In spite of the fact that the delayed rectifier of the squid axon is one of the most well known examples of excitable K channel, the ion-selectivity of this channel has only been determined qualitatively (Oxford & Adams, Biophys. J. 33:70a; 1981, Matteson & Swenson, J. Gen. Physiol. 87:795-816, 1986) but not quantitatively. We found several complications in making such a determination: (1) When we employed an instantaneous I-V method (such as those used by Matteson & Swenson), the reversal potential was often influenced by the accumulation of K ions in the periaxonal space. This was particularly a problem when the test ion in the external solution was Na^+, Li^+, or NH_4^+. (2) In some cases the current passing through the delayed rectifier does not obey the so-called "ion-independence" principle. External Cs^+, Rb^+ and K^+ all interfered with outward K current. We have partially overcome these problems by (1) minimizing the K current when we activated the delayed rectifier for instantaneous I-V measurements (i.e., by decreasing $[K]_i$, lowering the depolarizing potential and shortening the depolarizing time); (2) in addition to measuring the reversal potential when the test ions were placed in the external solution, we also measured the relative amplitude of delayed current when the internal K^+ was replaced by test ions. Our best estimated values for the relative permeabilities of K^+, Rb^+, NH_4^+, Na^+ and Li^+ through the delayed rectifier are 1 : 0.25 : 0.08 : 0.05 : 0.04, respectively. Cs^+ appears to be practically impermeable to the K channel. Its permeability is less than 0.01 of that of K^+. (Work supported in part by ONR contract N00014-85-K-0424 and NSF grant BNS-84-06932.)

M-Pos14 IONIC PERMEABILITY OF Ca^{2+}-ACTIVATED K^+ CHANNELS IN RAT PANCREATIC B-CELLS. J. Tabcharani, L. Falke and S. Misler. The Jewish Hospital, St. Louis, MO, 63110.

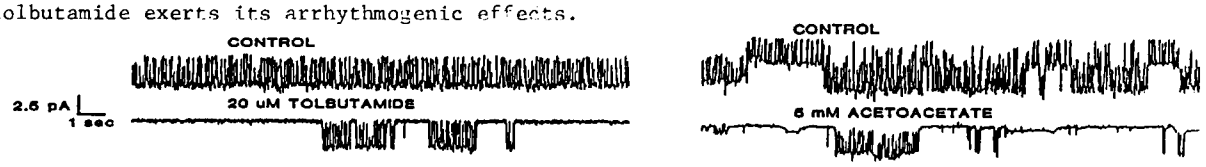
In B-cells, Ca^{2+} activated K^+ channels may contribute to the repolarization phase of individual or cycles of Ca^{2+} -dependent action potentials which are linked to insulin secretion. Aspects of their permeability were studied, chiefly in inside-out excised patches: external solution 138mM KCl and $\leq$ 20μM $CaCl_2$; the cytoplasmic solutions KCl (30-900mM), 0 or 2mM $MgCl_2$, 20-100μM $CaCl_2$ (both buffered to pH 7.0 or 7.3 with K^+ HEPES). 1. Increasing [KCl]i's shifted the reversal potential ($V_{I=0}$) towards more negative Vm's by 57mV per 10-fold increase in [KCl]i. 2. Single channel conductance measured around Vm=0mV rose asymptotically with increasing [KCl]i to a maximum of 350pS (K_D=40mM). 3. $P_{Tl}:P_K:P_{Rb}:P_{NH4}:P_{Li,Cs,Na}$ was calculated as 1.3:1.0:0.5:0.09:$\leq$0.05, from the Goldman-Hodgkin-Katz equation based on the shift in $V_{I=0}$ seen on complete substitution of each test cation for K_i. 4. P_{Rb}/P_K was constant with various mixtures of Rb_i + K_i. 5. On addition to 70mM Ki, Cs>Rb>Li>Na reduced the outward current through the channel. 6. The reduction in outward current produced by Na_i was a function of K_o: in outside-out excised patches replacing 138mM $NaCl_o$ with 118mM NaCl +20mM KCl nearly relieved the reduction in outward current (and abolished the region of negative slope conductance at Vm>+30mV) caused by addition of 5mM $NaCl_i$. 7. TEA produced voltage dependent reduction in single channel current from either surface. K_D was 50mM for TEA_i; 0.3mM for TEA_o. These results are strikingly similar to those obtained in excised patches from myocytes and chromaffin cells. While results 2, 4, 5, and 7 are quantitatively consistent with a "one ion" channel model, result 6 may be more easily fit by a "two ion" channel model.

M-Pos15 MODULATION OF ACTIVITY OF Ca^{2+}-ACTIVATED K^+ CHANNELS IN ADULT RAT PANCREATIC B-CELLS. S. Misler, J. Tabcharani and K. Gillis, The Jewish Hospital, St. Louis, MO, 63110.

One hypothesis of stimulus-secretion coupling in B-cells is glucose metabolism → ↑Ca_i^{2+} sequestration → ↓ activity of Ca^{2+} activated K^+ channel (K(Ca)) → membrane depolarization. In this scheme, hyperpolarization caused by ↑pHi or metabolic poisoning is attributed to ↑K(Ca) activity. Initial patch clamp data suggested that while an ATP_i sensitive K^+ channel, K(ATP), is the predominant species active around Vm = Vrest ≅ -70mV, in excised patches from neonatal cells, K(Ca) was the one modulated by pHi. We have investigated this apparent disparity in dispersed adult rat B-cells. 1. In stable cell attached patches, bath and pipette both containing 138mM KCl and no added Ca, K(Ca)'s were not detected around Vm = Vrest; the threshold voltage for detection, V_t, was at least 70mV depolarized from Vrest. Above V_t, average channel activity (I/i) increased e-fold per 13-15mV. Addition of 2μM A23187 and 2mM Ca to the bath, increased I/i 2-fold but shifted V_t less than 10mV. 2. Addition of 25mM glucose or glyceraldehyde to the bath reduced I/i of K(Ca) by 2-fold, at Vm's > V_t, while reducing I/i of K(ATP) up to 100-fold. 3. Addition of 3mM Na azide increased I/i of K(Ca) 3-4 fold at Vm's > V_t while increasing I/i of K(ATP) up to 20-fold at Vm = Vrest. 4. Bath addition of NH_4Cl (20mM) at constant pHo had little effect on K(Ca) but increased I/i of K(ATP) up to 10-fold. NH_4Cl washout transiently suppressed K(ATP) activity without effecting I/i of K(Ca). Varying pHi from 6.6 - 7.5 had no major consistent effect on I/i of K(Ca) in our excised patches. This suggests that while K(Ca)'s may accurately detect changes in Vm or Ca_i, they do not contribute significantly to the sustained changes in Vm seen with changes in cell metabolism.

M-Pos16 TOLBUTAMIDE INHIBITS AN ATP SENSITIVE K^+ CHANNEL IN CARDIAC MYOCYTES. William Gee and Stanley Misler, Depart. of Medicine, Jewish Hospital, St. Louis, MO, 63110.

Cardiac myocytes and pancreatic islet B-cells have time and voltage independent plasmalemma K^+ channels whose activity in membrane patches is increased by metabolic inhibition (cell attached), decreased by a variety of metabolic substrates (cell attached in B-cell) and decreased by cytoplasmic ATP (intracellular dialysis or excised patch). In the B-cell tolbutamide selectively blocks this channel in cell attached and excised patches. We report here a cardiac myocyte channel of similar description whose activity is also decreased by tolbutamide. Enzymatically dispersed neonatal rat cardiac myocytes were briefly pretreated with dinitrophenol and iodoacetic acid and then bathed in a 5.5 mM K^+ extracellular solution and patched with a 138mM K^+ pipette. These cells often show a 60 pS, voltage independent, irregularly bursting channel with a reversal potential of Vm≅+50mV in the cell attached patch. Average activity of this channel is decreased by 2.5-5mM Na acetoacetate or by 20μM tolbutamide added to the bath, with obvious long pauses appearing between the discrete bursts (see figure). These preliminary results raise the possibility that tolbutamide is a specific inhibitor of the ATP sensitive class of K^+ channels and suggest a mechanism by which tolbutamide exerts its arrhythmogenic effects.



M-Pos17 OPPOSITE ACTIONS OF TWO STRUCTURALLY SIMILAR SULFONAMIDES ON AN ATP SENSITIVE K^+ CHANNEL IN ADULT PANCREATIC B-CELLS AND RINm5F INSULINOMA CELLS. K. Gillis, W. Gee, L. Falke and S. Misler. The Jewish Hospital, St. Louis, MO, 63110.

Tolbutamide (TOL) depolarizes B-cells by reducing P_{K+}, thereby triggering Ca^{2+} AP's and insulin release; diazoxide (DZ) hyperpolarizes B-cells by increasing P_{K+}, thereby inhibiting glucose-induced AP's and insulin release. Patch clamping of B and RIN cells reveals that TOL selectively decreases average activity (A) and DZ selectively increases A of a 60 pS K^+ channel (in symmetric 138mM KCl), but neither drug effects channel conductance. Activity of this channel, which is unaltered by Vm or $[Ca^{2+}]$, is reduced, in the cell attached patch (c.a.p.), by application of glucose to the exterior of the cell and, in the inside-out excised patch (i.o.p.), by applying μM - mM ATP to the cytoplasmic surface. Onset of AP's coincides with nearly complete closure of these channels. TOL applied to the bath rapidly (within 30 sec) and reversibly reduces A (and induces spike activity) in the c.a.p. (K_D≅3μM); it increases both the pauses between the shortened channel bursts and the closed intervals within the burst. Similar results, though often less reversible, are seen with TOL applied to i.o.p. in the absence of ATP . This suggests that TOL is directly affecting a conformational state of the channel. Conversely DZ applied to the bath reversibly increases A in c.a.p. (K_D≅20μM), chiefly by reducing the length of the pauses between bursts. DZ has little effect on A in the i.o.p. in the absence of ATP_i but inhibits ATP-induced channel closure. The DZ effect can be overcome by increased ATP_i. This suggests that DZ may be acting as a "competitive" inhibitor of ATP binding at a channel site. Neither agent affects the activity of Ca^{2+} activated K^+ channels.

M-Pos18 CHARYBDOTOXIN KNOCK OFF BY POTASSIUM IN A CALCIUM ACTIVATED POTASSIUM CHANNEL. R. MacKinnon and C. Miller. Dept. of Biochemistry, Brandeis Univ., Waltham, MA 02254

The interaction of charybdotoxin (CTX) with single Ca^{2+} activated K^+ channels inserted into neutral phospholipid bilayers was studied. CTX blocks the channel from the external solution, and internal K^+ relieves the block. Increasing $[K^+]$ on the internal side of the channel increases the rate of dissociation of CTX from its blocking site. At high internal $[K^+]$ the CTX dissociation rate approaches a maximum value. The "knock off" of CTX from its blocking site by the permeant ion K^+ is independent of the channel open probability ($0.1<P_o<0.9$). The results suggest that K^+ influences the CTX block time by binding to a site in the pore which is in equilibrium with the internal solution. The voltage dependence of the knock-off suggests that K^+ from the inside traverses the entire applied transmembrane electric field when it moves to the site. A simple model consistent with these results is one in which CTX plugs the pore from the outside and can be repelled by the presence of K^+ on an adjacent binding site within the pore. (Supported by NIH Grants HL07044 and GM31768.)

M-Pos19 A VOLTAGE- AND CALCIUM-SENSITIVE INWARD RECTIFYING POTASSIUM CHANNEL ACTIVE IN THE RESTING MEMBRANE OF CULTURED MOUSE HIPPOCAMPAL NEURONS. J.M. Sullivan, Depts. Physiology-Biophysics and Anesthesiology, Mount Sinai School of Medicine, New York, N.Y., 10029.

Cell-attatched patch (CAP) clamp with high Ko levels illustrated a resting membrane potential (RMP)-active channel with an inward rectifying (IR) single channel (SC) I-V relationship. Ramp voltage clamp showed the rapid development of the rectification. SC conductance was dependent on the square root of Ko and reversal potentials were consistent with a K-selective process. Cs^+ and Ba^{2+} were both found to block the IR channel.

Probability of opening (P(o)) vs. voltage was steep around RMP, decreased with hyperpolarization and increased with depolarization. SC's isolated into isotonic KCl with 10 nM calcium (Ca) remain active but activity disappears at 1 nM. Three closed states and one open state are essential to explain CAP channel kinetics. Voltage-dependence of P(o) was related to the voltage-dependence of the burst length, as well as short and long closed times. A time- and voltage-dependent inactivation was found for hyperpolarization from near RMP for both ensemble clamped SC's and in whole cell voltage clamp consistent with SC kinetic measurements. Using P(o), g = f(Ko) and the I-V curves under ramp clamp, calculation of the expected steady-state I-V curve under conditions of physiological Ko (5.3 mM) shows significant outward conductance at RMP. These studies suggest that IR contributes to RMP and its unique voltage- and Ca-sensitivity pose it for a role in the after-hyperpolarization current known to exist in hippocampal pyramidal neurons.

JMS is a trainee on Medical Scientist Training Grant GM-07280 from the NIH.

M-Pos20 MODULATION OF K CHANNEL ACTIVITY IN AORTIC SMOOTH MUSCLE BY BRL34915 AND A SCORPION TOXIN. K. KUSANO, F. BARROS, G. KATZ, M. GARCIA, G. KACZOROWSKI,J.P. REUBEN, Merck Inst. Therp. Res., Rahway, NJ 07065

Smooth muscle cells from rabbit and bovine aorta, cultured for a limited number of passages, were examined by patch and whole-cell voltage/current clamp. Whole-cell recordings in both preparations revealed two types of K pathways, an inward rectifier (P_{Kin}) and a Ca-activated (P_{KCa}). BRL34915 (0.1 to 1.0 uM), a putative K channel agonist, hyperpolarized cells with low resting potentials by as much as 40-60 mV and increased K currents. Only P_{KCa} has been examined on the single channel level. This channel in both cell types has a conductance of $\sim$200 pS (150 mM K/150 mM K) and portrays the conventional sensitivity to external tetraethylammonium ions (blocked by 1-10 mM). In 3 on-cell patches with 150 mM K in the electrode and either 150 mM K or 135 mM Na in the bath, BRL34915 increased open time 3.5 to 6.3 fold for the same channel currents. BRL34915 did not change channel conductance. Of the 7 excised patches examined (both inside-out and outside-out), only 2 showed an increase in open time of 1.5 and 2.0 times. However, only in these 2 cases was the inside surface of the patch exposed to GTP (50-100 uM) and MgATP (1-2 mM). Clearly, additional excised patches must be tested to ascertain whether GTP and/or MgATP is required for the agonist-like effect of BRL34915 on P_{KCa} channels. Scorpion toxin (Leiurus quinuestriatus; 10-100 ug/ml), a blocker of PK_{Ca}, increased the silent periods between bursts when applied to the external surface of excised patches. The characteristics of the channel activity during the bursts were not affected by the toxin. The latter finding is in accord with that for skeletal muscle P_{KCa} channels incorporated into lipid bilayers (Miller et al. Nature 1985, 313;316).

M-Pos21 PANDINUS IMPERATOR SCORPION VENOM BLOCKS K CHANNELS IN GH3 CELLS. M.T. Lucero & P.A. Pappone, Animal Physiology, Univ. of Calif. Davis, CA 95616.

We studied the effects of venom from the scorpion Pandinus imperator on delayed rectifier K channels in cultured GH3 cells. Cells were whole cell voltage clamped using gigohm seal methods. Measurements were made in high K external solution. Block of K currents by venom was voltage dependent. 50-500 µg/ml of crude venom blocked 50%-80% of the peak inward K current at -20 mV, but blocked only 15%-60% of the outward K current at +50 mV. This seems to be a voltage-dependent effect of the venom, rather than a selective block of inward current, since the shape of the instantaneous current voltage relation was unaffected. The venom had only minor effects on K channel kinetics, slowing opening and speeding channel closing slightly. The largest kinetic effect was to increase the amount of inactivation of the current occuring during depolarizing pulses. In control records 60% of the peak K current had inactivated by 200 msec at +30 mV, but in 50 µg/ml venom over 90% of the peak current had inactivated. Block of K current by venom was essentially irreversible in high K solution. There was virtually no recovery of K currents following washout of the venom even after ~40 min in the venom-free solution. Zinc contained in crude Pandinus venom cannot account for these effects since effects of 0.2 mM Zn were completely reversed within 5-7 min. We conclude that Pandinus venom contains toxin(s) which can bind tightly to voltage-gated K channels. Supported by NIH Grant AM34766.

M-Pos22 Chemical Modification of Amino Groups on Calcium Activated K^+ Channels of GH3 Cells. A. Michael Frace and D. C. Eaton* Dept. of Physiology and Biophysics, Univ. Texas Medical Branch, Galveston, Tx. 77550 and *Dept. of Physiology, Emory University, Atlanta, Ga. 30322

The effects of amino-group specific reagents were examined on single channel currents of large conductance, Ca-activated K^+ channels in excised patches from GH_3 cells. The reagents used include trinitrobenzene sulfonic acid (TNBS), 4,4'-isothiocyano-stilbene-2,2'-disulfonic acid (DIDS) and its 4-acetamido derivative (SITS), and sulfophenyl isothiocyanate (SPIT). These reagents react covalently with terminal amino groups or lysine residues. The reaction replaces the positively charged amino group with negatively charged groups (SITS, DIDS, SPIT) or an uncharged group (TNBS). 0.1 - 1.0 mM reagent applied to inside-out patches irreversibly enhances channel open probability (in 1 uM Ca^{++} at +30 mV with symmetrical KCl: control = 0.1; after reagent = 0.85). Analysis of open interval distributions, fit with two exponentials, show this effect is primarily due to an increase in the mean open time of the longer distribution events (9 vs 15 mS; 1 uM Ca^{++}; +40 mV; symmetrical KCl). Short distribution events remain unaffected (mean open time = 1.0 vs 0.96 mS). Single channel conductance and channel voltage dependence are unaffected. After modification, channel activity is unresponsive to any reduction in Ca^{++} concentration (0.1 nM Ca^{++} perfusion for 1 hr). Channels can, however, respond to increases in Ca^{++} above the concentration present during modification. These actions suggest that amino-group modification produces a more favorable local charge environment for Ca^{++} interaction and greatly slows a Ca^{++} dissociation step from the channel. These reagents contrast with the effect of N-bromoacetamide which irreversibly removes nearly all channel activity (Pallotta, JGP:86, 1985). (Supported by DHHS-DK-38830.)

M-Pos23 CHARYBDOTOXIN BLOCK OF THE CALCIUM-ACTIVATED POTASSIUM CHANNEL FROM MAMMALIAN MUSCLE. C. Anderson and C. Miller. Dept. of Biology and Graduate Dept. of Biochemistry, Brandeis University, Waltham, MA 02254

Charybdotoxin (CTX), a protein component of scorpion venom, strongly inhibits the conduction of K^+ ions through high conductance Ca-activated K^+ channels. The mechanism by which CTX blocks current through this channel from rat t-tubule membrane was studied by incorporating single channels into planar lipid bilayers. CTX blocks conduction by binding to the outside of the channel with a high affinity in a simple bimolecular reaction (K_D 5nM). The K_D for the binding was found to be sensitive to changes in the voltage across the membrane, the gating state of the channel, and the ionic composition of the solutions surrounding it.

Depolarizing the membrane increases both the on and off rates for CTX binding. Since increasing the voltage also activates the channel, a possible explanation for the effect on the on-rate is that the toxin interacts more readily with the channel when it is in an open configuration. When the membrane voltage is held constant and the open probability of the channel is changed by varying the Ca^{++} concentration, the on-rate increases with increasing open probability. The off-rate is unaffected by changes in the open probability. Increasing the concentration of K^+, Na^+ and arginine on the external side of the membrane from 20mM to 300mM reduces the on-rate by almost two orders of magnitude, with little effect on the off-rate. Supported by N.I.H. GM31768.

M-Pos24 SLOW POTASSIUM CURRENT IN SKELETAL MUSCLE FIBERS OF THE RAT. I.D. Uribe and R.F. Valdiosera (Introduced by Roberto Valle). Dept. Physiology and Biophysics. CINVESTAV-IPN, Apdo. Postal 14-740, México, D.F.

Ca^{++} currents in rat muscle fibers are preceded and followed by outward currents that remain even in the presence of K^+ channel blockers. Although the early outward current seems to be carried by delayed rectifier channels not completely blocked by the TEA^+ present in bath, the late outward current seems to be less satisfactorily explained. We have measured Ca^{++} currents of the omohyoid with the vaseline gap voltage clamp since this technique allows the control of the internal ionic composition. Recordings were made at room temperature (22-24°C) in an external solution that contained in mM: $TMACH_3SO_3$ 106, $TEACH_3SO_3$ 40, $Ca[CH_3SO_3]_2$ 2, TMAMOPS 5, Glucose 12 and TTX .001. Fiber ends were cut in an intracellular medium containing KASP 125, KMOPS 5, K_2EGTA 20 and K_3Citrate .125, pH was adjusted to 7.35 in both solutions. The experiments were designed to test first of all this current crosses the membrane through a K^+ channel or through a Ca^{++} channel made permeable to monovalent cations by depletion of the tubular Ca^{++} concentration. We have discarded the latter possibility because in contrast with frog, there is no outward current when there is no Ca^{++} in the external solution and the delayed rectifier is blocked with 40 mM TEA^+ in the internal solution with K^+ as the main intracellular cation. Four observations support the view that the channel involved is the Ca^{++} activad K^+ channel: this potassium current is linked to Ica because when the Ca^{++} current is blocked with nifedipine (10 μM) or with cadmium (2 mM) the K^+ current is not activated, lowering the EGTA concentration in the internal solution from 20 mM to 7.5 mM increases 4-5 times this current, is blocked by 40 mM TEA^+ in the internal solution and Ba^{++} in the external solution does not activate this current.

M-Pos25 TRANSIENT OUTWARD CURRENTS IN MATURE NEURONS ISOLATED FROM THE SALAMANDER SPINAL CORD
Arturo Hernandez-Cruz and Peter R. MacLeish (Intr. by Sanford M. Simon)
The Rockefeller University, New York, N.Y. 10021

A series of experiments were conducted to study the voltage-gated membrane conductances of individual spinal cord neurons from the adult salamander (Ambystoma tigrinum). Neurons were prepared as previously described (Hernandez-Cruz & MacLeish, Soc. Neurosc. Abs. 12:1348, 1986). Whole-cell voltage-clamp recordings were obtained within 5 days in culture from cells with a single short process or none at all. Two transient components of outward current were found. The first one had properties similar to that of the Na-activated K current IK(Na), described in quail neurons (Bader et al, Nature 317:540, 1985). The evidence is as follows: a) Its I-V curve resembles that of INa. b) Its amplitude changed under conditions affecting that of INa, and it was blocked by TTX or removal of external Na. c) It was suppressed by K-channel blockers TEA or 4-AP (20 and 3 mM). d) Its tail reversal potential was near the theoretical EK value. In addition, we found that its production was linked to the activation of two types of Na channels with different TTX sensitivities, and that it was also present when Li substituted for Na in the external medium. This current activated in less than 1 ms and its rate of decay was strongly dependent on the depolarization level. The second transient component is a 4-AP-sensitive transient current similar to the Ia current described in several neuronal preparations. Its size varied greatly among different cells, being absent in some of them. Evidence was obtained indicating that these currents play an important role in determining the size of the action potential and its rate of repolarization, as well as the firing properties of the cell.
Supported by the Fogarty International Center 1f05TW03631, NIH grant EY05201 and The Klingenstein Fund.

M-Pos26 ADRENERGIC MODULATION OF THE TRANSIENT OUTWARD CURRENT IN ISOLATED CANINE CARDIAC PURKINJE CELLS. T.Nakayama and H.A.Fozzard. (Intr. by A. Scanu)
Cardiac Electrophysiology Labs, Departments of Medicine and the Pharmacological & Physiological Sciences, The University of Chicago. Chicago, IL 60637.

β-adrenergic stimulation of heart muscle results in an increase in both slow inward Ca^{2+} current (Reuter and Scholz, J. Physiol. 264:49-62, 1977) and delayed outward current (Bennett et al., Biophys. J. 49:839-848, 1986). We find that the transient outward current was also modified by norepinephrine(NE). Single canine Purkinje cells were voltage clamped using a sealed pipette clamp method under Ca^{2+} free conditions. The depolarizing pulse induced transient outward current with two exponential time constants of inactivation (48±17 msec, 352±126 msec at +58 mV). More than 10^{-9} M NE modified only inactivation kinetics without affecting activation kinetics. The half maximum dose was 1.9 x 10^{-8} M and the effect saturated with 10^{-6} M. NE reduced the amplitude of the fast time constant component of the inactivation and increased the amplitude of the slow one without changing the time constants. The mechanism was β-adrenergic agonist activation of adenylate cyclase to produce cAMP. The intracellular cAMP may phosphorylate the protein of the transient outward current channel. (Supported by HL 20592).

M-Pos27 POTASSIUM CHANNELS IN OBESE MOUSE BETA CELL MEMBRANES DISPLAY AN ALTERED RESPONSE TO ATP AND CALCIUM, J.-L. Schwartz*, G.A.R. Mealing* and J.T. Braaten+, from *the National Research Council, Ottawa, Canada and +the University of Ottawa, Ottawa, Canada.

Single channel ionic currents were recorded from 5 to 12 day old cultured insulin secreting cells from obese mice (C57BL/6J, Jackson Laboratory, Bar Harbor, Maine) using the patch-clamp technique in the inside-out configuration in quasi-physiological conditions (high sodium in the pipette and high potassium in the bath). Two types of channels were observed. The first has a conductance of 60 pS. It is voltage-dependent and is activated by ATP. At 0 mV membrane potential, this channel is open less than 5% of the time. The opening probability is between 18 and 35% at 20 mV membrane potential and exceeds 35% at 40 mV. At 0 mV, when 2 mM ATP is added to the bath, the opening probability increases to 93%. The second type of channel is also voltage-dependent. It has a conductance of 20 pS. When calcium concentration is raised (0.6 and 2 μM) the conductance drops to 5 pS and the opening probability curves are shifted to the right, indicating a calcium inactivation of the channel.

This is the first observation of a channel that is both voltage-dependent and ATP activated. We also report for the first time the existence of a small conductance channel (about 5 times less than $g_{K\ Maxi}$ described by Findlay et al, J. Membrane Biology, 1985, 83, 169-175) that displays calcium-dependent conductance and calcium inactivation. This work shows that in ob/ob mouse beta cells, potassium channels display profound alterations in their response to intracellular ATP and calcium concentration changes. This modified potassium channel function may be directly related to altered glucose sensitivity of obese mouse beta cells (Rosario et al, Quat. J. Exp. Physiol., 1985, 70, 137-150; Schwartz et al, 1986, Biophys. J., 49,162a).

M-Pos28 EFFECTS OF LONG-CHAIN ALCOHOLS ON I_A IN APLYSIA. Steven N. Treistman and Andrew Wilson. Worcester Foundation for Experimental Biology, Shrewsbury, MA.

We have reported that high concentrations of ethanol (EtOH; 200-400 mM) have cell-specific actions on the A-current in Aplysia neurons. In the present study we examine the actions of longer-chain alcohols on I_A in three identified neurons of Aplysia. In particular, we were interested in determining whether the longer chain alcohols had effects similar to EtOH, which would suggest a similar mechanism of action, and whether they showed the same cell-specificity as EtOH. Butanol (BuOH) was tested at concentrations of 10 to 40 mM, and hexanol (HexOH) was tested at concentrations of 1 to 6 mM. Both BuOH and HexOH differed from EtOH in their actions on I_A. EtOH had little effect on I_A amplitude in any of the three cells, whereas BuOH and HexOH both reduced I_A significantly (40-60% at the higher concentrations). I_A decay time constant, which is greatly prolonged in cells R15 and MCC by EtOH, was only minimally prolonged in MCC by BuOH and HexOH, and was significantly reduced in R15 (40% in 40 mM BuOH). The decay time constant was similarly reduced by BuOH and HexOH in cell B1, although this parameter is EtOH-insensitive in this cell. Thus, the longer-chain alcohols differ from EtOH both in their qualitative actions on I_A, and in the cell-specificity of those actions. When the actions of the three alcohols were plotted as a function of membrane concentration or volume occupied, BuOH and HexOH were very similar, whereas EtOH showed a very different relationship. The data suggest that high EtOH concentrations act on I_A by different mechanisms than the longer chain alcohols.

M-Pos29 ISOPROTERENOL AND cAMP REGULATE THE ACTIVITY OF A K^+- CHANNEL IN AtT-20 CELLS. Martha C. Nowycky, Dept. Anatomy, Med. Coll. Penn., Phila., PA. 19129

AtT-20 cells are a tumor line derived from mouse ant. pituitary which secrete ACTH and β-endorphin. In culture, the cells exhibit spontaneous, rhythmical action potential (AP) activity which has both Na^+ and Ca^{2+} components. β-adrenergic agonists, such as isoproterenol, stimulate ACTH secretion in part by increasing AP frequency and Ca^{2+} influx. These effects may be mediated through the cAMP-dependent protein kinase system. Here, I describe a class of K^+ selective channels which may play a role in terminating the Na-dependent AP, and thus may influence AP frequency. Experiments were performed with cell-attached and excised patch recording techniques. In excised patches, the channels have a slope conductance of ~ 22 pS and an apparent reversal potential of ~- 80 mV (pipette solution: Tyrode with 3 mM K^+, bath solution: 140 mM KAspartate). Channel openings are first detected at -30 mV and increase with more positive test potentials (TP). At TP = +40 mV, the averaged current peaks at about 3 msec and then partially inactivates during a 70 msec test pulse. The channel kinetics are very complex with frequent "null" or empty sweeps interspersed among periods of intense activity at all TPs and elicited from a broad range of holding potentials. These characterisitics most closely resemble those of the "FK" channel of chromaffin cells (Marty, A. & Neher, E., J. Physiol. 367, 117-141, 1985).

Application of isoproterenol to the bath while recording in the cell-attached mode decreased the average activity of the channels. Preliminary results suggest that in sweeps which contain channel openings, the kinetic behavior of the channel is similar before and after drug treatment. The reduced average activity apparently results from an increased number of null sweeps. Application of cAMP (1 mM) to the bath while recording with excised, inside-out patches produced a similar decrease of average activity. Application of AMP-PNP, a competitive inhibitor of ATP, caused a large increase in channel activity in ~50% of the patches. These results suggest that part of the molecular pathway of β-adrenergic enhanced secretion in AtT-20 cells may involve inhibition of a specific K^+ conductance. Furthermore, excised patches from these cells may contain the molecular machinery necessary for transduction of the cAMP effect.

M-Pos30 SPONTANEOUS, TRANSIENT K^+ CURRENTS IN DISSOCIATED SMOOTH MUSCLE CELLS FROM CAT ESOPHAGUS ARE SUPPRESSED BY ACETYLCHOLINE. Stephen M. Sims, Joshua J. Singer and John V. Walsh Jr. Dept. of Physiology, Univ. of Massachusetts Medical School, Worcester, MA 01605.

The tight-seal whole-cell recording technique was used to study membrane currents of smooth muscle cells freshly dissociated from cat esophagus. When cells were held positive to -70 mV, transient, spontaneous outward currents (SOCs) were observed, as large as 500 pA at 0 mV. Two observations indicate that the SOCs were carried by K^+. First, they were depressed in a dose-dependent and reversible manner by tetraethylammonium. Second, with $[K^+]_{out}$ = 40 mM, they reversed direction from outward to inward at -30 mV, the calculated equilibrium potential for K^+. In 40 mM K^+ these currents decreased in amplitude as the potential was made increasingly more negative than -30 mV, even though the driving force on K^+ was increasing. This finding suggests that the conductance underlying the SOCs is voltage-dependent.

Following demonstration that acetylcholine (ACh) elicited a contraction in these cells, we investigated its effects on SOCs. Under voltage clamp conditions, ACh suppressed the SOCs in a reversible manner. Under current clamp conditions, spontaneous hyperpolarizations, presumably corresponding to the SOCs, were abolished by ACh, which also caused depolarization. Thus cholinergic excitation of these mammalian cells involves the suppression of a K^+ conductance. In this general respect cholinergic excitation resembles that found previously in amphibian cells where ACh suppresses M-current (J. Physiol. 367:503 1985). Suppression of one or more K^+ currents may be a common feature of muscarinic excitation in a variety of types of smooth muscle.

Supported by NSF DCB-8511674 and NIH DK 31620

M-Pos31 ISOPROTERENOL ACTIVATES OUTWARD CURRENT THAT IS SUPPRESSED BY ACETYLCHOLINE IN FRESHLY DISSOCIATED SMOOTH MUSCLE CELLS. Stephen M. Sims, John V. Walsh Jr. and Joshua J. Singer. Dept. of Physiology, Univ. of Massachusetts Medical School, Worcester, MA 01605.

Effects of the ß-adrenergic receptor agonist isoproterenol were studied on single smooth muscle cells from the stomach of the toad Bufo marinus. Both microelectrodes and patch pipettes were employed for voltage clamp study of macroscopic currents. Isoproterenol (ISO) and acetylcholine (ACh) were applied by pressure ejection from micropipettes of 1-2 μm tip diameter (50 or 100μM in the application pipettes). Previous studies (J. Physiol. 367:503. 1985; Am. J. Physiol, 251:C580 1986) revealed that ACh, muscarine or substance P suppress a K^+ current, termed M-current, whose voltage sensitivity leads to characteristic inward current relaxations at the onset of hyperpolarizing voltage jumps, and outward current relaxations at their offset. An outward current, qualitatively resembling M-current was induced following application of ISO, as indicated by greater constant outward current at depolarized potentials and larger current relaxations in response to voltage jumps. In some cells, little or no M-current was initially evident. In these cases ISO caused the appearance of outward current again resembling M-current. In all instances, these outward voltage-dependent currents could be completely suppressed by ACh. These observations indicate either that there are two populations of channels that are sensitive to ACh, one activated by ISO and the other not, or that ISO activates the same population of K^+ channels that are responsible for M-current. Recovery from suppression by ACh was observed without a second application of ISO, suggesting that ACh blocks, rather that reverses, the effects of ISO.

Supported by NSF DCB-8511674 and NIH DK 31620

M-Pos32 TOPOGRAPHY OF THE ACETYLCHOLINE RECEPTOR BINDING SITES REVEALED BY FLUORESCENCE ENERGY TRANSFER. Jeffrey M. Herz, David Johnson, R. Dale Brown and Palmer Taylor, Div. Pharmacology, Dept. of Medicine, Univ. of Calif. at San Diego, La Jolla, CA 92093 and Div. of Biomedical Sciences, Univ. of Calif., Riverside, CA 92521.

Fluorescent labeled agonist, antagonist, α-toxin and noncompetitive inhibitor (NCI) ligands have been utilized to map the distances between the agonist/antagonist and allosterically-coupled NCI site and between the two agonist sites. Dansyl-C_6-choline (agonist) and NBD-bisquatenary acyl-choline (antagonist) were employed as fluorescence energy donors. Ethidium, a specific probe of the NCI site, was used as the energy acceptor for both donors. Energy transfer was measured in steady-state titrations. Donor quenching was observed for both ligands in the presence of ethidium. No energy transfer was observed if the agonist/antagonist sites were blocked by α-toxin or excess carbamylcholine. The competitive displacement of ethidium the NCI site by phencyclidine eliminated energy transfer. There was a linear increase in transfer efficiency as a function of acceptor occupancy. The transfer efficiency is 30-40% for both donor-acceptor pairs, and from knowledge of the axial depolarization factors, intersite distances have been calculated. Decidum, a novel fluorescent bisquaternary analog of decamethonium, was characterized as an metaphilic anatagonist. It was used as an energy acceptor from mono-fluorescein labeled α-toxin. Fluorescence lifetime analysis of the donor indicated that no site specific energy transfer occurs between these ligands bound to the agonist/antagonist sites. Thus, these fluorophores must be separated by more than 49Å. From consideration of the known molecular dimensions of the receptor these studies suggest that the NCI site is located close to or in the plane of the membrane bilayer while the agonist sites are near the outer periphery of the receptor. Grant GM 24437 and AHA CA86-S10 (JMH).

M-Pos33 **ISOLATION AND CHARACTERIZATION OF THREE SITE-SPECIFIC FLUORESCEIN-LABELLED COBRA α-TOXINS.** **David A. Johnson and Rosemary Cushman*, Div. of Biomedical Sciences, U.C., Riverside, CA 92521.**

We labelled cobra α-toxin (Naja naja siamensis 3) with near stoichiometric quantities of fluorescein isothiocyanate (FITC). To reduce selective labelling of the $^{\epsilon}$N-lysine 23, the α-toxin was modified with citraconic anhydride before labelling with FITC. The citraconic anhydride was later removed with strong acid, and six mono-FITC-labelled α-toxin bands were resolved on preparative immobilized-pH-gradient isoelectric focusing gels. Three of the monoconjugates (Bands 1, 2, and 6) were present in sufficient amounts to be quantitatively electrophoresed from the gel and separated from the gel contaminants by high pressure liquid chromatography. Based on the sequence analysis of the first 23 amino acids, Bands 1 and 2 were not labelled at the N-terminus, lysine 12, or lysine 23. Band 6 was labelled at lysine 23 based on its comigration with a previously prepared $^{\epsilon}$N-FITC-lysine 23-α-toxin. Binding of bands 1, 2 and 6 to the membrane associated acetylcholine receptor (AChR) is associated with -7%, -28% and +140% change in fluorescein fluorescence, respectively. The bimolecular association rate constants for binding to the AChR are 2.7, 4.3, and 1.3 x 10^3 $M^{-1}s^{-1}$, respectively. Iodine quenching of steady state fluorescence and fluorescence lifetime analysis indicate that the fluorophores associated with each of these three FITC-toxin monoconjugates are solute accessible either when the monoconjugates are free in solution or bound to the AChR. The implications of these results for the disposition of the cobra α-toxin on the surface of the AChR will be discussed.

(Supported by US Army Medical Research and Development Command, contract No. DAMD 17-84-C-4187.)

M-Pos34 **PROTEOLYTIC FRAGMENTATION AND SEQUENCING OF THE ACETYLCHOLINE RECEPTOR FROM TORPEDO USING MASS SPECTROMETRY.** C.R. Moore, J.R. Yates, D.F. Hunt and D.S. Cafiso. Department of Chemistry and Biophysics Program, University of Virginia, Charlottesville, Va. 22901.

We have purified the Acetylcholine Receptor by affinity chromatography and reconstituted it into Egg Phosphatidylcholine vesicles at lipid to protein molar ratios of 400:1. We utilized tandem mass spectrometry (1) to sequence the proteolytically accessible regions of the AcChR in both the reconstituted and detergent-solubilized states, using trypsin and Staphylococcus aureus V8 protease. Preliminary results indicate that fragmentation occurs in many regions of the AcChR, with positive identification of the following sequences: α 60-66,110-117,171-188,403-416,437-443; β 158-170, 332-336, 395-402; γ 26-36, 76-81, 332-340; δ 74-81, 332-337, using the numbering system of Stroud and Finer-Moore (2). Some of these fragments correspond to portions of the proposed amphipathic helix. Our present work is aimed at characterizing AcChR structure in systems where the sidedness and orientation of the protein are more definitively established, as reconstitution at these lipid to protein ratios has been shown to result in some formation of sheets of AcChR (3). We are also characterizing AcChR structure under functional reconstitution conditions. This information is relevant in investigating the transmembrane topography of the AcChR as well as its tertiary structure. [This work was supported by NSF grant BNS 8604101]

1. PNAS 83:6233-6237 (1986). D.F.Hunt, J.R.Yates III, J. Shabanowitz, S.Winston, and C.R.Hauer.
2. Ann.Rev.Cell Biol. 1985. 1:317-51. Robert M. Stroud and Janet Finer-Moore.
3. Biochemistry 25:5809-5818 (1986). J.P.Earnest, H.P.Limbacher, Jr., M.G.McNamee, and H.H.Wang.

M-Pos35 MOUSE-TORPEDO ACETYLCHOLINE RECEPTOR SUBUNIT HYBRIDS EXPRESSED IN XENOPUS OOCYTES: EQUILIBRIUM PROPERTIES. K. Yoshii, L. Yu, K. Mixter-Mayne, N. Davidson, and H. A. Lester. Div. of Biology, Caltech, Pasadena 91125.

Messenger RNA encoding each subunit from both species is transcribed in vitro by SP6 polymerase from cDNA clones. All 16 possible combinations that include one mRNA for each of a, β, γ, and δ are injected into oocytes. After 2-3 d, we assay each oocyte for (1) receptor assembly, measured by α-bungarotoxin binding to the oocyte surface; and (2) ACh-induced conductances (g_{ACh}), measured by voltage-clamp I-V relations. All combinations yield detectable assembly and g_{ACh}. Among oocytes injected with the same combination, assembly and g_{ACh} vary proportionally over a > 10-fold range; thus the fractional activation (μS/fmol) has a coefficient of variation only ~ 0.3. Average binding ranges from 0.39 ($\alpha_T\beta_M\gamma_M\delta_T$, abbrev. TMMT) to 11.5 (TMTM) fmol/oocyte. γ_T gives greater assembly than γ_M for all 8 pairs of combinations where the other 3 subunits are constant; likewise for δ_M (6 of 8). Hill coefficients are near 2 in all cases. Responses are compared by noting the [ACh] that induces 1 μS/fmol at -60 mV, corresponding to a fractional opening probability of ~ 2×10^{-5}. This [ACh] ranges from 95 nM (MTMM) to 13.5 μM (TMTT); α_M and β_T give lower values (6 of 8, 7 of 8). Voltage sensitivity, measured as (g_{ACh}, -100 mV)/(g_{ACh}, +20 mV), ranges from 1.0 (TTTT) to 16 (MMMT); β_M gives the highest values (8 of 8). Thus receptor assembly, fractional activation, and voltage sensitivity are governed by different properties. Supported by NS-11756 and GM-10991.

M-Pos36 KINETICS OF CHOLINERGIC CHANNEL ACTIVATION IN XENOPUS MYOCYTES. Anthony Auerbach and Christopher Lingle, Department of Biophysics, SUNY, Buffalo, NY, and Department of Biological Sciences, Florida State University, Tallahassee, FA.

We have examined the kinetics of the predominant forms of large (g_{60}) and small (g_{40}) conductance cholinergic channels in stage 18-21 Xenopus myocytes kept in culture ~24 hrs at room temperature. Single ACh-activated channels from cell-attached patches were studied over the [ACh] 40 nM to 200 uM (24°C, -120 mV). For each form of channel, we determined a set of molecular rate constants (assuming the standard four state linear model) which was consistent with our data over the entire range of [ACh].

g_{40} and g_{60} channels differ in their gating rates. The channel opening rate for larger conductance channels (~50,000 s^{-1}) is almost 10 times that of smaller conductance channels (~6000 s^{-1}). The channel closing rate is also about 10 times faster in g_{60} (~4000 s^{-1}) compared to g_{40} channels (~300 s^{-1}). The equilibrium constants for gating for both forms of channel are about the same.

g_{40} and g_{60} receptors differ in their affinity for ACh. ACh binds to g_{60} receptors with an equilibrium dissociation constant (K_d) of ~45 uM; there is no indication of cooperativity in binding. ACh binds to g_{40} receptors with about a 3-fold higher affinity. Assuming no cooperativity in binding, we estimate that the microscopic K_d for g_{40} receptors is ~15 uM. The g_{40} data is somewhat better described by allowing positive cooperativity in binding (K_{d1}=65 uM, K_{d2}=7 uM).

Supported by grants from MDA, the Whitaker Foundation, and NSF.

M-Pos37 A NEW PICTURE FOR THE STOCHASTIC FUNCTIONING OF ACETYLCHOLINE RECEPTORS. A NEW METHOD FOR THE ANALYSIS OF MULTIEXPONENTIAL FUNCTIONS WHICH DOES NOT REQUIRE A PRIORI HYPOTHESES. E. Yeramian[+], P. Claverie[‡] & A. Trautmann[§] (Intr. by R. Horn). [+]Institut Pasteur, Dépt de Biol. Mol., 75724 PARIS CEDEX 15, [‡]Dyn. Intér. Moléc., Univ. PARIS-VI, [§]Neurobiol., E.N.S., Paris (France).

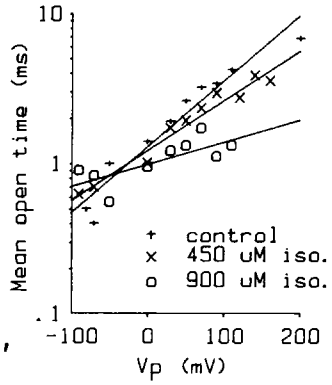
We have shown that resorting to stationary probabilities could be misleading for assessing whether ionic channels are mutually independent or not (one-time properties analyzed in terms of binomial or Poisson distributions). To address this general methodological question we have derived criteria based upon the short-time behavior of the two-time properties, e.g. transition probabilities, and we have applied them to the acetylcholine-activated channels (patch-clamp data from rat myotubes). Our analyses led to the conclusion that the multiple openings observed in the recordings correspond to functionally coupled channels (Biophys. J. 50:253-264, 1986).

For investigating in more detail the stochastic properties underlying the functioning of populations of acetylcholine receptors we wanted to analyze the full time-range behavior of the two-time probabilities. These functions are described by sums of exponentials. We devised a new method for the analysis of such functions which is not of the statistical least-squares type but relies on the use of the direct Laplace transform of the function and of Padé approximants (hence the name "Padé-Laplace"). This method allows the detection of exponential components (real and/or complex) without any hypotheses as to the number of components nor estimates for the various parameters. Several severe limitations reported in previous methods were shown to be overcome by our approach. This very general method was applied to analyze the correlation functions from patch-clamp data, further confirming the functionally-coupled receptors scheme.

M-Pos38 **DOSE-DEPENDENT REDUCTION OF THE VOLTAGE-SENSITIVITY OF SINGLE ACETYLCHOLINE RECEPTOR CHANNEL OPEN TIME BY A GENERAL ANESTHETIC.** K.E. Flanigan, J.P. Dilger and R.S. Brett, Department of Anesthesiology, SUNY, Stony Brook, NY 11794.

Volatile general anesthetics alter the kinetic properties of voltage- and neurotransmitter-activated ion channels in excitable membranes (1-3). We are investigating the effects of isoflurane, a recently developed polyhalogenated ether now in widespread clinical use, on nicotinic acetylcholine receptors in the BC3H1 cell line. Using standard patch pipettes containing 140 mM KCl, 5 mM MgCl2, 5 mM EGTA, 10 mM HEPES, 500 nM acetylcholine and 0-900 uM isoflurane, we formed on-cell membrane patches and observed unitary currents having slope conductances between 34 and 40 pS. Currents were filtered at 3KHz and sampled at 50 us/point.

Our initial examination of data obtained from patches hyperpolarized by 50 mV showed that isoflurane shortened the mean channel open time, as anticipated on the basis of analogy to several other volatile anesthetics (2,3). Extension of the analysis through a wide range of voltages revealed that isoflurane attenuates the normal voltage sensitivity of the channel open time. Patches formed on micropipettes containing 0, 450 and 900 uM isoflurane yielded mean voltage-sensitivity measurements of 99, 131 and 295 mV, respectively, per e-fold change in mean open time (figure).



1) Haydon et al, Mol & Cell Mech of Anesth, ed: Roth & Miller, Plenum 1986, 2) Lechleiter & Gruener, PNAS 81:2929, 1984, 3) Gage & Hamill, Neurosci Let 1:61, 1975. RSB acknowledges a grant from the ASA (431-3405A).

M-Pos39 NON-COMPETITIVE INHIBITORS REACH THEIR BINDING SITE IN THE ACETYLCHOLINE RECEPTOR BY TWO PATHS. Michael P. Blanton and Howard H. Wang, Department of Biology, University of California, Santa Cruz, Ca 95064

Using electron spin resonance spectroscopy (ESR) the interaction of the spin labeled tertiary and quaternary amine local anesthetic (abbreviated C6SL and C6SLMeI, respectively) with the nicotinic acetylcholine receptor (AchR) were examined. Using native membrane preparations of *Torpedo californica* binding of the two spin-labeled probes to the AchR has been shown to be charge dependent. C6SL and C6SLMeI binding to the AchR is titratable by phencyclidine (PCP) suggesting a common binding site. Binding of the tertiary amine analog C6SL to the AchR is unaffected by agonist preincubation. In contrast binding of the quaternary amine analog C6SLMeI to the AchR is decreased by agonist preincubation in a time-dependent manner. The data is best fit by a double exponential curve expressed by the equation ($y = 85e^{-K_1 t} + 15e^{-K_2 t}$) with K_1 and K_2 being 0.19 min^{-1} and 0.03 min^{-1}, respectively where t represents the duration of agonist preincubation. We have also determined that with an agonist preincubation time of 30 minutes, binding of the quaternary amine analog to the AchR required incubation times of 1 hour or more to reach full binding. This is consistent with equilibrium binding measurements conducted by us previously (*Mol. Pharmacology* 30:243-251, 1986). We interpret our results as suggesting that the permanently charged quaternary amine analog C6SLMeI is restricted but not excluded in its ability to interact with the desensitized state (D) of the AchR while the tertiary amine analog (7.2-7.4) is able to freely interact with the desensitized receptor. We propose two pathways to the anesthetic binding site on the AchR. A carb-induced open channel pathway available to both C6SL and C6SLMeI during the time between the open channel state and the desensitized (closed) channel state. An additional pathway may involved the uncharged form of the tertiary amine analog (C6SL) diffusing into the lipid bilayer and gaining access to the ion channel via a hydrophobic pathway. The anesthetic having reached the ion channel via either pathway binds in the charged state and is pharmacologically active.

M-Pos40 EFFECTS OF THE DISULFIDE REDUCING AGENT 2-MERCAPTOETHANOL ON XENOPUS ACETYLCHOLINE RECEPTORS. J. del Castillo, L. Rojas, A. Steinacker* and C. Zuazaga. Institute of Neurobiology, U. of Puerto Rico Med. Sci. Campus, San Juan, PR 00901 and *Dept. of Otolaryngology, Washington U. Sch. of Med., St. Louis, MO 63110.

If applied together with ACh, the disulfide (S-S) reducing agent 2-mercaptoethanol (2-ME) potentiates the depolarizations induced by electrophoretically applied ACh to eel electroplaques (1) and frog muscle (2). Dithiothreitol (DTT), a more powerful S-S reducing agent, decreases and eventually abolishes those responses (1,2,3,4). Simultaneous application of 2-ME and ACh to receptors rendered unresponsive by DTT, reverses the blockade and depolarizations occur. Previous reduction by 2-ME abolishes the responses to ACh, an effect similar to that of DTT. Single channel studies on ACh-activated channels in Xenopus myocytes were performed to investigate the mechanism of these effects of 2-ME. In cell-attached and inside-out configurations, 2-ME (2-7 mM) decreased channel conductance by ca 25%. Openings were prolonged and intervals between openings were shortened. The results suggest that channel opening rate increases and that channel closing rate and/or agonist dissociation rate decreases. These changes account for the enhancement of the response to ACh by 2-ME as, even if channel conductance decreases, the current evoked by a given quantity of ACh would increase. The different effects of DTT and 2-ME may be due to different sites of action or to differences in the reaction mechanism of the two reagents with S-S bonds. (Supported by NIH grants NS07464, NS14938, RR08102 and NSF grant BNS 8511022). 1) del Castillo et al. PNAS 69: 2081, 1972 2) del Castillo et al. Int. J. Neurosci. 1: 199, 1971 3) Karlin & Bartels BBA 126: 525, 1966 4) Ben Haim et al. Pflügers Arch. 355: 19, 1975.

M-Pos41 TIME-DEPENDENT BINDING OF PHOSPHONIUM IONS TO THE ACETYLCHOLINE RECEPTOR. S.C. Hartsel, C.R. Moore, D.E. Raines and D.S. Cafiso, Department of Chemistry, University of Virginia, Charlottesviile, VA 22901.

In receptor rich vesicles isolated from Torpedo, paramagnetic or fluorescent phosphonium ions bind to both the acetylcholine receptor (AcChR) and the receptor membrane. When added to receptor vesicles, 2 to 3 phosphoniums undergo a slow time-dependent binding to the AcChR. Approximately one phosphonium per receptor can be displaced by the addition of saturating concentrations of the high affinity histrionicotoxin derivative, isodihydrohistrionicotoxin, or by the addition of phencyclidine or quinacrine mustard. When a series of alkylphosphonium ions was studied, it was found that the rate of phosphonium binding to the receptor decreased with increasing hydrophobicity. This appears to be a function of the quantity of probe in the aqueous phase. The presence of agonist increases the rate but not the total number of probe binding sites. The phosphonium ions used here promote desensitization of the receptor as judged by the binding rate of the fluorescent agonist NBD-5-acylcholine or α-bungarotoxin. Preincubation of the receptor with isodihydro-histrionicotoxin virtually eliminates the phosphonium mediated desensitization. The phosphonium binding and agonist induced transitions observed here are not observed with a negative hydrophobic ion probe, or a negative surface amphiphile, indicating that changes in membrane electrostatics are not the source of the changes observed here. Thus, these labeled phosphoniums belong to a class of non-competitive blockers that bind to a "high affinity" blocking site and desensitize the receptor.

M-Pos42 AGONIST SELF-INHIBITION AT THE NICOTINIC ACETYLCHOLINE RECEPTOR: ADDITIVE EFFECTS OF OTHER INHIBITORS Stuart A. Forman, Leonard L. Firestone & Keith W. Miller, Departments of Pharmacology and Anesthesiology, Harvard Medical School and Massachusetts General Hospital, Boston, MA.

Agonist concentration-response curves for net integrated $^{86}Rb^{+}$ efflux have been measured in acetylcholine receptor-rich native Torpedo membrane vesicles. When the concentrations of acetylcholine, carbamylcholine, suberyldicholine, phenyltrimethylammonium, and (-)-nicotine were increased over a 10^{8}-fold range, all produced bell-shaped response curves due to stimulation of AChR-mediated ion flux at low concentration and inhibition at high concentration. Inhibition at high agonist concentration was not the result of a general non-specific membrane perturbation because lipid order parameters, determined from ESR spectra of 12-doxylstearate incorporated into Torpedo vesicle membranes, are not changed in the presence of self-inhibitory concentrations of agonists. Instead, Hill coefficients for agonist self-inhibition are compatible with a single saturable site of action. The additive effects of self-inhibitory concentrations of agonists with procaine or octanol indicate that these antagonists may decrease the apparent affinity of the inhibition site for agonists. Procaine's inhibitory action appears to be mutually exclusive with the agonist inhibition site, while octanol probably acts allosterically to lower the apparent affinity of agonists for the inhibitory site. (Supported by GM-15904).

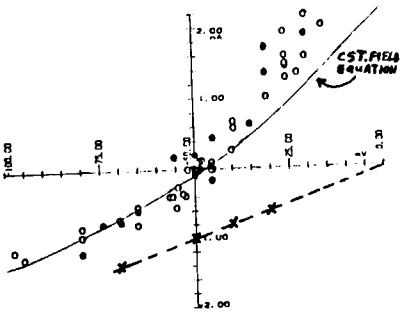
M-Pos43 EXCITATORY AMINO ACIDS INCREASE $[Ca]_i$ IN SINGLE CENTRAL NEURONES. Shawn N. Murphy, Stanley A. Thayer and Richard. J. Miller, Dept of Pharmacology & Physiology, Univ of Chicago, Chicago, Il 60637 (Spon M. Villereal).

We examined the effects of excitatory amino acids (EAA) on $[Ca]_i$ in single striatal and hippocampal neurones grown in monolayer cultures. $[Ca]_i$ was measured by taking the ratio of the 340/380 nm fluorescence intensities from the Ca sensitive dye fura-2. N-methyl-D-aspartate (NMDA), kainic acid (KA), quisqualic acid (Q), and glutamic acid (G) all caused rapid increases in $[Ca]_i$ in both striatal and hippocampal cells. None of these substances increased $[Ca]_i$ in Ca free media. In Mg free medium a maximally effective concentration of NMDA caused $[Ca]_i$ to rise from a a basal concentration of approx 40 nM to 400 nM. This effect of NMDA was completely blocked by Mg (1mM), by phencyclidine and by the NMDA antagonist AP5 The effects of NMDA were not inhibited by a concentration of La (30 μM) that inhibited the depolarization (50 mM K) induced rise in $[Ca]_i$ by >75%. Furthermore preliminary experiments show that when external Na was replaced by N-methyl-D-glucamine (NMDG) there was little change in the magnitude of the NMDA induced increase in $[Ca]_i$. These results indicate that the NMDA induced rise in $[Ca]_i$ is due to the influx of Ca through the NMDA receptor linked ionic channel. In contrast to the effects of NMDA, Mg had much less effect on the increase in $[Ca]_i$ produced by KA, Q or G. The effect of G was only partly inhibited by AP5 or Mg. Even in the presence of Mg, G caused a substantial increase in $[Ca]_i$. In Mg (1 mM), the effects of G or KA were not blocked by 30 μM La indicating that the rise in $[Ca]_i$ was not due to influx of Ca through voltage sensitive channels subsequent to depolarization. When external Na was replaced by NMDG the effect of G was partially blocked. This indicates that some of the effect of G was due to the entry of Na and may involve Na/Ca exchange. In addition however some Ca seeems to enter directly through a G activated channel which is different from the NMDA linked channel. Supported by DA 02121and MH-40165

M-Pos44 SYNAPTIC AND TOTAL CURRENTS ACTIVATED BY SEROTONIN AT A SYNAPSE BETWEEN IDENTIFIED NEURONS IN CULTURE. P.Drapeau and S. Sanchez-Armass, Neurosciences Unit, Montreal General Hospital Research Institute and McGill University, 1650 Cedar Ave., Montreal, Quebec, Canada H3G1A4.

Identified Retzius neurons reform an inhibitory serotoninergic synapse onto pressure-sensitive (P) neurons when the cells are removed from the leech and grown in tissue culture. We have voltage clamped P cells and compared the ionic currents induced by applied and synaptically released 5-HT to examine the function of receptors at newly formed synapses.

5-HT and synaptic release activated a Cl current (o in Fig.) that reversed near -50 mV,showed slight outward rectification and was not voltage-gated in the range of -10 to -100 mV. Higher Cli/Clo ratios shifted the I-V curve upfield. Single P cells superfused with an impermeant cation solution (160 Tris,5Cs,3Co,10 DAP) had a similar current in the presence of 5-HT(● in Fig.)that was blocked by 5-HT &Cl channel antagonists. High 5-HT (>100 uM) desensitized the Cl response and i) activated a faster, inward current (x in Fig.) that ii) was not synaptically activated and iii) was eliminated in the impermeant cation solution, consistent with 5-HT activation of a cation channel.



Thus, cultured P cells have two types 5-HT receptors, only one of which appears to be present at synapses.

Supported by a grant (PD) and awards fromthe MRC of Canada.

M-Pos45 VOLTAGE-DEPENDENCE OF GABA-GATED CHLORIDE CHANNELS IN CULTURED CHICK CEREBRAL NEURONS. David S. Weiss*, E.M. Barnes, Jr.+, and J.J. Hablitz*. (Intr. by Dan Johnston). Prog. in Neuroscience+*, Sect. of Neurophysiol.* and Dept. of Biochem.+, Baylor Col. of Medicine, Houston, TX.

Using the patch-clamp technique, we have been investigating the mechanism of the voltage-dependence of the gating of the GABA channel. We measured the whole cell GABA (10 μM) activated current (I_g) as a function of membrane potential (V_m) and found that the I_G/V_m plot was always nonlinear with an "outwardly-rectifying" appearance. However, the I/V relationship of individual GABA-gated Cl^- channels was found to be linear across a similar range of V_ms (conductance = 20 pS in isotonic choline-Cl^-). Knowing the single channel conductance, the number of GABA channels open at the peak of each I_G was calculated and semilogarithmic plots were constructed of channel number vs. V_m. These plots revealed that 80 ± 25 mvolts (± SD, n = 4) of depolarization yields an e-fold increase in the number of channels open at the peak of the I_G. We also held inside-out patches at various V_ms and calculated the probability of finding a single GABA channel open (P_o). Plots of P_o vs. V_m from four patches revealed that 80 ± 43 mvolts of depolarization yields an e-fold increase in P_o. Distribution of the open times of the GABA channel are best described as a sum of two exponentials: tau_f = .39 ± .14 ms, tau_s = 2.10 ± .88 ms (n = 9). V_m had no effect on the two tau values or on the relative amplitudes of the two components (n = 5). Thus, the observed nonlinearity in the whole-cell I/V relationship is due to a voltage-dependence of P_o rather than a voltage-dependence of the mean open time or single channel conductance. Possible sites of modulation by V_m within proposed kinetic models of the GABA channel will be discussed. (Supported by NS-11535, AM-17436).

M-Pos46 USE-DEPENDENT DEPRESSION IN INHIBITORY SYNAPTIC INTERACTIONS AMONG CULTURED RAT HIPPOCAMPAL NEURONS. N.L. Harrison, G.D. Lange and J.L. Barker (Intr. by Daniel L.Gilbert).
Laboratory of Neurophysiology, NINCDS, NIH, Bethesda, MD 20892.

We have combined monolayer culture of embryonic rat hippocampal neurons with simultaneous whole-cell presynaptic stimulation and postsynaptic recording to study the details of GABA-mediated synaptic inhibition *in vitro*. Patch pipettes contained gluconate in place of chloride; normal bathing medium contained 4mM Ca and 8 mM Mg. Monosynaptic inhibitory connections were often encountered, and voltage-clamp of the postsynaptic element at -40mV revealed Cl^--dependent inhibitory currents (IPSCs) that reversed at -60 to -70mV and were blocked by the GABA antagonist bicuculline.We conclude that these IPSCs are mediated by activation of postsynaptic $GABA_A$ receptors. No 'late' component of synaptic inhibition was observed. Synaptic transmission is quantal, as evidenced by random failures of transmission at very low $[Ca^{2+}]_o$ (Lange *et. al.*, Soc. Neurosci. Abstr., 12, 228.11, 1986). IPSC amplitude at -40mV was stable at 0.05-0.2Hz rates of presynaptic stimulation. High frequency trains (1-10Hz) elicited the use-dependent depression (UDD) characteristic of inhibitory signals in hippocampal slices (McCarren & Alger, J. Neurophysiol., 53, 557-571, 1985). We studied this phenomenon using a paired-pulse protocol (repetition 0.1Hz., interval 100msec.), and found a UDD characterized by a 34% depression of the 2^{nd} response with respect to the 1^{st}. Application of low Ca medium (0.5mM Ca; 11.5mM Mg) reduced the amplitude of the first response by 73% and reduced the second response proportionately less so that the UDD was now only 16%. Deisz & Prince (Soc. Neurosci. Abstr., 12, 9.12, 1986) have proposed that UDD results from synaptically released GABA acting back on the presynaptic cell leading to reduced subsequent GABA release. We applied the $GABA_B$ receptor agonist (-)baclofen (10μM) which produced no postsynaptic conductance change, but reduced the IPSC amplitude by 66%. This treatment abolished UDD, suggesting saturation by (-)baclofen of the mechanism mediating reduced GABA release. We suggest that use-dependent depression is indeed presynaptic in origin. It results from the action of synaptically released GABA at $GABA_B$ receptors located on the presynaptic terminals which leads to reduced calcium current (Dunlap, Br. J. Pharmacol.,74, 579-585, 1981) and hence to reduced transmitter output.

M-Pos47 ZINC IS A POTENT BLOCKER OF THE NMDA-ACTIVATED CONDUCTANCE ON HIPPOCAMPAL NEURONS. M.L. Mayer and G.L. Westbrook, Lab. Developmental Neurobiol., NICHD, NIH, Bethesda, MD 20892. (Introduced by C. Colton).

NMDA-receptor channels show open channel block with Mg^{2+} at physiological concentrations and with Co^{2+}, Mn^{2+} & Ni^{2+} applied experimentally. Ca^{2+}, Ba^{2+} and Sr^{2+} also reduce the NMDA-activated conductance at membrane potentials negative to 0 mV, but in addition produce a depolarizing shift of the reversal potential, reflecting permeation of these ions through NMDA-receptor channels. The interaction of Ca^{2+} and Mg^{2+} with NMDA receptor channels is of significance for the physiological role of these channels in the vertebrate CNS. The action of transition metal cations on responses to excitatory amino acids has not been examined. In view of the high concentration of Zn^{2+} within the mammaliam hippocampus, and its release during excitatory synaptic transmission from mossy fiber afferents, we have examined the action of the group IIB transition metal ions Zn^{2+}, Cd^{2+} and Hg^{2+} on the excitatory amino acid sensitivity of mouse hippocampal neurons in dissociated culture. Experiments were performed using whole cell voltage clamp recording.

We find Zn^{2+} to be a specific antagonist of responses to NMDA, with no blocking action on responses to kainic or quisqualic acids or to GABA. This action of Zn^{2+} occurs with concentrations of 5-10 μM, and is clearly not a competitive antagonism. However unlike the block produced by either Mg^{2+}like or Ca^{2+}-like divalent cations, the action of Zn^{2+} shows no voltage sensitivity. Cd^{2+} (up to 100 μM) has previously been found to have no action on responses to NMDA, however at mM concentrations Cd^{2+} also produces a Zn^{2+}-like block. The action of Zn^{2+} and Cd^{2+} does not appear to reflect an action at -SS- bonds, since Hg^{2+} is without effect on responses to NMDA.

M-Pos48 IONIC CHANNELS IN NEUROSECRETORY GRANULES FROM RAT NEURAL LOBE. José R. Lemos, Karen A. Ocorr, and Jean J. Nordmann. Worcester Foundation for Experimental Biology, 222 Maple Ave., Shrewsbury, MA 01545.

It is possible to separate and isolate on iso-osmotic gradients neurosecretory granules (NSG) from the rat neurohypophysis or neural lobe. We have been able to reconstitute neurosecretory granule membrane proteins and study their activity using tip-dip methods. The purity of such preparations makes possible the analysis of neurosecretory granule ion fluxes.

We have observed an anion channel in membranes prepared from isolated neural lobe neurosecretory granules. In asymmetrical salt gradients the channel appears to be permeable only to anions, such as Cl^-. This neurosecretory granule anion channel in symmetrical 200 mM KCl has a slope conductance of about 30 pS. The channel opens even in the presence of only 10^{-8} M free internal [Ca^{+2}] and shows little voltage-dependence. Furthermore, Ca-dependent hormone release is inhibited by anion channel blockers, such as SITS, from digitonin-permeabilized nerve terminals of the neural lobe. Characterization of this and other possible NSG channels is currently under way.

This data, and the previous report (Stanley et al, 1986, Bioph.J. 49:19a) that a Ca-activated K-channel exists in neurosecretory granule membranes, lends support to the theory that Ca^{+2} entry could lead to swelling of the NSG and thus promote fusion with the plasma membrane and release of the neurosecretory granule contents. Supported by NINCDS grant NS22542.

M-Pos49 PROPERTIES OF KAINATE ACTIVATED CURRENTS IN XENOPUS OOCYTES INJECTED WITH mRNA FROM RAT CEREBELLUM AND STRIATUM. J.C.R.Randle, E.Brault, P.Vernier, C.Batini* and R.T.Kado. Laboratoire de Neurobiologie Cellulaire et Moléculaire, CNRS Gif sur Yvette, France and Laboratoire de Neurophysiologie Pharmacologique U 191 INSERM, 2, rue de Alesia, Paris, France.

Injection of mRNA isolated from rat brain into oocytes of Xenopus laevis has been shown to induce sensitivity to the glutamate analog, kainate. Membrane currents observed when kainate is applied to voltage-clamped oocytes are presumably due to the activation of receptor-ionophore complexes that have been synthesized by the oocyte from the rat brain mRNA template and then installed in the membrane. Oocytes were recorded under two electrode voltage clamp conditions beginning two days after injection of the striatal or cerebellar mRNA. The permeability was examined by testing the response to Kainate in OR2 medium in which equimolar replacements of NaCl with other salts had been made.

Kainate (10^{-3} to 10^{-2}) induced a smooth inward current whose time-course followed that of the drug in the bath: no desensitization was observed during 2mn of application. Double logarithmic plots of peak current vs kainate concentration had slopes of 1.6+ 0.13 (n=6) for cerebellar mRNA and 1.9+ 0.05(n=3) for striatal mRNA. The current activated by kainate reversed at -4.5+ 0.2(n=18) mV. The current-voltage relation in oocytes injected with striatal mRNA was nearly linear at V_m positive to -100 mV. Oocytes injected with cerebellar mRNA showed a reduction in outward current at V_m positive to -20 mV. Kainate channels distinguished poorly between alkali earth metals; the permeability sequence in oocytes injected with cerebellar mRNA was Cs:Rb:K:Na:Li, for striatal mRNA; K:Rb:Cs:Na:Li. NH_4 was approximately 2 fold more permeant than Na and large cations such as tetramethylammonium, tetraethylammonium, choline and Tris were 4 to 12 fold less permeant than Na. Divalent cations Ca, Sr, Ba and Mg were also highly permeant.

These data indicate that the channels activated by kainate in oocytes injected with rat brain messenger RNA are large, water-filled pores similar to the cholinergic endplate channel. Consistent small differences in the behavior of the receptor-ionophore complexes synthesized from the different messengers suggests: 1) the existence of two subtypes of kainate receptors and/or 2) tissue specific differences in transcription, translation or post-translational processing of the product of a single gene.

M-Pos50 ASSOCIATION AND INTERACTION BETWEEN SYMPATHETIC NEURONS AND MAST CELLS IN VITRO.

M.G. Blennerhassett, R.H. Stead and J. Bienenstock, Pathology Department, McMaster University Health Sciences Centre, Hamilton, Ontario, Canada (Intr. by M.S. Kannan).

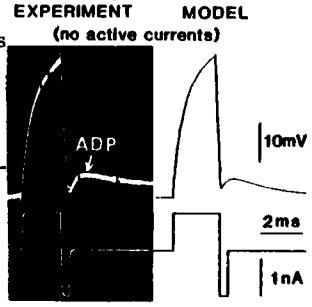
We developed a tissue culture model for the study of interactions between sympathetic nerves and mast cells (MC) in vitro, since the close association of peripheral nerve endings with MCs in vivo suggests functional innervation or trophic interactions may occur. Explants or isolated neurons from superior cervical ganglia (SCG) of neonatal mice were cultured with nerve growth factor. Spontaneous electrical activity and active responses to current injection were recorded from explants by 24 hours, but only from single neurons at ≥14 days in vitro as their membrane potential (E_m) hyperpolarized from -40 to -55mV. RBL-2H3 cells, a cell line considered homologous to mucosal MCs, were added to SCG cultures at 10^4 cells/dish, and time-lapse photomicrography and scanning electron microscopy used to study their interaction with advancing nerve processes. Contacts with RBL cells were quickly formed and were maintained over hours. Commonly, initial contacts resulted in branching so that a short extension terminating on an RBL persisted as nerve elongation continued. This contrasted with the normal linear outgrowth seen with no cell contact, of if contact occurred with fibroblasts or glial cells. Intracellular recordings showed that the E_m of RBL cells (-11.0±3.1 SD (38)mV) was similar to that of freshly isolated MCs: rat mucosal MCs, -11.2±3.1 (10)mV; peritoneal MCs, -15±5 (25)mV; dog lung MCs, -13.1±4.3 (24)mV. Further, the input impedance and membrane resistivity of RBL cells and mucosal MCs were similar (51±20 (26)MΩ; 300Ωcm). This suggests that a selective and preferential association between sympathetic nerves and mast cells is maintained in culture. (Supported by MRC of Canada).

M-Pos51 A PASSIVE COMPONENT OF THE AFTERDEPOLARIZATION (ADP) IN RAT HIPPOCAMPAL PYRAMIDAL CELLS.

J.F.Storm, L.Borg-Graham and P.R.Adams, Dept. of Neurobiology & Behavior, SUNY, Stony Brook, NY 11794, and Center for Biological Information Processing, MIT, Cambridge, MA 02139.

In pyramidal neurons, the action potential is followed by an ADP which may trigger extra spikes and contribute to bursting and epileptic activity. In the hippocampus, it has been suggested that the ADP consists of a monotonically decaying passive component,and a hump-like active component due to Ca and/or Na-currents. Here we report experimental and computational evidence that the ADP-hump is at least partly passive, due to redistribution of charge from the dendrites to the soma.

Experiments. CA1 cells were impaled with KCl electrodes in slices. The spike, elicited by current injection, was followed by a fast afterhyperpolarization (fAHP; due to the fast Ca-dependent K-current I_C; Neurosci.Abstr.16:764) and an ADP. When fAHP was blocked by Mn, the ADP-hump disappeared, but was restored when a 0.5ms hyperpolarizing pulse was used to substitute for fAHP. Even when all active currents were blocked (using Cs-electrodes and external TTX, Mn, Cd, TEA, Cs, Ba, 4-AP), an ADP-hump followed when a depolarizing/hyperpolarizing pair of current pulses were injected (to substitute for the spike and fAHP; see figure).



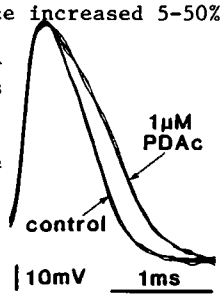
Computer simulations. Using a model based on electrophysiological and anatomical data from CA1 cells (lumped soma with a dendritic cable), a passive ADP-hump was reproduced in response to the stimuli described above. This ADP was due to redistribution of charge from dendrites to soma, following charging of the dendritic capacitance by the spike.[Supported by a Fogarty international fellowship and by NIH grant NS18579.]

M-Pos52 PHORBOL ESTERS (PEs) BROADEN THE ACTION POTENTIAL IN RAT HIPPOCAMPAL PYRAMIDAL NEURONS.

J.F.Storm, Dept. of Neurobiology & Behavior, SUNY, Stony Brook, NY 11794.

Long-term potentiation (LTP) of hippocampal synaptic transmission is associated with translocation of protein kinase C (PKC)[Akers & al,Science 231:587] and PEs which activate PKC, mimic LTP [Malenka & al., Nature 321:175]. A possible mechanism for synaptic potentiation is presynaptic spike-broadening, like the one mediated by protein kinase A during learning in Aplysia. Here I report that PEs broaden the action potential in CA1 pyramidal cells in hippocampal slices.

Cells were impaled with KCl electrodes, and spikes elicited by brief current pulses. In all 15 cells tested, 4β-phorbol-12,13-diacetate (PDAc;0.3-10μM) or 4β-phorbol-12,13-dibutyrate (PDBu;5-10 μM) reduced the rate of spike repolarization, while the spike upstroke and amplitude were essentially unaffected. The slow afterhyperpolarization was blocked, and the input resistance increased 5-50% In contrast to PDAc or PDBu, 4β-phorbol or 4β-phorbol-12,13-didecanoate, which do not activate PKC in other systems, were ineffective. Although the fast Ca- and voltage-dependent K-currents I_C and I_A contribute to spike repolarization in CA1 cells [Storm, Neurosci.Abstr.16:764], these currents did not appear to be substantially reduced by PEs, since (1) the fast afterhyperpolarization (which is due to I_C) was not blocked; (2) 1mM tetraethylammonium, which selectively blocks I_C, broadened the spike further after 5μM PDAc; (3) PEs did not block an I_A-dependent ramp potential; (4) 4-aminopyridine (4-AP;0.1mM), which blocks I_A, broadened the spike after PDAc; (5) PDAc had effect after 4-AP. Other possible mechanisms for the spike-broadening effect of PEs are reduction of I_K, I_{leak} or $I_{Cl(V)}$, or enhancement of I_{Ca}.



Supported by a Fogarty international fellowship and NIH grant NS18579.

M-Pos53 **ARE Ca-ACTIVATED K CHANNELS INVOLVED IN NEUROTRANSMITTER RELEASE?** George J. Augustine, Milton P. Charlton and Richard Horn. Dept. Biological Sciences, Univ. Southern California, Dept. Physiology, Univ. Toronto, Dept. Physiology, UCLA and MBL, Woods Hole.

Stanley & Ehrenstein (Life Sci. 37:1985, 1985) have proposed that Ca-activated K channels are present in synaptic vesicles and mediate Ca-dependent transmitter release at synapses. We have attempted to test this hypothesis by asking whether two known blockers of Ca-activated K channels, tetrapentylammonium (TPA) and Ba^{2+}, affect transmitter release at the squid 'giant' synapse. These agents were microinjected into the giant presynaptic terminal via iontophoresis while postsynaptic electrical responses were used as assays of transmitter release. TPA blocked transmitter release evoked by presynaptic depolarizations, but, as we have reported (Biol. Bull. 171, in press), simultaneously reduced voltage-gated Ca currents in the presynaptic terminal. Synaptic transfer curves, relating presynaptic Ca currents elicited by varied depolarizations to resultant postsynaptic responses, were measured before and after TPA injections. These curves were virtually superimposable, indicating that TPA has little effect upon Ca-dependent release beyond blockade of Ca channels. Ba^{2+} injections transiently reduced transmitter release evoked by presynaptic action potentials but also caused a massive increase in basal transmitter release, detected by a minutes-long decrease in the postsynaptic membrane potential. Because these two effects of Ba^{2+} injection had similar time courses, we propose that Ba^{2+} may decrease evoked release by depleting quanta available for release. In summary, none of our results provide any support for the hypothesis that Ca-dependent K channels are involved in transmitter release. The observations that TPA has little effect on transfer curves and that Ba^{2+} increases basal release are contrary to the predictions of the hypothesis. Supported by NIH grants to GJA and RH and an MRC (Canada) grant to MPC.

M-Pos54 **FURA-2 IMAGING OF LOCALIZED CALCIUM ACCUMULATION WITHIN SQUID 'GIANT' PRESYNAPTIC TERMINALS.** Stephen J Smith, Luis R. Osses and George J. Augustine (Intr. by Lou Byerly). Howard Hughes Medical Institute and Molecular Neurobiology Section, Yale Univ. Medical School, Dept. Biological Sciences, Univ. Southern California and Marine Biological Laboratory, Woods Hole.

We have used the dye Fura-2, combined with fluorescence imaging microscopy, to examine the spatial distribution of intracellular Ca ion concentration ($[Ca^{2+}]_i$) changes following stimulation of squid presynaptic terminals. Fura-2 was iontophoretically microinjected into presynaptic terminals to final concentrations on the order of 100 uM, levels which reduced, but did not eliminate, transmission elicited by presynaptic action potentials. $[Ca^{2+}]_i$ changes were induced by trains of 25 action potentials delivered at 100 Hz. and detected by fluorescence emission (> 520 nm) decreases associated with excitation at 380 nm and increases associated with excitation at 340 nm. Immediately following an action potential train, fluorescence changes were restricted to the side of the presynaptic terminal facing the synaptic cleft (i.e. the part of the terminal closest to the postsynaptic cell). However, 500 ms after such trains the fluorescence changes had spread uniformly throughout the 40-50 um width of the terminal. From these observations we conclude that Ca channels are restricted to the portion of the presynaptic membrane near the synaptic cleft and, thus, that the channels co-localize with sites of neurotransmitter release. The rapid redistribution of fluorescence changes following stimulation, presumably due to diffusion of Ca within the presynaptic terminal, should permit calculation of the diffusion coefficient for Ca but will require taking Fura-2 diffusion rates into account. Supported by Howard Hughes Medical Institute, Whitaker Foundation and NIH funds to SJS, a Fogarty Fellowship to LRO and NIH grant NS-21624 to GJA.

M-Pos55 THEORETICAL MODEL FOR BURSTING PACEMAKER NEURONS. Teresa Ree Chay, Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260.

The Eyring multi-barrier rate theory has been useful in the analysis of membrane ionic currents, since it is based on a microscopic theory and consequently has wider applicabilities than a Hodgkin-Huxley type model. A model based on the rate theory has been developed for the bursting pacemaker neurons. This model contains fast spike generating Na^+ and K^+ currents, in addition to a voltage-gated Ca^{2+} current which is inhibited by intracellular calcium ions. The intracellular calcium concentration was treated as a dynamic varaible, where influx of extracellular calcion ions is achieved by the Ca^{2+} channel and the efflux process is achieved by Ca^{2+}-ATPase activity. The model is seen to give a very good quantitative account of the many of experimental results reported in these neurons. In addition, it generates chaotic signals in response to variation in extracellular ionic compositions, certain chemicals, and the strength of applied depolarizing current.

M-Pos56 COMPARISON OF ANTICONVULSANT ACTION IN VOLTAGE & CURRENT CLAMP. J.M. Rendt and R.E. Furman*. Depts. of Physiology and Neurology*, Univ. of Pennsylvania, Philadelphia, PA.

The effects of the anticonvulsants 5,5-diphenylhydantoin (DPH) and carbamazepine (CBZ) on the electrical activity of aminopterin-treated N18 mouse neuroblastoma cells was examined with whole cell voltage and current clamp.

Under whole cell current clamp neuroblastoma cells respond to 2 ms depolarizations with TTx-inhibitable AP's with 25-30 ms durations at half amplitude. Both DPH and CBZ (80 uM) decreased action potential overshoot, maximum dV/dt, and repolarization rates. Under voltage clamp (V_h=-90) DPH and CBZ reduced peak inward sodium currents 29% and 15%, respectively, without affecting outward potassium currents. The sodium current block was highly voltage dependent. At V_h=-110 neither drug caused significant block (20-160 uM) while at V_h=-75 both drugs (80 uM) reduced the sodium current 45%.

N18 cells support trains of AP's driven by repetitive current stimuli (2 ms duration) to 20 Hz. In the presence of DPH or CBZ, the dV/dt and overshoot of the driven AP's show successively greater reductions with each AP compared to controls. Under repetitive, 25 ms voltage clamp steps (V_h=-75) to 0 mv at a 10-20 Hz rate, drug-treated cells showed a constant reduction in peak inward sodium current with successive depolarizations compared to controls suggesting that "use dependence" observed under current clamp results from cumulative, voltage-dependent block of the sodium current secondary to the increase in AP duration.

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M-Pos57 ELECTRICAL RESPONSES OF HUMAN EPILEPTIC CORTICAL TISSUE. Masukawa, L.M., Strowbridge, B.W., Kim, J., Spencer, D. D., and Shepherd, G.M. Section of Neuroanatomy, Yale University Medical School, New Haven, CT 06510.

Animal models of epilepsy have been extremely useful in studying the possible mechanisms controlling neuronal excitability. Examination of brain tissue from epileptic patients is potentially valuable to directly determine the validity of mechanisms that have been proposed. We have initiated an electrophysiological study of cortical tissue removed from patients with intractable seizures during routine surgical treatment for epilepsy.

We obtained intracellular recordings from neurons in brain slices from lateral temporal, cingulate and parietal cortex. Brain slices (400 and 700 um thick) were cut immediately after surgical removal from the patient. Within 10 minutes slices were placed in a recording chamber for eventual electrical examination. In general, neurons did not display abnormal electrical responses as reflected in spontaneous or orthodromically generated burst discharges. IPSPs were present and functional inhibition was intact as reflected by a truncation of the train of action potentials generated by a depolarizing current step. The resting electrical properties were within a normal range compared to neurons from animal tissue, i.e. a resting membrane potential of at least -60 mV, input resistance of 20 Megaohms, and a time constant of 5-10 msec. Of the tissue thus far examined,one biopsy contained neurons that responded to orthodromic stimuli with long, variable latency burst discharges. In this biopsy, IPSPs were diminished. Spontaneous synaptically driven activity was variably observed in approximate 20 % of the cases. These events were similar to those described by Schwartzkroin and Haugland (in press).

Throughout the slices studied, we have observed three types of neuronal responses comparable to those described by McCormick et. al. (1985) in guinea pig; regular firing, bursting and rapidly firing cells. Regular firing cells were found in laters 3 and 5, while bursting cells were only localized to layer 5,demonstrating a close correlation between neuronal responsiveness and location in human and guinea pig cortex. The general lack of hyperexcitability in brain slices from epileptic patients may be due to sampling of neurons from a relatively large population of normal cells; the abnormal cells may be in the minority and either not accessible to our recording technique, do not respond to the testing protocol or have not survived the slicing procedure. We are encouraged by the relative robustness of human brain slices and the similarities to animal brain tissue.

M-Pos58 ELECTROPHYSIOLOGICAL CHARACTERIZATION OF ISOLATED RAT CORTICAL NEURONS. N.W. Davies, M. Morad, R.A. Deisz and H.D. Lux. Max-Planck-Institute for Psychiatry, 8033 Planegg, FRG. Neurons were enzymatically dissociated from slices (500 μm) of neocortex of 10 day old rats in normal bicarbonate Ringer using a modified trypsin procedure (Huguenand et al., J. Neurophysiol. 56, 1986). Majority of cells were either round or pear-shaped, but 5-20 pyramidal shaped neurons with apical and proximal dendrites, were obtained per hemisphere. Na^+, K^+ and Ca^{2+} currents were recorded in most cells within 5 h using a whole cell patch clamp technique. Only 10% of cells survived in culture MEM-medium over night. Two types of Ca^{2+} currents similar to those described in DRG cells (Carbone & Lux, Nature 310. 501, 1984) were identified. When the membrane potential was held at -80 mV a low threshold I_{Ca} activated at -40 mV and inactivated almost fully within 60 ms. A second slowly inactivating I_{Ca} activated around -5 mV and had a peak amplitude of about 1 nA. Both channels could be blocked by Ni^{2+}, Cd^{2+} and diltiazem. Both currents were markedly suppressed when the proton concentration was raised to pH 6.7. A proton-induced Na^+ current was also recorded when the proton concentration was rapidly increased from pH 7.9 to 6.7 (Morad et al., J. Physiol., 131P, 1986). GABA induced a slowly decaying I_{Cl} which was blocked by bicuculline (50 μM). Both of these agents also reduced the voltage-gated I_{Ca}. Occasionally a second type of cell could be identified which appeared to lack I_{Ca} but had an inwardly rectifying K^+ current which was suppressed by glutamate (200 μM). Our studies show that viable cells for electrophysiological studies may be obtained from neocortex with enzymatic dissociation.

M-Pos59 POTASSIUM HOMEOSTASIS AROUND SQUID GIANT AXONS: A RE-EXAMINATION. Y. Pichon[+], N.J. Abbott[++] and Y. Larmet[+], [+]Department of Biophysics, C.N.R.S. Laboratory of Cellular and Molecular Neurobiology, F-91190 Gif-sur-Yvette (France) and [++] Department of Physiology, King's College, Strand, London WC2R 2LS (UK).

Potassium ions are known to accumulate outside the nerve membrane during nerve activity. This phenomenom is counterbalanced in vivo by several regulatory mechanisms. The nature and the efficacy of these regulatory mechanisms has been re-evaluated for two squid species: Alloteuthis subulata (A.s.) and Loligo forbesi (L.f.). The experiments were performed on healthy individuals as estimated from their appearance, their behaviour as well as their survival time in captivity (from a few days for A.s. to several months for L.f.). In a first set of experiments, giant axons of A.s. were impaled in situ in the superfused mantle and repetitive antidromic stimulation was found to have very little effect on the afterhyperpolarization or the afterdepolarization, indicating the absence of appreciable potassium accumulation. Rather unexpectedly, a similar absence of accumulation was found for freshly dissected axons from both squid species. The typical decrease of the afterhyperpolarization and increase of the afterdepolarization was observed in older preparations. This observation was confirmed quantitatively under voltage-clamp conditions in which the tail current which followed square membrane depolarizations was used as an index of potassium accumulation during the depolarization. These results suggest that, under physiological conditions, potassium accumulation is not present. This work was supported by the Royal Society and the British Council.

M-Pos60 MEMBRANE CONDUCTANCE CHANGES BY ANAPHYLACTIC STIMULATION IN RAT BASOPHILIC LEUKEMIA (2H3) CELLS. C. Barajas-López and J. D. Huizinga. Intestinal Disease Research Unit, McMaster University, Hamilton Ontario, Canada.

Involvement of membrane conductance changes and the role of extracellular Ca^{2+} in anaphylactic stimulation of mast cells and basophils is uncertain. Using conventional microelectrodes (60-120 Mohm) membrane potentials from -10 to -40 mV were recorded in 77% of the RBL-2H3 cells (n=44). The other 23% (n=13) exhibited two membrane potentials (one was -29 ± 2 mV and the higher was -70 ± 2 mV) Abrupt changes between membrane potentials were observed, occurring both spontaneously and after current injection. Current-voltage relationships were linear for outward-going current pulses and rectifying for inward-going current. Anaphylactic stimulation of the RBL-2H3 cells induced consistently a transient hyperpolarization (11.4 ± 2.1 mV; n=17) followed by depolarization, both associated with a decrease (between 15-55%) in input membrane resistance to the outward-going current. The depolarization reached 10.2 ± 2.6 mV above the control values in 9 out of 17 cells. These changes were not observed when the RBL-2H3 cells had not been previously sensitized with IgE (n=4). Above described effects of antigenic stimulation were not seen in Ca^{2+} free solution and they were mimicked by the Ca^{2+} ionophore A-23187. This suggests that the changes in ionic conductances were mediated by an increased concentration of intracellular Ca^{2+} which was dependent on extracellular Ca^{2+}. Interestingly, the anaphylactic stimulation did not have any effect when Co^{2+} (1-2 mM) was present, suggesting that a Ca^{2+} influx could be correlated with the membrane conductance changes induced by anaphylactic stimulation. This is the first report of IgE mediated conductance changes in RBL-2H3 cells. Supported by the MRC of Canada and the CFIC.

M-Pos61 POTENTIALS AND CURRENTS OF THE ACTIVE MYELINATED FIBER

N. Ganapathy and J.W. Clark, Dept. of Elec. & Comp. Engr., Rice University, Houston, TX

This paper is concerned with the accurate and rapid calculation of extracellular potentials and currents from an active myelinated nerve fiber in a volume conductor, under conditions of normal and abnormal propagation. The electrical behavior of the myelinated fiber is modeled in terms of an equivalent distributed parameter network. Solution of the partial-differential equation associated with this network provides a waveform for the spatial distribution of transmembrane potential V(z) at any instant of time. This model-generated waveform is then utilized as input to a model for the volume conductor that is based on the principles of electromagnetic field theory, and which allows one to calculate the potential and current density distribution everywhere in bathing medium surrounding the fiber. In addition, the field theoretic model may be utilized to calculate the total longitudinal current in the extracellular medium (I_L) and the transmembrane current per unit length (i_m); both quantities being defined in connection with the well-known core conductor model. The resulting currents and potentials correspond very well with experimental values reported in the literature. Paranodal demyelination at a single node of Ranvier is also simulated and its effects on potential and current waveforms as well as the conduction process are determined.

M-Pos62 EFFECTS OF AMBIENT LEVELS OF POWER-LINE-FREQUENCY ELECTRIC FIELDS ON A DEVELOPING ORGANISM. C. F. Blackman, D. E. House, S. G. Benane, W. T. Joines and R. J. Spiegel. Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711.

Fertilized eggs of Gallus domesticus were exposed continuously during their 21-day incubation period to either 50-Hz or 60-Hz sinusoidal electric fields at an average intensity of 10 Vrms/m. The exposure apparatus was housed in an environmental room maintained at 37 °C and 55-60% RH. Within 1.5 days after hatching, the chickens were removed from the apparatus and tested. The test consisted of examining the effect of 50- or 60-Hz electromagnetic fields at 15.9 Vrms/m and 73 nTrms (in a local geomagnetic field of 38 μT, 85°N) on efflux of calcium ions from the chicken brain. For eggs exposed to 60-Hz electric fields during incubation, the chicken brains demonstrated a significant response to 50-Hz fields but not to 60-Hz fields, in agreement with the results from commercially incubated eggs [Blackman et al, Bioelectromagnetics 6:1, 1985]. In contrast, the brains from chicks exposed during incubation to 50-Hz fields were not affected by either 50- or 60-Hz fields. These results demonstrate that exposure of a developing organism to ambient powerline-frequency electric fields at levels typically found inside buildings can alter the response of brain tissue to radiation-induced calcium-ion efflux. The physiological significance of this finding has yet to be established.

M-Pos63 **AN INTEGRATING PATCH-CLAMPING AMPLIFIER WITH ON-CHIP CAPACITORS AND RESET SWITCH.**
J. Prakash, D. N. Jensen, J. J. Paulos, A. O. Grant, and H. C. Strauss. (Intr. by M. Lieberman). Dept. of Elec. and Comp. Eng., North Carolina State Univ., Raleigh, NC. Dept. of Medicine, Duke Univ. Medical Center, Durham, NC.

Patch clamping is an established method for directly measuring ionic transport through cellular membranes with sufficient resolution to observe open/close transitions of individual channel molecules. However, conventional patch-clamping amplifiers have two serious limitations. First, the high-valued resistors used do not provide a frequency independent response due to internal and external shunt capacitances. Second, when a voltage step is applied at the membrane potential, conventional amplifiers saturate as the stray input capacitance is charged through the large-valued resistor. The first problem can be eliminated by using a capacitor as the transimpedance element, and the second problem can be eliminated by switching to a low-resistance element during the step transient. (Offner and Clark, Biophys. J. 47:142a, 1985) This paper describes an integrated-circuit, patch-clamping amplifier which uses on-chip capacitors for the transimpedance element and an on-chip reset switch to control saturation 'dead time.' The circuit also features programmable gain ranging from TTL-level inputs. A fully integrated approach is desired for two reasons. First, very small and precise capacitors (<0.1 pF)can be realized on-chip. Second, a small-geometry MOSFET can be used for the reset switch, thereby minimizing charge pumping at the switching times. The IC has been realized in the GTE 3 μm CMOS process. Test results for the IC will be presented along with a comparison of noise sources using conventional and capacitive techniques.
(Supported in part by Analog Devices Inc., the GTE Corp., and NIH Grant No. HL19216.)

M-Pos64 NOISE ANALYSIS OF THREE FUNDAMENTAL VOLTAGE CLAMP CIRCUITS.
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A comparative analysis of noise performance of three fundamental voltage clamp circuits was performed. The three circuits are of the inverting, non-inverting, and bootstrap type (Franz and Frazier, Bioph. J. 37: 73a, 1982). The analysis deals with (1) equivalent "membrane" currents generated by noise sources in the clamp amplifier and passive circuit elements; (2) the effect of summing point parasitics on equivalent "membrane" current and current-to-voltage conversion; (3) the effect of compensation circuits; and (4) variations in the membrane potential difference due to noise sources. The appropriate signal-to-noise ratios for membrane current and membrane potential difference are given.

M-Pos65 ^{13}C NMR STUDIES OF TRYPTOPHAN SIDECHAIN DYNAMICS IN HYDROPHOBIC OLIGOPEPTIDES. *Weaver, A. J., †Kemple, M., †Wassall, S., and *Prendergast, F. G. *Department of Biochemistry and Molecular Biology, Mayo Foundation, Rochester, MN; †Department of Physics, I.U.P.U.I., Indianapolis, IN.

tBoc-LAWAL-OCH_3 and tBoc-LALALW-OCH_3 were used to examine tryptophan sidechain dynamics in hydrophobic environments. In both peptides, the tryptophan sidechain was >95% enriched with ^{13}C at $C\delta_1$. T_1 and NOE were measured at 50.3 and 75.4 MHz for both peptides in CD_3OD, and at 75.4 MHz for tBoc-LALALW-OHC_3 in lysolecithin (from egg lecithin)/D_2O micelles. Steady-state fluorescence anisotropy data were collected for both peptides in CH_3OH and micelles. Relaxation data were analyzed by the "model-free" approach of G. Lipari and A. Szabo, (1982, J. Am. Chem. Soc., 104:4546-4559). A modification of this method allows information about internal motions to be obtained without also measuring relaxation parameters for a peptide backbone atom. Relationships between a sidechain correlation time τ_e, an overall correlation time for the peptide τ_m, and a generalized sidechain order parameter S, are shown by computer-generated curves. Analysis of ^{13}C-NMR results suggests that the tryptophan sidechain has τ_e somewhere in the range 20-70 ps with corresponding S values in the range 0.1-0.6. In lysolecithin micelles, τ_e was approximately 1 ns, and S ~ 0.75 for tBoc-LALALW-OCH_3. Interestingly, steady-state fluorescence anisotropy values suggest lower order parameters. The significance of the NMR results and the discrepancies with the fluorescence data will be discussed, as will their relevance to molecular dynamics studies. Supported by GM 34847.

M-Pos66 NATURAL ABUNDANCE ^{13}C NMR OF FROG SKIN. E.N. Gate, P.N. Venkatasubramanian, and M. Bárány. Department of Biological Chemistry, College of Medicine, University of Illinois at Chicago, IL 60612.

The skin of freshly killed frogs (Rana pipens) was removed in one piece, cleaned from any underlying tissue and used for NMR analysis without any bathing solution. Natural abundance ^{13}C spectra were recorded with a 12 mm probe at either 50.3 or 90.8 MHz in a Nicolet NMC 200 or 360 spectrometer, with a 45° pulse angle, recycling time of 1.2 seconds, sweep width of ± 110 ppm, 8K data points, and quadrature detection. Dioxane in a capillary was used as a reference of 67.4 ppm. Resonances characteristic of phospholipids were observed at 40.7 ppm (phosphatidylethanolamine N-methylene), 55.1 ppm (phosphatidylcholine N-methyls), 129.0 ppm and 130.3 ppm (poly- and monounsaturated carbons), and 174.9 ppm (esterified fatty acid carbonyls). In addition other resonances were seen which did not originate from phospholipids. After treatment of the skin with chloroform-methanol (3:1) the peaks arising from phospholipids could no longer be observed in the spectrum though other non-phospholipid resonances did remain. The spectrum of the chloroform-methanol extract revealed resonances characteristic of phospholipids. The most intense peak in the spectrum of the chloroform-methanol extract was from the $-(CH_2)_n-$ carbons of the fatty acyl chains which were not visible in the spectrum of the untreated skin. This indicates that in the intact skin the $-(CH_2)_n-$ carbons are restricted in their mobility when compared to the polyunsaturated carbons. Less prominent but still discernable peaks remained in the skin spectrum after extraction of phospholipids which may be derived from proteins. The spectrum of a perchloric acid extract of frog skin showed resonances from amino acid carbons in polypeptides and from metabolites such as creatine, glucose, lactate and glutamate. (Supported by MDA, CTR and RRC of the UIC).

M-Pos67 A ^1H NMR INVESTIGATION OF THE EFFECT OF POSITION AND NUMBER OF CHARGED AND UNCHARGED CO-ORDINATING RESIDUES ON THE METAL-BINDING AFFINITY OF SYNTHETIC CALCIUM-BINDING ANALOGUES OF SITE III OF RABBIT SKELETAL TROPONIN C. Brian J. Marsden, Robert S. Hodges, and Brian D. Sykes. Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7.

Calcium is known to regulate many cellular events, such as muscle contraction, by acting as a chemical signal that alters the structure of regulatory proteins such as troponin C and calmodulin. The calcium-binding site, which is a highly conserved region of these proteins, exists in a helix-loop-helix conformation. Within the twelve residue loop region there are six amino acids that co-ordinate the calcium ion. It is the type of liganding amino acid at these positions which is responsible for the different affinities that the calcium-binding sites have for the Ca^{2+} ion. To study the contribution made at each co-ordinating position to the metal-binding affinity of the site, ten 13 residue analogues of the calcium binding site 3 of rabbit skeletal troponin C have been prepared by solid-phase synthesis. Specific substitutions and permutations have been made at the X, Y, and Z metal-co-ordinating positions of the peptides. High field ^1H NMR spectroscopy has been used to investigate the events that occur within the calcium-binding site upon binding La^{3+} ions by the peptides. The chemical shift changes induced by the binding of La^{3+} were used to determine the metal-binding affinity for each analogue. These NMR results indicate that the peptide with the native sequence has the highest affinity, 4×10^{-6} M; and that the affinity decreases from 7×10^{-6} M to 1.1×10^{-3} M as the overall negative charge at the co-ordinating positions of the analogues decreases from -5 to -2. The results also show that the position of the negatively charged liganding residues is an important factor affecting the metal-binding affinity. (Supported by MRC and AHFMR).

M-Pos68 NUCLEAR MAGNETIC RESONANCE STUDY OF INTERACTIONS OF PHOSPHOLIPID MEMBRANES WITH POLYPEPTIDE ANTIBIOTICS. R. Zidovetzki[1], U. Banerjee[2], D.W. Harrington[1] and S.I. Chan[2]. [1]Department of Biology, University of California, Riverside, CA 92521; [2]Department of Chemistry, California Institute of Technology, Pasadena, CA 91125.

We report here the nuclear magnetic resonance (NMR) studies of interactions of three membrane-active polypeptide antibiotics (polymyxin B, gramicidin S and valinomycin) with model phospholipid bilayers composed of either dimyrystoyl lecithin (DML) or of a mixture of lipids with composition similar to that of the outer cell membrane of gram-negative bacteria. We used a combination of ^{31}P NMR and ^{2}H NMR measurements to deduce information about the perturbation of the lipid headgroups and the hydrophobic side chains of the lipid molecules. Our spectroscopic results indicated relatively small effects of antibiotics on DML bilayers. No significant interaction was detected between DML and polymyxin B. Valinomycin interacted with DML primarily at the water-lipid interface. Disruption of the DML bilayer was only evident in the case of gramicidin S. However, even here, the basic multilamellar bilayer phase of DML was apparently maintained at a gramicidin S:DML molar ratio of 1:5.7. Considerably more pronounced effects are observed upon addition of the antibiotics to the bilayers composed of a mixture of negatively charged and zwitterionic phospholipids. Polymixin B molecule is probably inserted into the membrane, which largely maintains the bilayer conformation. Both gramicidin S and valinomycin have a strong disruptive effect on the bilayer, inducing a significant part of the phospholipids to form micelles or small vesicles. Gramicidin S and valinomycin primarily perturb the structure of the phosphatidyl ethanolamine component of the model membranes.

M-Pos69 MAGNETIC INTERACTION BETWEEN THE TYROSYL FREE RADICAL AND THE ANTIFERROMAGNETIC IRON CENTER IN RIBONUCLEOTIDE REDUCTASE. M. Sahlin (1), L. Petersson (1), A. Gräslund (1), A. Ehrenberg (1) and J. Hyde (2), (1) Dept. of Biophysics, Arrhenius Lab., Univ. of Stockholm, S-106 91 Stockholm, Sweden, and (2) Natl. Biomed. ESR Center, Dept. of Radiol., Med. Coll. of Wisc., Milwaukee, Wisc. 53226.

Ribonucleotide reductase from *E. coli* and from mammalian cells are heterodimeric enzymes. The smaller subunits, proteins B2 and M2, respectively, contain iron and a tyrosyl free radical which both are required for enzymatic activity. The iron center in B2 is an antiferromagnetically coupled pair of high spin ferric ions with $J = -108\ cm^{-1}$. Above ca. 30 K several properties of the EPR absorption of B2 measured at 9.5 GHz deviate markedly from the corresponding properties of UV induced tyrosyl radicals. For B2 microwave saturation sets in at higher microwave power, and under non-saturating conditions, the signal amplitude decreases, while the linewidth increases. The effects, which are enhanced with increasing temperature, may be explained by magnetic dipolar interaction between the tyrosyl radical and the iron center. For the latter μ^2_{eff} increases with temperature. For M2 the effects are more pronounced than for B2 and a weak exchange interaction might also be operative in this case. For B2 progressive saturation was also measured at six microwave frequencies from 0.8 to 35 GHz at 100 K, and T_{1e} of the tyrosyl radical was measured by saturation recovery in the temperature range 100 - 200 K. The possibility to calculate from this data the distance between the radical and the iron center will be discussed. Exchange interaction suggests a route for electron flow and polarization which might be of importance for the enzyme reaction.

M-Pos70 STRUCTURE OF THE HEME CAVITY OF LIVER FLUKE (Dicrocoelium dendriticum) HEMOGLOBIN BY PROTON NMR SPECTROSCOPY. J.T.J. Lecomte, G.N. La Mar, J.D.G. Smit and K.M. Smith. Department of Chemistry, University of California, Davis, CA 95616, and Laboratorium für Biochemie I ETH-Zentrum, CH-8092 Zürich, Switzerland.

The body fluid of the lanceolate fluke Dicrocoelium dendriticum contains a monomeric hemoglobin (DdHb) characterized by a high oxygen affinity and a pronounced acid Bohr effect. Fragmentary sequence data have demonstrated the absence of distal His; the X-ray structure of DdHb is not available yet. We have undertaken the NMR study of the heme cavity of this primitive Hb in the high-spin metaquo form and the low-spin metcyano form. Several heme resonances are assigned by preparation of the apoprotein, reconstitution with deuterium-labeled hemins, and analysis of the resulting spectrum; additional heme and protein signals are identified through nuclear Overhauser effects (NOEs). The chemical shift patterns indicate that there is no heme rotational heterogeneity in the native material and that the proximal imidazole projection onto the heme plane passes through the nitrogens of pyrroles I and III. The high-spin species is shown to retain a coordinated water molecule below the pK of the Bohr transition, and NOEs, spin-lattice relaxation times, and chemical shift considerations are applied to determine the heme propionate side chain orientation. In the low-spin form, the NOEs are consistent with a rigidly held cis 2-vinyl and a more mobile trans 4-vinyl. Labile proton resonances arising from the proximal His residue are identified as well as signals from a distal side chain, believed to be a Tyr. The implications of a distal Tyr for the iron reactivity are discussed. It is also shown that in both complexes, the 7-propionate chain exhibits a pH response with a pK value similar to that of the Bohr transition.

M-Pos71 CHARACTERIZATION OF ^{7}Li NMR SHIFT REAGENTS SUITABLE FOR THE STUDY OF LITHIUM TRANSPORT IN HUMAN ERYTHROCYTES, Duarte Mota de Freitas, Mary C. Espanol, and Ravichandran Ramasamy, Department of Chemistry, Loyola University of Chicago, 6525 N. Sheridan Road, Chicago, IL 60626.

The standard experimental methods (atomic absorption and flame photometry) used to monitor lithium transport in human erythrocytes require physical separation of the intra- and extracellular compartments. We reported previously (Espanol et al. (1986) Biophys.J. 49, 326a) that Gupta's shift reagent, $Dy(PPP_i)_2^{7-}$ dysprosium (III) tripolyphosphate, resolves intracellular and extracellular $^{7}Li^{+}$ resonances and is therefore suitable for the non-invasive study of lithium transport in red cells. Dysprosium and thulium shift reagents, that had been previously used for ^{23}Na NMR studies, were investigated by us as to their potential as $^{7}Li^{+}$ NMR shift reagents. We found that although $Dy(PPP_i)_2^{7-}$ gave the largest chemical shift separation between the two $^{7}Li^{+}$ pools, the separation provided by $Dy(TTHA)^{3-}$ (where $TTHA^{6-}$ is triethylenetetraminehexacetate) is virtually independent of pH around physiological values and is not so strongly dependent on the concentration of divalent metal ions like Ca^{2+} and Mg^{2+}. The effects of these two shift reagents on energy metabolism and morphology of red cells have been investigated by ^{31}P NMR and scanning electron microscopy, respectively, and will also be addressed.

M-Pos72 STUDIES ON THE EXTENT OF NMR VISIBILITY OF ^{23}Na AND ^{39}K IN SOLUTIONS AND GELS OF POLYELECTROLYTES. B. Richey, B.A. Lewis, C.F. Anderson, T.C. Farrar and M.T. Record, Jr., Departments of Chemistry and Biochemistry, University of Wisconsin, Madison WI 53706.

The NMR signal from ^{23}Na and ^{39}K ions (I = 3/2) is reduced in intensity by approximately 60% in a variety of cells (1). This low degree of NMR visibility is generally attributed to quadrupolar splitting of the signal into three peaks, two of which account for 60% of the signal intensity and are presumably broadened into the baseline noise. Quadrupolar splitting of alkali metal ions has been demonstrated in some in vitro systems; examples include humid DNA fibers (2) and liquid crystals of soap (3). Since the metal ions in these condensed systems are associated with the polyions, it is not clear how applicable these results are to the in vivo situation where most counterions are thought to be free. In E. coli, the NMR visibility is observed to be 45% for $^{39}K^{+}$, and yet this ion serves as the primary osmolyte and must be to a large extent osmotically active and therefore unassociated. We have undertaken a series of experiments with polyelectrolytes in solution and copolymerized in rigid gels in order to investigate the extent of correlation between thermodynamic ion activity and measurable NMR parameters. Our results show that ^{23}Na and ^{87}Rb are 100% visible in solutions of DNA. The ^{23}Na signal from Na-DNA which is co-polymerized in rigid polyacrylamide gels is also 100% visible. Experimental results on these and other model systems should provide more information on the low degree of NMR visibility observed in vivo.

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M-Pos73 SOLID STATE ^{15}N NMR STUDIES OF UNIFORM AND SPECIFIC SITE ^{15}N LABELED GRAMICIDIN A´ IN AN ORIENTED LIPID BILAYER. F. Moll, L.K. Nicholson, P.V. LoGrasso, John C. Lay, C.A. Guy, J. Petefish, Gregg B. Fields H.E. Van Wart and T.A. Cross. Department of Chemistry and Institute of Molecular Biophysics, Florida State University, Tallahassee, FL 32306-3006.

Uniformly ^{15}N labeled gramicidin A´ has been produced by Bacillus brevis in a media containing antoclaved ^{15}N labeled E. coli cells. Specific site ^{15}N labeled gramicidin has been synthesized by solid phase peptide synthesis. These labeled gramicidins have been incorporated into hydrated bilayers of dimyristoyl-phosphatidylcholine. The samples have been characterized as bilayers by ^{31}P NMR, as having gramicidin in the channel conformation by circular dichroism. ^{15}N powder pattern spectra have been obtained for characterizing the dynamics of the channel forming dimer. The lineshape dependence of these spectra on lipidation, hydration and temperature have been studied. The results have been interpreted in light of both the overall rotational motion of the channel as well as local motions of the polypeptide backbone. Gramicidin containing bilayers have been uniformly oriented with the bilayer normal parallel with the magnetic field. The disorder in these preparations, is assessed by the range of angles that the bilayer normal makes with respect to the magnetic field. From both the ^{31}P and ^{15}N NMR spectra an estimate of less than +/-3° has been obtained. Spectra of uniformerly labeled gramicidin show as many as ten resonances for the 20 nitrogen sites. These chemical shifts can be interpreted to yield the orientation for the peptide linkage with respect to the gramicidin channel axis. There are three dominant resonances arising from multiple sites indicating a gramicidin structure which has similar orientations of several of the peptide linkages, but lacking the overall uniformity of previously published structural models.

M-Pos74 A ^{19}F NMR STUDY OF THE INTERACTION OF TRIFLUOPERAZINE WITH BOVINE BRAIN S-100B PROTEIN. P. L. Pingerelli, M. Molinaro, and H. Mizukami. Division of Regulatory Biology and Biophysics, Department of Biological Sciences, Wayne State University, Detroit, Michigan 48202.

Calcium modulated association of the drug trifluoperazine (TFP) with bovine brain S-100b has been studied in order to identify the structural changes of the hydrophobic regions. The ^{19}F NMR spectral features of the TFP/S-100b solution in the presence of excess Ca^{2+} were quite different from those observed in the absence of Ca^{2+}. In the presence of excess Ca^{2+}, there was a 6.5-fold increase in TFP linewidth from 8.7 Hz to 56.5 Hz. This change appears to be resulting from exchange broadening caused by a drug/S-100 interaction. Additionally, spin-lattice relaxation times (T_1) for TFP/S-100b solutions were obtained. The T_1 value of the free TFP solution (1.53 s) decreased to 0.86 s in addition to Ca^{2+} bound S-100b.

Comparing similar studies by others on TFP/calmodulin/Ca^{2+} solutions, the TFP linewidth for S-100b (56.5 Hz) is greater than that observed for Tetrahymena calmodulin (18.2 Hz) but less than what was observed for porcine calmodulin (110 Hz). However, the T_1 time of TFP in the presence of Ca^{2+} bound Tetrahymena calmodulin (0.42 s) is less than that observed for S-100b. In the case of S-100b, both TFP linewidth and T_1 time measurements suggests that the calcium bound protein has enhanced interaction with TFP resulting perhaps from a hydrophobic structural change. However, the calcium dependent interaction of TFP with S-100b appears to be unique from both Tetrahymena and porcine calmodulin. (Supported by WSU Graduate Research Award)

M-Pos75 PHOSPHATE UPTAKE BY MAIZE ROOT TIPS AS DETERMINED BY IN VIVO NMR SPECTROSCOPY. J. R. Cavanaugh and S.-I. Tu (Intr. by Leo D. Kahn). USDA, Agricultural Research Service, Eastern Regional Research Center, Philadelphia, PA 19118.

The extent of phosphate uptake measured by the cellular concentration of cytoplasmic P_i, vacuolar P_i, ATP, glucose-6-phosphate and UDPG was determined using in vivo ^{31}P NMR spectroscopy. Maize root tips were perfused with a solution containing 10 mM phosphate and either Na^+ or K^+ at pH 4.4 and 6.0 in the aerated state or under a nitrogen atmosphere. Concentrations of internal mobile phosphorus containing compounds were determined and comparisons were made between systems perfused with and without phosphate. Generally, the vacuolar P_i concentration increased significantly when the roots were perfused with phosphate in the aerated state but the cytoplasmic P_i remained nearly constant. Under a N_2 atmosphere, little change occurred in either P_i concentration. Effects of anions such as Cl^- and NO_3^-, cations such as Mg^{2+}, Zn^{2+} and Al^{3+}, metabolic inhibitors and protonophores on the uptake were also investigated. Results are interpreted in terms of the bioenergetics and the ion transport mechanisms applicable to root cell membranes.

M-Pos76 **A ^{31}P NMR STUDY OF METABOLIC AND COMPENSATED METABOLIC ACIDOSIS ON INTACT, PERFUSED BEATING RABBIT HEARTS.** Martin M. Pike, Robert S. Fitzgerald, and William E. Jacobus, The Johns Hopkins Medical Institutions, Baltimore, MD 21205.

The permeability characteristics of the sarcolemma to HCO_3^- are not well defined. In order to monitor intracellular [HCO_3^-] changes, rabbit hearts (3-5g) were perfused at constant flow in a Bruker W-H 180 NMR spectrometer (4.25T). The hearts were subjected to a 2hr experimental protocol with a 21min control period, a 30min intervention period, another 21min control period, a second 30min intervention period, and a final 21min control period. The control periods utilized a modified Krebs-Henseleit buffer with 24mM bicarbonate oxygenated with 95% O_2+5% CO_2. The two intervention periods exposed the hearts to metabolic acidosis (MA), (6mM HCO_3^-, 95% O_2+5% CO_2) or compensated metabolic acidosis (CMA), (6mM HCO_3^-, 98.72% O_2+1.28% CO_2). Buffer samples were analyzed for extracellular pH values: control period 1, 7.47 ± 0.04(SD); control period 2, 7.48 ± 0.03; control period 3, 7.47 ± 0.05; CMA, 7.34 ± 0.05; MA, 7.00 ± 0.07. NMR spectra (3min) were taken continuously. When CMA was introduced during the first intervention period, the intracellular pH (pH_i) decreased rapidly from a control value of 7.15 to 7.04 (n=4). The pH_i rapidly decreased upon introduction of MA during the second intervention period, falling from 7.15 to 6.94. The calculated intracellular [HCO_3^-] during the control periods was 13mM, and decreased to 2.5mM and 7.8mM during CMA and MA, respectively. The results for the reverse protocol were qualitatively similar (n=8). The depression of left ventricular developed pressure showed a direct relationship to the pH_i. The ATP and PCR intensities gradually declined during these protocols, to 85% and 75%, respectively. The coronary perfusion pressure always decreased markedly during MA, by approximately 30%. The pH_i changes during CMA suggest a rapid HCO_3^- efflux that overcompensates for the efflux of CO_2 (assumed to be in rapid transmembrane equilibrium). These data suggest that the sarcolemmal membrane contains an important mechanism for HCO_3^- transport.

M-Pos77 **NOVEL ^{31}P NMR SHIFT AND RELAXATION REAGENTS FOR INORGANIC PHOSPHATE.** Martin M. Pike, Myron L. Weisfeldt, and William E. Jacobus, Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

Nontoxic, anionic shift reagents could provide much useful information concerning anion distribution in cell and perfused organ systems. To approach this problem, we measured the frequency of inorganic phosphate (Pi) in a series of samples at varying concentrations of Pi in the presence of the lanthanide chelate NaPr(EDTA), (90mM NaCl, 25mM Hepes, pH 7.2). Using a Bruker W-H 180 NMR spectrometer (4.25T) which was field frequency locked to the water ^1H resonance, the resulting frequency shift with 8mM NaPr(EDTA) and 12mM Pi was 5.49ppm downfield with respect to controls. The halfheight linewidth was broadened from 19 to 55Hz. The magnitude of the shift was directly proportional to the concentration of the shift reagent up to 16mM NaPr(EDTA). Interestingly, in this buffer system, the shift showed little dependence on [Pi]. A sample with 3mM Pi displayed a shift of 5.97ppm. A series of samples with 8mM NaPr(EDTA) at varying pH (5.7 to 8.23) showed that the shifts were very pH dependent. The shift was 7.19ppm downfield at pH 8.23 and only 0.08ppm at pH 5.7 with respect to a sample without shift reagent (pH 7.22). The shifts were only slightly altered by divalent ions. The presence of 0.5mM $CaCl_2$ in a sample with 6mM Pi, 8mM NaPr(EDTA), 90mM NaCl and 25mM Hepes, pH 7.2, reduced the shift from 5.65ppm to 4.93ppm. A similar lanthanide chelate NaTb(EDTA) produced larger shifts. With 8mM NaTb(EDTA), (6mM Pi, 90 mM NaCl, 25mM Hepes, pH 7.34) one observed a 13.91ppm shift downfield, with a halfheight linewidth of 900Hz. In contrast, the chelate NaGd(EDTA) produced an upfield shift of 7.56ppm with a halfheight linewidth of 1100Hz. Finally, a sample of 8mM NaDy(EDTA), (5mM Pi, 125mM NaCl, 15mM MOPS, pH 7.2) exhibited a halfheight linewidth of 1500Hz. In summary, an appropriate reagent with minimal linebroadening could be used with various cell or perfused organs to distinguish intra- from extracellular Pi. On the other hand, relaxation reagents would be used to broaden an extracellular resonance, and thus render it indistinguishable from baseline. Both could be useful in in vivo studies.

M-Pos78 **EXTRACTING ROTATIONAL CORRELATION TIMES FROM cw-ST-EPR SPECTRA FOR SYSTEMS WITH TWO MOTIONAL COMPONENTS: VARIABLE ZEEMAN MODULATION FREQUENCY EXPERIMENTS.**
A.H. Beth, B.H. Robinson, C.E. Cobb, and T.E. Conturo, Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN 37232 and Department of Chemistry, University of Washington, Seattle, WA 98195.

In previous work we have shown that it is possible to theoretically model the effects of isotropic and anisotropic rotational diffusion on continuous wave saturation transfer EPR (cw-STEPR) spectra from spin labeled biomolecules (Beth et al., (1983) J. Phys. Chem. 87, 359-367). This methodology has been employed to extract rotational correlation times from spin labeled proteins in a single rotational motion environment. However, if more than one motional species is present in a system, such as will be encountered for a monomeric protein in equilibrium with an oligomeric complex, present data analysis approaches will only yield an ensemble average "effective" correlation time for all spin labeled species present. This limitation precludes obtaining valuable information present in the STEPR spectrum. We have explored the use of variable Zeeman field modulation frequencies, a parameter which can be conveniently varied in the process of recording experimental spectra, to provide a suitable means for separating multiple motional populations. The method stems from the fact that discrete motional species contribute differently to the composite STEPR spectrum at different modulation frequencies. The problem is cast in terms of simultaneous iterative least squares fitting of the motionally sensitive ratio parameters L"/L and H"/H (Thomas et al., (1976) J. Chem. Phys. 65, 3006-3024) from composite spectra obtained at the different modulation frequencies with the same parameters from a basis set of model spectra recorded at the same modulation frequencies. Results have indicated that it is possible to accurately extract the three independent variables f, τa, and τb; where f is the mole fraction of spin labels rotating with correlation time τa and (1-f) is the mole fraction rotating with correlation time τb, for a two component system. The appropriate range of reference modulation frequencies for X-band measurements of the second-harmonic out-of-phase absorption signal is 1 to 100 kHz for correlation times between 1 μsec and 1 msec. This approach is being employed to analyze the rotational diffusion of the anion channel in *intact* human erythrocytes for the existence of two components. Supported by: NIH HL34737 (AHB) and PCM 8216762 (BHR).

M-Pos79 ROTATIONAL MOTION OF BIOMOLECULAR MODEL SYSTEMS STUDIED BY SATURATION RECOVERY EPR. B.H. Robinson, T. Sugano, Chemistry, University of Washington, Seattle Wa. 98195, C. Mailer, Physics, The University of New Brunswick, New Brunswick, Canada, 5A2E3B, A.H. Beth, Molecular Physiology and Biophysics, Vanderbilt University, Nashville, Tenn, 37232.

Characterization of the rotational dynamics of soluble and membrane bound proteins and similar macromolecules is essential for elucidating structure-function-dynamics relationships. Measurement of the dynamics, particularly the rotational dynamics, of biomolecules has been limited to only a few techniques and remains one of the least characterized parameters of biophysical interest. Continuous wave EPR spectroscopy, using nitroxide spin labels, has provided a means for determining the rotational diffusion of macromolecules. However, limitations of this technique have been that it is not possible to directly extract rotational diffusion coefficients from experimental data nor to deconvolve overlapping spectra. Recent advances in EPR spectroscopy have opened new avenues for overcoming these limitations. Saturation recovery (SR), a technique which measures the time evolution of the Z-component of magnetization following a short rf pulse, has provided encouraging results on model organic and biological systems. We present our studies on model systems with well characterized rotational dynamics. In addition to the study of model spin labels, we have examined the satruation recovery of spin labeled anion channels in erythrocyte ghosts and spin labeled bovine serum albumin. We have developed a method of extracting the motional information from the overall recovery curve, accounting for the intrinsic relaxation of the spin system and the magnetic resonance parameters. We report that for these simple systems, the SR methodology gives motional rates which agree with the rotational rates calculated from known hydrodynamic sizes and shapes of the labeled species. The method appears to work well when the motion is slower than 1μsec but not more than 5 times slower than the intrinsic spin-lattice relaxation time (P. Fajer et al. Biophys. J., 49, 469a, 1986) and has the potential for determining overlapping species and anisotropic motion. Support by: PCM 8216762 (BHR) and HL34737 (AHB).

M-Pos80 DETECTION OF EPR SIGNALS ASSOCIATED WITH ISCHEMIC HEART TISSUE, Mark S. Crowder, John M. Luber, Jr.*, and P. S. Rao*. IBM Instruments Inc., Danbury, CT 06813 and Long Island Jewish Medical Center, New Hyde Park, New York 11042*.

We have used EPR spectroscopy to detect paramagnetic species in normal, ischemic, and reperfused heart. All the heart samples examined in this study had multiple EPR signals. Left ventricular myocardium biopsies of 1 month old swine were quick frozen with liquid nitrogen and examined by EPR at 10K. Normal heart tissue displayed a g = 2 EPR signal among several other EPR signals. The g = 2 signal was stable at room temperature. EPR difference spectra between ischemic and normal heart tissue, at 10K, showed an EPR signal in the g = 3.0-4.0 region and a well defined three line spectrum (g = 2.015, 2.005, 1.988). The splitting between the EPR lines were 2.25 mTesla. The three line spectrum may represent a nitrogen radical species or a triplet species. Microwave power saturation studies of ischemic heart tissue found that g = 2 signal saturates at much lower microwave powers than the three line EPR signal. The ischemia-induced EPR signals were irreversibly lost when the temperature of the sample was increased to 200 K. Reperfused heart tissue also showed the three line EPR signal, however, this signal was less predominant in reperfused heart than ischemic heart tissue.

M-Pos81 ELECTRON PARAMAGNETIC RESONANCE EVIDENCE ON THE CONFORMATIONAL CHANGES AROUND THE INTRINSIC METAL ION AND SUBSTRATE BINDING SITES OF *Escherichia coli* RNA POLYMERASE
Suresh Tyagi and Felicia Y.-H. Wu, Dept. Pharmacol. Sci., SUNY at Stony Brook, NY 11794

E. coli DNA-dependent RNA polymerase (RPase) contains 2 mol Zn/mol holoenzyme ($\alpha_2\beta\beta'\sigma$) with one Zn each in the β and β' subunits. We have replaced one Zn *in vitro* with Mn(II) in the β subunit under non-denaturation conditions by the treatment of enzyme with 0.25 M $NaNO_3$, 1 M NaCl and 1 mM 1,2-diaminocyclohexane tetraacetic acid. Spin label analog of ATP, (8-AmTEMPO)ATP, and Mn(3′-OCH_3)UTP, were synthesized. EPR dipole-dipole interactions of (8-AmTEMPO)ATP and Mn-Zn RPase caused the hyperfine broadening of 2.0 ± 0.1 G in the EPR spectra of (8-AmTEMPO)ATP, which was reduced to 1.6 ± 0.2 G in the presence of poly(dA-dT) and to 1.0 ± 0.1 G in the presence of poly(dA-dT) plus (3′-OCH_3)UTP. Based on hyperfine broadening, the distance calculated to be 4.1 ± 0.4 Å from Mn in Mn-Zn RPase to the (8-AmTEMPO)ATP and increased to 5.2 ± 0.1 Å by the addition of poly(dA-dT) plus (3′-OCH_3)UTP which may result in the formation of ppp(8-AmTEMPO)-ApU(3′-OCH_3) product. The addition of Mn(3′-OCH_3)UTP complex to the Mn-Zn RPase resulted in the hyperfine broadening of 11.0 ± 0.1 G in the EPR spectra of Mn(3′-OCH_3)UTP. This broadening was reduced to 9.0 ± 0.3 G after the addition of poly(dA-dT) and to 7.0 ± 0.2 G when both poly(dA-dT) and ATP were added. The distance was calculated to be 9.7 ± 0.2 Å from Mn in Mn(3′-OCH_3)UTP to Mn in Mn-Zn RPase and increased to 10.6 ± 0.1 Å in the presence of poly(dA-dT) and ATP. The increase in distances at both binding sites may be attributed to the movement around the metal ion caused by the conformational changes in RPase and the substrate binding sites due to alterations in hydrogen bonding between RNA product and template, during DNA transcription. Based on the Stokes-Einstein equation, the radius of gyration was calculated to be 52.1 Å and the molecular weight 472,481 dalton for (8-AmTEMPO)ATP - Mn-Zn RPase complex. Supported by NIH and NSF grants,

M-Pos82 PULSE FIELD-SWEEP EPR ON COPPER PROTEINS.
Charles P. Scholes, Chaoliang Fan, and Harold Taylor, Department of Physics, State University of New York at Albany, Albany, New York 12222.

Pulse Field-Sweep EPR (PFSEPR) is a low power pulse method to resolve underlying hyperfine structure from inhomogeneously broadened EPR lines. Saturating, "hole-burning" microwave pulses are followed by a magnetic field sweep at low microwave power to monitor the spread of saturation away from the original "hole". Satellite holes do occur, and it is the measurement of these and the relation of them to underlying hyperfine couplings that constitute this PFSEPR work. PFSEPR makes use of the standard microwave source and the field sweep unit built into standard commercial EPR spectrometers, low power microwave switching diodes, a home-built pulse programmer, and a standard signal averager. There is no complex double resonance equipment or kilowatt microwave pulses. PFSEPR has approximately ten times the sensitivity of standard ENDOR and a spectral resolution of about 0.3 Gauss. We have recently reported the application of PFSEPR to low spin ferric porphyrin systems where hyperfine transitions were observed from protons, deuterons, and nitrogens [Falkowski et al. J. Magnetic Resonance 68 (1986) 453-468]. We now find that PFSEPR is highly useful in resolving large hyperfine couplings of metal nuclei where high frequency ENDOR is inherently difficult. We report the resolution of copper nuclear hyperfine couplings from the blue protein stellacyanin and from the Cu_A signal of cytochrome c oxidase over regions of the respective EPR lines where no hyperfine structure is directly resolved by EPR. We will relate the observed PFSEPR to copper nuclear hyperfine energy levels.

M-Pos83 HIGH FIELD ^{15}N NMR ANALYSIS OF ^{15}N LABELED DNA IN SOLUTION. Chung H. Han and Laurel O. Sillerud, Los Alamos National Laboratory, Los Alamos, NM 87545.

Nitrogen-15 NMR methods have been applied to a study of the structure and dynamics of algal (A. Nidulans) DNA of 550 np median length highly-enriched (96%) in ^{15}N at all base nitrogens. Complete NMR signal assignments have been made at pH 7.0 - 7.5 for ^{15}N DNA by direct comparison with the resonances from mononucleotides. ^{15}N NMR gave a rapid, non-destructive and quantitative determination of the base composition and the amounts of ss (single-stranded) and ds (double-stranded) DNA; the DNA isolated from the algal cells was found to contain 41% A-T and 59% G-c pairs. Analyses of the ^{15}N NMR spectra of the ^{15}N DNA in solution gave a value of 43% ds DNA, which is consistent with the data obtained from a hyperchromicity assay. The spectra also gave direct identifications of base-paired DNA; ^{15}N resonances from ds C_3 and A_1 (hydrogen-bonding nitrogens) were found to resonate at 176.30 and 201.63 ppm respectively with respect to $^{15}NH_4{}^{15}NO_3$, i.e., 4.0 - 4.7 ppm upfield relative to ss DNA. ^{15}N signals from ds G_1 and T_3 (hydrogen-donor nitrogens) were found to resonate at 130.64 and 141.73 ppm, i.e., 4.06 - 4.58 ppm downfield relative to ss DNA. Quantitative information on base stacking has also been obtained from the ss DNA in solution; upfield shifts (0.1 - 1.3 ppm) are observed in the ^{15}N NMR spectra of the ss-DNA with respect to spectra of mononucleotides. We have measured, for the first time, the ^{15}N dynamics for ^{15}N DNA in solution. The relatively small line width (15 - 40 Hz) of the ^{15}N resonances from the protonated nitrogens of ss-DNA suggest fast internal motion. The width of the ^{15}N resonances from ds-DNA were twice as large as those of the ss-DNA. The ^{15}N NOE, at two different fields (4.7 and 9.4 tesla), was consistent with isotropic motions for the protonated nitrogens with τ_c of 0.1 to 1 nsec. Studies of the field dependent line widths of the ^{15}N resonances indicated that chemical shift anisotropy contributed to the relaxation of the nonprotonated nitrogens.

M-Pos84 ^{31}P NMR SPECTROSCOPY OF RED CELLS FROM HUMANS AND GROUND SQUIRRELS AS A FUNCTION OF TEMPERATURE. P. Ghosh, J. S. Willis, D. K. McFarlane and M. J. Dawson, Dept. of Physiology and Biophysics, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801.

Red cells from hibernating species such as the ground squirrel maintain active transport of Na^+ and K^+ over a wider temperature range than do red cells from human subjects. We are doing ^{31}P NMR spectroscopy in order to determine whether functional sensitivity to cold may be correlated with temperature effects on energetically important metabolites. Freshly obtained, washed red cells are incubated without oxygen in adenosine-containing medium at 15% hematocrit and studied over a temperature range of 2° to 30°C. Single-pulse ^{31}P NMR spectra (20 μs pulses at 1s intervals, 2000 FIDs) are obtained at 121.4 MHz on a GE/Nicolet GN300 wide-bore NMR spectrometer.

Peaks corresponding to 2,3-diphosphoglycerate and α, β and γ nucleotide phosphates are observed. Inorganic phosphate is below the limit of resolution in fresh cells from both species. In the spectrum from human red cells, the chemical shift difference between the α and γ nucleotide phosphorus is decreased and that between α and β is increased as the temperature is lowered. These effects are significantly smaller in the red cells from ground squirrels. The results are consistent with more dissociation of Mg from ATP and thus a lower free energy change for ATP hydrolysis in the human red cells at the lower temperatures. This finding may help to explain the difference in sensitivity to cold in the red cells of the two species.

M-Pos85 SIMULTANEOUS MEASUREMENT BY NMR OF O_2 TENSION and pH in CELL SUSPENSIONS. J. Taylor and C. Deutsch, Dept. of Physiol., Univ. of Penna., Phila., PA. 19104/6085.

Fluorinated hydrocarbon blood substitutes such as perfluorotributylamine (FTBA) and perfluorotripropylamine (FTPA) exhibit spin-lattice relaxation rates ($1/T_1$) which are directly proportional to the oxygen tension in the solution (1); they have been tested as O_2 imaging agents (2,3). We have used FTPA sonicated with egg lecithin to monitor O_2 concentration in cell suspensions during the course of NMR measurements of intracellular pH. FTPA relaxation rates were directly proportional to O_2 tension from 2% - 100% saturation in solutions and cell suspensions maintained in a 10 mm air-lift aeration cell in the NMR probe; a single measurement of O_2 tension requires < 2min. Paracoccus denitrificans, human lymphocytes, and rat insulinoma cells have been studied with ^{19}F NMR, using $1/T_1$ to monitor O_2 tension while measuring intra- and extra- cellular pH by the chemical shifts of α-difluoromethylalanine. We determined that aerobiosis was successfully maintained in fluorocarbon-containing suspensions of P. denitrificans consuming 0.51 mM O_2/min. for periods of 30 min to 1 hr. FTPA T_1s followed changes in O_2 tension produced by addition of CN^- or uncouplers to P. denitrificans suspensions. No cytotoxic effects from fluorocarbon or measurement conditions were observed for the cell types mentioned above, as determined by trypan blue exclusion and DNA replication. The fluorocarbon $1/T_1$ measurements described provide a means of direct monitoring of O_2 tension during high-resolution NMR studies of cell suspensions and perfused organs. (1) Parhami, P. and Fung, B., J. Phys. Chem. 87, 1928 (1983); (2) P.M. Joseph et al, J. Comp. Asst. Tomog., 1012 (1985); (3) B.G. Nichols, et al., Proc. of 4th Mtg. Soc. Mag. Res. Med. London 808 (1985).

M-Pos86 ^{31}P NMR STUDIES OF THE pH TITRATION BEHAVIOR OF 2-AMINOETHYLPHOSPHONIC ACID AND RELATED COMPOUNDS. Thomas O. Henderson, Patricio Meneses, Eileen Kleps, and Thomas Glonek. University of Illinois College of Medicine at Chicago and Chicago College of Osteopathic Medicine, Chicago, IL

2-Aminoethylphosphonic acid is a constituent of macromolecules from a variety of animals. ^{31}P NMR studies of the pH titration behavior of AEP derivatives and related compounds were carried out to aid in determining the functional groups of AEP involved in bond formation in these entities. Starting with authentic AEP, (I) $H_2N\text{-}CH_2CH_2\text{-}P(O)(OH)_2$, we prepared the following: (II) $Ac\text{-}NH\text{-}CH_2CH_2\text{-}P(O)(OH)_2$; (III) $Ac\text{-}NH\text{-}CH_2CH_2\text{-}P(O)(OH)(OMe)$; and (IV) $Ac\text{-}NH\text{-}CH_2CH_2\text{-}P(O)(OMe)_2$. The ^{31}P NMR chemical shifts (δ) of these compounds were determined as a function of pH between pH 1 and 13. For AEP, the change in δ showed 3 pK's, as reported elsewhere. Two of these are acidic (pK_1 = 1.2, pK_2 = 6.3), with δ moving upfield with increasing pH, and the third was basic (pK_3 = 11.1) and had a downfield change in δ. In comparison, Compound II exhibited 2 acid pK's, similar to pK_1 and pK_2 for AEP, and displayed upfield changes in δ for each, while Compound III had one pK, below pH 2, and an associated upfield change in δ. The δ for Compound IV did not change between pH 1 and pH 13. Phosphonoalanine, (V) $(HO)_2(O)P\text{-}CH_2CH(NH_2)\text{-}COOH$, had 4 pK's (1.2, 2.5, 5.8, and 10.7). Upfield changes in δ were observed for the $-P(OH)_2$ dissociations (1.2 and 5.8), whereas downfield shifts were associated with the dissociations of -COOH and $-NH_2$ (2.5 and 10.7, respectively). Ethanolamine phosphate, which has 3 dissociable protons, exhibits only 1 pK, at 5.6, by ^{31}P NMR titration, between pH 1 and 13. The change in δ is downfield with increasing pH, opposite to the direction for the $P(OH)_2$ dissociations in the phosphonates examined.

M-Pos87 ^1H NMR SPECTROSCOPY OF CHOLESTERYL ESTERS IN HUMAN ATHEROSCLEROTIC PLAQUE. Jaroslav Zajicek*, Justin D. Pearlman†, Theodore P. Trouard*, Carlos R. Ayers†, James R. Brookeman‡, and Michael F. Brown*. *Department of Chemistry and Biophysics Program, University of Virginia, Charlottesville, VA 22901 and Departments of †Internal Medicine and ‡Radiology, University of Virginia School of Medicine, Charlottesville, VA 22908.

NMR spectroscopy is a powerful tool for the study of tissue components at the molecular level, which we have implemented in characterization of the lesions of human atherosclerosis. Highly resolved ^1H NMR spectra have been obtained from the fatty plaque of human atheroma, despite its macroscopic solid appearance. The fraction of the total spectral intensity corresponding to the sharp ^1H NMR signals is temperature dependent, and approaches unity at body temperature (37°C). Extracted lipids from advanced fatty plaque as well as synthetic mixtures of cholesteryl esters (the major lipid component of fatty plaque) have been studied by ^1H NMR spectroscopy to identify the origin of the high resolution spectrum. The results show that the high-resolution signals from human atheroma are due to a mixture of cholesteryl esters whose liquid-crystalline to isotropic fluid phase transition is near, but somewhat below body temperature. Plaque samples were also characterized by measurement of their T_1 and T_2 relaxation times at different magnetic field strengths to aid in studies involving Magnetic Resonance Imaging (MRI). Work supported by NIH Grants EY03754 and EY00255, the Jeffress Trust, and the Alfred P. Sloan Foundation.

M-Pos88 NMR SATURATION FACTORS IN THE PRESENCE OF CHEMICAL EXCHANGE. R. S. Spencer, Harvard Medical School NMR Laboratory, Boston, MA

Rapid pulsing in NMR spectroscopy is frequently employed to increase the spectral S/N. In general, this results in a distortion of magnetization amplitudes. so that observed metabolite abundances must be corrected with saturation factors (SF's) to obtain true abundances. SF's may be derived from known T1's or obtained experimentally; however, an underlying assumption of either procedure is that there is no chemical exchange between metabolites with different T1's. This is almost never the case in biological samples, so that the usual application of SF's developed for systems which do not exhibit exchange must be modified in order to properly apply this powerful technique of S/N enhancement to biological NMR spectroscopy. Accordingly, we have extended the theory of SF's to allow for exchange and found that for systems with exchange, 1) saturation factors are not independent of abundances (in contrast to the nonexchanging case) and 2) in a particular case, the error in abundance measurements which results from the naive application of SF's appropriate only for nonexchanging systems depends on the reaction rates, on the difference in T1's of the exchanging metabolites, on the change in the ratio of metabolite abundances in different samples or during the time course of the experiments, and on the interpulse delay and flip angle used. For reasonable choices of kinetic and pulse parameters, this error can reach the order of 10% or more. In contrast, our development allows for a rigorous treatment of SF's so that abundances may be determined accurately. If the T1's and reaction constants are known, the true metabolite abundances may be derived from the observed apparent abundances; alternatively, pulse parameters may be defined which will result in a predetermined upper limit on the error resulting from neglect of exchange.

M-Pos89 ECHO ACQUISITION DURING TRAINS OF FREQUENCY SELECTIVE PULSES FOR ^{1}H-NMR SPECTROSCOPY OF METABOLITES IN MUSCLE. Ronald A. Meyer, Depts. of Physiology and Radiology, Michigan State University, East Lansing, Michigan, 48824.

Direct detection of lactate and other metabolites in mammalian skeletal muscle by ^{1}H-NMR is complicated by the need to suppress the much larger lipid resonances, as well as the water resonance. Although water suppression is easily accomplished by use of the binomial series of frequency selective composite pulses (P.J. Hore, J. Magn. Reson. 55:283, 1983), fat suppression has required difference spectroscopy or other spectral editing methods (e.g., S.R. Williams, et al, J. Magn. Reson. 66:562, 1986). This report proposes a method for fat and water suppression in proton spectra which depends upon the acquisition of the spin-echos formed in the steady-state during a train of equally spaced frequency selective pulses of the same amplitude and phase. It is shown that the excitation profile of the echos from such a series is flatter, and has a broader excitation null, than the profile of the FID from a single selective pulse. Using a stopped pulse version of the method (i.e., acquisition of the first echo after 21 selective pulses with 0.15 sec interpulse interval) fat was completely suppressed in spectra of rat skeletal muscle, enabling observation of lactate, creatine, and several other resonances. Moreover, on spectrometers which permit data acquisition immediately prior to a pulse, this method would allow very rapid data accumulation compared to standard echo methods, because the negative echos which form before each pulse could be continuously acquired once the steady-state is achieved. (Supported by NIH grant R23AM38235)

M-Pos90 SOLID STATE NMR METHODS TO MONITOR MOTIONS FROM MICROSECONDS TO SECONDS. S. D. Kennedy, S. Ganapathy, S. Swanson, R. G. Bryant, Departments of Biophysics and Chemistry, University of Rochester, New York, 14642.

Time frames for molecular motions in macromolecules have traditionally been studied by examining behavior at specific time scale windows provided by such observations as T_1, $T_{1\rho}$, and the line powder pattern line shapes of nuclei such as deuterium and carbon. Several multiple pulse methods will be presented that provide a detailed characterization at high resolution of the molecular motions from time scales of microseconds to seconds using solids NMR approaches. The virtue of the new methods is that the time scale investigated is in the control of the investigator; thus, motions may be characterized over a wide range of frequencies and time scales. Results will be presented for polyglycine and polyglutamic acid that demonstrate the effects of hydration and gross macromolecular conformation on polypeptide motions from nanoseconds to seconds.

M-Pos91 CALCIUM-43 HIGH RESOLUTION NMR IN THE SOLID BY CP-MASS METHODS. S. D. Kennedy, S. Ganapathy, R. G. Bryant, Department of Biophysics, University of Rochester Medical School, Rochester, New York, 14642.

Calcium-43 and magnesium-25 NMR in solution has been hampered by low resolution that results from small chemical shifts and broad lines caused by the nuclear electric quadrupole interactions. The line broadening caused by slow macromolecular motion may be defeated if the system to be studied is immobilized and solid state magic angle sample spinning methods are used; however, the major difficulty is signal to noise loss that results from the observation of only the central transition in the spin manifold. Cross polarization methods may provide large gains in signal to noise, of order a factor of 15 for calcium-43. We report the observation of calcium-43 in solids which demonstrates that the experiment is not only feasible, but that the resolution available may be greater than corresponding solution experiments. A summary of the practical aspects of the experiment will be presented including the range of chemical shifts, the contact times required, and the interplay between proton and calcium relaxation times that must be controlled to achieve maximum signal strength.

M-Pos92 A NEW APPROACH TO LEAST-SQUARES FITTING ANALYSIS OF NMR POWDER PATTERNS. Terrence G. Oas*,+, Cynthia J. Hartzell#, Frederick W. Dahlquist* and Gary P. Drobny#. *Institute of Molecular Biology, University of Oregon, Eugene, OR 97403. #Department of Chemistry, University of Washington, Seattle, WA 98195. +Present address: Francis Bitter National Magnet Laboratory, Massachusetts Institute of Technology, Cambridge, MA 02139.

A new approach to the extraction of NMR parameters such as the principal values and orientation of chemical shift tensors from powder patterns is described. The method is based on the least-squares fitting of first- and higher-order derivative spectra in selected regions of the powder pattern. This approach allows information to be extracted from powder patterns whose intensity across the spectrum is not ideal, i.e. not exactly simulateable. Examples of the application of this technique to the analysis of dipole coupled and uncoupled chemical shift and quadrupolar powder spectra are given.

M-Pos93 SIMULTANEOUSLY OBSERVING THE [^{13}C]-^{1}H AND [^{1}H]-^{1}H SIGNALS IN THE ^{1}H NMR SPECTRA WITHOUT AN X NUCLEUS DECOUPLER. T. Jue (Intr. by R.G. Shulman) Dept. of Molecular Biophysics & Biochemistry Yale University, New Haven, CT 06511

The information and sensitivity enhancement of ^{1}H over other nuclei has spawned many editing strategies to sort and select the pertinent resonances in the ^{1}H NMR spectra. Extant J modulation based methods (I) yield the ^{13}C -^{1}H signals upon subtraction of alternate, modulated spin echo experiments, but do not give edited ^{12}C-^{1}H signals upon addition--only the overall ^{12}C-^{1}H resonances.

For in vivo studies, both the edited ^{12}C-^{1}H and ^{13}C-^{1}H signals are required to fully understand the fractional enrichment question which is posed by the various sources of a metabolic pool. We have developed a broadband homonuclear editing scheme, in contrast to the selective one in practice, that permits the simultaneous detection of both the edited ^{12}C and ^{13}C proton signals under particular circumstances. It incorporates a selective 0/180 J modulation pulse that is asynchronous with the non-selective 180 spin-echo pulse (II).

I. ^{1}H: 90-t-180-t...Ag$\pm$
^{13}C 0/180

II. ^{1}H: 90...t_1...t_2...180...t_2...0/180...t_1...Ag$\pm$

Moreover, the broadband nature of the technique provides the flexibility and selectivity to extend the class of J modulation based editing methods to problems of chemical analysis, protein structure, and relaxation. It also permits the flexibility to use one rf source. Calibration of the spectrometer editing performance can be readily achieved with a broad observation window.