M-Pos175 MEASUREMENT OF Ca^{2+} TRANSIENTS IN ISOLATED MYOCYTES WITH QUIN 2. Mary A. Selak, Andrew P. Thomas, Norman S. Kato and John R. Williamson. Dept. of Biochemistry and Biophysics and the Dept. of Surgery (NSK), Univ. of Pennsylvania, Philadelphia, Pa. 19104 USA

The transient increase of cytosolic free Ca^{2+} elicited by electrical depolarization has been investigated using isolated rat ventricular myocytes. Typical preparations incubated in buffered medium containing 1.25 mM Ca^{2+} consisted of about 70% rod-shaped cells with well-defined striations and no terminal blebs. Synchronous contractions were obtained with electrical field stimulation using 70 volt pulses of 8 ms duration. Cells incubated with 2.5 mM Ca^{2+} were loaded with up to 1.5 mM Quin 2 using 2-20 nmol/mg dry wt of Quin 2 acetoxymethyl ester and an incubation time of 60 min. Intracellular Quin 2-Ca^{2+} fluorescence changes were monitored using 334 nm excitation and 490 nm emission interference filters and were recorded on a Brush recorder or displayed graphically after signal averaging successive Ca^{2+} transients using an A/D converter interfaced with an IBM PC. With Quin 2 loadings of < 0.4 nmol/mg dry wt, complete Ca^{2+} transients with contractions were obtained with stimulation frequencies up to 3/s, but at higher Quin 2 loadings, cells progressively failed to contract despite exhibiting small Ca^{2+} transients. The $t_{0.5}$ to peak Ca^{2+} (15 ms) was not affected by Quin 2 content but the $t_{0.5}$ for relaxation increased from 200 to 600 ms. Isoproterenol increased peak Ca^{2+} and decreased $t_{0.5}$ for relaxation at Quin 2 contents below 1.5 nmol/mg dry wt. The calculated cytosolic free Ca^{2+} concentration of resting cells was about 200 nM at low Quin 2 concentrations but decreased to below 100 nM with Quin 2 contents of 1 nmol/mg dry wt and above, and the Ca^{2+} transient was small. These studies indicate that although Quin 2 buffers the cytosolic free Ca^{2+}, it can serve as a useful indicator of stimulus-dependent Ca^{2+} transients as well as a means to manipulate intracellular calcium homeostasis. Supported by NIH grant HL-14461.

M-Pos176 INTRACELLULAR [Ca^{2+}] TRANSIENTS MEASURED BY TnC_{DANZ} FLUORESCENCE IN SINGLE BALUNUS NUBULIS MUSCLE FIBERS. C.C. Ashley+, P.J. Griffiths+, P.F. Strang*, and J.D. Potter* +University Laboratory of Physiology, Oxford OX 1 3PT, U.K., *Department of Pharmacology, Univ. of Miami School of Medicine, Miami, FL 33101. (Intr. by B. Pressman)

The calcium binding subunit of Troponin (TnC) can be labeled with dansylaziridine to produce a fluorescent species (TnC_{DANZ}) in which fluorescence increases with Ca^{2+} binding to the two Ca^{2+}-specific sites. Since Mg^{2+} does not affect TnC Ca^{2+} binding appreciably and since the on and off rates for Ca^{2+} are rapid, TnC_{DANZ} should make a potentially useful [Ca^{2+}] indicator. It has also been shown (Zot et al., Chim. Scrip. 21, 133, 1983) that the Ca^{2+} affinity of the Ca^{2+}-specific sites in TnC are essentially the same as those in whole Tn when it is associated with the thin filament, which should make TnC_{DANZ} a good indicator of Tn bound Ca^{2+} as well. We have injected TnC_{DANZ} into barnacle muscle fibres (final intracellular concentration 10-100 μM), and have studied changes in fibre fluorescence during stimulation under voltage clamp control. The injection of TnC_{DANZ} into the fibre causes the appearance of a fluorescence peak at ~520 nm on excitation at 325 nm. This additional fluorescence signal declines on either side of the peak wavelength to disappear outside the range 450 - 600 nm. Fibre autofluorescence is maximal and TnC fluorescence is negligible at 420 nm, therefore wavelengths close to this value were chosen to monitor non-specific changes in fiber fluorescence. After scaled subtraction of non-specific fluorescence changes at 440 nm from the signal at 520 nm upon electrical stimulation, a net signal was recorded whose amplitude was dependent on the intensity of stimulation. The halftime of rise of this signal generally preceded the halftime to peak force by 35ms (N = 15), while the halftime of the fall of fluorescence was 2.24 times longer than the halftime of mechanical relaxation (N = 11) at 12°C. Force and peak light were attained at about the same moment. These findings indicate a delay between formation of the Ca^{2+}-troponin complex and force generation. The slow fall of fluorescence is consistent with findings from other groups suggesting that the time course of mechanical relaxation may be much faster than the rate of decline of availability of actin binding sites for unattached cross-bridges (Huxley and Simmons, Cold Spring Harb. Symp. 37, 669, 1973; Ashley and Lignon, J. Physiol. 318, 10P, 1981). Supported by NIH HL226193A.

M-Pos177 Ca^{++} MEASURED IN A SINGLE SMOOTH MUSCLE CELL USING A NEW POWERFULLY FLUORESCENT DYE (FURA2) AND THE DIGITAL IMAGE MICROSCOPE. David A. Williams, *Roger Y. Tsien, & Fredric S. Fay, Physiol., U. Mass. Med. Ctr., Worcester, MA, and *Physiol.-Anat., U. Calif. Berkeley, CA

The approaches used to relate Ca^{++} and contractile activity in single skeletal muscle fibres are not suitable for use with smooth muscle due to problems related to the much smaller cell size. While the new fluorescent dye, quin2, has obviated some of those problems and provided some useful information, it does not readily lend itself to observation of Ca^{++} during contraction at the single cell level. We report here on the use of low light level digital imaging microscopy to monitor free cytoplasmic Ca^{++} in single smooth muscle cells (SMC's) of the toad (Bufo marinus) stomach utilizing a newly developed, highly fluorescent dye, fura2 which is readily loaded and deesterified. Fura2 behaved inside cells as expected from dye in solution. Excitation maxima shifted from 380 to 340 nm under conditions known (quin2 experiments) to increase $Ca^{++}i$ in SMC's. The ratio R of fluorescence at 340/380 nm, (directly related to Ca_i), was independent of cell geometry and free from movement artifact. Fura2 loaded SMC's contracted in response to carbachol or electrical stimulation with apparently normal kinetics, unlike with quin2 presumably the result of the lower (<20 times) dye levels required for similar fluorescent signals. The fluorescence in fura2 loaded cells was resistant to photobleaching, a marked improvement over quin2. Changing external [Ca^{++}] resulted in changes in $R_{(340/380)}$ suggesting direct effect on $Ca^{++}i$. SMC's showed variation in $Ca^{++}i$ (large $R_{340/380}$ range) in resting cells. These studies reveal that fura2 provides a stable measure of Ca^{++} in single cells which coupled with other measurements on SMC's should allow for a direct cellular and subcellular analysis of the role of changes in Ca^{++} in SMC function. Supported by HL14523 NIH and an MDA grant. DAW is an Aust. NHF research fellow.

M-Pos178 Ca^{++} TRANSIENTS IN ISOLATED SMOOTH MUSCLE CELLS IN RESPONSE TO EXCITATORY AND INHIBITORY STIMULI. David A. Williams & Fredric S. Fay, Physiol., U. Mass. Med. Ctr., Worcester, MA

As a step towards obtaining information about changes in Ca^{++} at the cellular level associated with smooth muscle contractile activity we have utilized the fluorescent Ca^{++} indicator, quin2, to measure changes in Ca^{++} simultaneous with contractility in smooth muscle cells in suspension. Smooth muscle cells (SMC's) from toad (Bufo marinus) stomach were loaded with quin2 under conditions determined previously which minimize buffering and/or reduction of cytoplasmic Ca^{++} in this cell type. Intracellular quin2 levels were approximately 0.5-1.0 mM as determined by fluorescence of cell suspension, estimates of cell volume and fluorescence calibrations from quin2 in solution. The dye is uniformly distributed in the cytoplasm as revealed by digital processing of images of the fluorescence of single cells. Free cytoplasmic Ca^{++} at rest was 125 ± 4 nM (n = 22) as calculated from the calibrated resting fluorescence. Both K^+ and carbachol caused increases in quin2 fluorescence which peaked rapidly (~ 1 sec) and then decayed with a $t^{1/2}$ of almost 10 secs. Contractile response to these agents (assessed with a Coulter Counter) was also transient, peaking at about 25 secs (slower by a factor of 2-3 than in unloaded cells), with the cells relaxing thereafter. Isoproterenol, (an SMC relaxant), marginally decreased resting fluorescence and had a pronounced inhibitory effect on carbachol-induced Ca^{++} transients. Results indicate that quin2-like compounds provide a reliable measure of free Ca^{++} in SMC's in suspension, and that changes in Ca^{++} appear to be linked directly to changes in contractility. Understanding of the precise linkage between Ca^{++} and contractile state will require measurements of contractility and Ca^{++} in the same cell, not readily attainable with quin2 but possible using new, more powerful fluorescent Ca^{++} sensitive dyes. Support by HL14523 NIH, MDA grant. DAW-Aust. NHF Res. Fellow.

M-Pos179 CALCIUM ENTRY AND THE REPRIMING PERIOD OF FROG TWITCH FIBERS. B.A. Curtis and R.S. Eisenberg, Depts of Physiology, Univ of Illinois College of Medicine at Peoria, IL 61656 and Rush Medical College, Chicago, IL 60612.

When a D600 paralyzed fiber is depolarized, the Ca entry can be described by two components, resting (0.5 pmol/min) and voltage sensitive (1.1 pmol/min). A third, e-c coupling related component (1.0 pmol/min) is observed in contracting fibers. These fibers were exposed to ^{45}Ca for 5 min; 3 min in Na and then 2 min in K. If the only application of ^{45}ca was for 5 min at 3°C in Na Ringer immediately following a K depolarization. Contracting fibers took up 9.3 ± 3.1 pmol Ca (8 fibers) and paralyzed fibers took up 4.4 ± 1.2 pmol Ca (5). Resting fibers took up 2.3 pmol. The voltage sensitive influx was reduced to 0.3 pmol/min. The contraction related influx was essentially unchanged at 0.8 pmol/min; the e-c coupling related Ca influx occurs after contracture. We believe this component is refilling a Ca store emptied to initiate the preceding contracture. After repolarization, the fiber's ability to undergo a second contracture increases with time and at 3-5°C requires 5 min for complete repriming. The observed e-c coupling related Ca entry occurs during this repriming or mechanically refractory period. When two fibers were exposed to ^{45}Ca for 2-3 min during the repriming period, the Ca uptake was reduced. These results suggest that the rate limiting process in the repriming period may well be Ca movement to refill a Ca store, possibly found in the t membrane. When 10 μM diltiazem was added to the Na Ringer after the first contracture, repriming was delayed. Supported by NIH Grants HL-20230 and NS-12038.

M-Pos180 INTRAMEMBRANE CHARGE MOVEMENT AND SR CALCIUM RELEASE IN FROG SKELETAL MUSCLE. B. Simon, G. Szucs, M.F. Schneider and W. Melzer. Department of Physiology, Univ. of Rochester, Rochester, NY and Gerontology Research Center, Baltimore, MD.

Myoplasmic free calcium transients (Δ[Ca]) and intramembrane charge movement (Q) were monitored in cut segments of single frog twitch fibers stretched to 3.5-3.8 m per sarcomere and voltage clamped at a holding potential of -100 mV using a double vaseline gap. The rate of calcium release during each measured Δ[Ca] was calculated as previously described (Melzer, Rios and Schneider, Biophys. J. 45: 637). The "threshold" voltage for calcium release (V_{th}) was taken as the pulse voltage at which Δ[Ca] during a 100 ms pulse reached a few percent of the Δ[Ca] level attained during a larger 100 ms depolarization that maximally activated calcium release. Using 100 ms pulses of various amplitudes, the peak rate of calcium release was proportional to $Q-Q_{th}$, where Q_{th} was the charge moved by a 100 ms pulse to V_{th}. Using pulses of a variety of durations and amplitudes, the peak rate of calcium release exhibited approximately the same proportional dependence on $Q-Q_{th}$ independent of the pulse amplitude. Thus, the degree of activation of SR calcium release appears to be uniquely determined by the amount of suprathreshold charge moved during a given depolarization.

M-Pos181 OPTICAL MEASUREMENTS OF VOLTAGE DEPENDENT Ca^{2+} INFLUX IN FROG HEART. G. Pizarro, L. Cleemann and M. Morad, Dept. of Physiology, University of Pennsylvania, Philadelphia, PA 19104. Ca^{2+} depletion transients in the extracellular space of the frog heart were measured using Antipyrolazo III (free Ca^{2+} around 50 uM). Ca^{2+} depletion was measured as a weighted average of light signals recorded simultaneously at three different wavelengths (Cleemann, L. et al., Science 226: 174, 1984). Ca^{2+} depletion had a maximal rate immediately after the upstroke of the action potential but prior to the onset of tension. Ca^{2+} depletion amounted to a 10 to 50 uM reduction in the total Ca_o. The initial rate of Ca^{2+} depletion had a bell-shaped voltage dependence, was enhanced by epinephrine and reduced by Ca^{2+} channel blockers (diltiazem and nifedipine). When I_{si} was inactivated with a prepulse to +8 mV the remaining Ca^{2+} influx was greatly reduced and had a voltage dependence resembling a hyperbolic sine function. Modification of the Ca^{2+} channel by epinephrine and Ca^{2+} channel blockers produced parallel changes in twitch force and the Ca^{2+} depletion. On the other hand the positive inotropic effect of strophanthidin and low K_o occurred without any change in the rate of Ca^{2+} depletion. These results support the idea that I_{si} is the major pathway for the inward movement of activator Ca^{2+} in the frog heart. The Na - Ca counter transport system does not contribute significant Ca^{2+} for activation of tension during an action potential, but may be involved in sequestration of Ca^{2+} during relaxation.

M-Pos182 EFFECTS OF INTRAVESICULAR CALCIUM ON VOLTAGE-DEPENDENT CALCIUM INFLUX MANIFESTED BY CARDIAC SARCOLEMMA PREPARATIONS. G.E. Lindenmayer, R.P. Serafino, R.H. Smith and W.P. Schilling. Departments of Pharmacology and Medicine, Medical University of South Carolina, Charleston, SC 29425, and Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550.

Previously, membrane vesicles in sarcolemma preparations from canine ventricle were found to manifest a 1.9-fold increase in calcium influx between -60 and +15 mV (J.Memb. Biol.79:163, 1984). Modifications in the protocol were tested to determine whether the voltage-sensitivity could be increased. Aliquots of the preparation were loaded for 15-18 hr at 5°C with 122 mM KCl, 30 mM N-methyl D-glucamine (NMDG), 10 mM Hepes/Tris (pH 7.4 for 22°C), 3 mM $MgCl_2$, 1 mM EGTA/Tris and 0.3 mM $CaCl_2$ with ^{45}Ca. A dilution was made into a medium at 22°C containing (final concentrations) of 0.1 µM valinomycin, sufficient KCl to yield potassium Nernst potentials of -88 and 0 mV, 1.2 mM $MgCl_2$, 10 mM Hepes/Tris, pH 7.4, 10 µM total $CaCl_2$ with ^{45}Ca, 10 µM EGTA/Tris and sufficient NMDG and/or sucrose to maintain osmolality equal to that of the loading medium. The initial velocity of calcium uptake was measured over 3 to 10 sec. At -88 mV, calcium flux = 6.8 ± 3.2 pmol/mg/sec. At 0 mV, flux = 61.3 ± 8.3 pmol/mg/sec. This equated to a 9.0-fold increase in calcium flux between -88 and 0 mV. Parallel studies (extravesicular total calcium = 31 µM; EGTA = 28.6 µM) yielded calcium flux = 29.0 ± 8.7 and 317 ± 47 pmol/mg/sec at -90 and 0 mV respectively. This equated to a 10.9-fold increase in calcium flux between -90 and 0 mV. Similar studies with no calcium in the loading medium, yielded a 2.4-fold increase between -70 and 0 mV. Thus, the presence of intravesicular free calcium appeared to enhance the voltage-sensitivity of calcium influx. (Supported by Grant HL29566 & a grant from the Keating Endowment.)

M-Pos183 ACTION OF DILTIAZEM ON CALCIUM CURRENTS FROM AMPHIBIAN AND MAMMALIAN SKELETAL MUSCLE. K.B. Walsh, S.H. Bryant, and A. Schwartz, Department of Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267

The calcium antagonist drug diltiazem potentiates mechanical activity in frog (Nature 298:292-294) and mammalian skeletal muscle (Biochem. Biophys. Res. Comm. 122: 1091-1096). This potentiation occurs at low concentrations of diltiazem (EC_{50}=0.1 to 1.0 µM) and is specific for the d-cis isomer of the drug. In this study we examined the effect of d-cis diltiazem on calcium currents (ICa^{++}) from frog (semitendinosus) and rabbit (sternomastoid) muscle fibers using the vaseline gap voltage clamp. Recordings were made at room temperature (21-23°C) in an external solution that contained in mM: $NaMeSO_3$ 120-140, $CaCl_2$ 10, Tris maleate 4, and 10-6 g/ml TTX. Muscle fibers were stimulated every 2 min from a holding potential of -100 mV. ICa^{++} measured had similar kinetics to those reported previously, were blocked by 0.5 mM $CdCl_2$ and were reduced by substitution of Mg^{++} for Ca^{++}. High concentrations of diltiazem were required to produce tonic block of ICa^{++} both in the frog (IC_{50}=200 µM) and the rabbit (IC_{50}=70 µM) muscle fibers. In the frog fibers partial block of ICa^{++} could be produced with 10 and 50 µM diltiazem by increasing the frequency of stimulation to .034 and .017 Hz respectively. With 1 µM diltiazem no block of ICa^{++} was observed in either the frog or rabbit fibers at any stimulation rate. The results demonstrate that ICa^{++} in skeletal muscle are less sensitive to block by diltiazem than ICa^{++} in other tissues. As in other tissues this block is frequency-dependent. The results also indicate that concentrations of diltiazem that enhance mechanical activity in skeletal muscle have no blocking effect on ICa^{++}. (Supported by NIH grants NS-03178 and HL-07382; K.B.W. is a fellow of the A.J. Ryan Foundation.)

M-Pos184 DECREASE IN TRANSVERSE TUBULAR CALCIUM CONCENTRATION IN BARNACLE MUSCLE WITH ELECTRICAL STIMULATION. David R. Van Wagoner, George R. Dubyak, and Antonio Scarpa. Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA 19104.

A spectrophotometric technique has been developed to measure the decrease of extracellular calcium contained in the clefts and tubules invaginating the surface membrane of a barnacle muscle fiber which occurs during depolarizing voltage clamp stimulation. The fiber is cannulated and injected with a solution containing high concentrations of EGTA and TEA^+. The dye tetramethylmurexide (TMX), used in extracellular concentrations of 100-200 μM in the artificial sea water (ASW), is continuously superfused over the muscle fiber. TMX can be used to measure apparent reductions in the [Ca] within the clefts/tubules even when the [Ca] in the bulk ASW is as high as 10 mM. Preliminary experiments have shown that the magnitude and rate of the calcium decrease are frequency dependent. In a fiber clamped to a holding potential of -40 mV, superfused with 1.5 mM calcium, and subjected to repetitive 500 msec duration depolarizing pulses to 0 mV, an approximate 30% steady state decrease of the cleft/tubular [Ca] was observed at 0.2 Hz, and a 60% decrease at 1 Hz. Studies are under way to characterize the effects of various ionic conditions and pharmacologic agents on the magnitude and kinetics of the decrease of extracellular calcium in this preparation. These studies should help to characterize the diffusion properties and the calcium buffering characteristics of the the cleft/tubular network. In addition, the simultaneous determination of I_{Ca} by voltage clamp techniques and optical measurement of decreases in extracellular calcium will help to further characterize the calcium channels in this preparation. This work was supported by NIH grants HL07499 and HL15835.

M-Pos185 LOW TEMPERATURE BLOCK OF CONTRACTION AND MEMBRANE CHARGE IN SUNFISH MUSCLE FIBERS. M.G. Klein and C.L. Prosser (Intr. by George N. Phillips), Dept. of Physiology and Biophysics, University of Illinois, Urbana, IL 61801

Charge movement currents were recorded from Green sunfish (Lepomis cyanellus) myotomal muscle fibers using the three-microelectrode voltage clamp technique. The fibers were bathed in Ringer solution containing (mM): TEA^+ 130, Rb^+ 5, Ca^{++} 2, Mg^{++} 1, Br^- 140, 4-Aminopyridine 1, HEPES buffer 4, pH 7.3. Charge movements recorded in hypertonic Ringer (350 mM sucrose) are qualitatively similar to those observed by others in frog fibers, the ON transient being comprised of a more rapid component, Q_β, and a slower one, Q_γ. The charge-voltage (Q-V) relation can be reasonably described by $Q(V) = Q_{max} / [1 + \exp -(V - V') / k]$ with Q_{max} = 28.6 ±3.9 nC/uF, V' = -39.9 ±6.8 mV and k = 6.4 ±1.2 mV (mean ±SE, 8 fibers, 7-9°C).

In about 60% of the experiments in which the preparation was maintained at low temperature (0-4 °C), contraction was spontaneously blocked. The membrane potential remained large (ca. -85 mV) and sodium currents appeared normal. The abolition of contraction is apparently manifest as a shift to the right in the contraction strength-duration relation, and is usually reversible upon warming. Charge movements in cold-blocked fibers bathed in isotonic Ringer exhibited only a fast component in the ON transient - the slow component was seemingly absent. The Q-V relation in this case is fitted by Q_{max} = 16.4 ±2.3 nC/uF, V' = -37.5 ±8.4 mV and k = 14.1 ±1.4 mV (8 fibers, 3 sunfish, 1-3°C). Upon warming, a variable portion of total charge was recovered along with contractile activity. The data suggest that the slower (Q_γ) component of charge movement is more intimately associated with contractile activation. (Supported by NSF PCM 82-19647 and PHS 5T32 GM7283-10).

M-Pos186 CALCIUM RELEASE AND CHARGE MOVEMENT IN SKELETAL MUSCLE FIBERS EXPOSED TO ZERO Ca AND LONG DEPOLARIZATIONS. Gustavo Brum, Eduardo Rios and John Goldman (Introduced by C. Schauf), Department of Physiology, Rush Medical School, Chicago.

Recent observations on the effect of Ca deprivation (cf. Rios et al., this meeting) and Ca channel blockers on skeletal muscle Excitation-Contraction (E-C) Coupling as well as a proposed role of extracellular Ca in repriming (Curtis and Eisenberg, in press) can be explained assuming that Ca^{2+}, bound to specific proteins of the T-tubule, endows them with charge essential to their functioning as voltage sensors that gate Ca release from the Sarcoplasmic Reticulum (SR) (Graf and Schatzmann, J. Physiol. 349:1, 1984). This model predicts that prolonged depolarization of a fiber in a very low extracellular [Ca^{2+}] will lead to loss of intramembrane mobile charge and permanent impairment of Ca release. We performed this intervention on voltage clamped cut skeletal fibers of the frog containing a Ca-sensitive dye (methods, cf. Rios et al., this meeting). A 10 seconds (s) depolarization to 0 mV in 2 mM Ca_e caused charge immobilization and suppression of Ca release. 27 s after repolarization 80% of the charge movement (Q) and 36% of the peak Ca release flux (R) had recovered. 12 minutes later 100% of Q and 85% of R had recovered. In an external solution with no Ca, 5 mM EGTA, and 3 mM Mg, the figures for recovery were: at 27 s, Q 60%, R 40%; at 12 minutes, Q 90%, R 89%. The time course and extent of the recovery are not essentially changed in zero Ca_e. This is not consistent with the referenced views. Bound Ca^{2+} may be a part of the voltage sensors; however, it doesn't leave them during long depolarizations. Repriming proceeds approximately normally even at very low extracellular Ca. Supported by NIH grant AM 32808.

M-Pos187 CALCIUM-ACTIVATED POTASSIUM CHANNELS INACTIVATE FROM A SHORT-DURATION OPEN STATE. Barry S. Pallotta, Dept. Pharmacology, University of North Carolina, Chapel Hill, N.C. 27514

Single-channel recordings from excised patches of membrane from rat muscle grown in culture were used to examine the time course of activation and inactivation of a large conductance (250 pS) calcium-activated potassium channel. Inside-out patches were bathed in solutions consisting of (mM): KCl, 140; TES buffer, 2; EGTA, 1 mM; pH 7.2. The intracellular surface solution contained in addition 0.6 μM free Ca^{2+}.

During depolarizing voltage steps (+30 to +80 mV) of 1 sec duration the averaged open probability was typically biphasic; a rapid activation phase (time-to-peak < 100 msec) was followed by a slower apparent inactivation (time constants ranged from 400-800 msec). Both the peak open probability and magnitude of the inactivating component increased with depolarization.

During steady-state recording, inactivation was apparent as relatively long duration shut intervals punctuated by one or more openings of short duration (< 200 μsec). The distribution of the lifetimes of those openings which immediately preceded an inactivated interval was composed almost entirely of short-duration openings. Those openings which occurred immediately after an inactivated shut interval were similarly almost entirely of short-duration.

This conditional probability analysis demonstrates that although most openings in the steady-state were to a state of relatively long duration (approximately 5 msec), transitions to and from the inactivated state occurred almost exclusively via a short-duration open state. This suggests that the rate at which the channel opened to the short-duration state might have been the rate-limiting step of the slow inactivation shown by the averaged open probability. Supported by NIH grant 1 RO1 GM32211.

M-Pos188 VOLTAGE-GATED CA-ACTIVATED K CHANNELS IN ISOLATED CANINE AIRWAY SMOOTH MUSCLE CELLS. John D. McCann and Michael J. Welsh, Dept. of Internal Medicine, Univ. of Iowa, Iowa City, IA.

The patch clamp technique was used to study K selective channels in collagenase-isolated canine airway smooth muscle cells. Single channel currents were studied in cell-attached and excised, inside-out patches of membrane. Single channel conductance was 266 in symmetrical 135 mM KCl solution. The channel was perfectly selective for K over Na, Cl, and Ca, as determined by the failure of gradients of these ions to alter the reversal potential. The probability of channel opening, as well as the duration of the open state, increased with membrane depolarization and with an increase in $[Ca^{++}]$ from 10^{-8}M to 10^{-6}M on the internal side of the membrane. Single channel currents were observed even in Ca-free ($<10^{-12}$M) solutions. Thus the channel is voltage-gated and Ca-activated. Addition of 10 mM Ba, Cs, Na, tetramethylammonium (TMA), or tetraethylammonium (TEA) to the internal surface of the membrane reversibly reduced the single channel conductance. Ba was the most effective internal blocker and the only internal blocker to overtly reduce the probability of channel opening. TMA was the second most effective internal blocker. Application of 10 mM Ba or 1 mM TEA to the external surface of the membrane also reduced single channel conductance. 10 mM external TEA abolished channel activity. Although this is the first report of a highly selective Ca-activated K channel in smooth muscle, similar channels have been reported in a variety of tissues including rat skeletal muscle. This voltage-gated, Ca-activated K channel may mediate cell membrane repolarization following contraction in airway smooth muscle. (Supported by the American Heart Association and the Council for Tobacco Research).

M-Pos189 EXPRESSION OF CALCIUM-ACTIVATED POTASSIUM CHANNELS IN HUMAN MONOCYTES WITH TIME AFTER CULTURE. Elaine K. Gallin, Physiology Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145

Human monocytes grown in culture exhibit large conductance (240 pS in symmetrical KCl) channels (Gallin, E.K., Biophys. J., 1984). These channels exhibit a subconductance state and are activated by both depolarization and intracellular calcium (in the range of 10^{-8} to 10^{-5} M). In this study the expression of these channels in human peripheral blood monocytes was examined at various times after cells were isolated and cultured, in order to determine if the expression of the channels changes during the period when the monocytes are differentiating into mature macrophages. Both cell-attached and excised inside-out patches were obtained using standard patch clamp techniques. No large-conductance outward channels were seen in response to depolarizing steps in cell-attached patches from monocytes cultured for 24 hours or less, while 80% of the cell-attached patches from monocytes cultured for over 7 days exhibited these channels. Similar results were obtained with excised patches recorded from in symmetrical KCl with 3×10^{-6} M $(Ca^{++})_i$. Only one in 32 excised patches from cells in culture $\leq$ 24 hr exhibited a calcium-activated potassium channel. While these channels were obtained in 16%, 66%, and 85% of the patches from cells cultured for 2-3, 5-6, or $\geq$ 7 days, respectively. The average number of channels per patch from cells cultured for 7 days or longer, estimated from the number of descrete current levels was 4.3. The channel conductance as well as its voltage and calcium sensitivity was the same regardless of the age of the cells. These data indicate that the expression of calcium-activated potassium channels on the monocyte surface increases with time during the first week in culture when the cultured monocytes are differentiating into mature macrophages. The increase in the number of calcium-activated potassium channels may be related to this maturation process.

M-Pos190 SINGLE CALCIUM-DEPENDENT POTASSIUM CHANNELS FROM AMPHIBIAN STOMACH SMOOTH MUSCLE CELLS. Brendan S. Wong, Department of Physiology, Baylor College of Dentistry, Dallas, TX 75246

Viable single smooth muscle cells were dissociated from the longitudinal and circular muscle layers of the stomach of Rana pipiens by repeated enzymatic digestion with trypsin and collagenase in a calcium-free solution. Dispersed and relaxed single smooth muscle cells ranging from 5-15 μm in diameter and 200-500 μm in length were obtained. Single-channel currents were recorded from these cells using excised patches and the patch-clamp technique. An outward, potassium-selective current was observed with a single-channel conductance of 95 pS under physiological potassium concentration gradients. When bathed in symmetrical 116 mM potassium solutions, the single-channel currents increased linearly with a slope conductance of 120 pS within the potential range of +100 mV to -100 mV studied. The probability of the channel being in the open state was increased by depolarizations of the membrane potential as well as by the presence of calcium ions at the cytoplasmic surface. The curve of the open-state probability of the channel as a function of membrane potential was found to translate along the voltage axis with changes in internal calcium concentrations. Also as the membrane potential was depolarized, the open-state lifetime was increased and bursts of channel openings were separated by longer periods of quiescent channel activity. Subconductance states with approximately one-third and two-thirds of the normal conductance were sometimes observed, most frequently amidst but could also precede or terminate with regular channel openings. These calcium- and voltage-dependent potassium channels were blocked reversibly in the presence of either extracellular or intracellular tetraethylammonium ions.

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M-Pos191 BLOCKERS OF Ca-ACTIVATED POTASSIUM CHANNELS FROM LENS EPITHELIUM. J.L. Rae; Department of Physiology, Rush Medical College, Chicago, Illinois.

Ca-activated potassium channels have been studied from Babcock chick lens epithelium using the extracellular patch voltage clamp technique. Seals were obtained using Corning #7040 and #8161 glasses which seal well to our membranes and have exceptionally low noise properties. Data was obtained from on-cell patches and from inside-out patches.

Several blockers were found for this channel. Decamethonium, Quinine, and Quinidine all produce a "flickery" block over the concentration range of .01-5 mM when applied to the internal surface of the membrane. Barium applied to the inside produces a long duration open channel block in the concentration range of .01-1 mM. Tryptamine HCl applied to the inside produces a reduction in the single channel currents at all voltages over the concentration range of .5-5 mM.

TEA (.1-5 mM) effectively blocks the channel from the outside and provides an effective way to "get rid" of the channel when one of the other seven potassium channels in this preparation is to be studied. With the possible exception of Tryptamine HCl, none of these blockers is specific for Ca-activated potassium channels since each of the blockers affects several of the potassium channels present in this epithelium.

All of the blockers studied are very voltage dependent in their action. (Supported by NIH grant EY03282, the Regenstein Foundation, and the Louise C. Norton Trust.)

M-Pos192 OUTWARD CURRENTS OF IDENTIFIED LACTOTROPHS IN CULTURES OF DISPERSED RAT ANTERIOR PITUITARY (AP). C.J.Lingle, S.Sombati, M.Truman & M.E. Freeman, Florida State U., Biol. Sci., Tallahassee, FL.

We have adapted the reverse hemolytic plaque assay to identify and characterize whole cell and single channel currents of lactotrophs in cultures of dissociated rat AP. Aliquots of AP cells (3×10^6/ml) were mixed with 12% suspension of sheep RBCs conjugated with protein A and spread on a poly-l-lysine-coated cover slip. The cell lawn was rinsed with media containing a specific rat prolactin (PRL) antiserum and guinea pig complement. Within 10 min, lysis of RBCs surrounding lactotrophs begins. Cover slips are transferred to saline for recording. Plaque-identified lactotrophs have resting potentials of -30 to -45 mV using whole cell recordings. In rare cases cell-attached patches show evidence of spontaneous cell spiking. To date, single channel recordings reveal 2 primary types of outward channels and a rarer outward channel. One channel activated on depolarization has a conductance of about 15 pS which rectifies at +100 mV above rest. Openings occur in bursts which are prolonged with depolarization. A Ca^{+2}-dependent K^+ channel, identified using outside-out patches with a 115 mM/1.6 mM K^+ gradient, had a conductance of 72 pS over inside negative potentials of 20-60 mV. The main feature of the third outward channel is excessive noise during openings. The minimum conductance is about 30 pS. The reverse hemolytic plaque assay is a useful technique for identification of specific secretory cells for electrophyiological studies.

M-Pos193 THE CALCIUM-ACTIVATED POTASSIUM CHANNEL HAS A BIPHASIC BEHAVIOR OF OPEN PROBABILITY AS FUNCTION OF POTENTIAL.J.Hidalgo (Spon. F. Snell), Dept. Biophysics, SUNY, Buffalo, N.Y. 14214

In contrast to most results in the literature, the calcium-activated K^+ channel present in tissue-cultured leg muscle cells from chick embryo shows a biphasic dependence on voltage for the probability of being open, Po. Po was obtained from either the amplitude histogram fitted to Gaussian distributions or from open and closed channel lifetimes fitted to sums of exponentials. Most experiments were done with outside-out excised patches with the intracellular face bathed in isotonic KCl, 10mM TES buffer (pH7.4) and with Ca^{2+} adjusted to pCa 3-7. The extracellular face was bathed in isotonic NaCl buffered with 10mM TES and containing 1mM Ca^{2+}. As the membrane was depolarized (cytoplasmic face more positive), Po increased until 0 mV whereupon it decreased ten-fold by +90mV. The decrease in Po was caused primarily by an increase in the lifetime of a longlived (>70ms) closed state. The decrease in Po at depolarized potentials maybe due to channel blockade by Ca^{+2} (cf. Vergara and Latorre, JGP,82:543,1983). The biphasic behavior with potential may limit the Ca-K channel's ability to repolarize a cell under Ca^{+2} loaded conditions.

---Supported by NINCDS-13194 to F. Sachs----

M-Pos194 THE LARGE CONDUCTANCE CALCIUM-ACTIVATED POTASSIUM CHANNEL IN CULTURED RAT MUSCLE HAS AT LEAST THREE OPEN STATES OF SIMILAR CONDUCTANCE AND SIX SHUT STATES. Owen B. McManus and Karl L. Magleby, Department of Physiology and Biophysics, University of Miami School of Medicine, Miami, FL 33101.

The patch clamp technique was used to study the effect of $[Ca^{2+}]_i$ on the kinetics of single large conductance Ca-activated K channels when Mg^{2+} was absent from the solutions. Hill plots of the % time open against $[Ca]_i$ gave an average slope of 3.6 ± 0.4 (8 experiments), suggesting that at least 4 Ca ions bind to the channel for maximum activity. Increased frequency resolution and maximum likelihood fitting has allowed us to identify additional open and shut states for this channel. The distribution of shut intervals was described by the sum of 5 exponentials plus an inactivated state, indicating at least 6 shut states, and the distribution of open intervals was described by the sum of 3 exponentials, indicating at least 3 open states of apparently similar conductance. In an experiment with 0.7 μM Ca_i (+30 mV) the channel was open 18% of the time. The probability density function for shut intervals was: 7.0exp(-t/.070 ms) + 0.77exp(-t/0.29 ms) + 0.056exp(-t/1.3 ms) + 0.0052exp(-t/40 ms) + 0.000063exp(-t/210 ms) + an infrequently occuring inactivated state of longer lifetime, and for open intervals was: 0.89exp(-t/0.11 ms) + 0.38 exp(-t/0.42 ms) + 0.19exp(-t/3.9 ms). The mean shut times of the components with initial 210 ms and 40 ms mean shut times were markedly reduced as $[Ca]_i$ was increased, and the mean open time of the longest open component was increased. A kinetic scheme is presented that can account for the Ca sensitivity of channel activity. Supported by Grants from the NIH (AM 32805) and the Muscular Dystrophy Association. OBM is a recipient of a Muscular Dystrophy Fellowship.

M-Pos195 ADJACENT STATE ANALYSIS OF THE LARGE CONDUCTANCE CALCIUM-ACTIVATED POTASSIUM CHANNEL IN CULTURED RAT MUSCLE INDICATES THAT OPEN STATES WITH SHORT MEAN LIFETIMES ARE ADJACENT TO SHUT STATES WITH LONG MEAN LIFETIMES, AND OPEN STATES WITH LONG MEAN LIFETIMES ARE ADJACENT TO SHUT STATES WITH SHORT MEAN LIFETIMES. Karl L. Magleby & Owen B. McManus. Dept. of Physiology & Biophysics, Univ. of Miami School of Medicine. Miami, FL 33101.

The relationship between the open and shut states of the large conductance Ca-activated K channel was determined by examining the conditional distributions of adjacent open and shut intervals determined from single channel recordings. The percentage of openings to each of the 3 exponential components of the open interval distribution was dependent (conditional) on the specified duration of shut intervals adjacent to the open intervals, while the mean lifetimes of the 3 components remained unchanged. With 0.7 μM Ca^{2+}_i (+30 mV), the % of openings in each exponential component of the conditional distribution of open intervals was:

Duration of adjacent shut interval	short open	intermediate open	long open
0-2000 ms (all shut)	10%	16%	74%
0-0.1 ms	7%	12%	81%
6-2000 ms	20%	19%	61%

These results suggest that open 'states' of short mean lifetime are adjacent to shut 'states' of long mean lifetime and open 'states' of long mean lifetime are adjacent to shut 'states' of short mean lifetime. ('State' includes compound states.) These results also indicate two or more entry pathways between open and shut states and rule out most strictly sequential models for this channel. Supported by grants from the NIH (AM 32805) and the Muscular Dystrophy Association. OBM is a recipient of a Muscular Dystrophy Fellowship.

M-Pos196 KINETIC PROPERTIES OF SINGLE CHLORIDE-SELECTIVE CHANNELS ACTIVE AT RESTING MEMBRANE POTENTIALS IN CULTURED RAT SKELETAL MUSCLE. Andrew L. Blatz and Karl L. Magleby. Dept. of Physiology and Biophysics, Univ. of Miami School of Medicine. Miami, FL 33101.

Resting mammalian skeletal muscle is more permeable to Cl^- than to K^+. We recently reported (Biophys. J., Jan. 1985) two novel Cl-selective channels with fast and slow kinetics and conductances of 45 and 60 pS (symmetrical 100 mM KCl) that are active at resting membrane potentials. We now report on the kinetic properties of the fast Cl^- channel. Single channel currents were recorded from excised inside-out membrane patches with 1 M KCl_i and 140 KCl_o. The distribution of all shut intervals was described by the sum of four exponentials plus an inactivated state, suggesting a minimum of five shut states. The probability density function (PDF) for shut intervals from an experiment at -40 mV was: 11.9exp(-t/0.028 ms) + 1.7exp(-t/.30 ms) + 0.088exp(-t/1.5 ms) + 0.00011exp(-t/68 ms) + about 1 out of every 10000 shut intervals was to an 'inactivated' state with a mean shut time of 10-20 s. The distribution of all open intervals was described by the sum of either two or three exponentials, depending on the experiment, suggesting a minimum of two or three open states of similar conductance. The PDF of open intervals for the above experiment was: 0.20exp(-t/.42 ms) + 0.92exp(-t/1.0 ms). In some experiments a faster distribution with a mean open time of about 0.050 ms was also observed and included about 15% of the openings. The percent of time the channel was open was voltage dependent, increasing from 5% at -80 mV to 60% at -20 mV. Short open intervals were more likely to be adjacent to long shut intervals than they were to short shut intervals, suggesting at least two separate entry pathways between open and shut states. Supported by grant AM 32805 from the NIH and a grant from the Muscular Dystrophy Association.

M-Pos197 PICROTOXIN AND BICUCULLINE ANTAGONISM OF GABA-ACTIVATED SINGLE CHANNEL CHLORIDE CURRENTS. Michael G. Weddle and Robert L. Macdonald. The University of Michigan, Ann Arbor, Mi.

We have studied the actions of γ-amino butyric acid (GABA)-antagonists picrotoxin (PICRO) and bicuculline (BICUC) on GABA-activated single channel chloride currents. Fetal mouse spinal cord and cortical neurons were maintained in dissociated cell culture for 2-6 weeks. For experiments, cells were transferred to a Hepes-buffered saline solution containing the glycine antagonist strychnine (100-500 nM.). Using the patch clamp technique, outside-out patches were obtained (intra-pipette solution contained the impermeable cation TRIS, a [Cl^-] similar to that of the bath, and an EGTA-Ca^{++} buffer). Patches were clamped at positive-inside potentials (+50 to +80 mV.), and broken-tipped micropipettes were used to approach the patches and apply GABA- and antagonist-containing solutions by diffusion. Spontaneous currents were present in all of the patches used in this study, and GABA was frequently applied to increase the occurance of this activity. The currents reversed at a membrane potential of 0 mV., and this reversal was shifted with a partial substitution of isethionate anions for bath chloride, indicating that Cl^- was the principal charge carrier producing these currents. The recorded activity was almost exclusively in the form of channel 'bursting', with open-state conductances falling predominantly into 3 groups -- 21-25 pS., 35-40 pS., and 47-52 pS.

The time distributions of channel openings and closures within bursts were analyzed. In the absence of antagonist, both distributions contained at least two populations of exponentially-distributed events. With increasing concentrations, the initial action of PICRO was the reduction of a population of long open times. Higher concentrations reduced short open times as well. BICUC did not noticeably affect the kinetics of intra-burst openings, but did decrease the burst length. This suggests that PICRO and BICUC antagonize GABA-activated Cl^- currents by different mechanisms.

M-Pos198 KINETICS OF MEMBRANE CHLORIDE CURRENTS IN CULTURED CHICK SKELETAL MUSCLE. J.A.Steele, Department of Physiology, University of Alberta, Edmonton, AB. T6G 2E1, and Department of Anesthesiology, Washington University School of Medicine, St. Louis, MO.

Membrane chloride currents were recorded under voltage clamp with the whole-cell version of the patch clamp technique from small diameter chick myoballs. The chloride currents are responsible for depolarizing the membrane potential to generate a long duration action potential. During step depolarizations from a holding potential of -60 mV, inward current development followed a single exponential time course. Time constants were voltage-dependent and decreased from about 200 ms at -30mV to about 75 ms above +10 mV. Following the onset of the voltage step, there was a delay of 5-10 ms before inward current started to develop, which suggests the presence of an additional closed state. Currents did not decline during maintained depolarization, indicating the lack of an inactivation mechanism. Upon repolarization to the holding potential, tail currents declined as the sum of two exponential components. The time constants for the two exponential components differed by a factor of about 10. The amplitudes of the two exponential components varied as a function of the duration of the depolarizing voltage step. The presence of the extremely slow tail current component suggests the presence of an additional, long-lived open state. All of the observations were consistent with a simple, linear kinetic model with two closed and two open states. Supported by the Alberta Heritage Foundation for Medical Research.

M-Pos199 POTASSIUM AND CHLORIDE CHANNELS IN CULTURED CENTRAL NEURONS OF RAT. F. Franciolini, M. Rizzo, and W. Nonner, Dept. of Physiology and Biophysics, University of Miami, Miami FL 33101.

Neurons from hippocampus or basal ganglia of 19 days old rat embryos were grown for 14 to 40 days in CSF-like medium supplemented with a serum fraction (Kaufman and Barrett, Science, 220, 1394-1396). Measurements of membrane currents from excised, inside-out patches revealed the presence of a variety of ionic channels. Among them are: 1. A potassium channel that is activated within the first 2 msec of a depolarization to 0 mV and then inactivates. Its steady-state inactivation is one half near -65 mV; the inactivation time constant is approximately constant (75 msec) at negative voltages, but decreases with positive voltages. Single-channel conductance is near 40 pS for symmetrical 150 mM KCl solutions. 2. A potassium channel activated by internal Ca ion (>10^{-6} M at 0 mV) to a conductance near 180 pS (150 mM KCl on both sides). 3. A potassium channel of the delayed rectifier type with conductance near 55 pS. 4. A chloride channel active at voltages between -60 and +60 mV. This channel rectifies (inward currents are smaller than outward currents), is not measurably permeant to sulfate ion, and has a conductance near 30 pS (150 mM Cl ion on both sides). Supported by NIH grant GM30377.

M-Pos200 ION CHANNELS IN THE PLASMALEMMA OF CARROT ROOT PROTOPLASTS.
N. Moran*, G. Ehrenstein*, K. Iwasa*, C. Bare# and C. Mischke#. *Lab. of Biophysics, NINCDS, NIH, Bethesda, MD 20205 and #Weed Science Lab, ARS, AEQI, Beltsville, MD 20705.

To the explosively growing list of animal cells now studied with the patch-clamp method we have recently added cells from higher plants- wheat leaf protoplasts (Science, in press), which became amenable to "patching" with the advent of enzymatic treatment techniques for the removal of their encapsulating cellulose walls. We now report that single ion channels are found in the two major groups of the flowering plants, namely the mono- and dicotyledons, and thus appear to constitute an omnipresent class of transport proteins in membranes of cells.

Voltage dependent channels have been observed in an on-cell patch configuration in plasmalemma of carrot root protoplasts grown in tissue culture. The conductance of the channels is app. 40 pS (with 50mM KCl, 1mM $CaCl_2$, 10mM EGTA, 20mM MES, pH6 and 0.4M sorbitol in pipette), which is similar to the voltage dependent type observed in wheat leaf protoplasts. Hyperpolarizing the patch caused channels to open, with inward current flow, while at depolarization no current fluctuations were seen. At least two or three time constants could be resolved in the duration histograms indicating the existence of more than one of each of the open or closed states. Increasing hyperpolarization lengthens the duration of open time (from the range of 15 to 150 ms to 2 to 700 ms) and shortens the closed time(from 15 to 700 ms to the range of 2 to 50ms). Two open channels of equal amplitude could be observed at -74 mV. As in the case of BTX-modified sodium channels in neuroblastoma (see an abst. by Iwasa et al. here), the level distribution departed severely from the binomial.
If the records represent two identical channels, then these channels do not act independently.

M-Pos201 POTASSIUM-SELECTIVE ION CHANNELS IN GUARD-CELL PROTOPLASTS OF BROAD BEANS.
J.M. Fernandez, J.I. Schroeder (Max-Planck-Institut für biophysikalische Chemie, D-3400 Göttingen, W.-Germany) and R. Hedrich (Pflanzenphysiologisches Institut, Universität Göttingen, D-3400 Göttingen, W.-Germany).

In higher plants, the gas exchange between the leaf and the atmosphere, is controlled by the turgor regulated stomatal pore. Opening and closing of this pore is associated with potassium ion fluxes through the guard cell plasmalemma. Patch-clamp techniques allow, for the first time, the separation of the electrical properties of the plasmalemma from those of the cell's vacuole membrane (tonoplast). These techniques have been applied to study the electrical properties of the plasmalemma of guard cell protoplasts of *Vicia faba* (broad bean). Cell free membrane patches revealed predominantly, a cation selective ion channel, with a permeability ratio P_{K^+}/P_{Na^+} greater than 11:1 and a single channel conductance of 37 pS in symmetrical 225 mM KCL solutions. The kinetics of this K^+ selective channel is voltage independent, having a mean lifetime of about 7 ms and an apparent mean closed time of 13 ms. It is estimated that up to 100 of these K^+ selective ion channels are present in a whole cell.

We propose that the K^+ selective ion channel described, is the principal pathway for the uptake and release of K^+ by guard cells during stomatal movement. The driving force for these K^+ fluxes is provided by the ionic gradients between the cell's cytoplasm and the cell wall, and by changes in the transmembrane potential caused by effectors on stomatal aperture such as light, CO_2 and abscisic acid.

M-Pos202 Ca CONCENTRATION-DEPENDENT RECTIFICATION OF α-LaTx-INDUCED CONDUCTANCE IN LIPID BILAYER MEMBRANES.
M. Fresia, M. Robello and S. Ciani. Istituto di Fisica dell' Universita' di Genova,Genova, ITALY and Dept. of Physiology, UCLA, Los Angeles, CA. 90024.

α-Latrotoxin, a presynaptic neurotoxin, induces a cation selective conductance in lipid bilayers, which increases stepwise (~200 pS in DOPC membranes and .1M NaCl), indicating the opening of pores with little tendency to close (Finkelstein et al. 1976, Science N.Y. 193, 1009; Robello et al.1984, Proc. R. Soc. Lond. B 220, 477). While the conductance in the presence of some univalent cations has been studied, little is known about the effects of the divalent ones.Using Montal membranes formed with lecithin-cholesterol mixtures, V_o measurements in Ca gradients conformed to 29 mV slopes. The single channel I-V relations in symmetrical $CaCl_2$ solutions showed a concentration dependent rectification with higher conductance when the toxin-containing solution was negative. From 5mM, where the ratio of the limiting conductances was about 7, up to .5M, where it was almost one, the conductance, normalized to its zero-current value, could be accurately described by a two-barrier, one-site Eyring type of model. Assuming the barriers to be located midway between the adjacent sites, only three parameters appear in the equations: e.g.the ratio of the two rate constants for entering the pore; the binding constant to the internal site; the ratio of the voltage drop between the solution facing the lowest barrier and the internal site to the total potential. The values: 2, $1M^{-1}$ and .3 for such quantities gave an excellent fit to 48 data points, corresponding to I-V curves between -100 and +100 mV at 3 $CaCl_2$ concentrations: 5mM, .3M and .5M. (Supported by funds from the Italian Government, MDA and the Univ. of California).

M-Pos203 VOLTAGE DEACTIVATION OF THE PORIN CHANNEL IN BLACK LIPID FILMS. Phillip G. Wood, Max-Planck Institut f. Biophysik, Frankfurt/M, FRG, Intr. by H. Eberle.
Several reports have indicated that porin (E. coli, K12) when incorporated into folded solvent-free bilayers displays voltage dependent inactivation. Others have shown that porin displays no voltage dependent deactivation in bilayers formed from a n-decane lipid solution. The apparent discrepancy implied that the residual solvent was responsible for the loss of this channel characteristic. However, the lipids and range of applied voltage also differed. In this study porin was incorporated into a n-decane black lipid bilayer (70% PE, 30% PS, 0.7 mm diameter) at low concentrations. A stepwise increase of conductance was seen until a steady state of unit conductance openings and closings was obtained (450 pS unit conductance in 200 mM KCl, 5-10 channels incorporated over 10-20 minutes). When the applied voltage was increased from 20 mV to 120 mV, the opened channels closed in a stepwise manner toward the porin-free lipid level. When the voltage was reduced to 60 mV, the channels again opened in a stepwise manner. The activation and deactivation could be repeated at will by either increasing or decreasing the applied voltage level. The channel seems to begin to inactivate above 80 mV. The apparent differences seem to be more likely the result of the range of voltages selected for observation and/or the choice of host lipid. The voltage dependent deactivation has been observed also under macroscopic conditions and in other phospholipids.

M-Pos204 HmT TOXIN FORMS CATION-SELECTIVE CHANNELS IN PLANAR PHOSPHOLIPID BILAYERS. Marcia Holden, Marco Colombini & Heven Sze, Depts. of Botany and Zoology, Univ. of Maryland, College Park, MD 20742.

Helminthosporium maydis, Race T, toxin is a causal factor of Southern Corn Leaf Blight. The toxin has been shown to uncouple oxidative phosphorylation and dissipate Ca^{2+} gradients in mitochondria isolated from susceptible, but not resistant, corn. The possibility that the toxin behaved like an ionophore was tested using a planar bilayer membrane system. Addition of the toxin to the bilayer system, under voltage clamp conditions, resulted in stepwise increases in current across the phospholipid bilayer. These channels had a lifetime in the range of seconds and there appeared to be an equilibrium established between toxin in solution and in the membrane (toxin could easily be washed out of the membrane with toxin free solution). The membrane conductance increased as a single power of the toxin concentration. The toxin channels are: (i) fairly uniform in conductance, (ii) ideally selective for K^+ over Cl^-, (iii) not voltage dependent over the range +100 mV to -100 mV, and (iv) most conductive to protons. In the linear range protons are 46 times more conductive than K^+ (17.7 pS versus 0.38 pS at 1mM activity). The channel showed the following selectivity for alkali metal cations: $Rb^+ > K^+ > Cs^+ > Na^+ > Li^+$ (16:9:7:3:1) based on the most frequently observed conductance in 1M chloride salts. Channel formation was also a property of a synthetic analog (Cmpd. IV) of the toxin. The ability of the native toxin to form channels in a planar bilayer may be a mode of toxin action on mitochondrial membranes from susceptible corn.
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Toxin and analogue were generous gifts of J.M. Daly, U. of Nebraska.

M-Pos205 VOLTAGE INDUCED CHANNEL-LIKE ACTIVITY IN PURE LIPID BILAYER MEMBRANES. A.Corcia and T.Babila. Dept.Membrane Research. The Weizmann Institute of Science. Rehovot. Israel.

Artificial lipid bilayer membranes formed at the tip of glass patch pipettes are assumed to be practically impermeable to ions unless a relatively high transmembrane potential (the breakdown potential) is applied. We have formed lipid bilayers at the tip of glass pipettes by a variation of the technique of Coronado & Latorre (Biophys.J.43,231-236,1983). Lipid vesicles were made up of soybean lipids, phosphatidylcholine and cholesterol in 12:7:1 molar ratio and added to a plastic well containing a small volume of salt solution (NaCl,150mM; $CaCl_2$,1.8mM; Hepes,10mM; pH=7.6) to give a final lipid concentration of 1.3mM. After a short time a lipid monolayer formed at the surface of the solution. Micropipettes were pulled from borosilicate glass to a tip diameter between 0.7-1 μm. No polishing or coating of the pipettes was necessary, and the pipettes were filled with the same solution as in the well. A lipid bilayer was formed at the tip of the pipette by simply pulling it out of the solution and reimmersing it. The resistance of these bilayers varied from a very few to almost one hundred gigaohms, with the most frequent values between 10-20 gigaohms. With no applied transmembrane potential these lipid membranes are very stable. The membranes were then clamped at increasing potential steps up to a maximum of 200mV. Each potential was maintained for 1-2 minutes and if the membrane remained stable a higher potential was applied. Many of the lipid membranes show, at some potential value well below the breakdown potential, current jumps that look indistinguishible from normal channel behavior. The amplitude of these 'channels' can vary from tens to several hundred pS and their opening times from a few milliseconds to several seconds. The channel activity reversibly disappears by decreasing the applied potential. The transmembrane potential at which this channel-like activity appears is linearly related to the resistance of the membrane.

M-Pos206 IMMUNOGLOBULIN-G INDUCED SINGLE IONIC CHANNELS IN MACROPHAGE MEMBRANES. D.J. NELSON, E.R. JACOBS, J.M.TANG AND R.C. BONE, DEPARTMENTS OF PHYSIOLOGY AND MEDICINE, RUSH MEDICAL COLLEGE, CHICAGO, IL 60612.

Although the binding of immune complexes to macrophage membrane receptors triggers a cellular response that initiates phagocytosis, the mechanism of signal transmission has been poorly understood. We studied the electrical properties of the macrophage membrane and its response to oligomeric immunoglobulin-G (IgG) using the patch clamp technique on human alveolar macrophages obtained by bronchoalveolar lavage and maintained in short term tissue culture. Cell resting potentials as determined from whole-cell tight seal recordings were -56 ± 4 mV (33 cells). Average input resistance was 3.3 ± 0.4 GΩ (27 cells). Exracellular application of heat-aggregated human IgG to cells voltage clamped at -70 mV resulted in peak inward currents of approximately 470 pA. The IgG single channel current response was investigated in a total of 70 membrane patches, 70% of which showed a response. We identified an IgG-dependent non-selective channel in both cell-attached and isolated membrane patches with a unitary conductance of around 350 pS and a predominant sub-conductance level of 235 pS in symmetrical NaCl solutions. Channel open times increased with both membrane depolarization and a rise in Ca_i. Channel opening involved transitions between a number of kinetic states and sub-conductance levels. Channel events recorded in cell-attached patches showed characteristic exponential relaxations implying a variation in membrane potential as a result of a single ion channel opening. These data support the hypothesis that this non-selective cation channel provides the link between the binding of immune complexes at phagocytic membranes and subsequent cellular activation. Supported by NIH grant NS 18587 and a grant from the Fry Foundation.

M-Pos207 AN IMPROVED AMPLIFIER FOR PATCH-CLAMP RECORDING. Franklin F. Offner and Brian Clark, Biomedical Engineering Division, Technological Institute, Northwestern University, Evanston, IL 60201.

The usual amplifier employed in patch-clamp experiments is a "current-to-voltage converter" using resistance feedback to the input. The source resistance with a good electrode seal may be of the order of 100 gigohms; to avoid degrading the signal-to-noise level the feedback resistor should be several times as high. Resistance units of such high values may no longer be treated as a pure resistance, their distributed capacitance and inductance distorting the frequency response in a complex manner. We have developed a current-to-voltage converter which employs a very small capacitor, of the order of 1 pf, preceeded by an integrator, to replace the feedbcak resistor. The capacitor adds no thermal noise, and provides a substantially pure reactance up to very high frequencies. It is thus possible to employ sufficient feedback to obtain a frequency response essentially flat to beyond 10 kHz without neutralization or frequency compensation. As a result the amplifier provides a signal to noise ratio superior to conventional patch clamp amplifiers up to frequencies where channel noise dominates. We have raised this frequency limit by using a single-ended FET input stage, rather than the usual differential amplifier; this results in a 3db improvement in channel noise, and thus raises the useful high frequency limit. The over-all circuit includes provisions for resetting the integrator and the zero-level of the amplifier before application of each voltage step to the preparation. Further improvement will require lowering of the input capacitance, the FET channel noise, or both.

M-Pos208 Stretch activated ion channel in heart cells of the snail, Lymnaea stagnalis. C.S.Morris, B.L. Brezden and D.R. Gardner. Biol. Depts., Universities of Ottawa and Carleton, Ottawa, Canada.

Permeability mechanisms of muscle cells of freshwater snails are of interest because they are a target for the molluscicide Frescon (Gardner & Brezden,1984,Pest.Biochem.Physiol.21:403). Patch clamp studies have therefore been initiated on the heart cells of the aquatic snail, Lymnaea stagnalis. Cells were isolated from ventricles of adult animals by enzymatic dispersion (trypsin, 0.25%, 1h; collagenase, 0.1%, 1h) in saline (50 mM NaCl, 1.6 mM KCl, 3.5 mM $CaCl_2$, 2.0 mM $MgCl_2$, 5 mM glucose, 5 mM HEPES, pH 7.6) containing penicillin-G and bacitracin. The same medium was used to maintain the cells (on coverships in Petri dishes) for up to 4d at R.T. At 2d the cells had resting potentials of -60mV, identical to that of cells in the intact ventricle. Patch clamping (cell-attached, Corning 7052 glass) revealed the presence of channels which reverse at about -70mV in normal saline and appear to be selective for K^+.

At rest with 100mM K^+ in the pipette, 3pA inward current bursts (mean duration ~3msec) were observed. All patches exhibited these currents, occurring at sufficiently low frequency to prevent doubles. When suction was applied, the frequency and duration of bursts increased and double events were seen. These increases were graded with the degree of stretch and sustained for the duration of the applied stretch. At resting potential with normal K^+ (1.6 mM) in the pipette, no currents were evident, either with or without stretch, although with depolarization, stretch-sensitive events were observed, presumably because of a more favourable driving force. The experiments are being repeated with quantitative control on stretch.

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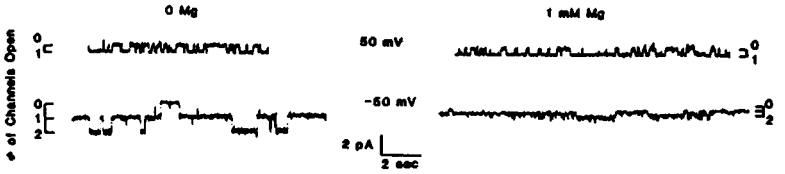
M-Pos209 **MAGNESIUM BLOCKADE OF A CATION-SELECTIVE, RECTIFYING CHANNEL FROM HEART MUSCLE.**

M.T. Nelson[1,3], J.F. Worley III[1], T.B. Rogers[2], W.J. Lederer[1]. Dept. [1]Physiology and [2]Biochemistry, Univ. Maryland, Baltimore, MD and [3]Dept. Pharmacology, Univ. Miami, Miami, FL

Currents through single channels from calf ventricular muscle were examined using planar lipid membrane techniques. When purified sarcolemmal vesicles isolated from calf heart (Rogers,T. J.Bio.Chem. 259,8106,1984) are added to one side ("cis") of a preformed bilayer, some types of channels incorporate with uniform orientation. One such channel has been observed (Lederer & Nelson, J.Physiol. In Press. 1984) and has the following properties: It selects for Na and K over Tris and anions, exhibits voltage-dependent kinetics, rectifies, and is not activated by calcium and not blocked by TTX. We have examined the effects of Mg^{2+} on this channel. Fig.1 shows that the addition of Mg^{2+} to the "cis" side of the bilayer produces virtually no effect on single channel currents while the addition of Mg^{2+} to the "trans" side reduces these currents. Half-maximal block ($K_{0.5}$) of these currents at -50 mV requires about 500 uM Mg^{2+} while at +50 mV there is no reduction of single channel currents even when trans Mg^{2+} was increased to 2 mM. Neither Ba^{2+} (10 mM) nor Ca^{2+} (100 uM) had any significant effect when added to the cis or trans sides of the bilayer. These results suggest that Mg-modulation of membrane currents could influence cardiac excitability.



M-Pos210 A THREE STATE MODEL FOR ALAMETHICIN CONDUCTANCE IN BILAYER MEMBRANES. L. J. Bruner, Dept. of Physics, University of California, Riverside, CA 92521.

A recently completed study of the effect of hydrostatic pressure on alamethicin conductance in bilayer membranes[1] has shown that, a), the onset of alamethicin conductance following a suprathreshold voltage step, which follows a simple exponential time course at one atmosphere, becomes distinctly sigmoidal at 1,000 atm, and, b) the activation volume for the onset of conductance is larger than that characterizing decay, even though the reaction volume associated with establishment of the conducting state is negligible. To account for these findings we have reexamined an old idea[2-4] in terms of a simplified reversible reaction scheme:

$$S \underset{\alpha k'}{\overset{\alpha k}{\rightleftharpoons}} P \underset{k'}{\overset{k}{\rightleftharpoons}} C$$

in which alamethicin moves from a nonconducting surface state, S, to a nonconducting precursor state, P, made more accessible from S by forward applied voltage, and then finally to a conducting state, C. The assumption of a decrease of the relative rate parameter, α, with increasing pressure accounts for the experimental observations. The model also relates this parameter to a geometric factor measuring the degree of sigmoidal response which can be evaluated directly from the data. Alternative models will also be considered.

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M-Pos211 THE EFFERENT TRANSMITTER OF THE VESTIBULAR HAIR CELL MODULATES OPENING RATES OF A K+ CHANNEL. A. Steinacker, Washington University School of Medicine

Sensory transduction in the vestibular system is modulated by an efferent synapse on the sensory hair cell and/or on the efferent fiber. The transmitter at this synapse is putatively acetylcholine, on the basis of immunohistochemical staining. The function of this synapse is unclear; some authors report excitatory and some inhibitory effects of efferent stimulation. This suggests that the transmitter may act by controlling the opening rate of an ionic channel with an equilibrium potential near the cell resting potential. These experiments were designed to test that hypothesis. Hair cells were obtained from the saccule of the toadfish. The macula was incubated in papain and gently triturated. 50 to 200 viable cells adhering to an uncoated plastic petri dish were obtained which retained their stereocilia. A cell attached patch was used with marine teleost Ringer extracellularly and 200 mM KCl in the pipette. The basolateral surfaces of the cells were used for recording. The experimental design was to record with zero holding potential, look for channels open at the voltage and bath apply ACh to the cell to assess an effect on channel opening rate. A channel was found that increased its opening after ACh or oxotremorine. This was a high conductance channel with a reversal potential between +20 to +40 mV with 200 mM KCl or K gultamate in the pipette and 0 mV in a cell detached patch with symmetrical KCl solutions. The channel open time and rate has little voltage sensitivity. Upon patch detachment (inside out), the opening rate of this channel increased markedly. Since bath applied oxotremorine increases the opening of the channel under the patch, a muscarinic receptor and an intracellular mediator control the opening rate of a K+ channel whose properties are suited for modulation of a transduction current.

M-Pos212 ACTION OF TRIFLUOPERAZINE ON MEMBRANE CURRENTS ON IDENTIFIED NEURONS OF *HELIX ASPERSA*. Bernal, J. and Alvarez-Leefmans, F.J. Department of Pharmacology. CINVESTAV-IPN. P.O. Box 14-740, México 07000 D.F.

The action of trifluoperazine (TFP) on Ca^{2+} inward currents (ICa^{2+}) and total outward currents was tested on identified neurons of *Helix aspersa*, using 2 microelectrodes voltage clamp. To suppress Na^{+} inward currents, the preparation was bathed with a solution containing (mM): N-methyl-D-glucamine, 75; $CaCl_2$, 15; KCl, 4; dextrose, 5; and HEPES, 5 at pH = 7.5. Command pulses from 10 to 100 mV were applied to the cells from holding potentials of -45 or -50 mV. The latter corresponded to the resting potential of these cells. Total outward currents were measured 20 ms after the onset of the command pulses while ICa^{2+} were measured at their peak values. 20 min. after TFP (20 µM) was applied to the bath, total outward currents were reduced by 10 to 50%. The TFP blocking effect could be removed by applying more positive command pulses. This became evident in the total outward current versus voltage curves which where shifted 7.6 ± .5 mV (mean ± S.E.; n = 5 cells) along the voltage axis, to more positive potentials. In contrast, peak ICa^{2+} were unaffected or slightly increased (5%) with respect to controls. The effects were reversible even when TFP was applied in doses of 200 µM. We are identifying the class of K^{+} channels affected by TFP. (J.B. was supported by CONACyT, México).

M-Pos213 CORRELATION BETWEEN BLOCKADE OF K CHANNELS IN SQUID AXONS AND AN INCREASE IN VENTRICULAR FIBRILLATION THRESHOLD (VFT) IN DOG HEARTS WITH QUATERNARY DERIVATIVES OF LIDOCAINE. John R. Clay and Marvin Bacaner* (Intr. by D.E. Goldman). Lab. of Biophysics, NINCDS, NIH, at the MBL, Woods Hole, MA 02543 and Dept. of Physiology, U. of Minn., Minneapolis, MN 55455.

We recently demonstrated that the antifibrillatory compounds, bretylium and bethanidine, produced a significantly greater blockade of K current in squid axons compared to the Class I antiarrhythmic compounds, lidocaine and procainamide, thereby suggesting a link between antifibrillatory drug action and K channel block in the heart (J.R. Clay and M. Bacaner, Biophys. J. 45:141a, 1984). We have further demonstrated this link using the quaternary derivatives of lidocaine, QX222, QX314, and QX572. We found that internal application of these drugs (1 mM) produced the following degree of block of K current: lidocaine, 15%; QX222, 40%; QX314, 70%; QX572, 85%. External application of lidocaine, procainamide, and QX572 also produced blockade of I_K, which suggests that these compounds are able to cross the membrane, whereas QX222 and QX314, like bretylium and bethanidine, are ineffective when placed outside the axon. (Cardiac membrane has an external receptor for K channel blockers (Kass et al., J.G.P. 79:1041, 1982); squid axons do not). Preliminary experiments on dog hearts indicate the following effectiveness of these drugs (5 mg/kg) in raising VFT: lidocaine, 53%; QX222, 65%; QX314, 250%; QX572, 300%. This additional correlation between blockade of I_K at the internal receptor site of the K channel in squid axons and a corresponding increase in VFT, independent of whether or not a given drug can cross the membrane, further supports our view that the site of antifibrillatory drug action is located on the outside of one or more types of K channels in cardiac membrane.

M-Pos214 **CHANNELS EXTRACTED FROM SQUID GIANT AXON.** R.M. Torres, R. Coronado, and F. Bezanilla, Dept. of Physiology, UCLA, Los Angeles, CA 90024, Dept. of Pharmacology, Univ. of North Carolina, Chapel Hill, NC 27513 and Marine biological Laboratory, Woods Hole, MA.

Channels extracted from the giant axon of the squid (Loligo pealei) have been studied in order to characterize their electrical and kinetic parameters. To extract the channels we used a modification of the dipping technique, described previously (Torres et al., 1984, Biophys. J. 45:38a),. The axon was wrapped on the tip of a pipette with the internal surface facing out to minimize the contact of the Schwann cells with the monolayer of lipids (L- -Dioleoyl PE or Pe:PS 1:1) as the axon was dipped through the lipid monolayer. Bilayers were then made on the tip of a patch pipette by passing the pitette twice through the monolayer. A preliminary analysis has revealed the presence of at least two different channels. One is a non or poorly selective channel with a conductance of about 10 pS with solutions containing 150 mM K//150 mM Na. Its reconstructed macroscopic current shows no kinetics during a positive voltage pulse (pipette held at virtual ground). The other type is a fast-flickering channel which shows a selectivity up to 5:1 for K over Na, with a conductance close to 20 pS with 150 mM K//150 mM Na. Its reconstructed macroscopic current shows a time dependence during positive voltage pulses and the rising phase of those currents becomes faster as the pulse is made more positive.

Supported by USPHS grants GM30376 and GM32824. RMT was a Grass Fellow.

M-Pos215 PRESENCE OF K^+ CHANNELS IN BILAYERS DERIVED FROM ISOLATED SEA URCHIN SPERM PLASMA MEMBRANES FORMED AT THE TIP OF A PATCH ELECTRODE. A. Liévano, J. Sánchez, and A. Darszon. Depts. of Biochem. and Pharmacol. CINVESTAV-IPN, Mexico City.

The acrosome reaction is a prerequisit for fertilization involving a complex series of events. Changes in the sperm plasma membrane permeability play a crucial role in this reaction. Egg jelly, the natural inductor of the acrosome reaction tirggers among other things, an increase in intracellular Ca^{++} and Na^+ and an efflux of H^+ and K^+. Considering the small size of sperm we have begun a study of its plasma membrane permeability properties by forming bilayers at the tip of a patch electrode (Suarez Isla, Biochem (1983) 22, 2319) from monolayers derived from a mixture of lipid vesicles and sea urchin sperm plasma membranes isolated according to Cross (J. Cell Sci (1982) 59, 13). Currents were recorded using the patch clamp technique (Hamil et al, 1981) with a 10 GΩ feedback resistance and voltage steps in the ±110 mV range were applied. Membranes with high resistance (1-30 gigaohms) could be formed in ~40% of the attempts (85) and from those 25% showed channel activity. Previously it was reported that in Mg^+ free sea water various channels were observed with different conductances (Darszon et al (1984) Biophys. J. 45: 308a). Using simples saline solutions we have initiated the characterization of the ionic channels. In symetric 0.1 M KCl, 10 μM $CaCl_2$ solutions we observed single channels with conductances of 24, 46 and 86 pS derived from linear I-V relations.When aKCL gradient was established we identified $3K^+$ channels (29, 88, 116 pS) from their reversal potential and one Cl^- channel (148 pS) which appears less frequently. Preliminary results indicate the possible presence of a Ca^{++} activated K^+ channel. The presence of K^+ channels in the plasma membrane of sea urchin sperm is consistent with results in intact sperm where TEA and 4A-P, well known blockers of K channels, inhibit the acrosome reaction.

M-Pos216 ION CONDUCTANCE, SELECTIVITY AND BLOCKAGE OF SINGLE K^+ CHANNELS IN CARDIAC CELLS. E. Rousseau, M.D. Payet and R. Sauvé*, Département de Biophysique, Université de Sherbrooke, J1H 5N4, *Département de Physiologie, Université de Montréal, H3T 1J7, CANADA.

The patch clamp technique was used to study the properties of single K channels from primary culture of rat myocardial cells. The experiments were performed at 22°C in McEven solution with KCl filled pipettes (5.4, 10.8, 75, 150 mM) and ionic strenght was maintained by NaCl addition. The I/V relationships were linear for voltages negative to zero-current voltages and show an inward rectification in 75 and 150 mM of KCl. The unit conductances ranged from 10 to 36 pS. Chloride permeability was ruled out by experiments performed in K acetate (150 mM) where the conductance was the same as in KCl (150 mM). The zero current voltages plotted as a function of $[K]_o$ were fitted by the Goldman-Hodgkin-Katz equation with a P_{Na}/P_K of 0.056 which indicated that Na^+ ions can permeate through the channel. Experiments performed with 20 mM KCl + 130 mM Choline chloride (without Na^+) inside the pipettes allow to calculate a conductance of 9 pS which could prove a facilitatory effect of Na ions on the unit conductance. The inward blocking agent, CsCl (0.5 mM) did not affected the unit conductance but induced a frequent flickering between zero level and the open state level.

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E.R. fellow and M.D.P. scholar of F.C.M.C.
R.S. scholar of F.R.S.Q.

M-Pos217 SINGLE "DELAYED RECTIFIER" POTASSIUM CHANNEL CURRENTS IN NEUROBLASTOMA. Stanley Misler and Lee Falke. Depts. of Medicine and Physiology, Washington Univ., St. Louis, MO 63110.

N1E115 neuroblastoma cells differentiated in DMSO display, on depolarization, a slowly inactivating delayed rectifier K^+ current, $I_K(V)$. We have investigated a single channel current which appears to be the unitary current correlate of the macroscopic $I_K(V)$. In cell attached patches formed with pipettes filled with 5.5 K extracellular solution (ES), we routinely see outward channel "bursts", activated within msec after depolarization (V_c), having a linear I-V curve with slope conductance (γs) 20-25 pS (rarely 32-35 pS) and zero current potential (E_{rev}) 15-20 mV negative to V_{rest} (measured as -45 to -50 mV in whole cell current clamping). In patches containing several channels, larger V_c's from V_{rest} initially open a greater fraction of available channels, which in turn close down with continued V_c, though channels are seen to open and close for the entire duration of pulses lasting seconds. Single channel currents averaged within several msec after the clamping pulse show a similar voltage dependence as the peak macroscopic $I_K(V)$; they are increased by hyperpolarizing pre-pulses and decreased by depolarizing pre-pulses. These features are maintained when (1) 5 mM Cd^{2+} is added to ES; (2) $(Cl^-)_o$ is replaced with F^- and (3) the region patched with ES is excised into IS, a 130 mM KF, ~10^{-8} M Ca bath. In the third case, E_{rev} is -60 to -70 mV implying that $P_K/P_{Na} > 5$. γs is reduced by half on addition of ~20 mM TEA to IS. In patches with ≤ 2 channels, τ_{open} and γs for a "burst" appear to be unchanged for the duration of the clamping pulse, while the "burst" frequency (ν) decreases with time. The voltage dependent features of a "burst" are the increased τ_{open} and initial ν with larger V_c's.
(Support: AHA, MO Affiliate & Jewish Hospital Research Endowment Fund).

M-Pos218 THE IONIC BASIS OF THE MEMBRANE POTENTIAL OF GLIAL CELLS SURROUNDING THE MEDIAL GIANT AXON OF THE CRAYFISH. Donald G. Brunder and Edward M. Lieberman, Dept. of Physiology, East Carolina University School of Medicine, Greenville, NC 27834.

The resting membrane potentials of the glial cells of the giant axons of the squid and crayfish are low (-40 to -45 mV) compared to potential measurements in many other invertebrate and vertebrate systems (-70 to -90 mV) (Smiley and Lieberman, Upsula J. Med. Sci. 85(1980)331; Villegas et al. J. Gen Physiol. 51(1968):47). With the exception of squid most glia studied behave as potassium electrodes. The response of the glial cells of the medial giant axon of the crayfish to altered $[K^+]_o$ varied from 52.1 + 1.1 mV in 0.1 mM K^+ to 17.8 + 0.7 mV in 54 mM K^+ - a response that is definitely non-Nernstian. Moreton analysis of this data estimates the intracellular K^+ concentration at about 60 mM. Crayfish and squid glia hyperpolarize in response to nicotinic agonists. Villegas (J. Exp. Biol. 95(1981)135) has shown that squid giant axon glia show a nearly Nernstian response to K^+ in the presence of carbachol. Crayfish glia hyperpolarize 3 - 13 mV in 10^{-7} M carbachol in a non-Nernstian manner. Reducing Na^+ (from 160 mM) to 5 mM results in a transient hyperpolarization of the glial cell membrane; removal of Cl^- has little effect upon membrane potential of the glial cells. The low membrane potential in glia of the crayfish medial giant axon may be due to a combination of K^+ and Na^+ permeabilities that are modified by carbachol. We have also observed a transient glial hyperpolarizataion in response to repeated action potential propagation in the giant axon, similar to that seen in the squid. The low intracellular potassium level and the carbachol-induced hyperpolarization may play a role in potassium ion homeostasis in the perineural space. ARO DAAG29-82-K0182.

M-Pos219 ROLE OF THE EPINEURIUM IN IONIC HOMEOSTASIS OF THE CRAYFISH CNS: AN ELECTROPHYSIOLOGICAL STUDY. E.M. Lieberman, P. Hargittai, J. Pascarella and N.J. Abbott. Dept. of Physiol. Sch. of Med., East Carolina Univ., Greenville, N.C. 27834 and Kings College, U. of London, U.K.

The permeability of the epineural sheath to ions was studied using the sheath (E_S) and medial giant axon (E_M) potentials as K^+ and Na^+-selective "electrodes". E_M, $f(K_o)$, and action potential (AP), $f(Na_o)$, were monitored with a cannulating electrode and E_S with an impaling micropipette. A rapid change of $[K]_o$ from 5.4 to 100 mM induced a transient E_S of 35 mV. The sheath response to 0.1 mM $[K]_o$ was ~-2 mV, indicating asymmetric permeability properties. Na^+ appeared to be totally restricted in that 6 min of Na depletion resulted in no significant change in AP amplitude. In a desheathed preparation the AP fell to a few mV in 2 minutes. Sheath resistance to inward K^+ movement decreased with increased $[K]_o$. The influx was always faster than the efflux. The influx and efflux rate constants with 12 mM/20 mM K^+ gradients were 3×10^{-3} sec^{-1} and 6×10^{-4} sec^{-1}, respectively. Estimates of intracord [K] was 10 mM and at the inner surface of the sheath 14-16 mM even though superfusate [K] was 5.4 mM. Substituting Cl^- with the impermeant ion, isethionate, had no effect on the K^+-induced sheath potential amplitudes but K^+ accumulation and washout decreased by a factor of 3 and 45, respectively. In Cl^- free solution the giant axon, surrounded by an intact sheath, showed a slow hyperpolarization due to outward co-movement of K^+ and Cl^- across the sheath. The periaxonal cell layer (desheathed cord) did not have asymmetrical diffusion properties. Desheathed preparations had 3X the influx rate and 65X the efflux rate of sheathed preparation. The sheath separating the nerve cord from the intrathoracic space behaves as a selective diffusion barrier to ions maintaining the ionic environment of the CNS. ARO DAAG29-82-K0182.

M-Pos220 INCREASED VOLTAGE-GATED K^+ CONDUCTANCE IN T-LYMPHOCYTES STIMULATED WITH PHORBOL ESTER. S. Lee, D. Krause & C. Deutsch, Dept. of Physiology, Univ. of Pennsylvania, Phila., PA 19104 (Intro. by David Wilson).

Previous work demonstrated the presence of a voltage-gated K^+-selective conductance in T-lymphocytes from human peripheral blood, and agents which blocked the current also inhibited lectin-stimulated proliferation (Matteson & Deutsch, 1984; DeCoursey et al., 1984). Using the whole-cell patch clamp method, we have looked at the K^+ current in human T cells one and two days after stimulation under mitogenic conditions with the tumor-promoting phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA), as well as plant lectins. By 20-24 hours after stimulation, the distribution of peak currents observed in TPA-activated cells is shifted to higher values, though the voltage-dependence, kinetics and pharmacological sensitivities are unchanged. However, the current did show sensitivity to intracellular pH not observed in unstimulated controls. At a pH more acidic (6.5) than the normal 7.2 used for the pipette filling solution, the current was slightly depressed, whereas at 7.8 it was increased. Cells activated with succinyl con A showed little or no increase in K^+-current at one or two days; and cells activated with the lectin, phytohaemagglutinin, actually had smaller peak current at 24 hours than control. Nevertheless, the K^+-channel blocker quinine was equally effective inhibiting proliferation regardless of which mitogen was used. This suggests that K^+-channel function may play a role in supporting mitogenesis, but increased K^+ conductance may not be a general requirement in mitogen-stimulated proliferation.

M-Pos221 CHARACTERIZATION OF IRON UPTAKE FROM HYDROXAMATE SIDEROPHORES BY CHLORELLA VULGARIS AND EVALUATION OF REDUCTIVE RELEASE AS A POSSIBLE UPTAKE MECHANISM. F. C. Thomas Allnutt and Walter D. Bonner, Jr. Dept. of Biochemistry and Biophysics and Department of Biology, University of Pennsylvania, Philadelphia, Pa. 19104

Utilizing Chlorella vulgaris, a unicellular green alga, for the study of iron uptake from microbially produced, ferric specific chelates, called siderophores, permitts flexibility not normally available for plant uptake studies. The siderophores studied, desferrioximine B (DFOB) and rhodotorulic acid (RA), have formation constants for the ferric chelates of about 10^{30}. Iron uptake from Fe^{3+}-DFOB and Fe^{3+}-RA by iron stressed cells was inhibited by 40μM but not by 20μM CCCP. Antimycin A (25μM) or 100μM cyanide did not inhibit iron uptake. Iron uptake was the same when measured in the dark and the light. The uptake rate saturates at about 5μM iron. Free DFOB does not compete with the chelated form even when 10 fold excess DFOB was present. Divalent cations Ni^{2+} and Co^{2+} were able to compete with the uptake mechanism while Mg^{2+} and Ca^{2+} had no effect. When [^{14}C]-RA was used as the chelator, no label was accumulated. Using electron paramagnetic resonance, uptake was correlated directly with ferric reduction. It was shown that reduction occurs exposed to the external medium by spectrophotometric determination of the presence of a Fe^{2+} specific chelator, bathophenanthroline disulfonate (BPDS), that was impermeable to the plasma membrane. Also, ferricyanide, an alternate electron acceptor that is impermeable to the plasma membrane, was able to inhibit uptake. However, reduction rates measured by the BPDS method were much faster than those seen using EPR. This suggests that only a fraction of the available reductive capacity is used for this reduction. Uptake and reduction were not found in non-stressed cells.

M-Pos222 TWO DISTINCT SITES RELEASE ^{86}Rb FROM THE Na/K PUMP. Bliss Forbush III, (Intro. by D. G. Shoemaker), Dept. of Physiology, Yale University School of Medicine, New Haven, CT 06510.

It is believed that the inward transport of K (or Rb) ions by the Na/K pump involves an "occluded state" in which the ions are tightly bound (Glynn and Richards, J. Physiol. 330:17,1982). We have reported that the rate of release of ions from the tightly bound form is rapid enough to be consistent with this hypothesis but that the time course of ^{86}Rb dissociation is markedly biphasic, particularly in the presence of K+MgPi (Biophys.J. 45:76a,1984, Proc. IUPAB(8), 299,1984). The biphasicity could be indicative of ^{86}Rb release from identical sites with cooperative interaction, or of release from non-identical sites; if the former were true, the two Rb ions should be indistinguishable. Our hypothesis is that stabilization of half of the occluded ^{86}Rb by K in the presence of MgPi is due to occupation of the second site by K. To test this, Na,K-ATPase was incubated with unlabelled Rb to form the occluded state and then incubated with ^{86}Rb and MgPi for 5 s at ~5°C. It was found that ^{86}Rb was tightly bound during the second incubation (~1/2 of maximal), indicating that the stabilizing Rb (K) ions themselves become occluded. Furthermore, as measured in the rapid filtration apparatus, most of the newly occluded ^{86}Rb was released with the time constant of the fast phase of normal dissociation, t_1~18 s^{-1}. When the protocol was reversed, that is occluded ^{86}Rb was formed followed by incubation in unlabelled Rb + MgPi, primarily the slow phase (t_2~1 s^{-1}) was observed. These findings clearly demonstrate that the two K sites are different. When deocclusion was promoted by ATP following the above procedure, it was found that ^{86}Rb ions at the "t_2 site" were released more rapidly than those at the "t_1 site", in contrast to the result with MgPi. These results are consistent with a simple model in which the first K ion bound to the extracellular face of the pump is the first one released from the intracellular face. (Supported by NIH GM31782).

M-Pos223 INTRACELLULAR pH AFFECTS EXTRACELLULAR POTASSIUM AFFINITY OF THE SODIUM PUMP. G.E. Breitwieser and J.M. Russell. Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550.

We utilized the technique of internal dialysis in squid giant axons to investigate the effects of variations in intracellular pH (pH_i) and extracellular pH (pH_o) on Na pump function. We previously reported that ouabain-sensitive Na efflux (Biophys. J., 41, 71a (1983)), and ouabain-sensitive K influx (Biophys. J., 45, 76a (1984)) are both sensitive to changes in pH_i and insensitive to changes in pH_o. Activation of ouabain-sensitive Na efflux by Na_i was examined at three pH_i's: 6.35, 7.35 ($_o$control), and 8.3. A decrease in pH_i by one pH unit to 6.35 caused a 33% reduction in V_{max}, but the K_m was essentially unchanged (7.35, K_m 29.1 mM; 6.35, K_m 31.5 mM). We also examined the ability of K_o to activate ouabain-sensitive Na efflux, at pH_i's 6.35 and 7.35. A decrease in pH_i of one pH unit, to 6.35, caused a decrease in the extracellular affinity for K, but no decrease in the V_{max} (7.35, K_m 6.5 mM, V_{max} 41.7; 6.35, K_m 15.4 mM, K_m 48.6). At $[K]_o$ = 10 mM, there was a 27% reduction in ouabain-sensitive Na efflux. This agrees well with the observed decrease in Na efflux obtained in the Na_i activation experiments. Decreasing pH_i causes a decrease in extracellular K affinity, and further, increasing K_o can fully reverse the Na pump flux inhibition caused by decreasing pH_i. The effect of alkaline pH_i on Na_i activation of ouabain-sensitive Na efflux is more complex. A shift in pH_i from 7.35 to 8.3 caused both a decrease in V_{max} and an increase in the K_m for Na_i (7.35, K_m 31.5, V_{max} 43.2; 8.3, K_m 45.9, V_{max} 19.4). Further evidence for such complexity comes from the alkaline limb of the pH_i dependence of both Na efflux and K influx, where there appears to be a change in the Na:K coupling ratio. Supported by NS11946 and NS13778.

M-Pos224 THE BEHAVIOUR OF THE OUABAIN INSENSITIVE Na EFFLUX IN BARNACLE MUSCLE FIBERS TOWARD PROCTOLIN. Jude Nwoga* and E. Edward Bittar. Department of Physiology, University of Wisconsin, Madison, WI 53706.

Earlier experiments with single barnacle muscle fibers have shown that external application of serotonin in a physiological concentration causes a transitory rise in the sodium efflux. This response is probably due to activation of cAMP-dependent protein kinase by newly formed cAMP. This work has now been extended to proctolin, a pentapeptide found in crustacean tissues and known to produce excitatory effects e.g. in muscle. External application of proctolin (10^{-8}M) to unpoisoned and ouabain-poisoned fibers always results in stimulation of the Na efflux. The kinetics of the response resemble those seen after injection of cAMP or Ca^{2+}, viz, the effect is rapid in onset and transitory. The concentration-response curve for proctolin is sigmoidal and the minimal effective concentration is considerably less than 10^{-9}M. Whereas concentrations of proctolin greater than 10^{-6}M cause shortening of the fibers to about one-fourth their original length, lower concentrations do not cause any visible shortening. Furthermore, experiments show that the response to proctolin wholly depends on external Ca^{2+}. Restoration of Ca^{2+}_e leads to transitory stimulation of the efflux. Substitution of Na_e by choline does not prevent the response to proctolin from occurring. Raising Mg^{2+}_e from 10 to 50 mM abolishes the response. To examine the possibility that proctolin action involves activation of Ca^{2+} channels, experiments were done with verapamil, Cd^{2+}, Co^{2+} and WB-4101. These substances prevent the response to 10^{-8}M-proctolin. Preinjection of 0.1M-EGTA abolishes the stimulation. Since forskolin, phosphodiesterase inhibitors, e.g. PMX (1-propyl-3-methyl-7-(5-hydroxyhexyl)-xanthine) and IAX (1-isoamyl-3-isobutyl xanthine), and preinjection with PKI fail to alter the response, stimulation of sodium efflux following proctolin is probably not cAMP-mediated.

M-Pos225 RESPONSE OF PRIMARY AND TRANSFORMED MAMMALIAN CELLS TO PULSED ELECTROMAGNETIC STIMULATION, C. T. Hanks, School of Dentistry, and W. C. Parkinson, Dept. of Physics, Univ. of Michigan, Ann Arbor, Michigan 48109.

It is known that appropriate pulsed magnetic fields[1] and dc electric fields[2] promote osteogenesis and wound healing, and that weak dc electric fields[3] cause a redistribution of membrane proteins and influence cell growth and motility in primary cell cultures. However, there is no evidence[4] for such effects for transformed cells. We have been studying the difference in the uptake of ^{3}H-TdR by cultures of primary (fetal rat calvarium) and transformed (L-929) mammalian cells in response to pulsed electromagnetic fields. Preliminary results indicate that the uptake is enhanced in proportion to the induced electric field for fields up to 1 volt/m for the primary cultures, but no statistically significant enhancement is observed for L-929 cells. Further the induced electric field, not the magnetic field, is the stimulating factor. Experimental details will be described and quantative results presented.

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M-Pos226 ^{3}H-OUABAIN AND ^{125}I-MONOCLONAL ANTIBODY BINDING TO NA,K PUMP IN HEART CELLS. L.A. Lobaugh and M. Lieberman. Dept. of Physiology, Duke University Med. Ctr., Durham, NC 27710

Receptor occupancy by ^{3}H-ouabain (^{3}H-O) is not strictly coupled to inhibition of Na,K pump activity in cardiac muscle, which suggests that ouabain may bind to a class of sites other than the functional Na,K pump (Werdan *et al.*, Biochem. Pharm. 33:55, 1984). Therefore, binding of ^{125}I-monoclonal antibody (AB) that recognizes chicken muscle Na,K-ATPase (Fambrough & Bayne, J. Biol. Chem. 258:3926, 1983) was compared with binding of ^{3}H-O to cultured chick embryo heart cells. Kinetic analysis of binding and dissociation of 0.1μM ^{3}H-O (0 .5K; 37C) showed $k_1=3\times10^4$/M·sec and $k_2=0.01$/sec. $K_D=0.25$μM can be calculated from the ratio of the rate constants. In 0.5K, equilibrium binding of ^{3}H-O (0.01-10μM; 30 min) revealed a single class of binding sites with $K_D=0.33$μM and $B_{max}=10.3\pm0.8$ pmol/mg prot (X±SEM; n=14). In 5.4K, ^{3}H-O bound with $K_D=5.0$μM and no significant change in Bmax. Ouabain inhibited the initial rate of ^{42}K-uptake in Na-loaded cells with ED_{50} = 0.9μM. By contrast, AB binding (13nM; 22C) was unaffected by K (1-13mM) or ouabain (1.6mM) in the medium and cells continued to beat in medium containing excess AB. AB (33nM) bound to monolayers with $t_{1/2} \simeq 10$ min @ 22C and less than 20% of bound AB dissociated into AB-free medium in 4 hrs. $B_{max}=10.0\pm0.4$ pmol/mg prot (n=6) was determined at a saturating AB concentration (33nM) after 4 hrs. We conclude that the number of Na,K pumps determined by specific AB and ^{3}H-O binding are equivalent ($p>0.8$) and represent 1.5×10^6 sites/cell ($4.0\pm0.2\times10^6$ cells/mg prot; n=8). Therefore, ouabain receptors not associated with Na,K pump inhibition are Na,K-ATPase molecules and may be nonfunctional Na,K pump sites. Supported by NIH grant HL27105. AB was kindly provided by Dr. D. Fambrough, Carnegie Inst. of Washington, Baltimore, MD.

M-Pos227 TEMPERATURE-DEPENDENT INHIBITION OF Na^+/K^+ ATPASE ACTIVITY BY MICROWAVE RADIATION. John W. Allis and Barbara L. Sinha, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711.

We report evidence for a temperature-dependent inhibition of the enzyme Na^+/K^+ ATPase in membranes from fresh human red cells during exposure to 2450-MHz microwave radiation. The inhibition occurs only near 25 °C within the range investigated, 23-27 °C. An Arrhenius plot of the log of the (unexposed) Na^+/K^+ ATPase activity vs. inverse temperature shows two linear regions with a break near 25 °C, indicating a change in the activation energy for the ATPase reaction. The change in activation energy and the effect of exposure to microwave radiation both occur at 25 °C. This coincidence is consistent with a mechanism in which the radiation affects the enzyme only during a temperature-induced conformational change. Although the Ca^{2+} dependent ATPase also showed a break in the Arrhenius plot near 24 °C, there is no evidence that 2450-MHz microwave radiation inhibited this enzyme.

Measurements were performed during exposure on a Cary 15 spectrophotometer with a wave-guide fitted into the sample compartment. Temperature was carefully controlled and continuously measured with a thermocouple installed through a frosted side of the spectrophotometer cell. Comparable exposed and unexposed samples were maintained at the same temperature.

M-Pos228 **TOPOLOGY OF THE *LAC* CARRIER PROTEIN IN THE MEMBRANE OF *ESCHERICHIA COLI*.** **N. Carrasco, D. Herzlinger, S. DeChiara, W. Danho* and H.R. Kaback, Roche Inst. Mol. Biol. and *The Biology Dept. of Hoffmann-La Roche, Inc., Roche Research Ctr., Nutley, NJ 07110**

The *lac* carrier protein, an intrinsic membrane protein encoded by the *lac y* gene that catalyzes H^+:lactose symport, has been purified to homogeneity in a functional state. The protein is a 46.5 Kd polypeptide of known sequence. A secondary structure model has been proposed based both on CD measurements indicating that the protein is ≈85% α-helix and on the hydropathic profile of the protein. Monoclonal antibodies against the protein have been prepared. One of the antibodies binds to an epitope on the external surface of the membrane, inhibiting H^+:lactose symport without altering the ability of the protein to bind substrate or catalyze exchange. Efforts to study the topology of the protein are focussed on the use of site-directed polyclonal antibodies. Polypeptides 10-15 residues in length corresponding to various portions of the protein have been synthesized, and antibodies against these peptides react with the *lac* carrier. Binding experiments with labeled antibodies demonstrate that loops 5, 7 and the C-terminus are on the cytoplasmic surface of the membrane as predicted by the model. Furthermore, some of the antibodies are able to discriminate between wild type and "uncoupled" permease molecules.

M-Pos229 PHOSPHORYLATION OF THE Ca^{2+}-ATPase PURIFIED FROM ERYTHROCYTE GHOSTS. Danuta Kosk-Kosicka, Sonia Scaillet and Giuseppe Inesi, University of Maryland School of Medicine, Baltimore, MD 21201

The phosphorylation of the erythrocyte ghosts Ca^{2+}-ATPase, purified on calmodulin column in the presence of $C_{12}E_8$ was studied. It was found that the enzyme can by phosphorylated either by [$^{32}\gamma$-P]ATP or ^{32}P-P_i in the forward or reverse direction of the cycle respectively, similar to the ATPases from other membranes. Maximal level of phosphorylation was 0.8 nmoles/mg protein, upon addition of [γ-^{32}P]ATP in ice in 20 sec. K_m for ATP was 8.7 μM. In rapid quench experiments it was established that formation of the phosphorylated intermediate is the first measurable event following addition of ATP to the enzyme preincubated with μM Ca^{2+}. P_i is then cleaved from the phosphoenzyme with a 4-5 sec^{-1} turnover at 25°, in the presence of 4 μM ATP. The pre-steady state kinetics include an overshoot of the phosphoenzyme level, and a lag period followed by an initial burst of P_i production. Phosphorylation with ^{32}P-P_i was obtained at equilibrium in the presence of 40% DMSO and the maximal EP level obtained was 0.5-0.9 nmoles/mg at 37°C. The enzyme can catalyze a very low rate of Ca^{2+} dependent exchange between orthophosphate and the γ-phosphate of ATP. The Ca^{2+} concentration required for half maximal activation of the ATP$\longleftrightarrow P_i$, exchange was around 10^4 times higher than that for Ca^{2+}-dependent ATP hydrolysis. (Supported by the National Institute of Health (HLBI) and the Muscular Dystrophy Association.)

M-Pos230 REGULATION OF ENERGY PRODUCTION IN RENAL CELL LINES. R.M. Lynch* and R.S. Balaban LKEM, NHLBI, NIH. Bethesda, MD. 20205

A close relation between active Na-K transport and glycolysis has been described in a variety of tissues. We have begun to study this coupling in epithelial cells by analyzing the energetics of two established renal cell lines, A6 and MDCK. ATP production was determined by measuring the rates of oxygen consumption (QO_2) and lactate production (Jlac). A6 cells produce ATP at a rate of 43.1 ±1.7 nmol/min·mg (16% via glycolysis) while MDCK cells produce ATP at a rate of 98.6 ±2.8 (22% via glycolysis), with glucose and glutamine as substrates. Ouabain was used to inhibit Na-K ATPase. Ouabain inhibited Jlac by 44% in MDCK and 43% in A6, whereas QO_2 decreased by less than 20% in both cell lines. Na-K ATPase was stimulated by the addition of arg-vasopressin (AVP) to A6 cells and nystatin to both cell lines. The addition of AVP leads to a doubling Jlac (+72.8%) without a significant effect on QO_2. Nystatin, which dissipates cell Na gradients, increased Jlac by 192.9% and QO_2 by 29.0% in A6 cells. In MDCK cells, nystatin elicited increases of 57.1% in Jlac and 10.3% in QO_2. AVP and Nystatin responses were inhibited by ouabain. These findings indicate that Jlac is more tightly coupled to Na-K ATPase than is QO_2 in these cell lines. This is directly supported by the observation that the addition of glucose stimulates the rate of ouabain sensitive K^+ uptake by K^+ depleted MDCK cells, which were respiring on glutamine alone.

M-Pos231 NA AND K EFFLUX AND [^{3}H] OUABAIN BINDING IN THE HUMAN LEUKEMIC CELL LINE K562. S. Dissing, Dept. of Clinical Chemistry, Aalborg Hospital South, P. Box 365, 9100 Aalborg, Denmark.

Na and K transport and Na/K pump densities in K562 cells with erythroid phenotypic features were studied in order to gain information about cation transport characteristics during hematopoietic cell differentiation. When K562 cells are suspended in Cl media ^{22}Na reaches equilibrium in less than a few seconds, but Na efflux can be greatly reduced when Cl is replaced by NO_3 or SO_4 on both sides of the membrane (Dissing et al., Am. J. Physiol. 16: C53-C60, 1984). When K562 cells contain 30 mM Na_i and are resuspended in NO_3 or SO_4 media containing Mg and K, the ouabain-sensitive Na efflux amounts to 7.3×10^{-9} mmole/cm^2/sec and is approximately 100 times faster than pump mediated Na efflux from human red cells at a similar Na_i. ^{42}K efflux is not affected by the anion substitutions, by the presence of 2 mM furosemide, and is in the absence of K_o reduced 85% resulting in K efflux of 1×10^{-9} mmole/cm^2/sec. In a K medium 0.4 mM quinine causes an 80% inhibition of K efflux. The number of Na/K pumps in K562 cells were determined by the specific binding of [^{3}H] ouabain in a K-free medium, and it was found that each cell at saturation binds 260000 ouabain molecules per cell. Thus, the K562 cell contains a Na/K pump density on the plasma membrane which is 120 times larger as compared to the mature erythrocyte when the surface area of the K562 is 6.75×10^{-6} cm^2 and the red cell contains 450 Na/K pumps per cell. This is in agreement with the observed ratio for ouabain-sensitive Na transport in the K562 cell compared to the mature red cell. An identical number for [^{3}H] ouabain binding sites per cell was obtained following induction with 3 μM 5-azacytidin for 4 days giving rise to hemoglobin synthesis and a greatly reduced rate of cell proliferation. (Supp. by The Medical Research Foundation, Northern Jutland).

M-Pos232 THE RELATIONSHIPS BETWEEN OUABAIN BINDING, Na-K PUMP MEDIATED K^+ TRANSPORT AND INTRACELLULAR Na^+ AND K^+ LEVELS IN ISOLATED FELINE VENTRICULAR MYOCYTES. Lewis H. Silver, Ethel L. Loew and Steven R. Houser. Department of Physiology, Temple University School of Medicine, Philadelphia, Pa. 19140

The initial event leading to the positive inotropic effect of the cardiac glycosides is thought to be binding to and inhibition of the sarcolemmal Na-K pump (the Na-K ATPase). The goals of this study were to : 1) characterize ^{3}H-ouabain binding to isolated adult calcium tolerant cardiac myocytes and 2) define the relationships between receptor (Na-K pump) occupancy, active K^+ transport and intracellular Na^+ (Na_i) and K^+ (K_i) levels. Scatchard plot analysis of specific binding revealed a single class of sites with an affinity (K_d) of 1.8 +/- 0.5 X 10^{-8} M and a binding capacity (B_{max}) of 1.9 +/- 0.7 X 10^6 sites/cell (n=6). The effects of ouabain on K^+ uptake and on Na_i and K_i did not follow the relationship between ouabain concentration and Na-K pump occupancy but instead were both shifted toward higher ouabain concentrations. Specifically, ouabain-sensitive ^{42}K uptake was maximally inhibited at 10^{-4} M ouabain with an ED_{50} value of 1.5 X 10^{-6} M. Ouabain produced a dose dependent (10^{-9} to 10^{-3} M) increase and decrease in Na_i and K_i, respectively. The ED_{50} value for the increase in Na_i was 1.0 X 10^{-6} M ouabain. These results support the idea that when a small fraction of pump sites are occupied, remaining non-inhibited sites increase their activity (functional pump reserve) to maintain active ion transport and cellular Na^+ and K^+ near normal levels.

(Supported by a grant from the American Heart Association, Southeast PA Chapter)

M-Pos233 CONVERSION OF E_1P TO E_2P IN NAK-ATPASE PROTEOLIPOSOMES. S. Yoda and A. Yoda, Department of Pharmacology, University of Wisconsin Medical School, Madison, WI 53706.

The membranous fragments of electric eel NaK-ATPase (Fr) mainly form the K^+-sensitive phosphoprotein (E_2P) with ATP in the presence of 10 mM Na^+, but they form the ADP-sensitive form (E_1P) in the presence of 100 mM Na^+. This E_1P from Fr is dephosphorylated by K^+. However, the E_1P is predominant (>70%) at various Na^+ concentrations in the NaK-ATPase proteoliposomes (Pl) prepared from Fr by using CHAPS. Since the disintegration of Pl by CHAPS shifted the E_1P and E_2P percentages close to those of Fr, this high E_1P content seems to be the characteristic feature of Pl. Another difference of Pl from Fr is that the E_1P of Pl was not dephosphorylated by K^+, even in the presence of Na^+ on both sides. These characteristics indicate that the closed lipid bilayer which separates the cytoplasmic side from the extracellular side of the NaK-ATPase particle interferes with the E_1P to E_2P conversion. Na^+ ionophores counteracted this closed bilayer effect, and Pl yielded the E_1P-rich EP in the presence of 20-50 μM monensin, nigericin, or monensin methyl ester in the presence of 10 mM Na^+. The proton ionophore, CCCP, did not change the content of E_1P in Pl but increased the content of E_2P. These effects suggest that there are at least three phosphorylated intermediates in the NaK-ATPase, as proposed by Nørby *et al.* (1983) (Supported by NIH Grant # HL16549).

M-Pos234 METABOLIC EVIDENCE FOR ACTIVE AMMONIUM UPTAKE BY Na^+-K^+-ATPase IN PROXIMAL KIDNEY TUBULE CELLS. Ira Kurtz and R.S. Balaban, (Introduced by M.B. Burg) LKEM, NHLBI, Bethesda, MD

The ammonium ion (NH_4^+) is capable of substituting for K^+ in the in vitro Na^+-K^+-ATPase hydrolysis reaction. However, little evidence is available that NH_4^+ may substitute for K^+ in the Na^+-K^+-ATPase reaction in intact cells. In this study we used the ouabain sensitive QO_2 of rabbit proximal tubule suspensions as an assay of Na^+-K^+-ATPase activity. Ouabain sensitive QO_2 and Na^+-K^+-ATPase pump activity in this preparation are tightly coupled with a stoichiometry of 18 K^+ pumped/O_2 consumed. The addition of 5 mM NH_4Cl to K^+-depleted tubules resulted in a 24.2 % ± 2.3 (n = 12) steady state increase in QO_2. This increase was completely inhibited by the addition of 5×10^{-5} M ouabain before or after the NH_4Cl addition. The addition of 5 mM KCl also stimulated the QO_2 of K^+-depleted tubules by 21.2% ± 5.7 (n = 7) and was ouabain sensitive. The effects of 5 mM NH_4Cl and KCl were non-additive suggesting that they were both affecting Na^+-K^+-ATPase in the same fashion. These results are compatable with the hypothesis that in the renal proximal tubule cell, NH_4^+ can be actively transported across the basolateral membrane by substituting for K^+ on the Na^+-K^+-ATPase.

M-Pos235 CALCIUM STIMULATED PHOSPHORYLATION OF THE Ca^{2+}-PUMP OF SQUID OPTIC NERVE PLASMA MEMBRANE. M. Condrescu and R. DiPolo, Centro de Biofísica y Bioquímica, Instituto Venezolano de Investigaciones Científicas, IVIC, Apartado 1827, Caracas 1010A, Venezuela.

Besides the Na/Ca exchange, an ATP-driven Ca^{2+}-pump has been shown to participate in maintaining a large electrochemical calcium gradient across the axonal membrane under resting conditions (DiPolo, Nature 274:390, 1978). A (Ca + Mg)-ATPase associated with calcium transport and exhibiting kinetic parameters similar to those found for the pump in dialyzed axons *in vivo* has been characterized in squid optic nerve plasma membranes (Beaugé *et al.* BBA 644:147, 1981; Condrescu *et al.* BBA 769:261, 1984; Osses Ph.D. Thesis, 1984). Experiments performed recently, indicate that this enzyme undergoes phosphorylation by ATP as a step in its reaction sequence. In fact, in the presence of [γ-^{32}P]ATP a rapid, Ca^{2+}-stimulated phosphorylation of the membranes could be observed, reaching a steady-state level in 30 sec. The concentration of Ca^{2+} for half-maximal effect was 0.38 μM. Addition of unlabeled ATP after a 30 sec. phosphorylation period resulted in a rapid loss of the Ca^{2+}-dependent labeling (half-time = 3 sec.) indicating that a rapid turnover is undergone by the phosphate in the protein. The dephosphorylation was independent of the presence of Ca^{2+} during this reaction step. In addition, the Ca^{2+}-dependent phosphorylation was sensitive to hydroxylamine, consistent with the formation of an acyl phosphate intermediate, as occurs in the case of other transport ATPases. (Supported by grants S1-1144 and RH 15-123 from CONICIT, Venezuela.)

M-Pos236 EFFECTS OF MULTIVALENT CATIONS ON THE ATPase ACTIVITY OF CORN ROOT PLASMA MEMBRANE. Shu-I Tu and Janine N. Brouillette. Eastern Regional Research Center, U. S. Department of Agriculture, Philadelphia, PA 19118.

Plasma membrane of corn root cells contains an oligomycin insensitive but vanadate sensitive Mg-ATPase activity. There is evidence to show that the enzyme functions as a transmembranous proton pump. Thus, the ATPase may play a key role in transporting ions from soil to plants. We are interested in examining the possible toxic effects of certain metal ions, e.g. Al^{+3} and Cd^{+2}, to the activity of this key transport ATPase. Using the plasma membrane isolated from corn root tips, we found that the presence of Ca^{+2}, Mn^{+2}, Cd^{+2}, or Al^{+3} significantly slowed down the catalyzed hydrolysis of Mg-ATP. The K_m's of the enzyme to Ca^{+2}, Mn^{+2}, and Cd^{+2}-ATP were only slightly different from that of Mg-ATP. Thus, it is likely that other interactions involving those metal ions and the membrane inhibit the enzyme activity. At pH 6.0, with the Al^{+3}/Mg^{+2} ratio as low as 0.01, the ATPase activity was still significantly inhibited ($\sim$ 40%). Thus, the observed Al^{+3} toxicity to plant growth may be in part attributed to its effect on plasma membrane ATPase.

M-Pos237 PLASMA MEMBRANE POTENTIAL GENERATED BY CATION PUMPING SUSTAINED BY ELECTRONEUTRAL ION MOVEMENTS AND LIMITED BY ANION LEAKS. C. Lindsay Bashford, Department of Biochemistry, Saint George's Hospital Medical School, Cranmer Terrace, London SW 17 ORE, United Kingdom.

Lettre cells, a mouse ascites tumour cell line, and human neutrophils maintain plasma membrane potentials, assessed with the optical indicator oxonol-V, in the range -50 to -80mV. The potential is scarcely affected by the addition of potassium but the cells are substantially depolarized by ouabain, dicyclohexylcarbodiimide (DCCD) and by removal of extracellular potassium without a concomitant change in their intracellular ion content. These results imply that cation pumps rather than cation (potassium) diffusion are responsible for the membrane potential of these cells. The cells possess electroneutral pathways for the transport of sodium, potassium, protons and chloride which can function as $Na^+(+Cl^-)$: $K^+(+Cl^-)$ and Na^+: H^+ exchanges. The coupling of sodium pump activity with Na^+: H^+ exchange provides the cells with what is, in effect, a proton pump; there may also be frank proton pump activity. The magnitude of the pump-generated potential is restricted by anion leaks (eg. chloride, bicarbonate and lactate). General models for the generation of membrane potential in cells with low passive permeability for sodium and potassium through the continuous action of cation pumps, which are balanced osmotically by electroneutral ion transport pathways and electrically by anion leaks, will be presented. This work was supported by the Cell Surface Research Fund.

M-Pos238 EFFECT OF UNSATURATED PE ON THE ACTIVITY AND MORPHOLOGY OF Ca-ATPase RECOMBINANT K.H.CHENG,S.W.HUI and A.SEN,Dept. of Biophysics,Roswell Park Memorial Institute,Buffalo,NY14263 and P.L.YEAGLE,Dept. of Biochemistry,State University of New York at Buffalo,NY14214

The effect of non-bilayer forming lipids on the activity and incorporation of sarcoplasmic reticulum Ca-ATPase was studied in reconstituted systems having varying amounts of unsaturated PE. Ca-ATPase was purified from rabbit muscle by solubilization in deoxycholate and 0.5 M NaCl. Reconstitution of Ca-ATPase with mixtures of soy PE and egg PC was achieved by dialysis in the presence of 0.4 M of potassium phosphate. The recombinants were active in both ATP-dependent Ca-transport and Ca-dependent ATP hydrolysis. The Ca transport activity for the recombinant peaked at 50% PE, and ATP hydrolysis was found to increase with decreasing PE content. Coupling efficiency (Ca/P) was low for the recombinant with low PE content but remains constant and high once PE content reaches 50%. Protein to lipid ratio was low for recombinant with low PC content. The ultrastructure and morphology of the recombinants were investigated by freeze-fracture EM and P-31 NMR. Results indicate that the recombinant with the highest PE content ($>$ 80%) exhibits non-bilayer hexagonal phase, while those recombinants with PE content less than 80% show predominantly closed vesicular type structures. The above results are interpreted in terms of the tendency of PE to destabilize the bilayer structure.

M-Pos239 INCORPORATION OF FUNCTIONAL SARCOPLASMIC RETICULUM CALCIUM ATPase INTO PHOSPHOLIPID VESICLES FORMED BY RAPID REMOVAL OF NONIONIC DETERGENT. Dwight W. Martin and Masaharu Ueno, Dept. of Physiology, Duke Univ. Med. Cntr., Durham, NC 27710.

Reconstitution of membrane transport systems into tightly sealed, well characterized liposomes should provide a valuable tool for investigation of the mechanisms of active transport and exchange. To accomplish these objectives, methodologies must permit unilamellar vesicle formation without loss of protein functionality. Ca^{++}ATPase was delipidated and solubilized from sarcoplasmic reticulum using n-dodecyl octaethylene glycol monoether ($C_{12}E_8$). The solubilized protein was incorporated into egg yolk phosphatidylcholine (EYL) vesicles by rapid removal of the detergent from a protein, detergent, lipid mixed micellar solution using hydrophobic beads as described by Ueno et al. (Biochemistry (1984) 23, 3070). Liposomes incorporating an average of from 1 to 4 ATPase polypeptides (MW 119000) were tightly sealed to permeation by Cl^-, K^+, and Ca^{++} with permeability coefficients of $\leq 10^{-9}$ cm/sec. Liposome permeability was most influenced by the ratio of $C_{12}E_8$ to EYL remaining after vesicle formation. Vesicle size was not significantly altered by protein incorporation, with mean vesicle diameters of $\sim$60-70 nm observed in all preparations. A significant fraction of the solubilized protein was removed upon interaction with the hydrophobic beads. The protein which incorporated into the liposomes was functionally active. ATPase activity was comparable to that of sealed SR vesicles. Addition of the Ca ionophore A23187 increased activity upto 4 fold, consistent with Ca^{++} accumulation coupled to ATP hydrolysis. Treating A23187 stimulated vesicles with 3mM $C_{12}E_8$ produced additional stimulation of ATPase activity suggesting an intravesicular orientation for some of the ATP hydrolysis sites.

M-Pos240 THE ROLE OF H^+ IN THE E-P + H_2O $\longrightarrow$ E + P_i REACTION OF SARCOPLASMIC RETICULUM (SR) ATPase. G. Inesi, D. Lewis, H. Scofano and H. Barrabin. Department of Biochemistry, University of Maryland School of Medicine, Baltimore, Maryland 21201.

In optimal conditions, the maximal levels of phosphorylated enzyme intermediate obtained by phosphoryl transfer from ATP to SR ATPase in the presence of Ca^{2+} are approximately the same, independent of pH variations between 6.0 and 8.0. Enzyme phosphorylation with P_i in the absence of Ca^{2+}, on the contrary, is highly H^+ dependent, and the maximal level of E-P formed at pH 6.0 is more than twice that obtained at pH 8.0. This difference is noted whether the substrate is considered to be total P_i or $HPO_4^=$, and therefore is attributed to protonation of enzyme residues. The rate constant of hydrolytic cleavage of the enzyme is favored by a low H+ concentration (pH 8.0). We have now established conditions for limited derivatization of SR with Dicyclohexylcarbodiimide (DCC), producing inhibition of the E-P + $H_2O \rightleftharpoons$ E + P_i reaction, without inhibition of calcium binding or enzyme phosphorylation with ATP. We attribute this specific inhibition to blocking of enzyme residues with pK near neutrality, which are involved in acid-base assisted catalysis of the E-P + $H_2O \rightleftharpoons$ E + P_i reaction. In their protonated form these residues assist the phosphorylation reaction serving as H^+ donors to form water with the oxygen derived from enzyme phosphorylation with P_i. In their dissociated form they assist phosphoenzyme cleavage by withdrawing H^+ from hydrolytic water, thereby favoring nucleophylic attack of water oxygen on the phosphorus atom. At neutral pH, these residues assist equally well incorporation of P_i onto the enzyme and hydrolytic cleavage of the phosphoenzyme, as shown by the pH dependence of $P_i \rightleftharpoons H_2O$ oxygen exchange (McIntosh and Boyer, 1983).

M-Pos241 STOPPED-FLOW FLUORESCENCE ANALYSIS OF A FLUORESCENT ATP ANALOGUE BOUND TO CA^{++}-ATPASE OF SARCOPLASMIC RETICULUM.
J.E. Bishop*, J.D. Johnson# and M.C. Berman*.
* MRC Biomembrane Research Unit. Univ. of Capetown Medical School, Capetown, South Africa
\# Dept. of Physiological Chemistry, Ohio State University Medical Center, Columbus, Ohio

The fluorescence of 2',3'-O-(2,4,6-Trinitrophenyl)-ATP (TNP-ATP) bound to the Ca^{++}-ATPase of sarcoplasmic reticulum (SR) is increased several fold when enzyme turnover is induced by ATP plus calcium (Watanabe, T. and Inesi, G., J. Biol. Chem. 257, 11510, 1982). We find that the fluorescence enhancement of TNP-ATP-SR correlates with the levels of phosphorylated enzyme intermediate (E$\sim$P) in the transient state, steady state and upon quenching turnover by Ca chelation. Our data suggest two types of TNP-ATP binding sites. 1. A low fluorescence nucleotide binding site with a Kon= $1.2x10^6 M^{-1}S^{-1}$, Koff=$1.2S^{-1}$ at which ATP binds competitively. 2. A high fluorescence "regulatory" nucleotide binding site (Kon=$5x10^7 M^{-1}S^{-1}$Koff=$100S^{-1}$), which forms ($97\text{-}130S^{-1}$) and decays ($0.22\ S^{-1}$) in syncrony with E$\sim$P. Presently, we cannot determine if these two sites are interconvertible. KCL decreases TNP-ATP-SR fluorescence (I_{50}=50mM) without affecting steady E$\sim$P levels.

These studies provide independent kinetic evidence for the existence of both catalytic and non-catalytic nucleotide binding sites on this Ca-ATPase. Further, they point out the utility of TNP-ATP fluorescence as a probe for the structural changes which occur at the regulatory nucleotide binding site during enzyme turnover.

Present address JEB: Dept. of Biological Chemistry, Univ. Maryland Medical School, Baltimore, MD.

M-Pos242 HIGH INTRACELLULAR pH INHIBITS Ca UPTAKE BY MYXICOLA AXON MITOCHONDRIA. Ronald F. Abercrombie. Department of Physiology, Emory University School of Medicine, Atlanta, Georgia 30322.

Microliter samples of axoplasm were surgically removed from giant axons of the marine annelid Myxicola infundibulum and placed in polyethylene tubes. The pCa (6.72 + 0.03, N=40) and, in some cases, the pH (7.51 + 0.01, N=7) of the isolated axoplasm were measured with ion-sensitive microelectrodes. The axoplasmic ionic milieu was subject to control by means of a dialysis capillary with a 6000 D molecular weight cut-off. Typically in these experiments the sample was dialyzed for 30 min using a metabolite-free medium, and the calcium activity [Ca^{2+}] then elevated to 10-50 µM with $CaCl_2$. Upon addition of 5 mM succinate to the axoplasm at pH 7.5, free Ca decreased. This decrease was presumably the result of mitochondrial Ca uptake since it could be blocked with ruthenium red or CN. Without these inhibitors, uptake continued at pH 7.5 until [Ca^{2+}] was below 1 µM. Uptake was slowed by elevating the axoplasmic pH to ~8.5. When the dialysis medium contained physiological (1 mM) concentrations of PO_4, the effect of high pH was reversible. In PO_4-free medium, this inhibition was not reversible. An ATP-dependent, ruthenium red-sensitive Ca uptake was also found in 1 mM PO_4 to be reversibly inhibited by pH ~8.5. When ruthenium red was added to axoplasm that had previously taken up Ca, the Ca activity gradually increased, suggesting ongoing release from storage sites. Increasing the axoplasmic pH to 8.5 did not increase the ongoing release over that found when ruthenium red was added. It is concluded that elevating intracellular pH to ~8.5 may favor the net release of Ca from mitochondria by slowing Ca uptake, which occurs against a background of ongoing Ca release. (Supported by USPHS NS19194.)

M-Pos243 THE EFFECT OF IONIC STRENGTH ON STILBENE-ANION INTERACTIONS IN HUMAN RED CELL GHOST MEMBRANES. Kevin R. Smith Department of Chemistry, SUNY, Binghamton, NY 13901

The inhibition of inorganic, monovalent anion transport in human red cells by disulfonic stilbenes is thought to involve a competitive binding of stilbenes and anions for a single binding site on band 3. Support for this view comes from a linear dependence of the apparent stilbene dissociation constant on anion concentration, and from linear Hunter-Downs plots of stilbene inhibition. These studies are usually done at varying ionic strength. We have measured, by fluorescence enhancement methods, the apparent dissociation constant of DBDS as a function of chloride concentration at varying ionic strength. In agreement with previous studies, we find a linear relation between the apparent DBDS dissociation constant and chloride concentration up to chloride concentrations of 600 mM. However, if ionic strength is held constant at 600 mM, the DBDS dissociation constant saturates at high chloride concentration indicating a non-competitive interaction. Ionic strength may account for the discrepancy between DBDS binding studies and other stilbene binding studies. Supported in part by NIH grant HL29488.

M-Pos244 EFFECT OF OSMOTIC AND IONIC CONDITIONS ON RED CELL MEMBRANE ELECTRIC POTENTIAL PROFILE. John D. Bisognano and James A. Dix, Department of Chemistry, State University of New York, Binghamton, NY 13901

A common assumption in calculating the electric potential in biological membranes is that the electric potential varies linearly across the membrane. A more detailed calculation involves consideration of membrane fine structure to resolve spatially the electric potential within different regions of the membrane; the potential is calculated within the Poisson-Boltzmann framework (Heinrich, Gaestel and Glaser, J. Theor. Biol. 96, 211-231 (1982)). For red cell membranes, there is a deep potential minimum near the inner surface of the membrane, primarily due to negatively charged phosphatidyl serine lipids. We have investigated the detailed electric potential profile of the red cell membrane in response to osmotic shocks of sodium chloride (in which osmolality, and intracellular and extracellular ionic strength are changed) and of sucrose (in which osmolality, intracellular ionic strength and Donnan equilibria are changed). Due to a separation of time scales of osmotic equilibria (10 msec) and Donnan equilibria (many sec to many min), the electric potential profile can exist in quasi-stationary states. If a positively-charged fluorescent probe of membrane potential partitions primarily in the potential minimum, then a time dependent potential profile may lead to time-dependent changes in probe fluorescence not correlated with trans-membrane potential. Supported by HL29488.

M-Pos245 NON-STEROIDAL DRUG BINDING TO HUMAN RED CELL MEMBRANES: CHARACTERIZATION BY FLUORESCENCE PROBES OF BAND 3 CONFORMATIONAL STATES. Sarah G. Finnegan and James A. Dix, Department of Chemistry, SUNY, Binghamton, NY 13901.

We have characterized the binding of three anti-inflammatory, non-steroidal drugs to band 3 in human red cell ghost membranes. The three drugs are flufenamic acid (N-(ooo-trifluoro-m-tolyl)-anthranilic acid), fenamic acid (N-phenylanthranilic acid) and TFA (3-trifluoromethyldiphenyl amine). We probed the band 3 binding site with two fluorescent inhibitors of anion transport, BADS (4-benzamido-4'-amino-2,2'-disulfonic stilbene) and DBDS (4,4'-dibenzamido-2,2'-disulfonic stilbene), and with intrinsic membrane tryptophan fluorescence. The apparent dissociation constant of DBDS and BADS increased linearly with drug concentration, suggesting simple competition between drug and stilbene for a single band 3 binding site. However, the inhibition constants of the drugs derived from this data depended on the probe used: for BADS, the binding constants of flufenamic acid, fenamic acid and TFA are (in uM) 0.61 $\pm$ 0.06, 14 $\pm$ 1 and 28 $\pm$ 5; for DBDS, the binding constants are 0.18 $\pm$ 0.01, 5.1 $\pm$ 0.2 and 40 $\pm$ 10, respectively. These results suggest separate or overlapping drug-stilbene binding sites. Supporting this picture are the results of kinetic studies, in which the effect of the drugs on stilbene binding occurs not on the rate constant of the bimolecular association, but on the rate constants of the unimolecular steps. Flufenamic acid quenches intrinsic membrane tryptophan fluorescence via a single bimolecular association, forward rate constant 7.3 $\pm$ 1.2 s^{-1} uM^{-1} and reverse rate constant 5.0 $\pm$ 0.6 s^{-1}. Supported by HL29488.

M-Pos246 **BUMETANIDE-SENSITIVE NA/K COTRANSPORT IN FERRET RED BLOOD CELLS.** R.W. Mercer and J.F. Hoffman, Dept. of Physiology, Yale Univ. School of Medicine, New Haven, CT 06510

Red cells from the ferret, unlike those from other carnivores such as the dog and cat, exhibit a high rate of bumetanide-sensitive Na/K cotransport (Flatman, J Physiol. 341:545, 1983). We have characterized bumetanide-sensitive cotransport in ferret red cells with respect to kinetic parameters, response to norepinephrine and cAMP, metabolic substrate depletion and volume regulation. Measurement of bumetanide-sensitive unidirectional Na influx as a function of external K yielded a Vmax of 87 mmol/l cell x hr and a Km for stimulation by K_o of 3.7 mM. The Na concentration causing half-maximal unidirectional K influx was 91 mM, with a maximal flux of 20.2 mmol/l cell x hr. Cotransport of K was dependent on Cl; SO_4 , or NO_3 substituting for Cl inhibited transport. The Ki for bumetanide inhibition of cotransport is 22 nM. Unlike cotransport in avian red cells that is stimulated by norepinephrine and cAMP, 1 uM norepinephrine exerted no effect on cotransport in ferret red cells while treatment of cells with 1 mM 8BrcAMP inhibited bumetanide-sensitive cotransport by 16%. Cotransport in ferret red cells is inhibited by metabolic depletion; decreasing intracellular ATP from 0.4 mM to 0.05 mM by treatment with 5 mM iodoacetamide inhibited uptake by 90%. Ferret red cells, like those of the dog, when placed in a hypotonic medium (247 mOsmol/kg) exhibited a 25% decrease in Na uptake while cells in hypertonic medium (394 mOsmol/kg), after a 5 minute lag, increased Na uptake by over 300%. This Na uptake, unlike the dog requires K_o and is bumetanide-sensitive. Experiments using ^{3}H-bumetanide have demonstrated that there are approximately 12000 bumetanide binding sites/ferret red cell. Thus ferret red cell membranes may prove of value in identifying and isolating the protein(s) responsible for cotransport. (Supported by HL-09906, AM-7259-08 and AM-17433-11.

M-Pos247 RELATION BETWEEN RED CELL ANION EXCHANGE AND TRANSPORT OF WATER AND ETHYLENE GLYCOL. Sung C. Yoon, Michael R. Toon and A. K. Solomon, Biophysical Laboratory, Dept. of Physiology and Biophysics, Harvard Medical School, Boston, MA 02115.

A new distilbene compound, DCMBT (4',4'-dichloromercuric-2,2,2',2'-bistilbene tetrasulfonic acid), has been synthesized for studies of anion and water transport in the human red cell. DCMBT, which combines features of both the stilbene anion transport inhibitor, DIDS, and the mercurial water transport inhibitor, pCMBS (p-chloromercuribenzene sulfonate), inhibits sulfate exchange almost completely. K_I is 15 μM, about 10 x greater than DIDS (K_I = 1.2 μM). DCMBT also inhibits water transport by ~20% with K_I = 8 μM, about 20 x smaller than pCMBS (K_I = ~ 0.15 mM). When the stilbene binding site is occupied by covalent reaction with DIDS, the DCMBT effect on water transport is reduced by 80 - 100%, suggesting that anion and water transport are mediated by the same protein.

Reithmeier (BBA 732, 122, 1983) reported that the SH-reagent, DTNB (5,5'-dithiobis(2-nitrobenzoic acid)) binds noncovalently to the human red cell and inhibits anion transport (K_I = 0.6 mM). When red cells are treated with NEM (N-ethyl maleimide) to block intracellular SH groups on band 3, we found that DTNB blocked 50% of sulfate transport, K_I= 4.2 mM. DTNB also blocks 60% of the ethylene glycol flux in NEM treated cells, K_I = 3.5 mM. Both inhibitions could be reversed by washing with buffer. These similarities suggest that ethylene glycol and anion transport are mediated by the same protein. (Supported by ONR N00014-83-0015 and NIH GM 34099).

M-Pos248 EVIDENCE FOR A Na/H EXCHANGE SYSTEM IN HUMAN RED CELLS (RC). Nelson Escobales and Mitzy Canessa. Department of Physiology and Biophysics, Harvard Medical School, Boston, MA 02115.

We have previously reported (Biophys. J. 45:82a,1984; J.Cell. Biol. 99:291a,1984) that RC possess an otherwise silent, Ca_i-activated, and Amiloride-sensitive (AS) Na influx pathway. The Ca_i-activation seems to depend on the metabolic integrity of RC: 1) It does not take place in ATP-depleted RC; 2) ATP-repletion of ATP-depleted RC fully restored AS Na influx; and 3) ATP-enrichment (ATP-RC) enhanced AS Na influx from about 1 to 2.3 mmoles/l cell x hr,(FU). A direct role of H^+ in the activation of AS Na influx was suggested by studies in ATP-RC which had a Cl_i/Cl_o =0.33 and a lower pH_i (6.9) than fresh RC (Cl_i/Cl_o =0.71, pH_i=7.2). Under basal conditions, ATP-RC demonstrated an increased Na influx which was AS (about 1 FU) and Ca_i-independent. Incubation of these cells in a low Cl medium (Cl_i/Cl_o=0.7, pH_i=7.2) markedly decreased AS Na influx (from 0.95 to 0.26 FU), but did not affect the Ca_i-activated component. To further define the role of H^+ on the activation of AS Na influx, we examined the effect of changes in pH_i and pH_o in RC treated with DIDS (125 uM) and Methazolamide (0.4 mM). AS Na influx into acid loaded cells (pH_i=6.3) markedly increased (from 0.1 to 3.0 FU) when pH_o was increased from 6 to 8. At pH_o=8, AS Na entry was abolished when pH_i was raised from 6.3 to 7.7. Moreover, mesurements of H^+ movements into an unbuffered medium indicated that the imposition of a Na gradient (Na_o=75, Na_i=12 mM), stimulated a net H^+ efflux of about 3.5 FU. Furthermore, with Na_i=Na_o=15 mM, an outward proton gradient (pH_i=6.3, pH_o=8.0) promoted a net AS Na uptake (about 0.8 FU) which was abolished at pH_o=6.0. These findings are consistent with the presence of an AS Na/H exchange in RC. Supported by the Porter Development Program of the Am.Physiol. Soc., Univ. of Puerto Rico (NE) and NIH-NHLBI grant 5R01-HL-29950 (MC).

M-Pos249 ^{31}P and ^{35}Cl NMR STUDIES OF INORGANIC PHOSPHATE AND CHLORIDE ION TRANSPORT BY HUMAN ERYTHROCYTES. Manfred Brauer, Carole Y. Spread, Reinhart A.F. Reithmeier and Brian D. Sykes. Department of Biochemistry and M.R.C. Group on Protein Structure and Function, University of Alberta, Edmonton, Alberta, Canada T6G 2H7.

Band 3 protein of human erythrocyte membranes catalyses Cl^- and HCO_3^- exchange across the membrane and plays a key role in acid-base balance and elimination of CO_2 from the body. The influx of inorganic phosphate (P_i) and efflux of Cl^- from erythrocytes were monitored by ^{31}P and ^{35}Cl NMR. Erythrocytes were incubated overnight at 4°C in 5mM P_i, 150mM NaCl, pH 7.4, collected by centrifugtion and mixed with an equal volume of various concentrations of P_i at pH 6.4 while maintaining a constant ionic strength and osmolarity. The difference in intra- and extracellular pH allowed us to resolve the ^{31}P resonances of intracellular P_i (P_i^I) and extracellular P_i (P_i^O). During transport the intensity of the P_i^O resonance decreased and that of P_i^I increased with time with transport rates consistent with literature values. Transport could be totally inhibited with 4,4'-diisothiocyano-2,2'-stilbene disulfonate (DIDS), a specific inhibitor of anion transport. From the chemical shift of the P_i^I and P_i^O resonances, intra- and extracellular pH were determined during transport. Intracellular pH fell and extracellular pH rose, consistent with the $H_2PO_4^-$ form being transported rather than the HPO_4^{-2} form. ^{35}Cl NMR studies showed a rise in the extracellular Cl^- resonance with time during transport. The Cl^- efflux observed coincided stoichiometrically with the initial rapid influx of P_i.

(Supported by MRC of Canada and AHFMR).

M-Pos250 INHIBITION OF PHOSPHATE TRANSPORT IN HUMAN ERYTHROCYTES BY A NON-PENETRATING CARBODIIMIDE INDICATES TWO CONFORMATIONS OF THE TRANSPORTER. James D. Craik and Reinhart A.F. Reithmeier, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7

Treatment of human erythrocytes with the water soluble carbodiimide 1-ethyl-3-(4-azonia-4,4-dimethylpentyl) carbodiimide (EAC) in the absence of added nucleophiles gives inhibition of phosphate transport. EAC does not penetrate the erythrocyte membrane or lead to significant intermolecular crosslinking of membrane proteins. At neutral pH in chloride free medium only about 50% of transport is rapidly and irreversibly inhibited (52% inhibition, 8.7mM EAC, 5 min, 37°C, pH 6.9) but at alkaline pH inhibition is more rapid and extensive. Treatment of cells in 154mM NaCl, pH 6.9, gave inhibition which partly reversed on incubation at 37°C for 1 hour. Protection from EAC inhibition is afforded by 4,4-dinitrostilbene-2,2-disulfonic acid (DNDS), a reversible competitive inhibitor of anion exchange. N-(4,azido-2-nitrophenyl)-2-aminoethylsulfonate (NAP-taurine), a reversible non-competitive inhibitor which stabilizes an outward facing transporter conformation, did not protect against EAC inhibition but prevented reversal of inhibition in saline medium. Transport inhibition with [^{3}H] EAC did not lead to stable incorporation of label into Band 3. The results suggest that EAC inhibition of anion transport is due to modification of a carboxylic acid residue(s) in or near the transport site accessible from the external face of the membrane. The fate of the modified carboxyl appears to be sensitive to the conformation of the transporter; an inward facing orientation promotes regeneration of the original carboxylic acid, while an outward facing conformation favors nucleophilic displacement and irreversible inhibition of transport. (Supported by the Medical Research Council of Canada [RAFR] and an Alberta Heritage Foundation for Medical Research Fellowship [JDC])

M-Pos251 "ANION EXCHANGE IS INCREASED IN AGED HUMAN ERYTHROCYTES". M.A. Zanner and W.R. Galey. Department of Physiology, University of New Mexico School of Medicine, Albuquerque, New Mexico 87131

Significant membrane alterations occur in the human erythrocyte as it ages in vivo or in vitro. One such alteration is the exposure of an antigen on senescent red blood cell membranes which may be involved in the removal of aged cells from the circulation (Kay, 1980). Kay et al., have presented evidence that the membrane protein from which this senescent cell antigen may be derived is band 3 (Kay, et al., 1983).

Since band 3 is the mediator of anion exchange across the RBC membrane, we have investigated whether there is a difference in the rate of anion transport in "young" and "old" human RBCs. In our studies red cells were separated according to density (and age, Murphy, 1973) and the anion exchange process across the membranes was studied utilizing $^{35}SO_4$ exchange at 37°C. Results show that the rate constant for $^{35}SO_4$ efflux (SO_4-Cl exchange) from "old" cells is approximately 20% greater than from "young", less dense cells. Incubation of both "young" and "old" RBCs with DIDS abolishes this difference.

Although aged red blood cells undergo immunological changes which are possibly associated with band 3, the anion exchange mechanism is not compromised and appears, in fact, to be enhanced.

M-Pos252 SYNERGISTIC EFFECT OF 20-HYDROXYCHOLESTEROL ON THE ACYL CHAIN IMMOBILIZING BEHAVIOR OF CHOLESTEROL IN HUMAN ERYTHROCYTE MEMBRANES M.W. Rooney and J.W. Kauffman, Biomedical Engineering Division, Northwestern University, Evanston, IL, S. Yachnin, Pritzker School of Medicine, University of Chicago, Chicago, IL, O. Kucuk, The Chicago Medical School, North Chicago, IL, L.J. Lis, Department of Physics, Kent State University, Kent, OH.

Vibrational molecular spectra (infra-red) revealed that 20-hydroxycholesterol insertion into human erythrocyte membranes (10% of total membrane sterol) immobilized membrane acyl chains to a degree that is equivalent to enriching total membrane cholesterol by 50% (J. Biol. Chem., 259: 8247-51). Erythrocyte membrane acyl chain gauche content (Raman spectra) was not altered by the presence of the 20-OH sterol indicating that chain immobilization was limited to an inhibition of lateral motion of acyl segments and was ineffective at altering translational conformation (trans/gauche content) within the acyl chains. Erythrocyte topology is unchanged in the presence of the 20-OH sterol however total membrane surface area is increased (Blood, 58: 317-25). Protein secondary structure (Raman) is not altered by the 20-OH sterol as are erythrocyte membranes enriched with cholesterol or inserted with 7-OH sterol, both of which undergo topologic rearrangement (J. Clin. Invest., 55: 115-26; Blood, 58: 317-25). Since 20-OH sterol and cholesterol have negligible differences in steric volume, the increase in erythrocyte membrane surface area and acyl chain immobilization are suggested to be caused by unique 20-OH/Cholesterol stoichiometries within packing subcells such that a synergistic effect is induced by the 20-OH sterol on the normal condensing or immobilizing behavior of cholesterol.

M-Pos253 SOME BIOPHYSICAL PROPERTIES OF SWOLLEN ERYTHROCYTES. Lawrence C. Cerny, Elaine L. Cerny, Charles L. Cerny and Darrell Pardi. Masonic Medical Research Laboratory and Utica college of Syracuse University, Utica, N.Y. 13502.

It is a well-established fact that the shape of an erythrocyte can be altered by controlling the osmolality of the suspending medium. Therefore it is possible to represent the shape of the red blood cell as a biconcave discoid, an oblate spheroid or a sphere. These shape changes play an important role in several biophysical measurements. Those studied here include viscosity, erythrocyte sedimentation rate, malonamide induced hemolysis and electrophoretic mobility as follows: 1) Viscosity determinations allowed evaluation of the shape factor from the intrinsic viscosity and its concentration dependence; 2) Erythrocyte sedimentation rates were determined in an automatic sedimentimeter. The shape factor had an effect on the final packing of the erythrocytes. It also was possible to superimpose all the data on a single curve if a reduced settling equation was used based upon the final settling value and the half life of the sedimentation process; 3) The rates of malonamide induced hemolysis depended strongly on the initial swollen state of the erythrocyte. However, it was possible to relate all the kinetics on a common scale with the half life and the kinetic breadth parameter; 4) Electrophoretic mobility measurements showed that motion (tumbling or rotation) depended upon the erythrocyte shape. Zeta potential calculations are also presented.

M-Pos254 DIRECT MEASUREMENT OF THE INTERNAL VISCOSITY OF SICKLED ERYTHROCYTES BY EPR-SPIN LABEL TECHNIQUES P. D. Morse II* and J. A. Warth, University of Illinois College of Medicine, Urbana, IL and Comprehensive Sickle Cell Center, Wayne State University, Detroit, MI.

The rotational dynamics of tempamine can be used to study directly the intracellular environment. The extracellular signal from TEMPAMINE is broadened away by the use of potassium ferricyanide which does not enter the cell. The EPR signal which results when 1 mM TEMPAMINE, 120 mM ferricyanide, and erythrocytes are mixed together arises from TEMPAMINE only in the intracellular aqueous space. The relative motion of TEMPAMINE in various control environments is: water at 37° C = 1; human plasma at 37°C = 1.1; washed erythrocytes or whole blood = 4.9 ± 0.8. Erythrocytes can be fractionated by density. In sickle cell anemia (SS), the percentage of cells with density > 1.128 g/ml is 20-40%, in normals (AA) and sickle trait (AS) ≤ 1%. Thus there are a relatively large proportion of dense erythrocytes in SS patients. By direct spin label measurements we show, for the firt time, that the internal aqueous viscosity of these dense erythrocytes is markedly elevated and density dependent. The increase is greater for SS erythrocytes than for AA or AS cells and is greater at 37°C than 20°C, especially in SS patients (data not shown). This difference at 37°C may indicate that intraerythrocyte HbS polymer is responsible for our findings in SS erythrocytes.

RELATIVE MOTION OF TEMPAMINE IN SICKLED ERYTHROCYTES

Number of Patients	Unfractionated Blood	Density g/ml 1.128	1.135	1.143	1.150	1.159
4	(AA) 4.9±0.3	4.7±0.6	5.9±0.1	7.2±0.3	-	-
3	(SS) 5.5±0.3	5.7±0.2	7.3±0.3	7.8±0.3	8.9±0.4	10.0±0.4
1	(AS) 5.0±0.4	4.5±0.1	6.2±0.1	-	-	-

all data relative to water at 37°C

M-Pos255 A Na^+-INDEPENDENT AMINO ACID TRANSPORT SYSTEM asc IS ALREADY PRESENT IN NUCLEATED PIGEON RED BLOOD CELLS LIKE THAT SEEN IN RED CELLS OF SOME SHEEP AND HORSES. HENCE THE LATTER APPEARS NOT TO ARISE BY LOSS OF Na^+-DEPENDENCY BY SYSTEM ASC. H.N. Christensen and J.V. Vadgama, Department of Biological Chemistry, The University of Michigan, Ann Arbor, MI 48109.

On the basis of its absence in transport-deficient phenotypes, Young et al. discovered in erythrocytes of sheep and horses a type of Na^+-independent transport system that resembles Na^+-dependent System ASC sufficiently in amino acid selectivity to suggest that the latter might have lost its Na^+-dependence in the course of erythrocyte maturation. Yet Na^+ plays a decisive role in amino acid selectivity by ASC. Hence we have looked for and found instead much the same Na^+-independent transport system already present in nucleated pigeon red blood cells; so we suspect it is present in erythroid precursors of our red cells. By a mutual and provisional decision Young and we have designated the new system asc to emphasize the similarity to ASC; we use conventionally (Nature, London, 311, 308, 1984) the lowercase letters to indicate the Na^+-independence of the newer system. Beyond its ASC-like transport of threonine, serine, and alanine, and also a characteristic sensitivity to cysteine inhibition, System asc of the erythrocyte of the pigeon (also where present in the horse) shows vigorous transport of valine, in sharp contrast to System ASC of the pigeon erythrocyte and the rabbit reticulocyte. Its response to lowered pH and non-inhibition by cysteinesulfinate also differentiate asc from ASC. The new system contrasts with System L not only in amino acid selectivity, NEM sensitivity, and pH sensitivity, but also in a much slower approach to the steady state of uptake. We conclude that Na^+-independent System asc is an independent biological entity whose full importance remains to be ascertained, and not merely a product of the terminal differentiation of the mammalian erythrocyte. Support acknowledged from Grant HD01233, NIH, U.S.P.H.S.

M-Pos256 STUDIES OF THE DIFFUSION BOUNDARY LAYER AROUND THE RED BLOOD CELL IN A STOPPED-FLOW APPARATUS. Julie B. Williams and Howard Kutchai. Department of Physiology and Biophysics Program, University of Virginia, Charlottesville, Virginia 22908.

Red cell ghosts were formed by osmotic hemolysis of human erythrocytes. The ghosts were labeled with the fluorescent probe tetramethyl rhodamine isothiocyanate (TRITC). Essentially all the TRITC was accessible to extracellular impermeant quenchers. The extracellular diffusion boundary layer was studied by determining the time-dependence of fluorescence when TRITC-labeled ghosts were mixed with 50 mM NaI (a collisional quencher of TRITC) in a modified Durrum-Gibson stopped-flow apparatus. TRITC fluorescence diminished with time after mixing with NaI with a half-time of about 40 msec. The decrease of TRITC fluorescence could be fit well with a monoexponential function of time. A spherical shell model of the diffusion boundary layer was solved analytically and used to analyze the experimental results. Using this model an average diffusion boundary layer thickness of 8 um fit the data best. (Supported by R01 HL 30900 from the National Institutes of Health.)

M-Pos257 ELECTROKINETIC EFFECTS OF NEUTRAL POLYMER ADSORPTION. Donald E. Brooks and Kim A. Sharp, Departments of Pathology and Chemistry, University of British Columbia, Vancouver, Canada V6T 1W5

The adsorption of neutral polymers to biological cells and some colloids can result in a large apparent increase in the zeta potential when electrophoretic mobility measurements are made in the equilibrated polymer solutions. A theory for this effect was proposed based on the idea that adsorbed polymer could partially exclude counterions from the surface region, thus increasing the electrical double layer thickness and, at constant surface charge, increasing the surface potential. The interpretation of a surface potential increase was supported by the polymer-induced aggregation/disaggregation behavior, which is strongly ionic strength dependent. More recently, however, a variety of types of equilibrium experiments have failed to show evidence of a surface potential increase in such systems. We have therefore extended a theory of the electrophoretic mobility of polymer coated particles (Levine et al. Biophys. J. 42:127, 1983) to include the effects of polymer in the suspending medium. By recognizing that the effective viscosity within and external to the adsorbed polymer layer is different we develop a treatment which rationalizes the observed mobilities without invoking large changes in the electrostatic potential profile.

M-Pos258 MICRO AND MACROENDOCYTOSIS IN CHLORIDE CELLS OF FISH GILLS. Anne CAROFF, Monique PISAM and Pierre RIPOCHE. Département de Biologie, C.E.N. SACLAY, 91191 GIF-SUR-YVETTE CEDEX, FRANCE.

To investigate the endocytic activity in chloride cells from fish gills, cationic ferritin (CF, 0,2 to 0,4 mg/ml and concanavalin A (Con A, 1 mg/ml) were added for 15 min to the medium of immersion and bound to the apical membrane. Con A was visualized by a gold-peroxidase complex. Animals were sacrificed and gills fixed at time intervals from 15 min to 17 h.

After 30 min, CF and Con A were in small vesicles and tubules in the apical region of the cell (= microendocytosis) and in wide and deep invaginations of the apical membrane (= macroendocytosis). From 30 min to 2h30, microendocytic vesicles were included in pale multivesicular bodies. Then, from 2h30 to 5h, the aspect of the multivesicular bodies was modified : the tracer particles accumulated to form dense masses which were found in between enlarged and rarer vesicles devoid of ferritin. In parallel, the macroendocytic invaginations lost, within 30 min and 2h30, their connection with the apical membrane ; they appeared as sheets like and tubes which might fuse to form rather complicated structures. From 2h30 to 5 h, numerous macroendocytic profiles were surrounded by a envelope containing cytidine monophosphatase (CMPase). Finally, after 5 hours of incubation, tracer particles were only found in small spherical structures containing "myelinic" figures and CMPase. CF and Con A were never seen inside elements of the Golgi apparatus.

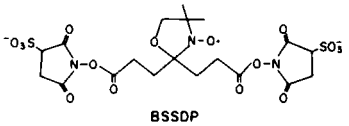
While microendocytosis was similar to that reported in numerous cells, the macroendocytosis was reminiscent of what has been so far only observed in macrophages.

M-Pos259 LIPOSOME-ENCAPSULATED HEMOGLOBIN: BIOPHYSICAL PROPERTIES OF A RED CELL SURROGATE. M. C. Farmer and B. P. Gaber, Naval Research Laboratory, Biomolecular Optics Section, Code 6510, Washington, DC 20375-5000

Liposome-encapsulated hemoglobin (LEH) is being developed as an emergency resuscitation fluid. Oxygenation of LEH is reversible and cooperative. Oxygen affinity can be modulated by co-encapsulation of organic phosphates. Our standard liposomal membrane composition has been dimyristoyl phosphatidylcholine:cholesterol:dicetyl phosphate in a 5:4:1 molar ratio. Other phospholipids have been substituted successfully. A dispersion of lipids in stroma-free hemoglobin is extruded through controlled pore size membranes to produce a nearly monodisperse suspension of largely unilamellar vesicles 0.4 microns in diameter. The encapsulation efficiency of this technique varies with the type and amount of negatively charged lipid and with the buffer conditions. Repulsive forces due to the negative charge on the dicetyl phosphate prevent significant aggregation or fusion during storage. Serum free Ca^{++} levels induce reversible aggregation but not fusion. No hemolysis is detected in a suspension of erythrocytes and LEH incubated at 37C for 8 hours. Circulation persistence to 50% of the initial dose of the standard LEH averages 5.5 hours in mice presented with a volume equivalent to 25% of blood volume, with 100% survival of the mice. Circulation persistence varies with phospholipid composition, and can be increased to at least 10 hours. Viscometry measurements show the LEH to have a pattern of non-Newtonian viscosity similar to that of erythrocytes. (Supported in part by the Office of Naval Research.)

M-Pos260 A MEMBRANE-IMPERMEANT, SPIN LABELED CROSS-LINKER FOR STUDIES OF THE DYNAMICS OF MEMBRANE PROTEINS: APPLICATION TO THE HUMAN ERYTHROCYTE ANION EXCHANGE CHANNEL. Albert H. Beth[+], Thomas E. Conturo[+], S. D. Venkataramu[+*] and James V. Staros[#], Departments of Physiology[+], Chemistry[*] and Biochemistry[#], Vanderbilt University, Nashville, TN 37232

We have synthesized bis(sulfosuccinimidyl)-4-doxylpimelate (BSSDP), a reagent which shows great potential for EPR characterization of the rotational dynamics of membrane proteins in the plasma membranes of intact cells. Preliminary studies with intact human erythrocytes indicate that BSSDP reacts covalently and with high specificity with the extracytoplasmic domain of the anion exchange channel (band 3). The EPR spectrum of BSSDP labeled erythrocytes is characteristic of a highly immobilized nitroxide probe. Saturation transfer EPR indicates that the spin labeled band 3 in intact erythrocytes exhibits rotational dynamics in the 0.1 msec correlation time range. Hemolysis of the spin labeled erythrocytes results in an increase in mobility of band 3. This reagent and its homologues are potentially useful for investigations of the dynamics of a number of membrane proteins using EPR techniques. Such investigations should provide physiologically meaningful characterizations of protein-protein interactions in the plasma membranes of intact cells. Supported by: The Chicago Community Trust/Searle Scholars Program, NIH HL30372, NIH GM07884 and NIH AM31880.



BSSDP

M-Pos261 PHOSPHATE ANALOG PROBES OF ERYTHROCYTE ANION TRANSPORT. Richard J. Labotka, Akira Omachi & Robert A. Kleps. The University of Illinois at Chicago.

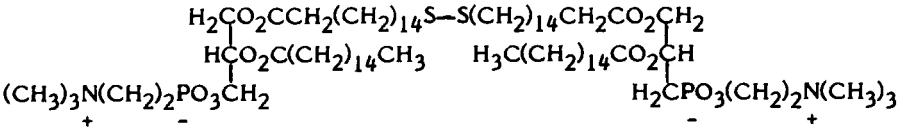
Phosphate analogs are compounds chemically related to orthophosphate, but have chemical substitutions for one or two oxygen atoms. Such substitutions result in unique changes in atomic structure, the number and acidity of titratible protons, and in the nuclear magnetic resonance (NMR) spectrum for each compound. These unique characteristics have been employed to study human red cell phosphate transport using ^{31}P NMR. Fresh red cells were placed in an isotonic HCO_3^- buffered NaCl solution (pH 7.4), containing 20 mM phosphate "P_i", phosphite (H_3PO_3) "HP", or methylphosphonate ($H_2CH_3PO_3$) "MeP". Each of these compounds has a ^{31}P chemical shift that is pH dependent near pH 7, allowing simultaneous observation of intracellular and extracellular signals, as a result of the transmembrane pH gradient. P_i and MeP both exhibited slow transport, requiring 90 min. for equilibration. Suprisingly, HP exchange was quite rapid (seconds), and HP was found to be distributed in a Donnan ratio across the membrane, reflecting the pH gradient. This rapid HP influx was confirmed by measuring the rates of colloid osmotic lysis of red cells suspended in isotonic (300 mOsM) NH_4^+ salts of these phosphate analogs. HP produced complete hemolysis within 3 min., while P_i and MeP showed only slight hemolysis after 10 min. The rapid HP transport is difficult to reconcile with the titratible charge model for anion transport, since HP is a stronger acid (pKa 6.4) than P_i (pKa 6.8), and exists to a larger extent as a divalent ion at pH 7.4 than does P_i, and would be predicted to undergo slower transport. Thus, the anion transport channel appears to be quite sensitive to variations in molecular structure of small anions.

M-Pos262 THE EFFECT OF UREA ON CHLORIDE TRANSPORT IN ERYTHROCYTES. O. Fröhlich and S. C. Jones. Dept. of Physiology, Emory University School of Medicine, Atlanta, GA 30322.

Chloride equilibrium exchange across the red cell membrane is inhibited 20% by 1 M urea. Experiments with urea-loaded and urea-free cells and with urea-containing and urea-free efflux media indicate that urea inhibits rapidly and that its inhibition is rapidly reversed (<2 s; urea net equilibration under these conditions occurs within a few seconds). Fifty percent inhibition of chloride transport was achieved by about 2.5 M urea and by about 500 mM thiourea (TU) or dimethylurea (DMU). These concentrations are at least 10 times larger than the apparent transport affinities of urea and TU for the urea transporter (Mayrand and Levitt, 1983). The inhibitory potency of the urea analogs increased with increased alkylation. Dixon plots of the inhibitory effect of DMU were nonlinear, indicating that more than one urea per band 3 binds and inhibits; however, no sigmoidicity was observed. The sidedness of urea inhibition was probed with the slowly transported TU, under isoosmotic conditions where Cl_o was reduced to 20 mM and substituted for by TU (280 mM) or sucrose, as necessary. Chloride transport was inhibited by both intracellular and extracellular TU, with maximal inhibition occurring when TU was present on both sides of the membrane. An interesting feature of the urea effect is that the effect of self-inhibition, i.e. the decreasing rate of anion transport at high anion concentrations, was reduced by urea and its analogs. It is concluded that the effect of urea on the red cell anion transporter is primarily nonspecific and a denaturation effect analogous to urea's known action on other proteins. Whether the effect on self-inhibition is through denaturation of monomers or through dissociation of band 3 dimers cannot be answered at this point. (Supported in part by NIH grant GM 31269.)

M-Pos263 DESIGN AND SYNTHESIS OF BIS-PHOSPHATIDYLCHOLINES. Elizabeth Ann Runquist and George M. Helmkamp Jr., Department Biochem. Univ. Kansas Med. Center, Kansas City, KS 66103

The chemical and physical states of membrane lipids play an integral role in protein - phospholipid interactions. In an approach to examine protein - phospholipid dynamics we have designed and synthesized a phosphatidylcholine derivative which has the potential to span a lipid bilayer and incorporate various probe molecules. The phosphatidylcholine is 1-(17,18-dithiatetratriacontandioyl)-bis(2-hexadecanoyl-sn-glycero-3-phosphocholine), or bis-PC_{34}.



The synthetic route involves esterification of GPC-$CdCl_2$ with 16-ethyldithiohexadecanoic acid, hydrolysis by phospholipase A_2 to yield the respective bis-lysophosphatidylcholine, and subsequent reacylation with a fatty acid of choice. Removal of the sulfhydryl protecting group was followed by thiol oxidation. Characterization of the bis-phosphatidylcholine has been achieved and studies are in progress to assess the ability of bis-PC_{34} to incorporate into vesicles. (This research was supported in part by NIH Grant GM 24035.)

M-Pos264 KINETICS OF CELL-SIZE LIPOSOME FORMATION. M.I. Angelova and D.S. Dimitrov, Central Laboratory of Biophysics, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria

Cell-size liposomes were formed by swelling dried egg lecithin in double-distilled water at 70°C for 4 hours. The kinetics of lipid swelling and liposome formation was observed under phase contrast. After approximately 15 minutes the lipid detached from the glass surface and formed a white fluffy cloud. The number of liposomes in the 3 to 5 μm diameter range continiously increased during swelling to reach saturation at the end of the 4 hours period. Their size distribution did not change for longer periods of time; at least for 10 days at 18°C and 3 months at 2°C. Liposomes of 30 to 150 μm diameter appeared after 30 minutes; their number increased during 60 to 90 minutes and after that it gradually decreased. There are only few of them after 36 hours at 18°C. Smaller liposomes, which got spherical shape after the 4 hours swelling, were observed inside the giant liposomes. The amount of non-liposome structures was almost constant, but slightly increased after 10 days at 18°C. The same sequence of phenomena was observed at 2°C temperature of swelling but at slower rate, e.g. giants appeared after 24 hours. Preliminary theoretical estimates, based on thin film dynamics, have shown that the rate of separation of lipid bilayers may be the determinant of the kinetics of the initial stages of liposome formation. The later stages, however, may involve liposome fusion and cleavage.