W-Pos297

STUDY OF MECHANISMS OF DNA TRANSFECTION BY ELECTROPORATION. T.-D. Xie & Tian Yow Tsong. Dept of Biochem, University of Minnesota, St. Paul, MN 55108

E. Coli (JM105) and the plasmid DNA, either PBR322 or PCU18, which carries an ampicillin resistance gene, were used to study DNA transfection using the electroporation method (PNAS 74, 1923-1927, 1977; Nature 268, 438-441, 1977). The plasmid DNA transfected E. Coli confers ampicillin resistance and transfection efficiency can be conveniently assayed by counting colonies in a selection medium containing ampicillin. ^{32}P labelled DNA was used to monitor its binding to cell surface. $CaCl_2$, $MgCl_2$ and NaCl, in mM conc, facilitated DNA binding also promoted transfection in proportion to their effects on binding. Square pulses of electric field up to 12 kV/cm and 8 ms were used. A transfection efficiency of $5x10^8$ per μg DNA was obtained under the best of conditions. Electroporation of E. Coli preceded the addition of plasmid DNA also resulted in transfection of $5x10^4$ per μg DNA, indicating that DNA entry into E. Coli is by surface diffusion not by electrophoretic movement. The efficiency of the transfection was at least 10^4 times higher for the circular plasmid DNA, either supercoiled or relaxed, than that of the linearized DNA.

W-Pos298

INTERACTIONS OF MUMPS VIRUS AND RECONSTITUTED MUMPS VIRAL ENVELOPES WITH GHOST ERYTHROCYTES AND CV-1 CELLS. Christopher Di Simone and John D. Baldeschwieler, California Institute of Technology, Pasadena, CA, 91125.

Mumps virus and Triton X-100 reconstituted viral envelopes were labeled with octadecyl rhodamine B selfquenching and NBD/Rd resonance energy transfer fluorescent probes. Tritium ^3H-labeled virus was also prepared using ^3H-Leucine. The kinetics of binding and fusion with ghost erythrocytes and CV-1 cells were studied. Saturation curves for viral fusion were obtained. The maximal number of fusion events with ghost erythrocytes is similar to Sendai (about 200 viral particles/ ghost erythrocyte). About 2000 viral particles were able to fuse with each cell. Under non-saturating conditions 20% of the virus fused in 30 minutes and 80% fused overnight. The rate of fusion of mumps virus with ghost erythrocytes appears to be slower than Sendai fusion with ghosts.

W-Pos299

VIDEO STUDIES OF INFLUENZA VIRION FUSION WITH PLANAR LIPID MEMBRANES. W.D. Niles and F.S. Cohen. Physiology, Rush Medical College, Chicago, IL.

We are using video fluorescence and transmission microscopy to study the mechanisms by which the surface hemagglutinin glycoprotein (HA) of influenza virus mediates membrane binding and fusion. To detect the fusion of individual virions with a planar membrane, the envelopes of virions are labelled with a self–quenched concentration of the lipophilic probe octadecyl rhodamine (R18). Virions are ejected from a pipette at a planar lipid membrane containing gangliosides to mimick the cell surface receptor for this strain. Fusion is detected as an increase in R18 fluorescence, that appears as a flash, due to dequenching of the probe's fluorescence as it diffuses from the viral envelope into the now–contiguous planar bilayer, followed by a decrease in brightness as the R18 becomes diluted. These flashes result from virion fusion rather than by non–fusion transfer of R18 to the planar membrane. Fusion will result in the reconstitution of HA into the planar membrane. Indeed, the number of erythrocytes (which possess surface receptors for HA) binding to membranes exposed to viruses is correlated with the fluorescence of the membrane. The rate of flashes is low at pH 7.4 (2/min) and increases as the pH is lowered (60/min at pH 5.0), consistent with the pH dependence of infection by influenza virus. Supported by NIH grant GM27367.

W-Pos300

ACID INDUCED BILAYER DESTABILIZATION OF LIPOSOMES COMPOSED OF 1,2-DIACYL-3-SUCCINYL-GLYCEROL. Ana M. Tari, David Collins and Leaf Huang, University of Tennessee, Knoxville, TN 37996-0840.

Bilayer liposomes were prepared by using pure DOSG (1,2-dioleoyl-3-succinylglycerol) or DPSG (1,2-dipalmitoyl-3-succinylglycerol) at pH 7.4. These liposomes undergo an acid-induced (pH<6) destabilization as shown by the entrapped content leakage and lipid mixing experiments. Electron micrographs of both DOSG and DPSG dispersions at pH 5.5 indicate large vesicular or lamellar structures with no evidence of any reverse micellar or hexagonal II phase. Phase separation at acidic pH was observed with DPSG dispersions by differential scanning calorimetry. DOSG immunoliposomes entrapping diphtheria toxin A chain (DTA) were used for cytoplasmic delivery. Our data indicate that DOSG and DPSG liposomes are pH-sensitive liposomes with cytoplasmic delivery activities and that hexagonal phase or its precursors are not necessary for such activity. Since DOSG liposomes are also stable in plasma, it may be applicable as an *in vivo* drug delivery vehicle. Supported by NIH grants CA24553 and AI25834.

W-Pos301

INHIBITION OF VIRUS FUSION TO BIOLOGICAL AND MODEL MEMBRANES BY BILAYER STABILIZING MOLECULES. James J. Cheetham*, Thomas D. Flanagan**, and Richard M. Epand*, *Dept. of Biochemistry, McMaster Univ., Hamilton, Ontario, Canada, and ** Dept. of Microbiology, SUNY Buffalo, Buffalo, NY, U.S.A.

Several amphiphilic molecules which raise the bilayer to inverted hexagonal phase transition temperature of phosphatidylethanolamines also inhibit Sendai virus induced hemolysis of human erythrocytes and fusion of virus to human erythrocyte ghosts and to phosphatidylethanolamine:ganglioside GD_{1A} large unilamellar vesicles. This does not imply that formation of hexagonal phase structures is required for fusion, but only that a correlation between stabilization of phosphatidylethanolamine membranes and inhibition of membrane fusion exists. The probable site of action of these antiviral bilayer stabilizers is within the target membrane. The charge of the amphiphile is not important as negative, positive and zwitterionic bilayer stabilizers all inhibit fusion. We are currently attempting to determine the specific process during the interaction of virus and target membrane that is inhibited by bilayer stabilizers.

W-Pos302

FUSION BETWEEN RESPIRATORY SYNCYTIAL VIRUS (RSV) AND MAMMALIAN CELLS. L.A. DOWNING, J.M. BERNSTEIN*, and A. WALTER. Intro. by R.W. Putnam. Wright State Univ. & VA Med. Ctr., Dayton, OH.

The RSV fusion protein is responsible for viral entry and cell-to-cell fusion but the molecular mechanism(s) of fusion are not understood. We have adapted a dynamic fluorescence assay to examine binding and fusion of RSV with cells. RSV (Long strain) was labeled with octadecylrhodamine (R18); at the density of labeling used, R18 self quenches, yielding little fluorescence. After virus-cell fusion, fluorescence increases due to probe dilution in the cell membrane. R18-RSV was incubated with Hela cells on ice and unbound virus removed by centrifugation. A small fraction of the virus bound (8±1%) at apparently low affinity. The extent of dequenching was less than 5% at 30 min but greater than that of heat inactivated controls. Fusion of RSV occurred at the cell membrane (pH 7). Neuraminidase treatment of RSV significantly increased binding (16±6%) and the extent of fusion (8%). A method of viral purification using a column technique will be evaluated.

W-Pos303

LONG-TERM MONITORING OF MEMBRANE CAPACITANCE CHANGES (ΔC_m) USING PERFORATED PATCH RECORDING. K. Gillis, R. Pun, and S. Misler. Washington Univ., St. Louis and University of Cinncinatti

Perforated patch recording (PPR), using the pore-forming antibiotic nystatin, permits stable recording of certain membrane currents which rapidly "rundown" during standard "whole cell" recording. Applying PPR and the phase detector technique to bovine adrenal chromaffin cells, we have recorded voltage dependent, high threshold Ca^{2+} currents (I_{Ca}) and depolarization-induced increases in C_m for up to 2 hours after patch perforation. ΔC_m is linked to I_{Ca}: it is abolished by the I_{Ca} blocker Cd^{2+} (100μM) and shows a similar dependence on clamping potential as I_{Ca}. Similarly evoked ΔC_m's of comparable size previously have been related to chromaffin granule exocytosis. PPR may prove useful in the study of otherwise labile messenger-mediated exocytosis by single cells. Support: NIH DK37380; NSF DCB8812562.

W-Pos304

A SPECIFIC TIME INTERVAL IS FOUND BETWEEN APPLICATION OF FUSOGENIC ELECTRIC PULSE AND FIRST EVIDENCE OF FUSION IN SINGLE EVENTS. Dimiter S. Dimitrov and Arthur E. Sowers. ARC/Holland Laboratory, Rockville, MD 20855.

Low light level video microscopy of electrofusion of DiI-labeled and unlabeled rabbit erythrocyte ghosts held in pearl chains by dielectrophoresis in 20 mM sodium phosphate buffer showed reproducible time intervals between the application of a pulse and first detection of fluorescence in the unlabeled adjacent membranes. This time interval increased from an average of 0.3 sec to 4 sec when the electric field strength was decreased from 1 kV/mm to 0.25 kV/mm. At a field strength of 1 kV/mm it increased from 0.56 sec to 3.3 sec as medium viscosity (adjusted with glycerol or sucrose) increased from 1.8 mP.s to 10 mP.s. The lag time in fluorescence transfer may reflect a long-lived intermediate state. It can be partly due to the mutual approach of membranes necessary to get molecular contact and/or relatively slow molecular rearrangements leading to membrane merging after the pulse. Support from American Red Cross (to DSD) and ONR grant N00014-89-J-1715 (to AES).

W-Pos305

ELECTROFUSION IN ERYTHROCYTE GHOSTS FROM DIFFERENT MAMMALIAN SPECIES: CLUES TO FUSION MECHANISM FROM RELATIONSHIP BETWEEN YIELD AND PULSE CHARACTERISTICS. Arthur E. Sowers, Yankuan Wu*, and Dimiter S. Dimitrov. ARC/Holland Laboratory, Rockville, MD 20855, *Dept. Biophys., U. Maryland Sch. Med., Baltimore, MD 21201.

Erythrocyte ghosts from seven mammalian species were used to obtain clues about the influence of membrane composition on the electrofusion mechanism. Dielectrophoresis was used as a non-chemical, reversible, and mild method of inducing close membrane-membrane contact and an exponentially-decaying electric pulse was used as a fusogen. The medium was 20 mM NaPi buffer (pH 8.5). Isofusion yield contours in pulse strength/duration plots showed not only species-dependent translational positional shifts but also qualitatively different shapes. For example, while fusion yields in human erythrocyte ghosts are proportional to pulse strength and duration, rabbit erythrocyte ghosts show a fusion yield saturation which is dependent on pulse strength but independent of duration. Support from American Red Cross (to DSD), ONR grant N00014-89-J-1715 (to AES) and UMAB Grad. Sch. (to YW).

W-Pos306

SOLUTE EFFECTS ON ELECTROFUSION YIELD. Arthur E. Sowers and Dimiter S. Dimitrov. ARC/Holland Laboratory, Rockville, MD 20855.

Yields in electric-pulse induced fusions of rabbit erythrocyte ghosts were found to be significantly higher when the ghosts were pink (about 0.5 % of intact cell hemoglobin) than when they were white (about 0.05 % of intact cell hemoglobin). Reloading white ghosts with hemoglobin restored the higher fusibility characteristic, thus suggesting that the fusion-modulating effect was not due to an influence from membrane preparation. Reloading white ghosts with bovine serum albumin or MW = 70 kD Dextran had a similar effect suggesting that the fusion modulating effect was due to a non-specific molecular effect on solution property. Reloading white ghosts to higher concentrations of hemoglobin had a fusion inhibiting effect. Use of low MW viscosity modifiers (glycerol, sucrose, ethylene glycol) in the medium only had the effect of reducing fusion yields and only at high concentrations of the solute (1% - 30%, v/v). Use of ethanol (up to 30%, v/v) had little or no effect on yield. Support from American Red Cross (to DSD) and ONR grant N00014-89-J-1715 (to AES).

W-Pos307

ELECTROFUSION OF MOUSE MYELOMA CELLS AND LYMPHOCYTES USING AC PULSES

by Y. Takahashi, K. Suzuki, T. Kano and S.Takashima, Department of Bioengineering, University of Pennsylvania, Philadelphia, PA. 19104-6392

A simple and effective technique of electrofusion induced by the short, high intensity alternating field pulses (AC pulses) instead of DC pulses was investigated. In addition, we used a mild centrifugation of high density cell suspension to enhance the cell-cell contacts in a vertical fusion chamber. The optimal frequency for cell fusion of mouse myeloma cells and lymphocytes was found to be 10 kHz and the monoclonal antibodies against HSA (Human Serum Albumin) were produced by the use of this fusion technique. The number of hybridoma colonies selected by HAT solution were counted and the monoclonal antibody production was tested by the Enzyme Immuno Assay.

Hybridoma colonies were detected in as high as 95% of the wells (The mean number per well was 7.4) and 33% of them proved positive against the HSA. This yield of monoclonal antibodies was twice higher than that of PEG technique. This research is supported by a grant from Olympus Optical Company, Tokyo, Japan.

W-Pos308

RAPID, CA-DEPENDENT FUSION OF CHROMAFFIN GRANULE MEMBRANES WITH PHOSPHATIDYLCHOLINE LIPOSOMES. E. Hildebrandt and J. P. Albanesi*, Pharmacology Dept., Univ. of Texas Southwestern Medical Research Center, Dallas, Texas 75235

Activity of Ca-dependent phospholipase A_2 (PLA2) in chromaffin granule membranes (GM) toward phosphatidylcholine liposomes (PC) is enhanced 10-fold upon addition of 40% ethylene glycol (EG). Because PC liposomes tend to resist fusion with other synthetic bilayers and because EG is fusogenic, we measured the rate of bilayer mixing between GM and PC, through decreases in resonant transfer between a fluorescent donor/acceptor pair coincorporated into PC liposomes. In the absence of Ca, fluorescence changes indicative of PC/GM fusion took place in 40% EG, requiring 60 min to reach completion at 37°C, but did not occur in water. However, in the presence of 2 mM Ca, PC/GM fusion was complete within 2 min both in water and in 40% EG. Fluorescent PC liposomes did not fuse with empty PC vesicles under any of the experimental conditions. The results demonstrate that acceleration of PC/GM fusion can not account for the dramatic effect of EG on PLA2 activity, and this enhancement may then reflect sensitivity of the enzyme to fluidity or other properties of the bilayer.

W-Pos309

THE EFFECTS OF CORD FACTOR ON PHOSPHOLIPID BILAYERS

B.J Spargo, J.H. Crowe, L.M. Crowe, B.L. Beaman, Univ. of Calif., Davis

Cord factor (CF), a trehalose dimycolate derived from pahthogenic bacteria such as Mycobacterium tuberculosis and Nocardia asteroides has been implicated indirectly in inhibition of fusion of lysosomes and phagosomes in cells infected with these bacteria. We have shown that CF inhibits Ca^{+2}-induced fusion between unilamellar vesicles. CF (10 moles phospholipid/ mole CF) inhibits mixing of trapped ANTS and DPX by as much as 25%. Furthermore, this effect varies between CF's that differ in length of hydrocarbon chain and degree of unsaturation. Differential scanning calorimetry and infrared spectroscopy suggest that the CF inserts into the bilayer, but the mechanism by which it inhibits fusion is not yet clear.

(Supported by NSF DMB 85-18194 to JHC and NIH 2R01 AI20900 to BLB)

W-Pos310

Peptide Inhibitors of Enveloped Virus Infection Inhibit Phospholipid Vesicle Fusion and Sendai Fusion with Phospholipid Vesicles.

Daniel R. Kelsey, Thomas Flanagan*, Joyce Young and Philip L. Yeagle

Department of Biochemistry and *Department of Microbiology, School of Medicine, State University of New York, Buffalo, New York 14214

Abstract:

Small, hydrophobic peptides which are structurally similar to the N-terminus of several known viral fusion proteins and are capable of inhibiting the fusion of Sendai virus with cells (Richardson, et al., 1980) are also capable of inhibiting fusion in a pure lipid system. Large unilamellar vesicles (LUV's) of pure N-methyl dioleoylphosphatidylethanolamine (N-methyl DOPE) containing encapsulated ANTS and/or DPX were formed by extrusion. Vesicle fusion or leakage was then monitored with an ANTS/DPX fluorescence assay. Sendai virus fusion with lipid vesicles was monitored by the relief of fluorescence quenching of virus labeled with octadecylrhodamine B chloride (R_{18}). The efficiency with which Z-D-PhePheGly, Z-PheTyr and Z-GlyPhe inhibit fusion in the model system directly parallels their previously known effectiveness in blocking virus-cell fusion. In addition, above a certain concentration threshold, they decrease the initial rate of leakage from lipid vesicles and inhibit virus-vesicle fusion. The observation that the fusion inhibitory activities of the peptides examined in this study are qualitatively similar in the virus-cell, virus-vesicle and pure phospholipid systems suggests that the structural intermediates in the fusion process may be similar as well.

Reference:

Richardson, C. D., Scheid, A. and Choppin, P. W. (1980) Virology 105, 205-222.

W-Pos311

Structural Intermediates in Membrane Fusion: ^{31}P NMR Studies of Lipid Phase Behavior in the Presence of Peptidic Fusion Inhibitors.

Daniel R. Kelsey, Thomas Flanagan* and Philip L. Yeagle

Department of Biochemistry and *Department of Microbiology, School of Medicine, State University of New York, Buffalo, New York 14214

Abstract:

Previous work (Ellens, et al., 1989) with N-methyl dioleoylphosphatidylethanolamine (N-methyl DOPE) vesicles has shown that there is a correlation between the initial rates of membrane fusion and leakage and the appearance of an isotropic phase, I_s, which is recognized by its characteristic resonance in ^{31}P NMR spectra. On the basis of these experiments it was hypothesized that I_s represents a necessary intermediate in the fusion process. It has also been shown (unpublished results) that a peptide inhibitor of viral fusion, Z-D-PhePheGly, is capable of inhibiting fusion between N-methyl DOPE vesicles. The experiments described here examine the effect of Z-D-PhePheGly and Z-GlyPhe on the ability of N-methyl DOPE vesicles to form I_s. The spectral characteristics of the isotropic phase were found to be altered upon addition of Z-D-PhePheGly at concentrations that were known to be sufficient to inhibit membrane fusion. The significance of these results for elucidating the mechanism of membrane fusion is discussed.

Reference:

Ellens, H., Siegel, D. P., Alford, D., Yeagle, P. L., Boni, L., Lis, L. J., Quinn, P. J. and Bentz, J. (1989) Biochemistry 28, 3692-3703.

W-Pos312

INTERACTION OF MELITTIN WITH LIPID MEMBRANES

S. Ohki, E. Marcus, D. Sukmaran and K. Arnold; Dept. of Biophysical Sciences, State University of New York at Buffalo, Buffalo, NY 14214

Interaction of Melittin, the principal toxic peptide of Bee Venom, with lipid membranes was systematically studied with regard to its adsorption onto membranes and its effect on membrane leakage, membrane adhesion, fusion and lysis, at various ratios of melittin to lipid molecules.

It was found that this peptide had a strong affinity of adsorption onto lipid membranes; for small unilamellar lipid vesicles, membrane leakage was observed at a ratio of 1/1000, membrane adhesions started to occur at a ratio of 1/500 and membrane fusion occurred at a ratio smaller than 1/300. At a ratio greater than 1/20, membranes lysed, and became micelles.

The above interaction of melittin with lipid membranes was slightly dependent on the types of lipids used and ionic strength of lipid vesicle suspension solutions.

The results infer a mode of interaction of fusion-inducing proteins with lipid membranes.

W-Pos313

THEORETICAL STUDIES OF DIFFUSION OF LIPID-LIKE MOLECULES BETWEEN MEMBRANES IN VIRUS-CELL AND CELL-CELL FUSION SYSTEMS.

Robert J. Rubin and Yi-der Chen, LMB, NIDDK, NIH, Bethesda, MD 20892

The kinetics of redistribution of lipid-like molecules between the membranes of two fused vesicles is studied by solving the appropriate diffusion equation. The effects on the redistribution rate of pore size at the fusion junction, vesicle size, and probe diffusion coefficient are examined. The purpose is to determine if the mixing of lipid-like probe molecules is rate-limiting in virus-cell or cell-cell fusion reactions. It is found that the redistribution rate constant decreases significantly as the relative size of the pore to that of the vesicles decreases. For typical values of diffusion coefficient, pore size, and virus and cell dimensions, the mixing half-time for the virus-cell and for the cell-cell cases is about 2 milliseconds and 200 seconds, respectively. Dequenching of fluorescent self-quenching probes is about twice as fast as probe mixing.

W-Pos314

DIPOLE INTERACTIONS IN ELECTROFUSION ELECTRIC POLARIZATION OF INTACT AND PRONASE-TREATED HUMAN ERYTHROCYTES.

David A. Stenger*+, Karan V.I.S. Kaler#, Alan K.C-Tai#, and Sek Wen Hui*. *Membrane Biophysics Laboratory, Roswell Park Memorial Institute, Buffalo, New York 14263, #Dept. of Electrical Engineering, University of Calgary, Calgary, Canada T2N 1N4, and +Bio/Molecular Engineering Branch, Code 6190 Naval Research Laboratory, Washington, DC 20375.

The low-conductivity (15-650 μS/cm) effective polarizability of intact and pronase-treated human erythrocytes was examined using dielectrophoretic levitation. Intact cells exhibited two dominant polarization mechanisms: 1) a cell surface charge-mediated mechanism in the low frequency range (10 Hz-10^3 Hz) and 2) interfacial polarization in a variable range of frequencies between 10^4 and 5×10^7 Hz. Pronase-treated cells did not levitate in at low frequencies but gave otherwise identical spectra above 10^4 Hz, allowing their polarization to be modelled as a first-order relaxation. The frequency domain polarizability is used to calculate the temporal development of electric pulse-induced dipole moments in pronase-treated cells.

W-Pos315

STOPPED FLOW MEASUREMENTS OF pH-ACTIVATED MEMBRANE FUSION OF INTACT VIRUS WITH CELLS.

Michael J. Clague, Christian Schoch, Loren Zech and Robert Blumenthal. LMMB, NCI, NIH, Bethesda, Md.

Fusion of intact Vesicular Stomatitis Virus and Influenza Virus with human erythrocyte ghosts was monitored spectrofluorometrically with a 50 ms time resolution using an assay based on mixing of the lipid fluorophore, octadecylrhodamine. At 37 °C and pH values near the threshold for fusion, a lag phase of a few seconds was observed. The lag time decreased steeply as pH decreased, while the initial rate of fusion showed the reverse functional dependence on pH. The observed rapid fluorescence changes resulted from fusion of virus bound to the target, and the time lags were not due to association-dissociation reactions between virus and target. The results were fitted to a multistate model similar to that resulting from ion channel gating kinetics. The model allows testing of hypotheses concerning the role of cooperativity and conformational changes in viral spike glycoprotein-mediated membrane fusion.

W-Pos316

CONFORMATIONAL CHANGES AND FUSION ACTIVITY OF INFLUENZA HEMAGGLUTININ OF THE H2 AND H3 SUBTYPES

Anu Puri, Frank Booy, Robert W. Doms, Judith M. White and Robert Blumenthal. NIH, Bethesda, Md. and UCSF, San Francisco, CA. (Intr. by R. Jernigan).

Marked difference were observed between the H2 and H3 strains of influenza virus in their sensitivity to pretreatment at low pH. While viral fusion and hemolysis mediated by X-31 (H3 subtype) was inactivated by pretreatment of the virus at low pH, A/Japan/305/57 (H2 subtype) retained those activities even after 15 min incubation at pH 5.0 and 37 °C. Fusion of intact virions and of CV1 cells expressing influenza hemagglutinin (HA) in the plasma membrane with erythrocytes was measured using the octadecylrhodamine dequenching assay. HA from the two strains assumed different conformational states after exposure to low pH as indicated by their susceptibility to protease digestion, release of fusion peptide, and by Electron Microscopy of unstained, frozen, hydrated virus. The relationship between conformation of HA and its fusogenic activity is discussed.

W-Pos317

EVALUATION OF THE MINIMAL NUMBER OF FUSION PROTEINS AT THE SITE OF MEMBRANE FUSION.

Joe Bentz. Dept. Bioscience & Biotechnology, Drexel University, Philadelphia, PA, 19104.

A basic question about the mechanism of membrane fusion between viruses and cells is how the assembly of proteins within the fusion site creates the local physical environment which leads to fusion. Knowing the minimal number of fusion proteins at the fusion site is the first step toward answering this question. In Ellens et al. (1989, preceding poster), we found that influenza hemagglutinin (HA) expressing fibroblasts fuse with a small constant fraction of bound glycophorin bearing liposomes (0.5 μm diameter), following a brief low pH incubation. We also found that two or more HA's were required at the fusion site. In that analysis, it was assumed that surface aggregation of the HA (perhaps induced by the low pH conformational change) was rapid compared with the kinetics of the fusion. Here, a more general analysis proves that surface aggregation of the HA can occur on any time scale with respect to fusion, and the experimental protocol will produce the same estimates for the minimal fusion unit. While the fusion mechanisms for other viral families will differ in details, it appears that a configuration of two or more fusion proteins is quite conducive to physicochemical descriptions of the merging of two membranes. (Supported by NIH grant GM31506).

W-Pos318

DIVALENT HISTAMINE INDUCES RECONDENSATION OF EXOCYTOSED MAST CELL GRANULES.

M. Villalon, P. Verdugo & J.M. Fernandez, Ctr. Bioengineering, Univ. of Washington, Seattle, WA and Dept. of Phys. and Biophys., Mayo Clinic, Rochester, MN

An important post-transcriptional modification of secretory products is their condensation into secretory granules, and their decondensation during exocytosis. The physicochemical principles that govern condensation/recondensation in secretion are still largely unknown. We have proposed that the molecular mechanism of condensation/decondensation in regulated secretion is a polymer gel phase transition. In this study we have used video image analysis to monitor the volume changes of individual mast cell granules of bg^J/bg^J mice. Results show that after stimulation with 10 μg/ml of 48/80, the exocytosed granules can be readily and reversibly recondensed by exposure to solutions containing histamine at acidic pH. Recondensation is both [histamine]-dependent (critical range 1-10 mM at pH 3) and pH-dependent (critical range pH 5 to 7 at 150 mM histamine)]. At pH 3, monovalent cations (Na^+ or K^+) produced negligible recondensation. Conversely, Ca^{++} was found to readily recondense the granule although not as effectively as histamine. These observations suggest that histamine, by acting as a divalent cation at low pH, can effectively screen the polyionic charges of the heparin polymer network triggering a polymer gel phase transition that leads to its recondensation. This is the first demonstration of recondensation of secretory products triggered by conditions that mimic the environment found in the Golgi and condensing vacuoles during granule formation. These experiments provide a powerful model for the understanding of sorting and condensation of secretory products.

W-Pos319

THE EFFECT OF GTPγS AND Ca^{++} ON THE KINETICS OF EXOCYTOSIS OF SINGLE SECRETORY GRANULES IN PERITONEAL MAST CELLS.

G. Alvarez de Toledo & J.M. Fernandez, Dept. Phys. & Biophys., Mayo Clinic. Rochester, MN 55905 (sponsored by A. Oberhauser).

We have studied individual exocytotic events in degranulating mouse peritoneal mast cells by monitoring the cell membrane capacitance. In well resolved reversible ("flicker") and irreversible fusion events we have quantified the dwell time in flicker and the latency between irreversible fusion events. The probability distribution functions of the flicker duration and the latency between irreversible fusions were fitted by single exponentials giving τ_f and τ_i, respectively. τ_i, was inversely proportional to [GTPγS] in a range between 0.2 and 50 μM. $[Ca^{++}]_i$ over 1 μM increased the sensitivity to GTPγS by shifting the τ_i vs [GTPγS] relationship so that less [GTPγS] was required to reach the same value of τ_i. In contrast, τ_f was unaffected by GTPγS and Ca^{++}. These results suggest that the mechanisms involved in flicker are different from those that cause the irreversible release of granule contents, which also appears to be the GTPγS and Ca^{++} dependent rate limiting step. A simple model with three states, U (unfused), F (flicker) and S (secretory) mimics the experimental data when the last step is made irreversible and dependent on [GTPγS] and $[Ca^{++}]_i$, whereas the F to U transition is assumed to have a constant rate. This kinetic analysis allows for the separation of processes in the late stages of exocytosis, and may help elucidate their nature. Supported by NIH grant 38857.

W-Pos320

POSSIBLE ROLE FOR MEMBRANE FUSION IN TUMOR CELL INVASION AND METASTASIS.

Raouf A. Guirguis+*, Ki Min Eum* and Jeong Soon Kim*, Cancer Diagnostics Inc.*, Rockville, MD 20852 and Georgetown Univ.+, Wash., DC 20007

Pseudopodia protrusion is a prominent feature both of actively motile cells *in vitro*, and of invading tumor cells *in vivo*. Whole cell migration through gelatin-coated polycarbonate membranes (>5μm diameter) was observed in human breast cancer cell lines (MDA-435s and HS578T) in response to their conditioned media. However, in case of small pore diameter filters (<5μm), tumor cell behavior was surprisingly different. Individual cells migrated through more than one pore in the filter and then reformed on its lower surface. Pseudopodia fusion was dose- and time-dependent, and changed significantly with the size and density of the pores in the membrane (0.8μm to 5μm). On the other hand, isolated intact pseudopodia fragments were refractory to fusion when subjected to same conditions. Pseudopodia protrusion but not fusion was observed in case of normal breast cell line (HS578N) in response to HS578T conditioned medium. This suggest that pseudopodia fusion is triggered by activation of an intrinsic membrane fusion mechanism which is associated with the process of cancer cell invasion. The observed formation and fusion of cell pseudopodia may faciliate *in vivo* both extravasation and intravasation of the cencer cells.

W-Pos321

ATTRACTION, DEFORMATION AND CONTACT OF ERYTHROCYTE MEMBRANES IN 60 Hz ELECTRIC FIELDS. Dimiter S. Dimitrov, Mariana A. Apostolova and Arthur E. Sowers. ARC/Holland Laboratory, Rockville, MD 20855.

The time-dependent separation distance, deformation and shared contact area between erythrocyte ghost membranes induced by a 60 Hz AC electric field were measured by video microscopy. The approach time increased with a decrease in the field strength and an increase in the medium viscosity, adjusted by adding glycerol. The membranes deformed at close approach before making contact. The contact area increased with time until reaching the equilibrium state. The ghosts became visibly separated within 1 min after switching off the field. The force of attraction was calculated from the experimental data for the separation distance. It varied from about 10^{-14} N at large separations to about 10^{-12} N at close approach and contact and depended on separation as predicted for dipole-dipole interactions down to separations of the order of 1 μm. These data and theoretical estimates suggest that lag times in membrane fusion kinetics include a time interval needed for the membranes to reach a contact. Support from American Red Cross (to DSD) and ONR grant N00014-89-J-1715 (to AES).

W-Pos322

BILAYER CURVATURE PROMOTES POLY(ETHYLENE GLYCOL)-INDUCED FUSION OF UNILAMELLAR DPPC VESICLES. Barry R. Lentz, Derek J. Parks, Julie C. Yates, & Donald Massenburg. *Biochemistry Dept., Univ. of North Carolina, Chapel Hill, NC 27514.*

Unilamellar vesicles of varying and uniform size were prepared by the extrusion procedure and sonication. Vesicle size was determined by quasielastic light scattering (QEL) using a standard cummulant analysis. Different vesicle preparations had mean diameters of: 1340Å, 950Å, 680Å, 550Å, and 350Å (sonicated). Mixing of bilayer lipids following treatment by PEG was monitored utilizing the DPHpPC fluorescent lifetime probe. Vesicle contents leakage and mixing was examined using the ANTS/DPX assay. Vesicle size after PEG treatment was obtained by QEL following removal of PEG by size exclusion chromatography. By all three measures, no fusion was detected for vesicles of diameter greater than 680Å. Vesicles of this size or smaller experienced 100% lipid exchange at PEG concentrations greater than 25 wt%, suggestive of fusion. Mean vesicle diameter also increased dramatically following treatment with greater than 25wt% PEG. Below 25wt% PEG, the extent of lipid exchange increased with increased vesicle curvature (decreased diameter). Leakage of vesicle contents also increased dramatically at and above 25wt% PEG. This and the small trapping volume of small vesicles made it impossible to confirm fusion by detection of contents mixing in all but the 680Å vesicles. We conclude that high bilayer curvature demonstrably destabilizes extrusion vesicles in a way that encourages fusion of closely juxtaposed bilayers. *Supported by USPHS Grant GM32707 to BRL.*

W-Pos323

REVERSIBLE CROSS-LINKING OF THE 60 kDa PHOSPHOPROTEIN IN SKELETAL SARCOPLASMIC RETICULUM (SR). Qing Tian, Arnold M. Katz & Do Han Kim, Dept. of Medicine, Univ. of Connecticut Health Center. (Intro. by Victor Skita)

Ca/calmodulin-dependent phosphorylation of a 60 kDa protein (60 kDa-P) in skeletal SR has been reported to regulate Ca release. To define the molecular mechanism for this regulation, we attempted to cross-link the 60 kDa-P to associated proteins. Junctional SR was phosphorylated in the presence of 5 mM Ca, 1 uM calmodulin and 0.5 mM Mg.[^{32}P]ATP and cross-linking carried out by addition of 2 mM DSP. At various times, the cross-linking reaction was stopped by addition of 100 mM lysine. Upon cross-linking, the 60 kDa-P labeled with ^{32}P disappeared and reappeared in high molecular weight region (>800 kDa). Incubation of the cross-linked SR with 20 mM DTT led to reappearance of the 60 kDa-P. The results presented indicate that the 60 kDa-P could be associated with high molecular weight protein(s). Supported by NIH (HL-33026) & AHA-CT. DHK is an Established Investigator of AHA.

W-Pos324

MOLECULAR CHARACTERIZATION OF A 60 kDa PHOSPHOPROTEIN IN JUNCTIONAL SARCOPLASMIC RETICULUM (JSR). Young Sup Lee and Do Han Kim, Dept. of Medicine, Univ. of Connecticut Health Center.

In light of evidence that Ca/calmodulin-dependent phosphorylation of a 60 kDa protein (60 kDa-P) in JSR could inhibit Ca release induced by several Ca release stimuli (Kim, D.H., Biophys. J. 419a, 1988), the 60 kDa-P in 0.5% CHAPS was isolated by sequential column chromatography of DEAE, heparin-agarose and hydroxylapatite. Amino acid composition and sequence analyses, ion-exchange chromatography, and immunoblots suggest that the 60 kDa-P could be an isoform of phosphoglucomutase (PGM) bound to JSR. Using oligo probes and cDNA library in λgt 11, four cDNA clones (2.0 - 3.0 kb) were identified. The cDNA clones in pBluescript are currently being sequenced. The results indicate that multiple functions could be regulated by a single gene. Supported by NIH (HL-33026) & AHA-CT. DHK is an Established Investigator of AHA.

W-Pos325

EFFECTS OF AZUMOLENE ON THE KINETICS OF Ca RELEASE FROM NORMAL (N) AND MALIGNANT HYPERTHERMIC (MH) SARCOPLASMIC RETICULUM D.H. Kim, Y.S. Lee, F.A. Sreter, T. Ohkusa, M. Dershwitz & N. Ikemoto, U. of Conn. Health Ctr, Boston Biomed. Res. Inst., MGH, Harvard Med. Sch.

Primary cause for MH symptoms has been postulated to be abnormal Ca release from SR such as elevated Ca release rates (Kim et al., BBA 775, 320, 1984). Dantrolene Na has been used to prevent MH episode, however, use of the drug for in vitro studies has been limited due to its low solubility in water. The present study characterized effects of azumolene, a water soluble analog of dantrolene, on the kinetics of caffeine-induced Ca release from N and MH pig skeletal SR monitored by stopped-flow spectrophotometry. Azumolene inhibited Ca release from both N and MH SR by preferentially reducing the rate constants. [Azumolene] for half max. inhibition (ug/ml) was much lower in MH SR (31) than N SR (59), indicating that MH SR has higher sensitivity to the release inhibitor, azumolene than N SR. Supported by NIH (HL33026, AR16922), AHA-CT & MDA. DHK is an Established Investigator of AHA.

W-Pos326

VOLTAGE AND TIME DEPENDENT GATING OF FROG SARCOBALL CHLORIDE CHANNELS.

I. Zahradnik, A. Zahradnikova, and P. Palade, Univ. Texas Med. Branch, Dept. Physiology & Biophysics, Galveston, TX.

Patch-clamped frog skeletal muscle sarcoplasmic reticulum chloride channels studied in sarcoballs exhibit a steep voltage dependent decrease in P_o (*Hals et al., J. Gen. Physiol. 93: 385-410, 1989).* With excised multichannel patches in symmetrical ionic conditions (200 mM TrisCl) averaged currents decline exponentially after steps from V_h=0 mV to both plus and minus voltages. Two time constants, needed to fit the current decline, decreased from the 100 ms and 10 ms ranges to the 10 ms and 1 ms ranges, respectively, with increasing $|\Delta V_m|$ from 10 mV to 80 mV. In two-pulse experiments test pulse current amplitudes decreased with amplitude and duration of conditioning voltage steps, but only if both pulses were of the same polarity. Application of pulses of opposite polarity did not decrease test currents. Fast I_{Cl} decline correlates with channel closing from the fully opened state. Open channel substates or fast flickering delay long lasting closures. This channel behavior might exert a modulatory effect on Ca release and uptake during the contraction cycle.

W-Pos327

INOSITOL TRISPHOSPHATE -INDUCED Ca^{2+} RELEASE FROM SARCOPLASMIC RETICULUM VESICLES. Alice Chu. Dept. of Medicine, Cardiovascular Sciences, Baylor Coll. of Medicine, Houston, TX.

Terminal cisternae (TC) fractions of sarcoplasmic reticulum, as determined by high affinity [^{3}H]-ryanodine binding among other criteria, were passively loaded with $^{45}Ca^{2+}$. Fast-twitch (f-TC), slow-twitch (s-TC) skeletal and cardiac (c-TC) muscle TC all release $^{45}Ca^{2+}$ in the presence of myo-inositol-1,4,5-trisphosphate (IP_3) (no Ca^{2+}). In all muscle types, the rate of Ca^{2+} release with IP_3 is much slower than with Ca^{2+}, and the total amount of Ca^{2+} released by IP_3 is less. The total Ca^{2+} released by IP_3 in less than one min is about 30, 40-50, and >50% from f-TC, s-TC and c-TC, respectively, as compared to the Ca^{2+} released in the presence of 10 mM Mg^{2+}. IP_3-induced Ca^{2+} release from TC is concentration dependent, inhibited by ruthenium red, and is minimal in light SR fractions. At present, it is still unknown whether IP_3 is involved in excitation-contraction coupling.

[Supported by an AHA-Texas Affiliate grant and NIH BRSG to A.C. and an NIH grant (HL13870) to M.L.E.]

W-Pos328

THE MECHANISM OF STIMULATION IN CALCIUM UPTAKE OF CARDIAC SARCOPLASMIC RETICULUM VESICLES BY MONOCLONAL ANTIBODY AGAINST PHOSPHOLAMBAN

Y. Kimura, M. Inui, M. Kadoma, *J. H. Wang, and M. Tada Osaka University School of Medicine, Osaka, Japan, *Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada.

The monoclonal antibody (mAbA1) against phospholamban (PLN) was reported to stimulate Ca uptake by cardiac sarcoplasmic reticulum vesicles (SRV). To determine whether this stimulation of Ca uptake is brought by stimulation of Ca pump ATPase or by other mechanisms such as channel activity of PLN, we examined the effects of mAbA1 on Ca uptake and ATPase activity of SRV. When SRV were preincubated with mAbA1, Ca uptake was activated, shifting K_{Ca} to lower Ca concentration. Since the Ca uptake was fully activated by mAbA1, no further stimulation was observed with cAMP-dependent protein kinase treatment. The Ca-dependent ATPase activity was also stimulated by mAbA1. The K_{Ca} was shifted from 1.2 μM to 0.7 μM. No significant change in stoichiometry between Ca and ATP was observed under these conditions. The binding of mAbA1 to PLN thus produces essentially the same mode of action on Ca pump ATPase as that by PLN phosphorylation, in that mAbA1 and PLN phosphorylation de-suppress the inhibitory action of PLN on Ca pump ATPase, resulting in stimulation of Ca pump.

W-Pos329

EFFECTS OF THE DIHYDROPYRIDINE (DHP) RECEPTOR-SPECIFIC AGENTS ON DEPOLARIZATION-INDUCED CA^{2+} RELEASE FROM SR IN VITRO T. Ohkusa[a], H.M. Smilowitz[b], and N. Ikemoto[a,c] (a, Dept. Muscle Res., Boston Biomed. Res. Inst.; b, Dept. Pharmacol., Univ. Conn. Health Ctr.; c, Dept. Neurol., Harvard Med. Sch.)

Ionic replacement of the isolated triad system induces Ca^{2+} release from SR in two kinetically distinguishable phases by mediation of the T-tubule depolarization (Ikemoto et al., J. Biol. Chem., **250**, 13151, 1984). We investigated effects of nifedipine and a monoclonal antibody (mAb) directed to the DHP receptor α_1 subunit (Chang and Smilowitz, Ann. N.Y. Acad. Sci. **560**, 59, 1989) on the depolarization-induced Ca^{2+} release. Both nifedipine and mAb inhibited the slow phase of Ca^{2+} release, while the fast phase was often (but not always) activated. They had no appreciable effect on Ca^{2+}- nor caffeine-induced Ca^{2+} release, which is triggered by direct stimulation of the SR. Thus, both the fast and slow phases of the depolarization-induced Ca^{2+} release are controlled by the T-tubule via different mechanisms that are distinguishable in the sensitivity to the DHP receptor-specific agents.

(Supported by NIH and MDA)

W-Pos330

STRUCTURAL ANALYSIS OF THE CALSEQUESTRIN (CSQ)-FOOT PROTEIN (FP) INTERACTION

A. Tarcsafalvi[a], J.H. Collins[b], J.J. Kang[a], and N. Ikemoto[a,c] (a, Dept. Muscle Res., Boston Biomed. Res. Inst.; b, Dept. Biol. Chem., Univ. Maryland; c, Dept. Neurol., Harvard Med. Sch.)

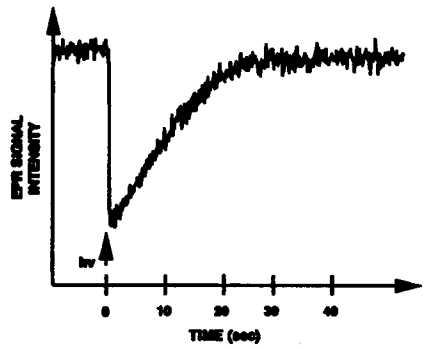
To gain further insight into the mechanism by which the functions of Ca^{2+} release channels of SR are regulated by CSQ (Ikemoto et al., Biochemistry, **28**, 6764, 1989), following studies were performed. Tryptic fragments of CSQ were screened for their ability to bind to the junctional face membrane (JFM). Partial amino acid sequencing of the JFM-binding and non-binding peptides allowed us to localize the critical binding site in the region encompassing residues 86-191, the portion of the CSQ molecule that is most highly enriched in α-helix. Studies on the reconstituted JFM-CSQ complex with a reversible cross-linking agent, sulfosuccinimidyl 2-(m-azido-o-nitrobenzamido)-ethyl-1,3'-dithiopropionate, suggest that the FP is the major CSQ binding protein of the JFM, and in the FP-CSQ interacting region there are highly reactive FP-SH group(s) and an occluded compartment.

(Supported by NIH and MDA.)

W-Pos331

TIME-RESOLVED EPR DETECTION OF TRANSIENT CONFORMATIONAL CHANGES IN SPIN-LABELED Ca-ATPase USING CAGED-ATP. James E. Mahaney and David D. Thomas. Dept. of Biochemistry, Univ. of Minnesota Medical School, Mnpls., MN 55455.

We have used laser-induced photolysis of caged-ATP in order to detect transient conformational changes in the SR Ca-ATPase, with a time resolution of 100 msec. Distinct conformations were detected using EPR spectroscopic analysis of the Ca-ATPase labeled with an iodoacetamide spin label. The EPR spectra of spin-labelled Ca-ATPase consisted of two resolved spectral components, the mole fractions of which depended on ligand environment and, therefore, ATPase conformation. Immediately upon photolysis of caged-ATP, which produced 1.6 mM ATP, the EPR signal changed within 300 msec to a level indicating decreased probe mobility. Following a 2-3 sec steady-state period, the signal returned to the original value. These signals correspond to conformational transients, which correlate well with known steps in the Ca-ATPase kinetic cycle.



W-Pos332

DIFFERENTIAL EFFECTS OF CIS/TRANS pH CHANGES ON NATIVE Ca^{2+} RELEASE CHANNELS. **E. Rousseau* and J. Pinkos, Dép. Physiol/ Biophys., Univ. of Sherbrooke, Qué. Canada.**

Sarcoplasmic Reticulum vesicles from muscles were isolated and fused into Planar Lipid Bilayers. Single channel recordings were obtained in a large asymmetric Ca^{2+} Buffer-System (50 mM Ca^{2+} Trans/2.5 μM Ca^{2+} ± 2 mM ATP cis) at pH 7.4. The pH was independently varied on each side of the channel. Acidification of the cis-chamber (7.4 to 6.6) induced a reversible modification of the gating behavior, resulting in a decrease of the Po due to longer closed states and fewer open events. Parallel isotopic flux measurements confirm that acidic pH reduced the % of Ca^{2+} release. However, 10 fold increases of cis Ca^{2+} (25 μM) do not prevent the pH dependent inhibition. The P.L.B. set-up allows a direct access to the luminal face of the channels (trans side) which was perfused with either Ca/HEPES or Ca/PIPES solution. Acidification of the trans-chamber (7.4 to 6.8) induced a reduction of the unitary conductance of the SR Ca^{2+} release channel without modification of the gating behavior. *E. R. is a scholar of C.H.F.

W-Pos333

BINDING OF [^{3}H]RYANODINE TO AND RAPID Ca^{2+} RELEASE FROM SR Ca^{2+} RELEASE CHANNELS OF CARDIAC AND SKELETAL MUSCLE: A COMPARATIVE STUDY. I. ZIMANYI and I.N. PESSAH, Dept. Vet. Pharm. Tox. Univ. Calif., Davis, CA 95616

SR membrane vesicles from rat heart (RH) and rabbit fast skeletal muscle (RSM) have been prepared. The binding properties of [^{3}H]ryanodine to the SR Ca^{2+} release channel of RH and RSM is compared by equilibrium and kinetic binding studies, as well as the Ca^{2+} release. Both preparations exhibit two binding sites. The K_d of high and low affinity sites of both preparations are nanomolar, neither RSM nor RH exhibit micromolar affinity sites. Ruthenium red is a competitive inhibitor on the binding of ryanodine in nanomolar concentrations with a lower IC_{50} in RSM than in RH. Dissociation of [^{3}H]ryanodine from its sites induced by 100-fold dilution, 50 nM ryanodine, 1 μM ruthenium red or 10 μM 4,4'-dithiobispyridine showed significant differences in the two preparations. RH SR is more sensitive to the inhibitory effect of Mg^{2+} on the [^{3}H]ryanodine binding than RSM SR. The results obtained from binding studies are directly correlated to Ca^{2+} release measurements by antipyrylazo III. Supported by NIH grant ES 05002.

W-Pos334

INTERACTION OF A SYNTHETIC PHOSPHOLAMBAN PEPTIDE WITH THE CARDIAC SARCOPLASMIC RETICULUM (SR) Ca^{2+}-ATPase. J. Cuppoletti*, H.W. Kim, and E.G. Kranias. Depts. of Physiology & Biophysics* and Pharmacology & Cell Biophysics, Univ. Cinti., Cinti., OH.

The Ca^{2+}-ATPase (Ca-A) in cardiac SR is under regulation by phospholamban (PLB). Using purified reconstituted systems, we have shown that both PLB and a synthetic peptide corresponding to amino acids 1-25 of PLB are inhibitors of the Ca-A, and phosphorylation of PLB may relieve this inhibition. To determine whether regulation of the Ca-A is mediated through direct interaction between the Ca^{2+} pump and the hydrophilic portion (AA 1-25) of PLB, a photoaffinity probe ([^{125}I]NHS-ASA PLB peptide) was used to label Ca-A. Radioactive labeling appeared to be specifically associated with the Ca-A in either the purified enzyme or cardiac SR membranes. Controlled tryptic digestion of the photolabeled Ca-A resulted in fragments of 55, 45, 35, and 25 kda. Only the fragments of 55, 35, and 25 kda were labeled. These findings suggest that the hydrophilic portion of PLB may directly interact with the Ca-A and the region of interaction on the Ca^{2+} pump appears to be within the 25 kda tryptic fragment. (Supported by NIH HL26057 and DK38808.)

W-Pos335

STUDIES WITH A MONOCLONAL ANTIBODY THAT INHIBITS THE Ca^{2+}-ATPase OF THE SARCOPLASMIC RETICULUM.

Howard Kutchai*, Lisa M. Geddis*, & Kevin P. Campbell+. *Dept. of Physiology & Biophysics Program, University of Virginia, Charlottesville, VA 22908 and +Dept. of Physiology & Biophysics, University of Iowa, Iowa City, IA 52242

A monoclonal antibody, designated VIE8, that is directed against the Ca^{2+}-ATPase of the sarcoplasmic reticulum (SR) of rabbit fast skeletal muscle was tested for its influence on the function of the Ca^{2+}-ATPase. Preincubation of SR with VIE8 led to pronounced inhibition of both Ca^{2+} uptake (oxalate supported) and ATP hydrolysis by the SR. The inhibitory effects of VIE8 increased with increasing ratio of VIE8 to SR protein in the preincubation mixture. Preincubation of SR with monoclonal antibodies against other SR proteins, such as calsequestrin, and the 53 and 160 KDa glycoproteins, did not result in inhibition of Ca^{2+} uptake and Ca^{2+}-ATPase activity. Experiments in progress aim at determining the location of the epitope of VIE8 in the Ca^{2+}-ATPase molecule. In other experiments we found that the ability of VIE8 to inhibit Ca^{2+}-ATPase activity and Ca^{2+} uptake by SR can be greatly potentiated by either of two monoclonal antibodies against the 53 KDa glycoprotein (GP-53) of the SR. The molecular mechanisms by which VIE8 inhibits Ca^{2+}-ATPase activity and Ca^{2+} uptake remain to be elucidated.

W-Pos336

STRUCTURAL DYNAMICS OF THE Ca^{2+}-ATPase OF SARCOPLASMIC RETICULUM (SR). I. Jona, J. Matko and A. Martonosi (Intro. by Thomas J. Csermely), Dept. Biochem, SUNY HSC, Syracuse, NY 13210.

The temperature dependence of fluorescence polarization and Forster type resonance energy transfer (Somogyi, et al., Biochemistry 23, 3403-3411, 1984) was analyzed in the Ca^{2+}-ATPase of SR using protein tryptophan and IAEDANS, FITC, TNP-AMP or lanthanides (Pr^{3+}, Nd^{3+}) as probes. The normalized energy transfer efficiency between AEDANS bound at cysteine 670 and 674 and FITC bound at lysine 515 increases with increasing temperature (10-37°C), indicating the existence of a flexible structure in the region that links the AEDANS to the FITC site. Thermal fluctuations were also evident in the energy transfer between FITC linked to the nucleotide binding domain and Nd^{3+} bound at the Ca^{2+} transport sites. By contrast the normalized energy transfer efficiency between AEDANS and Pr^{3+} was relatively insensitive to temperature, suggesting that the region between cysteine 670 and the Ca^{2+} site is relatively rigid. The "soft" and "rigid" domains are tentatively allocated within the ATPase contours defined by crystallography. (Supported by grants from the NIH, NSF and MDA.)

W-Pos337

VANADATE-CATALYZED, CONFORMATIONALLY SPECIFIC PHOTOCLEAVAGE OF THE Ca^{2+}-ATPase OF SARCOPLASMIC RETICULUM (SR). M. Vegh, E. Molnar and A. Martonosi (Intro. by Edward D. Lipson), Dept. Biochem., SUNY HSC, Syracuse, NY 13210.

Vanadate-sensitized photocleavage of the Ca^{2+}-ATPase of rabbit SR was observed upon illumination of SR vesicles or the purified Ca^{2+}-ATPase by ultraviolet light in the presence of 1 mM monovanadate or decavanadate. When the [Ca^{2+}] is maintained below 10 nM by EGTA, the photocleavage yields fragments of ≈ 87 and 22 kDa, while in the presence of 2-20 mM Ca, polypeptides of 71 and 38 kDa are obtained as cleavage products. The site of photocleavage is in the A fragment near the T2 cleavage site in the absence of Ca^{2+}, and in the B fragment between lysine 515 and aspartate 650 in the presence of 2-20 mM Ca^{2+}. The proximity of the T2 cleavage site to the site of vanadate binding may explain the photocleavage and the inhibition of the tryptic cleavage at T2 in the absence of Ca^{2+}. The photocleavage in the presence of Ca^{2+} is consistent with the existence of an ATPase-Ca^{2+}-vanadate complex (Markus et al., Biochemistry 28, 793-799, 1989). (Supported by grants from the NIH, NSF and the MDA.)

W-Pos338

THE BINDING OF MONOCLONAL AND POLYCLONAL ANTIBODIES TO THE Ca^{2+}-ATPase OF SARCOPLASMIC RETICULUM (SR). N. W. Seidler, E. Molnar, I. Jona and A. Martonosi, Dept. of Biochem., SUNY HSC, Syracuse, NY 13210

We analyzed the interaction of 14 monoclonal and 5 polyclonal anti-ATPase antibodies with the Ca^{2+}-ATPase of rabbit SR. Of the seven antibodies directed against epitopes on the B tryptic fragment of the Ca^{2+}-ATPase, all except one reacted with the enzyme in native SR vesicles in both the E_1 and E_2V conformations. Several of these antibodies interfered with the crystallization of Ca^{2+}-ATPase and increased the polarization of fluorescence of FITC-labeled Ca^{2+}-ATPase, suggesting dissociation of ATPase oligomers. Of the five antibodies with epitopes on the A_1 tryptic fragment of the Ca^{2+}-ATPase only one reacted with the native enzyme, but solubilization of the membrane with $C_{12}E_8$ rendered the antigenic sites fully accessible. Two monoclonal antibodies that interfered with crystallization also inhibited Ca^{2+} transport. Other antibodies that interacted with the native Ca^{2+}-ATPase produced little or no inhibition of activity, suggesting that their antigenic sites do not undergo major movements during Ca^{2+} transport. (Supported by grants from the NIH, NSF and MDA.)

W-Pos339

NATIVE ARCHITECTURE OF THE CALCIUM CHANNEL/FOOT STRUCTURE FROM SKELETAL MUSCLE. M. Radermacher, T. Wagenknecht, R. Grassucci, J. Frank, A. Saito[1], M. Inui[1], C. Chadwick[1], S. Reif[1] and S. Fleischer[1]. Wadsworth Ctr. for Laboratories & Research, New York State Dept. of Health, Albany, NY and [1]Dept. of Mol. Biol., Vanderbilt University, Nashville, TN.

Previously we determined the three-dimensional shape of the calcium release channel/foot structure (JCC, junctional channel complex) from sarcoplasmic reticulum of skeletal muscle by three-dimensional reconstruction from electron micrographs of negatively stained specimen (Nature (1989) 338, 167). In order to further characterize the structure of JCC we have begun to analyze electron micrographs of specimens that are in a frozen-hydrated (non-stained) state. This technique does not suffer from the potential artefacts associated with the negative stain technique and should reveal the native structure of JCC. Although a three-dimensional analysis is not yet complete, a two-dimensional analysis already shows that the major structural features detected in stain are present in the presumably native frozen-hydrated JCC. [Supported by NIH GM29169 (J.F.), DK14632 and MDA (S.F.).]

W-Pos340

ANNEXIN VI MODULATES THE GATING BEHAVIOR OF THE Ca^{2+}-RELEASE PROTEIN OF THE SARCOPLASMIC RETICULUM. M. Diaz-Munoz, M.A. Kaetzel*, P. Hazarika*, J.R. Dedman* and S.L. Hamilton. Departments of Molecular Physiology & Biophysics, Baylor College of Medicine, Houston, Texas 77030 and *Physiology & Cell Biology, University of Texas Health Science Center, Houston, Texas 77030. Annexins are a family of proteins that show calcium-dependent binding to membranes. The annexin family consists of at least seven distinct but closely related proteins, each of which is composed of a repeat motif of 60-70 amino acids. Annexin VI, a 67 kDa protein, is found in many tissues but is particularly enriched in skeletal muscle. Using an anti-annexin VI antibody it has been determined that annexin VI co-purifies with heavy sarcoplasmic reticulum (SR), specifically with terminal cisternae fractions. This protein appears to be localized in the lumen of isolated SR vesicles. In an effort to elucidate the physiological role of annexin VI, we tested the effect of this protein on the activity of Ca^{2+}-release channel from SR incorporated into phospholipid bilayers. Our findings are: a) Annexin VI at concentrations of 5 nM and above modifies the gating of Ca^{2+}-release channels; b) The effect of annexin VI occurs only when it is added in the TRANS chamber (corresponding to the intraluminal surface); c) The Ca^{2+}-release channel modified by annexin VI is opened by ryanodine.

These studies suggest the possibility that annexin VI or a similar molecule controls the gating properties of Ca^{2+}-release channel. This effect could be indirect through a high affinity interaction with either lipids or another protein localized on the luminal surface of the SR. Annexin VI, is therefore, a candidate for a modulator of excitation-contraction coupling.

W-Pos341

PATCH CLAMP RECORDING OF CALCIUM CHANNELS OF SARCOPLASMIC RETICULUM (SR) INCORPORATED INTO LIPOSOMES. **J. Sierralta, P. Vélez, A. Escobar[1] & B. A. Suárez-Isla[2]. Dept. Fisiol. Biofísica. Fac. Medicina, U. de Chile, [1]Inst. Biología Celular, Fac. Medicina, U. Buenos Aires & [2]Centro de Estudios Científicos de Santiago, Casilla 16443, Santiago, Chile.**

We applied patch clamp techniques to liposomes formed by the hypoosmotic swelling method (PE:PS=7:3 + frog skeletal muscle SR membranes; 0.5 M to 0.25 M NaCl, or CsCl or KCl, pH 7.4, 20 mM HEPES Tris; Correa & Agnew, BJ, 54, 1988). In excised patches we detected a 500 pS cationic channel (symmetric 250 mM CsCl; filter at f_c:2-10 kHz,-3dB and sampling at 5xf_c) with voltage dependent gating. P_o decreased from 0.90 at +10 mV to 0.42 at +60 mV. Records at 10 kHz revealed a very fast gating (at +40 mV: τ_{open}=0.3 and 1.8 ms and τ_{closed}=1.2 and 3.4 ms). The conductance sequence in symmetrical chloride salts (250 mM, 0 mV) was Cs^+>Na^+>K^+ and helped to distinguish this channel from a K^+selective SR channel. A smaller channel (68 pS, 250 mM CsCl) was activated by 1 mM ATP and blocked by 2 mM Mg^{2+} and 100 nM ruthenium red. These findings reveal new aspects on the fast gating of Ca^{2+} channels present in SR.

Supp. by FONDECYT #902, 927 and NIH-GM35981.

W-Pos342

THE EFFECT OF NEGATIVELY CHARGED PHOSPHOLIPIDS ON THE RECONSTITUTED Ca^{2+}-ATPase FROM SKELETAL SARCOPLASMIC RETICULUM.
G. Szymanska, H.W. Kim and E.G. Kranias, Dept. of Pharmacology and Cell Biophysics, Univ. of Cincinnati, Cincinnati, OH 45267.

The purified Ca^{2+}-ATPase from skeletal sarcoplasmic reticulum (SR) was reconstituted into liposomes at a cholate/protein/phosphatidylcholine (PC) ratio of 4.5/1/40. The highest rates of Ca^{2+}-uptake were 0.96 ± 0.05 (n=4) μmol Ca^{2+}/min/mg reconstituted vesicles compared to 1.6 ± 0.08 (n=4) μmol Ca^{2+}/min/mg native SR. The Ca^{2+}-ATPase activity assayed under identical conditions was 2.4 ± 0.1 μmol Pi/min/mg reconstituted vesicles compared to 1.5 ± 0.04 μmol Pi/min/mg native SR. When acidic phospholipids were used in combination with PC, the activity of the reconstituted Ca^{2+}-pump was stimulated. In the presence of phosphatidylinositol phosphate (PIP) (PIP:PC of 23:77) maximal stimulation of both Ca^{2+} transport and Ca^{2+}-ATPase was 50%, while in the presence of phosphatidylserine (PS) (PS:PC of 50:50) Ca^{2+} transport was increased by 300% and Ca^{2+}-ATPase activity by 50%, compared to PC-proteoliposomes. These findings suggest that negatively charged phospholipids may be involved in the activation of the SR Ca^{2+}-ATPase. (Supported by NIH HL26057 and HL22619).

W-Pos343

RECONSTITUTION OF SARCOPLASMIC RETICULA (SR) Ca^{2+}-ATPases FROM CARDIAC AND SKELETAL MUSCLE. Hae Won Kim, G. Szymanska, N.A.E. Steenaart, and E.G. Kranias. Dept. of Pharmacology and Cell Biophysics, Univ. of Cincinnati, Cincinnati, OH 45267.

Reconstitution of the cardiac and skeletal SR Ca^{2+}-ATPases into liposomes required different conditions for optimal enzymic activity. In the presence of phosphatidylcholine (PC) maximal activity was obtained at cholate/Ca^{2+}-ATPase/PC ratio of 2/1/80 for the cardiac SR Ca^{2+}-ATPase (C-CaA) and 4.5/1/40 for the skeletal SR Ca^{2+}-ATPase (S-CaA). Reconstitution of the C-CaA did not require the presence of oxalate in the reconstitution buffers, while this was essential for the S-CaA. The maximal rates of Ca^{2+} uptake by reconstituted vesicles were 700 nmol/mg/min for C-CaA and 960 nmol/mg/min for S-CaA. Partial substitution of phosphatidylserine (PS) for PC did not have any effect on the reconstituted C-CaA but it was associated with a 3-fold stimulation of the Ca^{2+} uptake by the reconstituted S-CaA. These findings suggest that although the Ca^{2+}-ATPases from cardiac and skeletal SR have similar amino acid sequences (80% homology) and phospholipid composition they require different conditions for reconstitution. (Supported by NIH HL26057 and HL22619).

W-Pos344

ANTHRAQUINONES SENSITIZE CARDIAC SR Ca^{2+} RELEASE CHANNELS BY INFLUENCING OXIDATION OF CRITICAL THIOLS OF THE RYANODINE RECEPTOR. **I.N. Pessah and I. Zimanyi, Dept. of Veterinary Pharm. and Tox., Univ. of Calif., Davis, CA 95616.**

Rat cardiac membranes enriched in biochemical markers of the junctional region of SR and exhibiting ligand-induced rapid Ca^{2+} release have been prepared. Doxorubicin (DXR) and seven congeners are shown to enhance the binding of [^{3}H]ryanodine to its receptor by enhancing its rate of association and reducing the K_d. DXR enhances rapid Ca^{2+} release from SR vesicles in a dose and Ca^{2+}-dependent manner. These effects are competitively and noncompetitively antagonized by caffeine and ruthenium red, respectively. The acute effect of DXR on the cardiac Ca^{2+} release channel is fully reversible, however long-term treatment (up to 24 hr) with DXR followed by its removal increases the sensitivity of the channel to subsequent acute challenge with DXR or Ca^{2+} and appears to be the result of a receptor-mediated shift in the redox equilibrium of specific thiols at the ryanodine receptor complex. These results identify a receptor-mediated mechanism for the cumulative sensitization of SR Ca^{2+} release channels by anthraquinones.
Supported by NIH Grant ES05002.

W-Pos345

MEASUREMENT OF Ca2+ UPTAKE BY CARDIAC SARCOPLASMIC RETICULUM IN SITU. M. A. Movsesian, J. Krall and William H. Barry, University of Utah, Salt Lake City, UT

ATP-dependent, oxalate-supported Ca^{2+} uptake was measured in isolated sarcoplasmic reticulum (SR) and in dissociated ventricular myocytes prepared from eighteen-day chick embryo hearts and permeablised with 50 µM digitonin. Kinetic parameters (with respect to Ca^{2+}) were:

	V_{max} (nmol/mg-min)	$K_{0.5}$ (µM)	n_{Hill}
Myocytes	16	.52	1.9
SR vesicles	330	.57	2.0

Ca^{2+} uptake at 5.0 µM Ca^{2+} was stimulated 1.5-fold in isolated SR and 1.7-fold in myocytes by the addition of 0.5 mM ryanodine. The characteristics of Ca^{2+} uptake measured in situ in cardiac myocytes thus correspond to those measured in isolated SR. To demonstrate the applicability of these methods, Ca^{2+} uptake was measured in myocytes cultured in the presence and absence of thyroxine. At 2.0 µM Ca^{2+}, uptake was 18.2 nmol/mg-min in cells cultured in the presence of thyroxine and 8.1 nmol/mg-min in cells cultured in its absence. These results show that effects of hormonal interventions on SR Ca^{2+} uptake can be measured in situ in cultured cells.

W-Pos346

TWO DISPOSITIONS OF JUNCTIONAL FEET IN THE TRIADS OF STRIATED MUSCLE FROM INVERTEBRATES. **K. Loesser*, L. Castellani#, C. Franzini-Armstrong*, *Depts. Anat. & Biol., Univ. Penn., Philadelphia, PA; #Rosenstiel Ctr., Brandeis Univ., Waltham, MA.**

In striated muscle of both vertebrates and invertebrates, Ca^{2+} release from the sarcoplasmic reticulum (SR) is graded and depends on the external surface membrane potential. E-C coupling, however, differs between vertebrates and invertebrates in its dependence on extracellular Ca^{2+}. We have examined the SR feet in junctional plaques isolated from muscles of four invertebrates. The cytoplasmic domains of the foot proteins, visualized by electron microscopy, display the typical four-fold symmetry observed in vertebrates. The disposition of the feet, however, differs: in crayfish and scallop, all feet abut side by side, but in grasshopper and scorpion, adjacent feet have two distinct orientations. Two-dimensional reconstructions from thin sections of scorpion muscle show two orientations for the four-subunit feet, as observed in isolated junctional SR. The differences in disposition are probably dictated by the cytoplasmic domain of the foot protein and may therefore indicate a variation in this part of the molecule.

W-Pos347

X-RAY MICROANALYSIS OF SUBCELLULAR CALCIUM DISTRIBUTION IN CONTRACTED AND RELAXED CARDIAC MUSCLE.

Christine S. Moravec and Meredith Bond, Cleveland Clinic Foundation, Cleveland,OH.

We have used electron probe X-ray microanalysis to measure Ca^{2+} in the junctional sarcoplasmic reticulum (jSR) of hamster papillary muscle rapidly frozen at the peak of contraction or during relaxation. Ca^{2+} in the jSR was 12.4±1.6 (SEM) mmol/kg dry wt in relaxed muscles (n=36 cells) and was significantly lower in contracted muscles, averaging 7.2±1.2 mmol/kg dry wt (n=30) ($p<.03$). Pre-treatment of muscles with 10^{-7} M ryanodine, followed by freezing during relaxation, resulted in an average jSR Ca^{2+} of 6.9±1.2 mmol/kg dry wt (n=11). We also determined the concentration of Ca^{2+} in mitochondria (MT) during contraction and relaxation, since it has been proposed that MT Ca^{2+} uptake during contraction activates certain enzymes of the TCA cycle. In relaxed muscles, MT Ca^{2+} averaged 0.1±0.2 mmol/kg dry wt (n=43), which did not differ from the average of 0.4±0.2 mmol/kg dry wt in contracted muscles (n=38). These results provide a direct measurement of Ca^{2+} release from the jSR during contraction and also indicate that the Ca^{2+} load of the jSR is decreased by ryanodine. The data do not, however, support the hypothesis of MT Ca^{2+} uptake during contraction.

W-Pos348

MOLECULAR CLONING OF cDNA ENCODING THE Ca2+ RELEASE CHANNEL (RYANODINE RECEPTOR) OF RABBIT CARDIAC SARCOPLASMIC RETICULUM:

Kinya Otsu, *Huntington F. Willard, and David H. MacLennan, Banting and Best Dept. of Medical Research, U. of Tor., Charles H. Best Institute, Tor., Ont., M5G 1L6, and *Dept. of Medical Genetics, U. of Tor., Tor., Ont., M5S 1A8, Can.

We screened a cDNA expression library from rabbit cardiac muscle with monoclonal antibodies against the purified cardiac muscle ryanodine receptor (kindly provided by Drs. T. Imagawa and M. Shigekawa). The amino acid sequence from cDNA sequence has extensive homology with that of the fast skeletal muscle. Analysis using a panel of human-rodent somatic cell hybrids indicates that the genes encoding these two forms of the ryanodine receptor are localized on different chromosomes. Northern blot analysis of mRNA from a variety of tissues demonstrates that the cardiac form is expressed in heart and brain, but not in fast and slow skeletal muscle. (Supported by the OHSF, MRC and MDAC).

W-Pos349

THE INCREASE IN INORGANIC PHOSPHATE ASSOCIATED WITH SHORT TERM HYPOXIA DEPRESSES Ca^{2+} UPTAKE BY CARDIAC SARCOPLASMIC RETICULUM.

V. Perlitz, M.E. Long, R.J. Adams, and T.M. Nosek, Dept. of Physiology & Endocrinology, Medical College of Georgia, Augusta, GA 30912

Short term hypoxia significantly changes the intracellular milieu of the myocardium (Kammermeier et al., J. Mol. Cell. Cardiol., 14:267, 1982). We have previously described how these changes depress the contractile properties of skinned cardiac muscle fibers (J. Physiol., 412:155, 1989). The current study used Fura-2 and fluorescence spectroscopy to measure the effect of these milieu changes on Ca^{2+} uptake by canine cardiac membrane vesicles enriched in SR. Solutions mimicking the hypoxic state depressed the rate of Mg-ATP-stimulated Ca^{2+} uptake (4.74 μmol Ca^{2+}/mg protein sec) by 69 ± 4%. Increasing the Pi content of the control solution (0.88 mM) to that mimicking the hypoxic state (17.36 mM) inhibited the rate of Ca^{2+} uptake by 63 ± 4%. In all solutions, vesicles were able to completely take up 25 μmol Ca^{2+} added to the bathing medium. Pi also inhibited the maximum Ca-ATPase activity of the vesicles (linked enzyme assay). We conclude that the milieu changes with short term hypoxia not only depress contractile function, but also inhibit the ability of the SR to transport Ca^{2+}.(Supported by NIH HL/AR 37022)

W-Pos350

COMPLETE SEQUENCE OF HUMAN CARDIAC PHOSPHOLAMBAN DETERMINED BY cDNA CLONING

Terrence L. Scott and Ghazala Ali, Dept. of Muscle Research, Boston Biomedical Research Institute, Boston, MA 02114.

The complete sequence of human cardiac phospholamban cDNA has been determined. The transcript is 2.3 kbp in length, with a coding region of only 156 bp. The 5'-untranslated region is extremely long and shows extensive homology to a number of mammalian repetitive sequences. Both the 5' and 3' (presumed) untranslated regions contain several open reading frames. Northern blots indicate that human cardiac muscle contains several distinct phospholamban transcripts of widely different sizes. While there are significant differences in the untranslated regions, the deduced amino acid sequence is highly homologous with that of canine phospholamban (Fujii, et al, J. Clin. Invest., 79, 301, '87). Supported by NIH GM-32247 and an Established Investigatorship of the AHA to TLS.

W-Pos351

UTILIZATION OF 3'-DEOXY ATP BY THE SR ATPase. Jose Amaral*, Sergio Verjovski-Almeida*, and Carol Coan+, *Dept. of Biochemistry, Inst. of Biomed. Sci., Universidade Federal do Rio de Janeiro, Rio de Janeiro 21910, Brasil, and +Dept. of Physiology, Univ. of the Pacific, San Francisco, CA 94115.

3'-d ATP, and most 3' substituted derivatives of ATP, exhibit very low levels of activity with the SR ATPase. Utilizing ^{32}P derivatives, we have compared levels of E-P formation, as well as rates of hydrolysis, of 3'-d ATP to those of ATP, 2'-d ATP and 3'-amino ATP. We find 3'-d ATP to give substantial levels of E-P with a Km of 20 μM while the rate of hydrolysis is negligible in this range. Alternatively, 3'-amino ATP exhibits substantial levels of E-P and high levels of hydrolysis, as does 2'-d ATP. This suggests that the 3' hydrogen of the ribose moiety is important for the proper turnover of E-P but not essential for utilization of the substrate to form E-P.

W-Pos352

POLARIZED INFRARED ATTENUATED TOTAL REFLECTANCE SPECTROSCOPY OF THE Ca^{2+}-ATPase OF SARCOPLASMIC RETICULUM (SR). Rene Buchet, Dept. of Biochem. Mol. Biol., SUNY HSC, Syracuse, N.Y. 13210.

Thin films of dry SR can be deposited on the surface of Ge or ZnSe crystals from a solution of 50 mM KCl, 0.5 mM $MgCl_2$, 5 mM imidazole, pH 7.4, 10 mM dithiothreitol, and 10 mg SR protein/ml. The Ca^{2+}-ATPase is preserved in the oriented multilayers for several days under nitrogen at 2-4°C, and more than 90% of its native ATPase activity can be recovered after rehydration. The mean orientation of the protein secondary structures and side-chain groups was determined from polarized FTIR spectra. The dichroic ratios from ZnSe and Ge crystals and the mean angles between the transition moment and the normal of the film plane were: -C=O (protein Amide I, 1650 cm^{-1}) 1.74, 1.90, -56°; -NH (protein Amide II, 1543 cm^{-1}) 1.71, 1.82, 57°; -CO (phospholipid, 1739 cm^{-1}) 1.77, 2.14, 53°; $-CH_2$ (lipid and protein, 2923 cm^{-1}) 1.80, 1.97, 55°. Changes in orientations related to Ca^{2+} transport will be analyzed. (Supported by a Fellowship from the American Heart Association and by grants to A. Martonosi from NIH, NSF and MDA).

W-Pos353

CARDIOTOXIN STIMULATES Ca-Mg-ATPase ACTIVITY AND AFFECTS THE SR CALCIUM RELEASE CHANNEL J-L. Huang and W.R. Trumble, Dept. Bact. & Biochem., Univ. of Idaho, Moscow, ID 83843

Cardiotoxin, a peptide component of cobra venom, induces contracture of heart and skeletal muscle by an unknown mechanism. Isolated membrane vesicles from bovine ventricular tissue, highly enriched in either SL or SR membrane, were used to examine the effects of cardiotoxin (CTX) on Ca^{2+} transport mechanisms. In SL vesicles, CTX (1-10μM) had no effect on the Na-Ca exchanger, but stimulated Ca uptake (50-100%) by the Ca-Mg-ATPase. This stimulation was not affected by ouabain (100μM) but was reduced to 50% of control levels by 0.5 mM DCCD. The effects of CTX on SL do not appear non-specific since Ca-efflux from the SL was not affected. A CTX-mediated increase in Ca-uptake by the Ca-Mg-ATPase was supported by the observation of a CTX-mediated (1-10μM) increase in Ca-stimulated, Mg-dependant ATP hydrolysis (50-75%). In isolated SR vesicles, CTX (1-10μM) similarly increased Ca-uptake into the vesicles by the Ca-Mg-ATPase and stimulated ATP-hydrolysis. Notably, however, CTX (5-10 μM) induced Ca^{2+} efflux from either passively or actively-loaded SR vesicles. Ruthenium Red (RR), at 10 μM, blocked the CTX-induced Ca efflux. Preparations of rabbit skeletal muscle "heavy" and "light" SR were made to assess the effects of CTX on the SR Ca-release channel. CTX (5 μM) induced Ca-efflux from passively loaded "heavy" SR (blocked by 1 μM RR) but did not induce Ca-efflux from "light" SR. We suggest CTX interacts with isolated SR vesicles to maintain an "open" Ca-release channel.

W-Pos354

ALTERED PHOSPHOLAMBAN PHOSPHORYLATION IN CARDIAC MICROSOMES OBTAINED FROM SENESCENT RATS. M. A. Kirchberger, E. Zhen, C. Kasinathan. Mount Sinai School of Medicine, New York, NY 10029.

Cardiac microsomes prepared from senescent (S) (23 mo) Fisher 344 rats showed higher ^{32}P incorporation into 27 kDa phospholamban (PLN) when normalized for a marker for sarcoplasmic reticulum than microsomes from young adult (YA) (3 mo) rats ($p<0.05$). No significant differences were found in (K^+, Ca^{2+})-ATPase, ouabain-inhibitable (Na^+, K^+)-ATPase, and azide-sensitive ATPase activities. Native S microsomes contained more PLN in the unphosphorylated form ($p<0.02$). ^{32}P-labelling in PLN decreased to 81% and 41% in YA and S microsomes, respectively, when the microsomes were incubated in the presence of $MnCl_2$ for 30 min ($p<0.001$). S microsomes, moreover, exhibited phosphoprotein phosphatase activity that was 2.5 fold that obtained in YA microsomes with ^{32}P-histone as substrate. Our data suggest that increased phosphoprotein phosphatase activity in the S rat heart leads to lower levels of phosphorylated PLN and perhaps to decreased responsiveness to catecholamines whose actions are mediated by phosphorylation reactions. (HL15764)

W-Poe355

COMPARATIVE STUDIES OF THE CONFORMATIONAL STATES OF CARDIAC AND SKELETAL SARCOPLASMIC RETICULUM CA,MG-ATPASE. THE EFFECTS OF CATIONS AND NUCLEOTIDES.

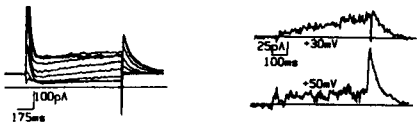
F. Mandel and S.S. Gupte. The Upjohn Company, Kalamazoo, Michigan and Department of Biochemistry, Uniformed Services University of Health Sciences, Bethesda, Maryland.

The conformational states of Ca,Mg-ATPase isolated from dog cardiac sarcoplasmic reticulum (DCSR) and rabbit skeletal muscle sarcoplasmic reticulum (RSSR) were compared using the fluorescent sulfhydryl probe 2-(4'maleimidylanilino) naphthalene 6-sulfonic acid (MIANS). The rates of MIANS binding to the Ca,Mg-ATPase were examined as functions of Ca^{2+}, Mg^{2+}, K^{+}, ATP, and ADP concentrations. The addition of all ligands resulted in a decrease in the rate of probe binding to RSSR as compared to a reference solution containing only EGTA, EDTA, and Tris maleate (pH 6.8) buffer. This result is in contrast with purified lamb kidney Na,K-ATPase (NKA) where the presence of all ligands studied caused a large increase in the rates of probe binding. The results with DCSR were intermediate in that under some conditions (e.g., Mg^{2+}) DCSR behaves like NKA while other ligands yield results similar to RSSR.

W-Pos356

DELAYED RECTIFIER POTASSIUM CURRENT, Ik, IN CAT VENTRICULAR MYOCYTES. T. J. Colatsky and C.H. Follmer. Wyeth-Ayerst Research, Princeton, N.J.

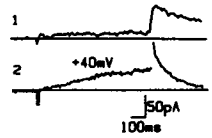
The delayed rectifier potassium current (I_k) was studied in cat ventricular myocytes (CVM) using single suction pipette voltage-clamp techniques (normal HEPES buffer with Cd^{++} to block Ca^{++} currents, T=31-32C). Voltage-clamp steps from -40mV to test potentials (Vt) between 0 to +70mV elicited a time-dependent outward current (I_k-Vt). "Tail" currents (I_{kt}) observed on repolarization to -30mV activated near 0mV, were 0.5 maximal at +28mV, and saturated near +60mV (120±14pA, n=13). I_k-Vt time-course had little voltage-dependence except for a rapid "jump" at start of clamp step. Rectification of I_k was revealed in fully-activated I_{kt} I-V relation (Vt=+60mV for 875ms): peak I_{kt} was maximal near -30mV and 0 near +20mV. Time-dependence of I_k-Vt at +40mV did not conform to the "envelope test" since I_{kt} activated faster (τ=215ms) than I_k-Vt (τ=450ms) in some cells. I_k properties were confirmed by analysis of "difference-currents" obtained using I_k selective blockers, E-4031 (1uM) or amiodarone (0.2 uM, below right). In conclusion, I_k in CVM appears to rectify in a time- and voltage-dependent manner, resulting in a complex voltage-dependent time-course. Non-conformity to the "envelope-test" may reflect these properties although the existence of multiple channel types cannot be ruled out.



W-Pos357

FLECAINIDE BLOCK OF THE DELAYED RECTIFIER, I_k, IN CAT VENTRICULAR MYOCYTES. C.H. Follmer, Y.W. Chen, N.J. Lodge, T.J. Colatsky. Wyeth-Ayerst Research, Princeton, NJ.

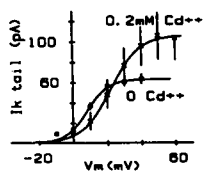
Flecainide (F) block of the delayed rectifier (I_k) was studied in cat ventricular myocytes (CVM) using single suction pipette voltage-clamp techniques (normal HEPES buffer with Cd^{++} to block Ca^{++} current, T=31-32C). I_k tails (I_{kt}) were studied using clamp steps from -40mV to selected test potentials (V_t,-10 to +60mV, 750ms). Flecainide (1μM) produced a 43±9% (n=4) decrease in maximal I_{kt} but had no effect on current during the depolarization (I_k-V_t). Measurement of the F-sensitive current (fig.) demonstrated that a higher F concentration (10 μM) blocked a small I_k-V_t with slow I_{kt} deactivation (I_{ks}, trace 1) in some cells and a time-dependent I_k-V_t with fast deactivation (I_{kf}, trace 2) in others. I_{kf} time-dependence during V_t had essentially no voltage-dependence (+20 to +70mV). Time-dependence of I_{ks} was seen between 0 and +30mV but not from +40mV to +70mV. In conclusion: F is a potent blocker of I_k in CVM and differences in F-sensitive current may underlie normal action potential disparity in the myocardium.



W-Pos358

MODULATION OF THE DELAYED RECTIFIER, I_k, BY CADMIUM IN CAT VENTRICULAR MYOCYTES. N.J. Lodge, C.H. Follmer, H. Mao, T.J. Colatsky (Intro. by L.H.Frame) Wyeth-Ayerst Research, Princeton, NJ.

The effects of Cd^{++} on the delayed rectifier (I_k) were studied in cat ventricular myocytes using single suction pipette voltage-clamp techniques (normal HEPES buffer, T=31-32C). Cd^{++} (0.2mM) increased peak tails (I_{kt}) and shifted activation (act) to positive potentials (Vm) by 14mV (fig.). Maximum I_{kt} increased from 64±5 (+40mV, n=5) to 106±23pA (+60mV, n=8). Time-constants for deactivation (I_{kd}, -40mV) decreased from secs to ms. Voltage ramps (30s, -100 to +60mV) showed I_k activated near -15 mV and rectified (became flat) positive to +10mV. Wash-in of Cd^{++} increased I_k above +10mV by shifting rectification to more positive Vm. This increase occurred before shifting I_{kt} act. Mg^{++} (10mM) was similar but less potent. Lanthanum (50μM) was also effective. In conclusion, Cd^{++} increases I_{kt} by reducing inward rectification, and speeds deactivation probably by favoring the closed-state of I_k. These effects appear to be distinct from surface charge effects.



W-Pos359

ARACHIDONIC, LINOLEIC, AND OTHER UNSATURATED FATTY ACIDS ENHANCE K^+ AND DEPRESS Na^+ AND Ca^{2+} CHANNEL ACTIVITY. G. Katz, L. Roy-Contancin, T. Bale, and J.P. Reuben, Merck Sharp and Dohme Research Laboratories, P.O. Box 2000, Rahway, NJ 07065.

Single channel (high conductance $P_{K,Ca}$; bovine aortic smooth muscle) and whole-cell (Na, Ca_L, & Ca_T; GH_3 cells) currents are modified by fatty acids in the same concentration range (50 nM to 10 μM). The average open time and openings/sec of $P_{K,Ca}$ channels increased for external and internal (more potent) applications. The amplitude of I_{Na}, I_{CaL}, and I_{CaT} decreased (see Leibowitz these abstracts) while I_{KA} increased. Kinetics were also modified. These data and those of recent reports reveal that the activity of a wide variety of membrane proteins is modified by fatty acids. Many types of K^+ channels are potentiated while those that convey inward currents are depressed. This suggests a physical mode of action such as a perturbation of membrane lipids which modifies lipid-protein interaction and consequently channel activity. Enhancement of membrane fluidity has been suggested to be a critical variable. However, temperature-induced (10^0-35^0C) changes in activity of $P_{K,Ca}$ channels within excised patches indicate that factors besides membrane fluidity must be involved.

W-Pos360

BACULOVIRUS-MEDIATED EXPRESSION OF SHAKER K^+ CHANNELS.

K. Klaiber*, N. Williams#, T. Roberts#, and C. Miller*. HHMI, Dept. of Biochemistry, Brandeis Univ., Waltham, MA, and #Dana Farber Cancer Institute, Harvard Medical School, Boston, MA

Most ion channels are minor membrane components that are difficult to isolate and analyze at the protein level. In view of the recent outpouring of cloned ion channel genes, it would be desirable to have available a high-level expression system for producing ion channel proteins. To this end, we have constructed a recombinant baculovirus containing the "Shaker" K^+ channel gene under control of the polyhedrin promoter. A cell line, Sf9, derived from the army-worm caterpillar Spodoptera frugiperda, when infected with the recombinant virus, expresses fully functional Shaker K^+ currents, as assayed by whole-cell recording. Shaker currents begin to appear about 15 hours post-infection, and they grow over the next 3 days. Over the same period of time, a 75-80 kDa band appears on SDS gels stained with Coomassie blue. These results give hope that the baculovirus system, which has been successfully used for high-level expression of soluble proteins from higher eukaryotes, may be appropriate for producing large amounts of integral membrane proteins in general, and ion channels in particular.

W-Pos361

BLOCK OF Ca^{2+} ACTIVATED K CHANNELS IN SMOOTH MUSCLE FROM CANINE GI-TRACT BY CHARYBDOTOXIN.

A Carl, ML Garcia, JL Kenyon and KM Sanders. Dept of Physiology, University Nevada School of Medicine, Reno, NV 89557, USA

Dispersed smooth muscle cells from canine proximal colon and gastric antrum express 250 pS Ca^{2+} activated K channels. The precise physiological role of these channels in electrical activity *in situ* is unknown and it would be usefull to have a specific probe for this channel. Therefore, we studied the effect of Charybdotoxin (ChTX), known to block Maxi K channels with a K_D between 2 and 5 nM, in excised patches. 100 nM ChTX applied to the bath solution of outside-out patches decreased the open probability from 54.2 % to 29.8 % in the presence of 140 mM K^+ in the pipette. 50 nM ChTX in the pipette solution of inside-out patches caused a block that depended strongly on the K^+ concentration in the bath solution. Open probability was reduced from 68.6 % in 140 mM K^+ to 5.7 % with 70 mM K^+ and 1.4 % with 5.9 mM K^+. These data suggest, that nanomolar concentrations of ChTX are not effective in blocking Ca^{2+} activated K channels in canine GI-tract unless intracellular K^+ is removed.

Supported by NIDDK P01-DK41315.

W-Pos362

CHARIBDOTOXIN BLOCKS Ca^{2+}-ACTIVATED K^+ CURRENTS IN LARVAL MUSCLE FIBERS FROM *Drosophila*.

Ricardo Delgado, Pedro Labarca, Enrico Stefani and Ramon Latorre. Dept. of Biology, Faculty of Sciences, Univ. of Chile, Centro de Estudios Cientificos de Santiago, Santiago, Chile, and Dept. of Physiology and Molecular Biophysics, Baylor College of Medicine, Houston, TX 77030.

Four outward K^+ currents have been described in the wild type larval muscle membrane of *Drosophila melanogaster*[1]. Two of these currents are fast and transient (I_A and I_{Acd}). The other two are slower potassium currents that do not show appreciable inactivation (I_K and I_C). I_{Acd} and I_C are calcium-activated currents[1,2]. Using the two microelectrode voltage clamp technique we found that the scorpion toxin charibdotoxin (CTX), added to a final concentration of 100 nM, partially blocks both transient and steady-state components of the outward currents. Steady-state current-voltage relationships are N-shaped. After treatment with Co^{2+} or CTX the N-shape disappears. Since Co^{2+} is a calcium current blocker and the N-shape appears at voltages in the range between +30 to +60 mV, the results suggest that CTX completely blocks the slow Ca^{2+}-activated current present in *Drosophila* larval muscle. Furthermore, CTX appears to abolish the transient component of the outward current due to I_{Acd}.

[1] Gho and Maillart. Pflugers Arch. 407: 526-533 (1986)
[2] Elkins et al PNAS 83: 8415-8419 (1986)

Supported by NIH and FONDECYT 1167/88 and 451/88.

W-Pos363

EFFECT OF pH IN A Ca^{2+}-ACTIVATED K^+ CHANNEL [K(Ca)] FROM RAT SKELETAL MUSCLE.

Laurido,C.*, Wolff,D.** and Latorre,R.**. *Departamento de Química, Facultad de Ciencia, Universidad de Santiago. **Departamento de Biología, Facultad de Ciencias, Universidad de Chile, and Centro de Estudios Científicos de Santiago.

We have studied the effect of pH on the activation of the K(Ca) channel isolated from rat skeletal muscle incorporated into planar lipid bilayers. Experiments were done at a constant applied voltage of +30 mV and at different intracellular calcium concentrations and pH's. a) At constant $[Ca^{2+}]$, changes in pH modified channel kinetics only from the intracellular face of the channel. b) At constant $[Ca^{2+}]$, intracellular acidification induced a decrease in the open probability of the channel. c) The mean open time (T_o) of the channel was a linear function of $[Ca^{2+}]$ and the mean closed time (T_c) was a linear function of $1/[Ca^{2+}]^2$. d) Plots of T_o versus $[Ca^{2+}]$ and T_o versus $1/[Ca^{2+}]^2$ at different pH's showed a decrease in T_o and an increase in T_c. e) Changes in the internal $[H^+]$ modified the slope, but not the intercept of the relations T_o versus $[Ca^{2+}]$ and T_c versus $1/[Ca^{2+}]^2$. On the basis of these results we discuss two possible mechanisms of the proton action. Protons can compete with calcium for the calcium binding sites of the channel, or they could interact with an allosteric site changing the calcium affinity.

Supported by FONDECYT 0451/88, Tinker Foundation and NIH grant GM-35981.

W-Pos364

BLOCKADE OF AXONAL FLOW INDUCES THE APPEARANCE OF SK CHANNELS IN RAT SKELETAL MUSCLES. María Isabel Behrens, Ramón Latorre and Cecilia Vergara. Centro de Estudios Científicos de Santiago, Casilla 16443, Santiago 9 and Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile.

Small conductance Ca-activated K (SK) channels in skeletal muscle are responsible for the late afterhyperpolarization that follows action potentials in the denervated muscle. Binding measurements with ^{125}I apamin, a toxin that specifically blocks SK channels, have shown that these channels are not present in innervated muscle and appear after denervation. We have studied the effect of blockade of axonal flow on the SK channels of muscle. Blockade of axonal flow was achieved by applying colchicine to the sciatic nerve of adult Whistar rats. Four to 35 days after application of the drug we measured ^{125}I apamin binding to membrane fractions of the hind leg. Binding is observed since day 4, reaches a maximum around day 8 and has disappeared at day 35. No binding is detected in control rats in which only buffer was applied to the nerve. Maximal binding (4 to 15 fmoles/mg protein) and affinity binding constant (~80 pM) are similar to those obtained in denervated muscle. These results suggest that the number of SK channels in skeletal muscle is regulated by a factor that travels along the nerve by axonal flow.

Supported by NIH, FONDECYT 451/88, 296/89 and Tinker Foundation.

W-Pos365

EFFECTS OF K$^+$ OPENERS, PINACIDIL AND BRL34915 ON K$^+$ CURRENT OF AORTIC SINGLE CELLS. **Economos, D., M. Peyrow, D. Esconde[1] and G. Bkaily. Dept. of Physiology and Biophysics, Fac. Medicine, Univ. of Sherbrooke, Sherbrooke, Que. Canada. J1H 5N4. [1]Dept. Pharmacol. Rhône-Poulenc Santé, Vitry-sur-Seine, France.**

The effect of the antihypertensive potassium channel opener, pinacidil and BRL 34915 were studied in single cells of rabbit aorta using the whole-cell voltage clamp technique. Pinacidil was found to increase the delayed outward K$^+$ current of aortic cells in a dose dependent manner (10^{-8} - 10^{-6} M) and high concentration of (10^{-5}M) pinacidil decreased the current activated by 10^{-6}M. Also, using the whole-cell current clamp technique, low concentration of pinacidil hyperpolarize the membrane by 12 mV. The effects of pinacidil was blocked by TEA and barium. BRL 34915 was found to be less potentent activator of aortic VSM than pinacidil. The increase of K$^+$ current and hyperpolarizing of the membrane by pinacidil may account for the vasorelaxant effect of this drug. This work was supported by FQMC grant to Dr. Bkaily, a scholar of the CHF and M. Peyrow is a Ph.D. fellow of the CHF.

W-Pos366

ANF OPEN A cGMP SENSITIVE K+ CHANNEL IN VASCULAR SMOOTH MUSCLE CELLS. **Carpentier, A., M. Hakim, M. Peyrow and G. Bkaily. Dept. of Physiology and Biophysics, Fac. of Medicine, Univ. of Sherbrooke, Sherbrooke, Que. Canada. J1H 5N4.**

The effect of ANF on microscopic and macroscopic K$^+$ channel was studied in order to see if ANF relaxe vascular smooth muscle by opening a K$^+$ channel. Our results demonstrate that addition of 10^{-8}M of ANF increased a delayed outward K$^+$ current in aortic single cells of rabbit. The K$^+$ opener bethanidine did not further stimulate I_K in presence of ANF. Nitroprusside stimulated the bethanidine sensitive I_K. Bethanidine, ANF, and nitroprusside activated I_K was sensitive to cGMP. ANF was found to increase the frequency of opening of the bethanidine sensitive K$^+$ channel and to increase the mean open time of this channel.

These results demonstrate that ANF acts as a K$^+$ opener in VSM and this effect is initiated via increasing $[cGMP]_i$. The vasorelaxation effect of ANF is due to hyperpolarization of the membrane. This work was supported by MRCC grant No. Ma 8920 to Dr Bkaily who is a scholar of the CHF. A. Carpentier is a fellow of U.P. John, M. Peyrow is a Ph.D. fellow of the CHF.

W-Pos367

ANF [illegible] A I_K AND BLOCKED ONE TYPE OF I_{Ca} IN CARDIAC SINGLE CELLS. **Perron, N., D. Jacques, A. Sculptoreanu and G. Bkaily. Dept. of Physiol. and Biophys., Fac. Medicine, Univ. of Sherbrooke, Sherbrooke, Que. Canada.**

The effect of ANF was studied on K$^+$ and Ca^{2+} currents of single heart cells.

ANF was found to increase the delayed outward K$^+$ current in a dose dependent manner. At 10^{-10}M of ANF, the amplitude of I_K was doubled and then a further decrease was obtained at 10^{-9}M. At high concentration (10^{-6}M), there was a larger increase of I_K. Addition of 8-Br-cGMP of further increased I_K. ANF was also found to block the high threshold type of Ca^{2+} current.

These results suggests that ANF activates a K$^+$ current by increasing the probability of opening a K$^+$ channel that is cGMP sensitive. The negatif inotropic effects of ANF in heart muscle could be due to the activation of I_K which may hyperpolarize the membrane and also to blockade of an L-type I_{Ca} in heart muscle. This work was supported by MRCC grant No. MT 9816 to Dr Bkaily, a scholar from CHF. D. Jacques is a Ph.D. fellow of the Fac. of Med. and A. Sculptoreanu is a Ph.D fellow of the CHF.

W-Pos368

VOLTAGE-DEPENDENT GATING OF INWARD-RECTIFYING POTASSIUM CHANNELS IN AORTIC ENDOTHELIAL CELLS.

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We have observed a K^+-selective channel in vascular endothelial cells from bovine descending aorta using the patch clamp technique. The extrapolated zero-current potential was found to be dependent on external K^+ concentration in a manner expected for a K^+-selective channel, shifting approximately 30mV for a 3-fold change in extracellular K^+ concentration. Inward current steps were observed at potentials more negative than Ek; no current steps were observed at potentials more positive than Ek demonstrating strong rectification of the channel. In symmetrical K^+ (150mM KCl external) unitary channel conductance was 25pS. Application of hyperpolarizing voltage steps (2720ms) to multi-channel patches produced more channel openings at the beginning of the test pulse than in the steady state. When many current responses were averaged, inactivation of the mean current was stronger and faster at more negative pulse potentials. Steady state open probability (P_o) was determined from patches with multiple channels and found to be smaller at more negative potentials. Calculated Po for single channels ranged from .20 at -20mV to .03 at -90mV. Channel openings were grouped into complex bursts. At -60mV at least 3 exponentials were needed to fit the frequency histogram of closed state lifetimes (.45, 18, & 1330ms). Open times were fit by a single exponential at slightly negative potentials (38ms at -20mV), but at more negative potentials 2 exponentials were needed to fit the open times (1.3 & 261ms at -60mV).

W-Pos370

Rb SLOWS K CHANNEL CLOSING BY ACTING AT A SITE IN THE CHANNEL.

D.R. Matteson and S. Sala. Univ. of Maryland, Dept. of Biophysics, Baltimore, MD 21201. We have used the whole-cell variation of the patch-clamp technique to study the effect of Rb^+ on K channel gating in toadfish pancreatic islet cells. The K channels studied are similar to "A-current" channels. At +70 mV they activate to a peak in 10 ms and then inactivate along the sum of two exponentials: τ_{fast} = 27-38 ms, and τ_{slow} = 200-250 ms. Rb^+ slows K channel closing in a voltage-dependent manner, suggesting that Rb^+ produces this effect at a site in the channel. In the presence of 100 mM external Rb^+, K channel closing is slowed by a factor of about 8 at -60 mV, but by only 2.5 times at -120 mV. With Rb^+ present internally, one can remove Rb^+ from the channel by making the membrane potential sufficiently negative. We have found that in the presence of internal Rb^+, K channel closing is slowed by a factor of 2.7 at -50 mV, but the effect is nearly abolished by making the membrane potential more negative: at -100 mV closing kinetics are decreased only 1.2 times. Thus, removing Rb^+ from the channel by applying negative voltages decreases the effect of Rb^+ on K channel gating, suggesting a site of action in the channel. Supported by NIDDK grant DK 33212 and the Generalitat Valenciana.

W-Pos369

IONIC CURRENTS IN RAT PANCREATIC β-CELLS RECORDED WITH THE PERFORATED PATCH TECHNIQUE.

A.S. Cohen, D.R. Matteson, R.V. Parsey and S. Sala. Univ. of Maryland, Dept. of Biophysics, Baltimore, MD 21201.

We used the perforated patch (PP) technique developed by Horn and Marty (JGP 92:145,1988) to record ionic currents in rat pancreatic β-cells. Access resistance, estimated by analyzing capacitive currents, gradually declined to a steady-state level as low as 10 MΩ. PP development was confirmed by measuring voltage-dependent K currents generated by voltage clamp steps from -80 mV to voltages positive to -20 mV. As a final test of the recording configuration we ruptured the patch and observed a large increase in steady-state K conductance due to loss of ATP through the pipette and unblock of K_{ATP} channels. The resting K conductance under PP conditions was 3.86 ± 0.83 nS (n=10), and 95.6 ± 42 nS (n=8) under whole-cell conditions without ATP. If all resting conductance is due to K_{ATP} channels, ~4% of them are open in the intact cell. The PP technique was also used to record voltage-dependent Na and Ca channel activity, and will be useful for investigating regulation of ionic channel activity by insulin secretagogues. Supported by NIDDK grant DK 33212 and the Generalitat Valenciana.

W-Pos371

MODULATION OF VASCULAR Ca^{2+}-ACTIVATED K^+ CHANNELS BY CROMAKALIM, PINACIDIL, AND GLYBURIDE.

C.H. Gelband, J.R. McCollough, and C. van Breemen. University of Miami School of Medicine, Miami, FL 33101.

Cromakalim and pinacidil relax vascular smooth muscle by membrane hyperpolarization due to opening of K^+ channels. Glyburide, an ATP-sensitive K^+ channel blocker, antagonizes the effects of cromakalim and pinacidil in vascular smooth muscle. We have investigated the effects of these drugs at the single channel level using high conductance Ca^{2+}-activated K^+ channels isolated from rabbit aorta incorporated into planar lipid bilayers. Cromakalim (0.05-10 μM) and pinacidil (0.1-10 μM) dose-dependently shifted the P_{open}-voltage relationship at 1 μM $[Ca^{2+}]_i$ in the hyperpolarizing direction. Glyburide alone (1-20 μM, internal) had no effect on P_{open} of the channel. However, after maximal stimulation of K^+ channel activity by intracellular cromakalim or pinacidil (10 μM), glyburide (10 μM, *cis*) shifted the P_{open}-voltage curve in the depolarizing direction. V_o, potential where P_{open} = 0.05, under control conditions (1 μM $[Ca^{2+}]_i$) was -10 mV ± 0.6 and this was shifted to -47 ± 1.1 and -42 mV ± 1.4 in the presence of cromakalim and pinacidil respectively (n=3). The subsequent addition of glyburide shifted the V_o to -20 ± 0.8 and -22 ± 1.0 mV respectively (n=3). We conclude that glyburide, in addition to its effects on ATP sensitive K^+ channels, also inhibits Ca^{2+}-activated K^+ channels which are activated by cromakalim or pinacidil. Supported by NIH HL-07188.

W-Pos372

BRADYKININ-INDUCED TRANSIENT ACTIVATION OF LARGE CONDUCTANCE Ca^{2+}-ACTIVATED K^+ CHANNELS IN AIRWAY SMOOTH MUSCLE.

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Agonists, which contract smooth muscle by increasing cytosolic Ca^{2+}, might additionally activate Ca^{2+}-activated K^+ channels in the plasma membrane serving as a negative feedback mechanism. We studied bradykinin (Bk) effects on whole-cell and single-channel K^+ currents in smooth muscle cells cultured from rabbit trachealis. In the whole-cell configuration, Bk (10μM) increased outward currents elicited by depolarising voltage pulses. Outward currents increased 10-15s after Bk application and decayed within 30-60s below control values. In the cell-attached configuration, using a physiological K^+ gradient, Bk (10μM) stimulated single channel outward currents with a similar time-course. In excised inside-out patches, the channel previously activated by Bk in the cell-attached mode, proved to be sensitive to the Ca^{2+} concentration (0.1-10μM) at the internal side of the membrane and the membrane potential. The slope conductance of the channel was 150pS at 0mV and the reversal potential was found to be close to the K^+ equilibrium potential. These experiments demonstrate the ability of Bk to activate a large conductance K^+ channel in airway smooth muscle presumably due to a marked, transient increase in the cytoplasmic Ca^{2+} concentration close to the plasma membrane.

W-Pos373

Tityus serrulatus VENOM PEPTIDES SELECTIVELY BLOCK NON-INACTIVATING K^+ CHANNELS IN RAT BRAIN SYNAPTOSOMES. R.S. Rogowski, R.K. Yip, K.J. Schneider, B.K. Krueger, & M.P. Blaustein, Physiol. Dept., Univ. Maryland Med. Sch., Baltimore, MD 21201.

More than 15 polypeptides were separated from the venom of the Brazilian scorpion, T. serrulatus, by cation exchange HPLC. Two polypeptides showed potent K^+ channel block when tested on depolarization-activated ^{86}Rb efflux in rat brain synaptosomes. In time-course studies, both toxins inhibited only the non-inactivating component of the K-stimulated ^{86}Rb efflux; neither affected the Ca-dependent component. This contrasts with toxins from the Old World scorpion, Leiurus quinquestriatus, which inhibit only the rapidly inactivating and Ca-dependent components of the ^{86}Rb efflux. The latter toxins displace [^{125}I]-α-DaTX (dendrotoxin), a blocker of both inactivating- and non-inactivating K^+ channels, from its high-affinity binding site. Neither toxin from T. serrulatus displaced bound DaTX; however, two other polypeptides, with little or no K^+ channel blocking activity, did displace DaTX. Our data suggest that scorpion venoms contain a variety of polypeptides that block K^+ channels; Old World and New World scorpions differ in the types of K^+ channel blockers they produce.

W-Pos374

FUNCTIONAL EXPRESSION OF A RAT CARDIAC K CHANNEL cDNA G-N Tseng, Dept Pharm, Columbia U, New York, NY 10032. J Tseng-Crank, Dept Pharm & Cell Biophys, U Cincinnati, Cincinnati, OH 45267-0575. M Tanouye, Div Biol, CalTech, Pasadena, CA 91125 A full-length K channel cDNA (RHK1) was isolated from a rat heart library. The deduced amino acid sequence revealed a structure similar to that of other voltage(V)-gated K channels: 6 hydrophobic potential membrane-spanning domains and an amphipathic S_4 domain. RHK1 channel activity was examined in the Xenopus oocyte expression system. RHK1 encoded a protein gating a 4-aminopyridine(4AP)-sensitive transient outward current (I_{to}), with an activation threshold of -40 mV and displaying an outwardly rectified peak I-V. The average peak current at +60 mV was 276.8$\pm$125.6 nA (n=6). The steady-state inactivation of RHK1 had a sigmoidal V-dependence, with a half-maximum voltage of -47$\pm$5 mV and a slope factor of 5.8$\pm$0.4 mV (n=6). The current decayed during depolarisation with a V-sensitive single exponential time course (τ=27$\pm$14 and 19$\pm$3 ms at -20 and +80 mV, n=6). Res-titution (R) of the current followed a single exponential time course (τ=1.94$\pm$0.25 s at -80 mV). At 2 mM $[K]_o$, the instantaneous I-V of RHK1 showed an outward rectification with a reversal potential (E_{rev}) of -58$\pm$7 mV (n=6). Elevating $[K]_o$ to 20 and 40 mM shifted the E_{rev} to -30 and -10 mV, and reduced the degree of outward rectification. RHK1 is similar to the 4AP-sensitive I_{to} described for rat ventricular (V) cells (Josephson et al, Circ Res 1984;54:157) in the V-dependence of activation and pharmacology, but different in the V-dependence of inactivation and kinetics of inactivation and R. Elevating $[K]_o$ from 2 to 20 mM accelerated the R of RHK1 in oocytes by 3-fold (n=3), but did not affect the rate of R of the 4AP-sensitive I_{to} in dog V cells, suggesting that different isoforms of I_{to} channel respond differently to changes in $[K]_o$.

W-Pos375

FURTHER EVIDENCE FOR A DIFFERENTIAL EFFECT ON Na AND K CHANNELS BY CAMPHORQUINONE-10-SULFONIC ACID (CSA) J.R. Clay and K.E. Krebs, Biophysics, NINDS, NIH, Bethesda, MD

The effects of the arginine modifier CSA on I_{Na} and I_K were studied using internally perfused squid axons. The addition of CSA to the internal perfusate (20 mM) reduced peak I_{Na} amplitude by ~75% with a slowing in time to peak by ~ 30%. 50 mM CSA virtually eliminated I_{Na}. In contrast, 50 mM CSA had little effect on I_K amplitude with a modest slowing of I_K kinetics (~30%), which was voltage independent. All CSA effects were irreversible. The general conclusion from these results concerning a differential effect on I_{Na} and I_K by CSA is similar to that of Fohlmeister and Adelman (Biophys. J. 51: 194a, 1987), although our results differ considerably in detail from their work. Similar results were obtained with phenylglyoxal and *p*-hydroxyphenylglyoxal (at lower concentrations than with CSA), although these compounds also reduced the I_K amplitude. Consequently, a differential effect of these arginine-specific reagents on I_K and I_{Na} was less readily apparent than with CSA.

W-Pos376

THE INACTIVATION CURVE OF THE DELAYED RECTIFIER IN SQUID AXONS IS SHIFTED ALONG THE VOLTAGE AXIS BY CHANGES IN pH_i. J.R. Clay, Biophysics, NINDS, NIH, Bethesda, MD 20892.

Several years ago Wanke, et al. (Biophys. J. 26:319, 1979) reported that the I_K conductance in squid axons was reversibly reduced without a change in kinetics when pH_i was reduced ($5.2 < pH_i < 10$). I have found that this effect is dependent upon the holding potential (HP) in steady-state conditions ($\gtrsim 3$ min at each holding level). No effect of pH_i was observed either for HP < -70 or HP > -30 mV, whereas a clear effect was observed for -70 < HP < -30 mV. In particular, I_K was quite labile to changes in pH_i in the physiological range ($6.8 < pH_i < 7.5$) with HP = -60 mV. Moreover, a hyperpolarization of the resting potential was observed with an increase of pH_i, which is suggestive of a role for pH_i in the regulation of rest potential. All of these results are consistent with a pH_i dependent shift along the voltage axis of the steady-state inactivation curve of the delayed rectifier (Clay, Biophys. J. 55:407, 1989), possibly by a surface charge effect. These results may provide the simplest example of such an effect, in that no other effect of pH_i on I_K was observed. In contrast, the effects of pH on I_{Na} (either pH_i or pH_o, Wanke, et al., Nature, 287:62, 1980); the effects of pH_o on I_K (Shrager, J.G.P., 64:666, 1974); and the effects of Ca^{+2} on either I_{Na} or I_K (Shoukimas, J. Memb. Biol. 38: 271, 1978; Armstrong and Matteson, J.G.P. 87:817, 1986) all appear to be more complicated than a simple shift of a gating parameter.

W-Pos378

TWO CLASSES OF VOLTAGE-DEPENDENT K-CHANNELS IN ORGANELLES FROM THE SQUID GIANT AXON

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We have continued study of K-channels in axoplasmic organelles extruded from giant axons of *Loligo pealei* and fused with planar lipid bilayers (*Biophys. J.*, 55:317a). Two classes of K-channels were distinguished by their conductance and gating. Small conductance (35-50 pS in symmetric 500 mM KAc) K-channels exhibited strongly voltage-dependent gating. The fractional open time (F_o) was near zero at potentials hyperpolarized relative to -90 mV and increased as a steep function of voltage with depolarization to potentials between -80 mV and -60 mV. The channels opened in bursts of rapid gating, with the burst duration lengthened and the interburst interval shortened by increasing depolarization. Some channels inactivated when the membrane potential was held at depolarized potentials. This inactivation could be removed by hyperpolarization to -90 mV. Currents activated by repeated depolarizing voltage steps were averaged, and the average current closely resembled the macroscopic delayed rectifier current of the giant axon. Large conductance K-channels (110-150 pS in symmetric 500 mM KAc) exhibited only weakly voltage-dependent gating, with open durations slightly shorter at negative potentials. F_o was very low (<1%) at all potentials. We surmise that voltage-dependent, small conductance K-channels contribute to the macroscopic delayed rectifier current whereas large conductance K-channels might contribute a small resting K current. (supported by the Alberta Heritage Foundation for Medical Research.)

W-Pos377

VERAPAMIL APPLIED EXTERNALLY BLOCKS OPEN K CHANNELS IN RAT ALVEOLAR EPITHELIAL CELLS IN THE WHOLE-CELL CONFIGURATION BY PERMEATING THE MEMBRANE IN UNCHARGED FORM AND BLOCKING FROM INSIDE THE CELL IN CHARGED FORM, AS REVEALED BY VARYING THE INTERNAL OR EXTERNAL pH. **by Thomas E. DeCoursey, Department of Physiology, Rush Medical Center, Chicago, IL.**

We reported last year (*Biophys. J. 55*:540*a*) that long chain tetraalkylammonium ions block whole-cell delayed rectifier K currents in alveolar epithelial cells when applied externally at 0.1–10 μM. Block resembles that in nerve for internal application; we proposed that the blockers cross the membrane and act from the inside. This possibility is now demonstrated for verapamil, whose block is similar to that by quaternary compounds. Applied externally, verapamil produces time-dependent block of open K channels; $1/\tau$ is proportional to $[drug]_o$. At pH_o 4.5, 7.4, 10, respectively, the relative block-rate $(1/\tau)$ is $<0.3 : 6.5 : 100\%$, which correlates with the fraction of *UNCHARGED* $[drug]_o$ (pKa 8.5), $0.01 : 7.4 : 97\%$. When pH_i is 7.2 or 10, relative block-rates are $100 : 6.4\%$, correlating with the fraction of *CHARGED* drug inside the cell, $95 : 3.1\%$. *Ergo* the title. *Supported by NIH grants HL01928 and HL37500.*

W-Pos379

THE ELECTROPHYSIOLOGY OF PLASMA CELLS: A PATCH CLAMP STUDY. P.R. Brink and B. Walcott, S.U.N.Y. Stony Brook.

Plasma cells isolated from the avian lacrimal gland were whole cell patched. The whole cell currents are characterized by an outward rectifying current with a slow inactivation time course of 1-3 seconds. No inward currents were detectable in cells bathed in RPMI media. These plasma cells are approximately 10-15 microns in diameter and peak outward whole cell currents can be in excess of 3000 pA. Inside-out and inside-in patch records revealed maxi K channels with single channel conductances in symmetric K solutions on either side of the pipette of 200-240 pS. Voltage steps applied to inside-in patches often revealed multichannel activity. The number of open states or channels open declined in time after the onset of a step with an inactivation time of 1-5 sec. The similarity between multichannel activity and whole cell current leads to the conclusion that the maxi K channel is the dominant membrane channel in plasma cells. Grant #31299.

W-Pos380

CHARYBDOTOXIN BLOCKS THE CA-ACTIVATED K-CHANNEL (KCa) AND INCREASES BURST FREQUENCY WITHOUT AFFECTING AVERAGE SPIKE FREQUENCY IN PANCREATIC B-CELLS.

M. Kukuljan and * A.A. Gonçalves (Intro. by I. Atwater). NIDDK, NIH, Bethesda,MD 20892

Modulation of membrane potential oscillations is important for glucose sensing in the B-cell. To test the hypothesis that KCa regulates burst duration, we studied the effects of venom from the scorpion, Leiurus quinquestriatus (LQV), which contains charibdotoxin, a selective blocker of KCa. Cultured rat B-cells were studied using the patch clamp technique (pipet solution, mM: 140 KCl, 0.2 CaCl2, 10 NaHEPES, pH 7.4). LQV selectively blocked KCa (200 pS) in outside-out patches. Inhibition was reversible and dose dependent (half max ca. 5 µg/ml). In whole-cell mode, LQV (10 µg/ml) blocked the slow activating and inactivating outward current, without affecting the delayed rectifier current. Mouse B-cell membrane potential oscillations were recorded in 11 mM glucose. LQV (50 µg/ml) increased burst frequency from 3 to 8/min without changing fractional active phase duration or spike frequency and amplitude. The results indicate that KCa does not regulate the glucose-sensitive component of B-cell electrical activity

(* Partially supported by CNPq, Brazil)

W-Pos381

APPEARANCE OF LARGE CONDUCTANCE INWARD RECTIFYING K CHANNELS DURING POSTNATAL DEVELOPMENT OF THE RAT VENTRICLE.

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Two types of inward rectifying K channels have been demonstrated in adult rat ventricular cells, but only the small (low conductance) type has been reported in cultured neonatal (1-3 day) rat ventricular cells. In cell-attached patches (pipette: 150 mM KCl), typical large (42 pS) and small (25 pS) conductance K channels were observed in 6 of 6 patches from adult (3-6 mo) rats. In cultured neonatal (5-6 day) cells, no large conductance channels were observed (0 of 3 patches); however, in cultured later neonatal (9-11 day) cells, large channels (43 pS) were observed in 3 of 3 patches, albeit rather infrequently. Conclusions: (1) short-term culturing of cells is not responsible for the reported absence of high conductance K channels in neonatal rat ventricular cells, and, (2) functional high conductance inward rectifying K channels appear relatively early during postnatal development of the rat ventricle. (Supported by AHA-Metropolitan Chicago).

W-Pos382

SKELETAL MUSCLE ATP-SENSITIVE K^+ CHANNELS RECORDED FROM SARCOLEMMAL VESICLES OBTAINED BY A NEW NON-ENZYMATIC METHOD.

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The presence of a basal membrane and a tight connective tissue matrix restricts the use of the patch-clamp technique to study surface membrane ionic channels of non-cultured skeletal muscle fibers. Usually, prolonged treatment with collagenase and protease is needed, probably not without adverse effects on the various proteins involved in ionic conduction and regulation.

We have found that, when a frog skeletal muscle fiber is split in half in a Ca^{++}-free relaxing solution, large hemispherical vesicles grow spontaneously within minutes without any enzymatic treatment. These vesicles readily form high-resistance seals with patch pipettes and were found to contain a variety of channels. Among those we have identified ATP-sensitive K^+ channels similar to those already reported in skeletal muscle. These channels are highly selective for potassium, have a conductance of ≈47 pS in 130 K^+, display inward rectification, and are blocked by mM MgATP. More than 50 channels could be seen in a patch of membrane in the absence of MgATP. In excised patches and 0 MgATP, channels ran down within several minutes, an effect which could be partly reversed by application of MgATP. Furthermore, we show that these channels are reversibly blocked by glibenclamide (0.1-1µM, MgATP in bath), an anti-diabetic sulphonylurea known to be a specific blocker of ATP-sensitive K^+ channels in cardiac and pancreatic cells. Presence of such channels in some vesicles shows that these vesicles are made up, at least in part, of plasma membrane.

W-Pos383

A VOLTAGE-DEPENDENT POTASSIUM CURRENT FROM RABBIT CORONARY ARTERY VASCULAR SMOOTH MUSCLE CELLS

Volk, K.A., Matsuda, J.J. & Shibata, E.F. Dept. of Physiology & Biophysics, Univ. of Iowa, Iowa City, IA 52242

Single coronary artery vascular smooth muscle cells exhibit large net outward currents when depolarized. In an effort to begin separating the components of this net current, the single pipette whole-cell voltage clamp technique was employed while blocking the calcium current with cadmium chloride and chelating internal calcium with EGTA. This strategy presumably eliminated calcium-activated currents and allowed study of another outward current that was voltage-activated. This current was activated from a holding potential (HP) of -60 mV using test pulses to -20 mV and above. Long voltage steps (5 sec) revealed relatively slow inactivation. The inactivation process began at HP=-40 mV and was complete at HP=0 mV. The deactivation process was best fit with two exponential components. The predominant charge carrier was identified as the potassium ion using reversal potential/ion substitution experiments. A minor portion of the current may also be carried by chloride ions. The known potassium channel blockers 4-aminopyridine and tetraethylammonium were equally effective in blocking the current at millimolar levels.

Supported by NIH HL 41031 and HL 14388.

W-Pos384

BLOCKING K CHANNELS INHIBITS THE THERMOGENIC RESPONSE OF BROWN FAT CELLS.
P.A. Pappone & M.T. Lucero, Department of Animal Physiology, Univ. of Calif., Davis CA & Hopkins Marine Station, Stanford Univ., Pacific Grove CA.

Brown fat cells respond thermogenically to adrenergic stimulation by substantially increasing their metabolic rate. We have shown previously that cultured rat brown fat cell membranes have two K currents; an adrenergically-activated current, $I_{K,NE}$, that is probably gated by increases in intracellular Ca^{+2} levels, and a voltage-gated current, $I_{K,V}$, that is activated by membrane depolarization. $I_{K,NE}$ can be blocked by apamin, charybdotoxin, TEA, or 4AP, while $I_{K,V}$ is sensitive to block by TEA and 4AP but not apamin or charybdotoxin. In the present experiments we examined the effects of K channel blockers on the metabolic response of isolated brown fat cells measured isothermally using a microcalorimeter. Stimulation of the cells with 4 μM norepinephrine in the absence of K channel blockers resulted in an average five-fold increase in heat production. Blocking both $I_{K,NE}$ and $I_{K,V}$ with TEA (20 mM) or TEA + apamin (700 nM) reduced steady-state norepinephrine-stimulated heat production by 15-45% compared to controls. Apamin alone, which should selectively block $I_{K,NE}$ had little effect on thermogenesis. These data indicate that functional voltage-gated K channels, but not Ca-activated K channels, are necessary for full thermogenic activity in brown fat cells. Supported by NIH AR34766 and NSF 84-21163.

W-Pos385

ZINC MODULATES THE VOLTAGE DEPENDENCE OF TRANSIENT OUTWARD POTASSIUM CURRENTS.
Zalman S. Agus, Iain Dukes and Martin Morad. Depts of Medicine and Physiology, Univ of Pa Sch Med, Phila Pa.

In studies of transient outward current (I_{to}), Cd^{2+} and Co^{2+} are frequently used to block the activation of I_{Ca}. Since divalent cations shift the steady state inactivation of both I_{Ca} and I_A, we investigated whether I_{to} is similarly modified by divalent cations.

Isolated rat ventricular myocytes were whole cell voltage clamped and dialyzed with a KCl based solution containing 14 mM EGTA and 10 mM free Mg^{2+} to block I_{Ca}. Inactivation curves were constructed by applying test pulses to +60mV from conditioning potentials of -100mV to -20mV. Steady state activation was studied using the reversal potential and peak current at +60mV. All the divalents studied caused parallel shifts in both steady-state activation and inactivation curves, but at markedly different concentrations. In the mM range, a rightward shift of 20mV was produced by 10 Ca^{2+}, 15 Mg^{2+}, 5 Co^{2+} and 5 Ni^{2+} whereas μM concentrations of 10 Cd^{2+}, 10 Zn^{2+} and 100 Cu^{2+} produced an equivalent shift.

The shifts in voltage dependence by μM concentrations of divalent cations are inconsistent with surface charge theory previously used to explain such shifts and may indicate a regulatory divalent binding site in the vicinity of the voltage sensor of the channel. Similar effects were observed in hippocampal neurons where μM concentrations of Zn^{2+} markedly shifted the voltage dependence of I_A and activated it at a holding potential of -50mV. Thus, Zn^{2+} may play an important physiologic role in allowing activation of I_A in cells with a resting potential of -50mV, where I_A is normally inactivated.

W-Pos386

EVIDENCE FOR TWO TYPES OF DELAYED RECTIFIER K CURRENTS IN GUINEA PIG CARDIAC MYOCYTES. Kevin Chinn. (Intro. by R.D. Goldman).

Delayed rectifier K currents were studied using the whole cell patch clamp technique. Calcium currents were blocked using 0.1 mM Cd and Ca_i was buffered in some cells with EGTA. Delayed outward currents were activated by depolarizing voltage steps from a holding voltage of -40 mV. After a 7 s pulse (LP), tail currents were observed which showed two decay time constants (T_{fast} 50-90 ms, T_{slow} 400-600 ms). T_{fast} was often a small (<20%) component of the tail. With 200-300 ms pulses (SP) T_{fast} became more prominent (sometimes >75%). In one cell, LP currents were observed but SP currents were not. 2 mM 4AP reduced SP tails 15% more than LP tails. V_{rev} of both LP and SP tails was approximately -65 mV. The ability of differing pulse durations to alter the proportion of T_{fast} and T_{slow} and the different sensitivity to 4AP indicates the presence of more than one delayed rectifier type.

W-Pos387

PINACIDIL OPENS ATP-DEPENDENT K+ CHANNELS IN CARDIAC MYOCYTES IN AN ATP- AND TEMPERATURE-DEPENDENT MANNER
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Pinacidil is an antihypertensive agent which has been found to increase potassium conductance. This study examined the type of K channel affected by pinacidil in cardiac myocytes. Pinacidil shortened action potential duration in papillary muscle. The effect was reversible upon addition of glyburide, a known IK_{ATP} blocker. The effect of pinacidil was temperature-dependent. Action potential duration was shortened more rapidly and to a greater extent at 37°C than at 23°C. Whole-cell experiments showed that I-V curves lost rectification after pinacidil treatment, similar to that observed by others after treatment with metabolic inhibitors which activate IK_{ATP}. As with the action potential experiments, the effect was more rapid at 37°C than at 23°C. Rectification was restored after exposure to glyburide. The effect of pinacidil was also ATP-dependent. Addition of 5 mM ATP to the internal solution prevented activation of IK_{ATP}. These data indicate that pinacidil activates IK_{ATP}. The ATP data indicate that pinacidil may act to alter the sensitivity of IK_{ATP} channels to ATP.

W-Pos388

SYNAPTOSOMAL K^+ CHANNELS AND REGULATION OF EXCITABILITY Gareth Tibbs, Oliver Dolly and David Nicholls*. Dept. of Biochemistry, Imperial College, London and *University of Dundee, Dundee.

The K^+ channel blockers dendrotoxin (DTX, 100 nM) and 4-aminopyridine (4-AP, 1 mM) cause a partial depolarization (5-8 mV) of the synaptosomal plasma membrane, stimulate efflux and ouabain-sensitive accumulation of K^+ and increase the cytosolic concentration of Ca^{2+} ($[Ca^{2+}]_c$, by 72±2 and 131±5 nM, respectively). These actions are mediated by an increase in Na^+ channel activity as demonstrated by sensitivity to tetrodotoxin (TTX). Charybdotoxin (CTX, 100 nM) and tetraethylammonium (TEA, 25 mM) also elicit TTX-sensitive increases in $[Ca^{2+}]_c$ (52±1 and 42±3 nM, respectively). Whereas no additivity is observed if DTX and 4-AP are present together, in the presence of either of these ligands CTX elicits a further, though not additive, rise in $[Ca^{2+}]_c$. In contrast, TEA elicits a synergistic increase in $[Ca^{2+}]_c$ (143±4 nM) in the presence of DTX. These results suggest that a K^+ channel sensitive to DTX, CTX and 4-AP is involved in regulation of excitability while a TEA-sensitive channel(s) play a significant role in repolarization at the central nerve terminal.

W-Pos389

ISOPROTERENOL (ISO) BLUNTS QUINIDINE-INDUCED INHIBITION OF THE CARDIAC DELAYED RECTIFIER I_K. J Turgeon, JR Balser, PB Bennett, DM Roden. Vanderbilt University, Nashville TN.

We have previously shown that quinidine (Q) blocks I_K in guinea pig ventricular myocytes (VM). Since ISO and other mediators which raise kinase activity stimulate I_K and since ISO is used to treat some forms of Q toxicity, we hypothesized that ISO pretreatment would alter Q block of I_K. Current was measured in acutely isolated VM using the whole-cell mode of the patch-clamp technique. The extracellular solution was Tyrode's modified to eliminate I_{Na}, I_{si}, and I_{to}; ISO (2μM) was added ≥30 min prior to electrode application in 5/12 experiments. I_K tail amplitudes were measured at -30mV following 5 sec clamp steps to +10 to +60 mV at baseline (BL) and during 50μM Q. The %Δ I_K (Q vs BL) was compared in cells pretreated with ISO to those not exposed to ISO (mean±SD; *p<0.05):

V_m (mV)	NO ISO	ISO
+10	-38±22%	-18±10%
+30	-45±15%	-25±11%*
+60	-46±12%	-30±11%*

We conclude that quinidine block of I_K is inhibited by isoproterenol; we speculate that phosphorylation of the channel or regulatory elements modulate the effect of quinidine.

W-Pos390

ENDOGENOUS AND EXPRESSED K^+ CURRENTS IN *XENOPUS* OOCYTES

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This study demonstrates an endogenous Ca^{2+}-independent and quinine-sensitive K^+ current and a Ca^{2+}-activated K^+ current induced by injection of size-fractionated mRNA from cultured rabbit medullary thick ascending limb (MTAL) cells in *Xenopus* oocytes. The endogenous Ca^{2+}-independent K^+ current was activated by depolarization and inactivated by prepulsing Em from -50 to 0 mV. Amplitudes of the K^+ current varied from 30 to 400 nA at +30 mV with an activation time course of 100 ms. The time constant of inactivation at -10 mV was 16.48 s. The E_R was shifted by changing external $[K^+]$, as expected for a K^+-selective channel. A Ca^{2+}-activated K^+ current was observed in oocytes injected with mRNA from MTAL cells with a mean amplitude of 32±3 nA at +30 mV. Charybdotoxin (CTX) at 10 nM reduced these currents by 46%. Our results indicate that Ca^{2+}-activated K^+ channels from MTAL cells can be expressed in *Xenopus* oocytes and are distinguished from the endogenous K^+ current.

W-Pos391

INHIBITION OF A CALCIUM ACTIVATED K^+ CHANNEL BY γ-INTERFERON. F. Strickland, M. Diaz-Munoz, M.J. Hawkes and S.L. Hamilton "(Intro. by H.J. Pownall)". Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, Texas 77030. The binding of γ-interferon (γ-Ifn) to its plasma membrane receptor alters the metabolic state of target cells. The mechanism whereby the binding of γ-Ifn at the cell surface initiates this series of events is for the most part unknown. The effect of γ-Ifn ion channel activity was investigated in synthetic lipid bilayers using purified plasma membranes from the rat insulinoma cell line, RIN5mF. Recombinant rat γ-Ifn inhibited the activity of a Ca^{2+} activated K^+ channel at a concentration of 1 nM. The channel was also blocked by charybdotoxin, TEA and EGTA, characteristics of the Ca^{2+} activated maxi-K^+ channel. The γ-Ifn receptor was partially purified (500-fold) from RIN5mF membranes and found to contain γ-Ifn-sensitive Ca^{2+} activated K^+ channel activity. The binding of $[^{125}I]$-γ-Ifn to RIN5mF membranes and the effects of recombinant human γ-Ifn on Ca^{2+} activated K^+ channels in the human macrophage cell line THP-1 were also investigated.

W-Pos392

METHADONE BLOCK OF NEURONAL K CURRENT, F.T.Horrigan (Intro. by W.F.Gilly), Hopkins Marine Station of Stanford University, Pacific Grove, CA 93950. Methadone (a synthetic opiate narcotic) reversibly blocks K current (I_K) and, to a lesser degree, I_{Na} and I_{Ca} when applied externally to patch clamped neurons from the squid giant fiber lobe. Block is independent of μ-opiate activity. Methadone is structurally similar to local anaesthetics (LA), and I_K block is consistent with LA-type models of open channel block and drug trapping. Upon depolarization I_K activates normally (little if any resting block) and is blocked in a time dependent manner (τ=5ms at $K_{1/2}$=50um, 10°C, pH=7.4). Recovery from block is slow and can be described by two time constants (200 and 4000ms at -80mv). Recovery rate is markedly accelerated at more positive voltages or by elevated external K. A permanently charged derivative shares methadone's action, but only if applied internally. Methadone blocks I_K in GH3 cells and in chick myoblasts more effectively than in squid ($K_{1/2}$=20um at 20°C); this difference may in part be explained by a temperature sensitivity of block (Q_{10}=2 in squid).

W-Pos393

PHOSPHORYLATION INCREASES THE DENSITY OF SURFACE CHARGES IN THE CYTOPLASMIC SIDE OF THE DELAYED RECTIFIER K^+ CHANNEL

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It has been shown at the single channel level and with macroscopic current experiments, that the delayed rectifier channel can be phosphorylated, producing a current increase. This increase is a consequence of a shift in the activation and inactivation parameters towards more positive potentials. Such a shift can be explained assuming that the transfer of a phosphate occurs from ATP to the channel, increasing the density of negative surface charges near the voltage sensor of the channel. Voltage clamped and internally dialyzed axons were used, and the voltage shifts were estimated (before and after phosphorylation) by changing the internal Mg^{++} concentration. The shift of the activation and inactivation produced by Mg^{++} is increased by phosphorylation. For Mg^{++} concentrations ranging from 0 to 100 mM, the local potential increases by at least 7 mV for the *activation* curve and by at least 9 mV for the *inactivation* curve. Changes in internal pH in the absence of ATP can partially mimic the phosphorylation effect; low pH_i reduces the current and speeds-up the turn-on kinetics, and high pH_i potentiates the current amplitude with a slowing-down in kinetics. These results, when combined with single channel data from the same preparation, can explain the K channel modulation produced by ATP on the basis of electrostatic interactions. Supported by MDA and NIH grant GM30376.

W-Pos394

ARE TYPE 'l' K^+ CHANNELS IN LYMPHOCYTES THE SAME AS 'g_{f2}' K^+ CHANNELS IN FROG NODE OF RANVIER? *by Mark S. Shapiro, Department of Physiology, Rush Medical Center, Chicago, Il 60612.*

Delayed rectifier currents in frog node of Ranvier have been separated into 3 components on kinetic and pharmacological bases (g_s, g_{f1}, g_{f2} currents; Dubois, *J. Physiol. (1981), 318: 297-316*; Plant, *J. Physiol. (1986), 375: 81-105*). Type 'l' K^+ currents ('l'; DeCoursey et al, *J. Gen. Physiol. (1987), 87: 379-404*) were studied here in mouse lymphocytes and appear very similar to g_{f2} currents in voltage dependence ($V_{1/2}$ ≈ -10 mV for 'l' and ≈ -15 mV for g_{f2}), kinetics (τ_{tail} at -90 mV = 1.4 ms for 'l' and 1.7 ms for g_{f2}; act. $t_{1/2}$ at +30 mV ≈ 2 ms for both), inactivation (1-2 sec and incomplete for both types) and TEA sensitivity (K_i = 80-100 μM for 'l' and 160-400 μM for g_f). The permeation of Rb^+ through both channel types is similar: P_{Rb}/P_K ≈ 0.8 and g_{Rb}/g_K ≈ 0.5 for both, Rb_o^+ increases τ_{tail} by ~ 10X for 'l' and ~ 5X for g_{f2} and shifts g_K 10-15 mV to the left for both. External or internal Rb^+ has no effect on activation kinetics for both types. Other common pharmacology are block by capsaicin (*cap*) and naloxone (*nx*). K_i for *cap* ≈ 25 μM for 'l' s.s. currents. K_i for *nx* ≈ 500 μM for 'l' peak currents. *Cap* block was reversible with time- and voltage-dependence similar to block in node (Dubois, *Brain Res.* 245 *(1982): 372-375*), suggesting open channel block. *Nx* block was time- and voltage- independent and was only partially reversible. Both compounds reportedly block g_{f2} (but not g_{f1} or g_s) in node at *ca.* the same concentrations. We conclude that, considering differences in milieu and experimental technique, 'l' and g_{f2} are very similar. *Supported by NIH.*

W-Pos395

ω-CONOTOXIN RECEPTOR OF RAT BRAIN: PARTIAL PURIFICATION AND CHARACTERIZATION OF THE SYNAPTOSOMAL CALCIUM CHANNEL. M.W.McEnery, A.M.Snowman, A.H.Sharp, and S.H.Snyder Dept. Neuroscience, Johns Hopkins Sch. of Med., Baltimore, MD 21205

ω-Conotoxin (ω-CgTX), a peptide toxin isolated from the marine snail Conus geographus has been shown by other investigators to block voltage-dependent N- and L-type Ca^{++} channels from nerve cells. The binding site for ^{125}I-CgTX VIA has been localized exclusively to neuronal tissues, and more specifically to the synaptosomal membranes as determined by subcellular fractionation of rat brain. We report the partial purification of the ^{125}I-CgTX receptor from rat brain synaptosomes. This preparation is 50-100 fold enriched in receptor compared to total rat brain membranes. The soluble and purified receptor is similiar to the native, membrane-bound receptor with regard to its affinity for ω-CgTx, and sensitivity to Ca^{++}, organic and inorganic salts, and polyamines. We are attempting to reconstitute this preparation into phospholipid vesicles to determine if the solubilized ω-CgTX receptor possesses voltage-dependent Ca^{++} channel activity.

W-Pos396

DIHYDROPYRIDINES MODULATE CA^{++} CHANNELS BY ALTERING THEIR AVAILABILITY TO PROTEIN PHOSPHORYLATION AND ITS REMOVAL

David Armstrong[1] and Daniel Kalman,[2]
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[2]Department of Biology, UCLA, Los Angeles, CA 90024

We reported previously that 1 μM BAY K 8644 slows rundown and Ca^{++}-dependent inactivation of the large-conductance, high-threshold, fast-deactivating calcium channels in a rat pituitary tumor cell line, GH_3 (Armstrong *et al* '87 *Biophys.J.* 51:223a). Both rundown and inactivation can be reversed by cAMP-dependent phosphorylation (Armstrong & Eckert '87 *PNAS* 84:2518; Kalman *et al* '88 *J.Gen.Physiol.* 92:531). Thus, in addition to prolonging the mean channel open time, BAY K 8644 also appears to inhibit dephosphorylation of the channel or a closely associated membrane protein. We hypothesized that other dihydropyridines might inhibit the channel by selectively binding to its dephosphorylated, or inactivated, state and reducing the probability of its rephosphorylation (Armstrong '88 *Biomed.Res.* 9, Suppl 2, 11-15). We now report that the sensitivity of these channels to inhibition by nimodipine, and the apparent voltage-dependence of its inhibition, can be reduced substantially in intact cells and cell-free patches by reagents that promote cAMP-dependent phosphorylation. These results support our hypothesis and suggest a novel strategy for the rational design of calcium channel ligands in the treatment of human cardiovascular disorders.

W-Pos397

G-PROTEINS MODULATE CALCIUM CURRENTS IN PARAMECIUM AND HELIX NEURONS. J. Bernal and B.E. Ehrlich (Intro. by A.M. Katz), Depts. of Medicine and Physiology, Univ. of CT, Farmington, CT.

We reported previously that backward swimming behavior and the calcium action potential in Paramecium are prolonged by activation of G-proteins (Biophys. J., 53:21a,1988; 55:39a,1989). Here we report that the calcium currents of Paramecium are also increased. But, surprisingly, the current modification was in the opposite direction as that seen in Helix neurons. When compounds of interest were injected into the cells by pressure, we found that GTPγS, which activates G-proteins, enhanced the magnitude of the calcium current in Paramecium by 40%; whereas GTPγS reduced the calcium current in Helix neurons by the same amount. GDPβS, which inactivates G-proteins, had effects opposite to those of GTPγS in Paramecium as well as in Helix. These results suggest that G-proteins may activate the T-type calcium channel that is found in Paramecium and may inhibit the L-type calcium channel found in Helix neurons. JB is a Fellow of the AHA, CT Affiliate. BEE is a PEW Scholar in the Biomedical Sciences.

W-Pos398

VARIABLE MODES OF INHIBITION OF CALCIUM CHANNEL BLOCKERS ON A CALCIUM TRANSPORT ENZYME.

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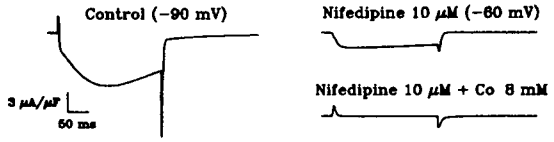
The availability of chemically heterogeneous calcium channel blockers permits targeted therapy in numerous pathophysiologic states. In this study, we have used a modification of the procedure of Jarrett and Kyte (1979) to obtain kinetics constants for human erythrocyte membrane calmodulin activated calcium ATPase in the presence of two calcium channel blockers, namely, diltiazem and verapamil. We have also estimated the approximate Ki from the 50% inhibition of enzyme activity observed by comparing control with inhibitor at several reliable data points. This measurement was made under two conditions. In the first, drug was allowed to react with the membrane alone before other components of the assay medium were added, and in the second the drug was added just before the reaction was initiated with ATP. With Diltiazem the approximate Ki is not dependent on when the drug was added. However, with verapamil the apparent Ki is lower when drug is added first to the membrane, suggesting that the drug bound to the membrane. The mechanism in this case might be non-competitive, even though a competitive mechanism is obtained when drug is added last. This difference between diltiazem and verapamil suggests that the drug delivery system could change the therapeutic efficacy, and might provide a rationale for the use of sustained-release drug formulations.

W-Pos399

ISOLATION OF FAST Ca^{2+} CURRENT OF FROG SKELETAL MUSCLE. A. J. Avila-Sakar, J. García & E. Stefani. Dept. Molecular Physiology & Biophysics. Baylor College of Medicine, Houston, TX, 77030.

Voltage clamp experiments were performed in cut segments of single muscle fibers of the frog (*R. pipiens*) with the vaseline gap technique. Recording solutions contained: TEA-methanesulfonate 105 mM, $CaCl_2$ 2 mM, HEPES 5 mM, 3,4-DAP 1 mM, TTX 0.2 μM, and 9-anthracene carboxylic acid 1 mM, pH 7.0. Under these conditions, a two-component inward current is readily recorded with depolarizing pulses from a holding potential of -90 mV. The slow component has a peak time of 75-125 ms and an amplitude of 6.7 ± 2.0 $\mu A/\mu F$ at 0 mV. It is partially inactivated at a holding potential of -60 mV, without major changes in the fast component. Nifedipine (10 μM) abolishes or greatly reduces the slow component, being more effective under conditions of partial depolarization. The remaining component consists of the fast activating current, which has a peak time of 10-30 ms and an amplitude of 3.2 ± 0.5 $\mu A/\mu F$ at 0 mV, from a holding potential of -60 mV. This component is completely blocked by cobalt (8 mM). Supported by NIH.



W-Pos400

SINGLE-CHANNEL ANALYSIS OF T-TYPE CALCIUM CHANNELS IN EMBRYONIC VENTRICLE. Seiko Kawano and Robert L. DeHaan, Emory University, Atlanta GA 30322. T-type (I_T) calcium channels were studied in cell-attached patch electrode recordings from 14-day chick ventricle. All experiments were performed in the absence of Ca^{2+} with Na^+ (120 mM) as the charge carrier. I_T channels were distinguished from L-type (I_L) channels by their more negative activation and inactivation potential ranges, their small unitary slope conductance (26 pS), and their insensitivity to isoproterenol or D600. Inactivation kinetics were voltage-dependent. The time constant of inactivation was 37 ms when the membrane potential was depolarized 40 mV from rest (R+40 mV), and 20 ms at R+60 mV. The frequency histogram of channel open-times (τ_o) was fit by a single-exponential curve while that of closed-time (τ_c) was bi-exponential. τ_o was the same at R+40 mV and R+60 mV whereas τ_c was shortened at R+60 mV. The open-state probability (P_o) increased with depolarization: 0.35 at R+40 mV, 0.8 at R+60 mV and 0.88 at R+80 mV. This increase in P_o at depolarized potentials could be accounted for by the decrease in τ_c. (Supported by NIH P01-HL27385)

W-Pos401

IMMUNOLOGICAL CHARACTERIZATION OF THE IP_3 RECEPTOR IN BRAIN AND PERIPHERAL TISSUES. A.H.Sharp, R.J.Mourey, C.D.Ferris, C.A.Ross and S.H.Snyder, Johns Hopkins Univ. School of Medicine, Dept. of Neuroscience, Baltimore, Maryland 21205

The IP_3 receptor is a 260 kDa membrane protein that has been isolated from rat cerebellum and has been shown to mediate IP_3 stimulated Ca^{2+} channel activity after reconstitution in phospholipid vesicles. Little is presently known of the IP_3 receptors in forebrain and peripheral tissues. We have now developed polyclonal antibodies in rabbits against the rat cerebellar IP_3 receptor that cross react with proteins in forebrain and a variety of peripheral tissues. Anti-IP_3 receptor antibodies were affinity purified using an IP_3 receptor affinity column. The affinity purified antibodies recognized a single band of 260 kDa on Western blots of crude cerebellar membranes. A 260 kDa band was also detected on Western blots of membranes from other brain regions and other organs. The relative intensity of staining parelleled binding activity for ^3H-IP_3 in various brain regions and organs. The anti-IP_3 receptor antibodies are now being used to localize the IP_3 receptor in peripheral tissues by immunohistochemical techniques.

W-Pos402

REPETITIVE DEPOLARIZATION POTENTIATES THE L-TYPE, NOT THE T-TYPE CA^{2+} CHANNEL CONDUCTANCE; MEMBRANE POTENTIALS AND INTRACELLULAR CA^{2+}, NOT CA^{2+} THROUGH THE CA^{2+} CHANNELS ACTIVATE THE POTENTIATION. Kai S. Lee, The Upjohn Company, Kalamazoo, MI.

Conductance of the L-type Ca^{2+} channel can be potentiated by repetitive depolarization. Three possible interpretations exist: (1) contamination by I_{to}; (2) T-type, not L-type Ca^{2+} current is potentiated; (3) potentiation of the L-type. The first two possibilities were ruled out because (1) replacing all K^+_i with Cs^+_i, plus 20 mM internal TEA and 5 mM 4-AP (external solution contained 5 mM Ca^{2+}, 250 mM mannitol and 5 mM glucose throughout) removed all I_{to} but without effect on potentiation; (2) repetitive depolarization inactivated T-type but potentiated L-type; and (3) Ni^{2+} or nifidipine reversibly removed L-type and the potentiation. Prepulse depolarizations of 5 seconds beginning at -80 mV increased I_{ca} progressively, reaching maximum at -40 mV before declining as inactivation was increased. Short prepulse of 1 msec to +80 mV that elicited negligible Ca^{2+} influx nevertheless elicited strong potentiation. Reduction of Ca^{2+} current by Cd^{2+} or replacement of Ca^{2+} with Ba^{2+} produced minimal effect on potentiation whereas reduction of $[Ca^{2+}]_i$ by 10 mM BAPTA, 0 mM Ca removed potentiation. These data imply potentiation is dependent both on voltage and internal Ca^{2+}, but probably not on Ca^{2+} through the L-type Ca^{2+} channels.

W-Pos403

ACCELERATION OF CALCIUM CHANNEL RECOVERY FROM INACTIVATION BY ISOPROTERENOL.
K. Mubagwa and A.J. Pappano, Dept. of Pharmacology, University of Connecticut, Farmington, CT. (Introd. by R. Sha'afi).

Both an acceleration or a slowing by β-adrenergic receptor stimulation of the recovery of Ca channels from inactivation have been reported. This problem was reinvestigated in guinea-pig ventricular myocytes. Using whole-cell voltage clamp, currents carried by either Ca or Na through L-type Ca channels were measured at 0 mV, with other channels inactivated (holding potential -40 mV) or blocked (by external Cs and internal Cs + TEA). Recovery from inactivation occurred with comparable half-times ($t_{1/2}$ = 50-150 ms) with Ca or Na as charge carrier. Isoproterenol (ISO; 0.03-3 μM) decreased the $t_{1/2}$ of I_{Ca} recovery (control: 90 ± 11.2 ms; ISO: 65 ± 6.5 ms; mean ± SEM; n=6), but had no effect when Na was the charge carrier (103 ± 18.0 ms vs 107 ± 18.7 ms; n=4). ISO had no effect on the recovery of I_{Ca} when ryanodine (10 μM) was added to the external or the pipette solution. Thus, β-adrenergic stimulation accelerates the recovery process, probably by increasing Ca release from intracellular stores.

W-Pos404

EFFECTS OF HALOTHANE (HAL) ON CA++ CHANNEL CURRENTS IN CANINE CARDIAC PURKINJE CELLS. *Hanna Eskinder, Franjo D. Supan, Nancy J. Rusch, John P. Kampine and Zeljko J. Bosnjak. Departments of Anesthesiology and Physiology, The Medical College of Wisconsin, Milwaukee, WI 53226*

Inhalational anesthetic agents were reported recently to depress L-type Ca++ current in canine cardiac ventricular cells (Bosnjak and Rusch, Anesthesiology 69(3):A452, 1988). However, the effects of these anesthetics on T-type Ca++ current are unknown. The objective of this study was to examine the effects of HAL (0.45 and 0.9 mM) on L- and T-type Ca++ channel currents in single canine cardiac Purkinje cells. Cells were dialyzed with pipette solution containing CsCl, and superfused with a 10 mM $BaCl_2$ solution. Voltage steps from a holding potential (HP) of -70 mV elicited a rapidly inactivating, low-threshold inward current at -40 to -30 mV, which was maximally activated at -20 to -10 mV, and blocked by Ni++ (120 μM) but not by nifedipine (1 μM). In contrast, depolarizing steps from -40 HP elicited a sustained inward current, which was maximally activated at +12 mV and was nifedipine sensitive. Low and high concentrations of HAL suppressed peak T-type current by 33% and 56%,and peak L-type current by 34% and 63%, respectively. HAL did not shift the voltage-dependency of the I-V curves of these currents. L-type current elicited from an HP of -40 or -70 mV was suppressed the same amount by HAL, while nifedipine (1 μM) suppressed these currents by 91% and 58%, respectively. These results suggest that HAL, unlike nifedipine, suppresses both L- and T-type Ca++ channel currents and the suppression of L-type current by HAL is voltage independent.

Supported by NIH Grants HL34708 and HL01901 (ZJB).

W-Pos405

EFFECTS OF NIMODIPINE ON MULTIPLE POPULATIONS OF VOLTAGE DEPENDENT CA^{2+} CHANNELS IN FRESHLY DISPERSED MAMMALIAN NEURONS. R.T. McCarthy, Dept. Cell Biol., Yale Univ., New Haven, CT 06510 U.S.A.

Multiple populations of Ca^{2+} channels have been characterized in dorsal root (DRG) and sympathetic ganglia (SG) cells from rabbit. In whole cell records (DRG), two types of high threshold Ca^{2+} channel currents which differed in the voltage dependence of inactivation also differed in sensitivity to nimodipine (NIM). Sustained current ($V_{1/2}$= -28 mV) is potently inhibited by NIM (K_I= 5.3nM; n=8). High threshold current which inactivates at a more negative potential ($V_{1/2}$= -46mV) is not inhibited by NIM (10,20,50 nM; n=3). Low threshold current elicited from more negative potentials ($V_{1/2}$= -78 mV) is also resistant to NIM (20 nM; n=3). In single channel records of DRG's, an 8 pS conductance requires V_H<-60mV while 16 and 28 pS conductances remain available at V_H = -40 mV. The 28 pS channel is modulated by dihydropyridines (DHPs). The predominant conductance in SG cells (16 pS) remains available at V_H= -30 mV. This channel is insensitive to BAY K 8644. These results show that only specific subtypes of voltage dependent Ca^{2+} channels can be modulated by DHPs.

W-Pos406

THE RATE OF HORMONE SECRETION IS CORRELATED WITH THE ACTIVITY OF FD Ca CHANNELS IN CULTURED LACTOTROPES. M. Hiriart*, J. L. Torres, A. Navarrete and G. Cota. *Dept. of Neurosciences, IFC-UNAM, and Dept. of Physiology, CINVESTAV-IPN, Mexico, D.F.

Prolactin-secreting cells (lactotropes) in primary cultures of the pituitary pars distalis from adult male rats were detected with the reverse hemolytic plaque assay. Measurements of plaque area, an index of the relative amount of prolactin released per unit time, revealed a bimodal frequency distribution that was composed of lactotropes forming small plaques and others forming large plaques. The large plaques were 3-4 times greater in area than the small plaques. Whole-cell patch clamp experiments were then performed on the lactotropes to analyze the activity of Ca channels (20 mM external Ba, HP -80 mV, activating pulses to +20 mV). We have previously shown that these cells express two Ca channel types, FD and SD. The amplitude of the SD current, normalized by cell capacitance, did not significantly differ between the two subpopulations of lactotropes. In contrast, the mean current density through FD channels in large-plaque lactotropes was 3.3 times bigger than in small-plaque cells.

W-Pos407

TWO DIFFERENT CALCIUM CURRENTS IN HELIX ASPERSA NEURONS ARE DIFFERENTIALLY EFFECTED BY DOPAMINE. Young-kee Kim and Michael L. Woodruff, Department of Chemistry and Biochemistry, Southern Illinois University, Carbondale, Ill 62901-4409.

Inward calcium currents in Helix aspersa neurons were measured by single electrode voltage-clamp using a perfusion pipet. The voltage-activated calcium current in neurons F-14 to F-16 and F-28 to F-32 has two components. One is a quickly activating, transient current and the other is a slowly activating, sustained current. Both currents are blocked completely by adding cobalt ions in the bath solution. Transient current has its peak inward current between +10mV and +20mV and the sustained current is maximal between +30mV and +40mV. Above +40mV of membrane potential, most of transient current disappears and only sustained current remains.

When the neurons are treated with 5 uM dopamine both calcium currents decay about 30%, but then recover as the cells desensitize. Interestingly the slow sustained current recovers more rapidly than the transient current. Sustained current was recovered completely after 20 min but transient current was recovered only 70% in the same time period. This result suggests that these two calcium currents are modulated differently.

W-Pos408

RUN-DOWN OF CARDIAC CALCIUM CHANNEL PREVENTED BY THE ENDOGENOUS PROTEASE INHIBITOR CALPASTATIN. Chr. Romanin, P. Grösswagen and H. Schindler

Single L-type calcium channel activity from cardiac myocytes, though clearly observable in the cell attached patch, is rapidly lost upon patch excision into standard intracellular solutions. We investigated calcium currents in inside-out patches under different conditions in an attempt to prevent run-down. Neither the inclusion of agents promoting phosphorylation (e.g. ATP) nor of protease inhibitors (pepstatin A, leupeptin) to the intracellular solution was sufficient to prevent run-down of channel activity in excised patches. Complete stabilization, however, was obtained in the presence of cardiac cytosol supplemented with ATP + GTP, giving rise to the question about components involved. The employment of both protease inhibitors and ATP + GTP already led to a distinct deceleration of run-down, confirming the simultaneous involvement of proteolyses and dephosphorylation in the regulation of cardiac calcium channel activity. Substitution of these unspecific protease inhibitors by calpastatin, the endogenous inhibitor of calpain, resulted in pronounced stabilization of channel activity similar to that obtained by the application of cytosol. Furthermore, run-down was remarkably slowed in standard intracellular solution subsequent to an effective stabilization of channel activity obtained in the preceding presence of calpastatin plus ATP + GTP. We suggest calpastatin as one essential regulatory component provided by cardiac cytosol. (supported by Austrian Res. Fonds: S-45)

W-Pos409

REMOVAL OF SIALIC ACID ALTERS BOTH T- AND L-TYPE CA CURRENTS IN CARDIAC MYOCYTES. B. Fermini and R. D. Nathan, Dept. of Physiology, Texas Tech Univ. Health Sciences Center, Lubbock, TX 79430.

Sialic acid (NANA), a carbohydrate that comprises as much as 10-12% of the alpha subunit, appears to be necessary for the normal gating and conductance of Na channels. These anionic residues might also influence the function of Ca channels. Whole-cell Ca currents ($i_{Ca,T}$ and $i_{Ca,L}$) were recorded from cultured pacemaker cells isolated from the rabbit sinoatrial node. Interfering currents were eliminated by including (mM) 120 CsCl and 20 TEA in the patch pipette and 20 CsCl, 4.0 4-AP and 0.03 TTX in the bath (36°C). A highly purified enzyme, neuraminidase (Neur), was used to remove the surface NANA. Neur (1.0 U/ml) increased $i_{Ca,T}$ in 5/9 cells and $i_{Ca,L}$ in 3/6 cells. In the cells that did *not* exhibit such an increase, Neur *reduced* $i_{Ca,T}$ but had no effect on $i_{Ca,L}$. Activation- and inactivation-voltage relationships were measured to begin to investigate the basis for these changes. Neur shifted the activation curve for $i_{Ca,T}$ to more negative potentials and increased the slope of its inactivation curve; the enzyme had no significant effect on either the activation or inactivation of $i_{Ca,L}$. The effects of Neur were maximal within 5 - 10 min. The enhancement of $i_{Ca,T}$ and $i_{Ca,L}$ could not be mimicked by adding Neur to the patch pipette or by adding phospholipase C to the bath (phospholipase C is a contaminant in the Neur preparation). Our results suggest that the removal of NANA might alter both T- and L-type Ca channels. Additional experiments will be required to characterize the underlying mechanisms. Supported by the Canadian Heart Foundation (BF) and NIH grant HL 20708 (RDN).

W-Pos410

A HYPERPOLARIZATION-ACTIVATED CALCIUM CURRENT THAT IS INHIBITED BY BARIUM IN PARAMECIUM TETRAURELIA. R.R. Preston and Y. Saimi, Lab. of Molecular Biology, Univ. of Wisconsin, Madison, WI 53706

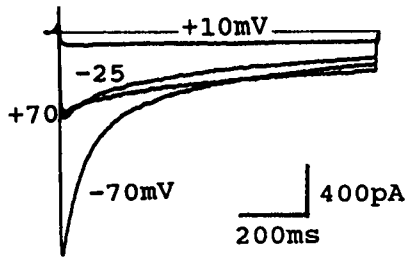
Under conditions where K^+ currents are suppressed (by use of CsCl electrodes and by TEA^+ extracellularly), hyperpolarization of P. tetraurelia under voltage clamp in 1 mM Ca^{2+}, 10 mM TEA^+, 1mM HEPES, pH 7.2 activates an inward current. The current elicited by stepping from -40 mV to -110 mV peaks at about 30-40 ms with an amplitude of about -4 nA, and inactivates in the subsequent 50-250 ms. This inactivating current is carried by Ca^{2+}, since it is unaffected by removing TEA^+, HEPES, or Cl^- from the bath and/or the electrodes. Inactivation of the current contains a Ca^{2+}-dependent component. The putative hyperpolarization-activated Ca^{2+} channel passes Ca^{2+} exclusively. Divalent cations (Ba^{2+}, Mn^{2+}, Co^{2+}, Mg^{2+}, Sr^{2+}) inhibit the current in a voltage- and dose-dependent manner. Ba^{2+} inhibits the current with an IC_{50} of approx. 100 µM. The Ca^{2+} current is insensitive to conventional Ca^{2+} channel antagonists, but is inhibited by amiloride ($IC_{50} \approx 400$ µM). Supported by NIH.

W-Pos411

HIGH-THRESHOLD Ca CURRENTS WHICH DIFFER IN MECHANISM OF INACTIVATION AND IN SENSITIVITY TO OPIOIDS

J.E. Schroeder, M. Mamo, E.W. McCleskey
Dept of Cell Biology, Washington Univ. St. Louis, MO.

The existence and appropriate criteria for identification of different kinds of high-threshold Ca channels is a recurring controversy. The figure shows how we distinguish these currents. Each record is evoked by a test pulse to +10mV that is preceded by a 3-second-duration prepulse to the indicated voltage. A prepulse to -20mV evokes no current but inactivates the transient component in the ensuing test pulse. In contrast, inactivation of the sustained component only occurs when there is a current during the prepulse: stepping to the reversal potential (+70mV) induces no inactivation, whereas the greatest inactivation occurs with maximal inward current during the prepulse (+10mV). Evidently, the transient component undergoes voltage-dependent inactivation and the sustained component undergoes current-dependent inactivation. Using these criteria, we find that the transient component is inhibited by the mu-opioid agonist, DAGO, whereas the sustained component is spared. This pharmacological difference supports the notion that the transient and sustained components pass through distinct ion channels.



W-Pos412

TWO TYPES OF CALCIUM CHANNELS IN HUMAN NEUROBLASTOMA CELLS SH-SY-5Y. E. Reuveny and T. Narahashi (Intro. by Heidi Hamm). Dept. of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

Multiple types of Ca^{2+} channels have been found in different vertebrate neurons. We have found two types of Ca^{2+} channels in the human neuroblastoma cell line SH-SY-5Y by the whole cell patch clamp technique. A step depolarization from a holding potential of -80 mV was associated with a transient current followed by a steady-steady current. Both components of current were activated at potentials more positive than -10 mV. Thus the transient and steady-state currents appear to mimic N-type and L-type calcium channel currents, respectively. The two types of channels showed different permeability profile to the divalent cations, Ba^{2+}, Sr^{2+} and Ca^{2+}, with permeability ratios 1:0.6:0.35 for the L-type channels, and 1:0.5:0.5 for the N-type channels. Cd^{2+} was equally potent in blocking both channels (K_d~3 μM). The two types of channels were equally and reversibly blocked by Pb^{2+} with K_d=1 μM. The peptide toxin ω-conotoxin reduced the N-type and L-type current amplitudes by 50% and 20%, respectively, at a concentration of 40 nM. Supported by NIH grants NS14144 and AA07836.

W-Pos413

PYRETHROID INSECTICIDES BLOCK CALCIUM AND NMDA-ACTIVATED CURRENTS IN CULTURED MAMMALIAN NEURONS. J. Frey and T. Narahashi. Dept. of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

Pyrethroid (PYR) insecticides have been shown to interact with a variety of ion channels. Using the whole cell patch clamp technique, we have found that the α-cyano PYRs deltamethrin and fenvalerate and the non-cyano PYR allethrin block voltage-activated calcium channel currents and NMDA-activated currents in rat hippocampal and neocortical neurons maintained in culture. At 50 μM, deltamethrin and allethrin blocked the peak amplitude of calcium channel currents by ~30 and ~50%, respectively. Low threshold non-inactivating and high threshold inactivating currents were both suppressed. All of the PYRs tested inhibited the NMDA (100 μM)-induced current by 35-60% at 10-50 μM. The rate of desensitization was often accelerated. Deltamethrin blocked the NMDA current only when applied together with NMDA. The addition of glycine (1 μM) attenuated the PYR block of NMDA current. It appears that PYR blocks the NMDA channels in their open configuration. Supported by NIH grant NS14143.

W-Pos414

SELECTIVE INCREASE OF T-TYPE Ca CURRENT IN ATRIAL MYOCYTES FROM ADULT RATS WITH GROWTH HORMONE SECRETING TUMORS. Xiaoping & P.M. Best. Dept. of Physiology, Univ. of Illinois, Urbana, IL 61801

Growth hormone secreting tumors were induced in adult, female Wistar-Furth rats by subcutaneous injection of GH_3 cells. Compared to controls, tumor rats showed large increases in body (40 g/week) and heart weight (0.2 g/week) beginning about 20 days after injection. 8 weeks after injection Ca currents of atrial cells from tumor rats (415±17 g, $\bar{x}$±s.d., n=9) and age matched controls (178±8 g, n=6) were studied using whole-cell patch-clamp technique. T-type Ca current density is tripled in atrial cells from tumor rats, 1.24±0.51 pA/pF (n=23) compared to 0.40±0.25 pA/pF (n=23) in controls, while L-type Ca current density is unchanged in tumor and control rats, 9.8±2.8 pA/pF (n=18) vs. 9.7±3.2 pA/pF (n=18). The steady state activation and inactivation of T- and L-type Ca current are the same for both tumor and control rats. The increase of T-type Ca current density precedes by several days any noticeable increase in heart weight and cell size, suggesting T-type Ca current may play some role in atrial cell growth. Supported by NIH AR32062, RR-5861 & Ill. Heart Assoc.

W-Pos415

DIFFERENTIAL EXPRESSION OF LOW VOLTAGE THRESHOLD CALCIUM CHANNELS IN VISUAL CORTICAL NEURONS. K. Giffin & J.M. Nerbonne, (Intro. by R. Wilkinson) Dept. of Pharmacology, Washington Univ. Med. School, St. Louis, MO. 63110. The waveforms of Ba^{++} currents (I_{Ba}) through voltage-gated Ca^{++} channels in isolated callosal-projecting (CP) and superior colliculus-projecting (SCP) visual cortical neurons have been characterized using the whole-cell recording technique. Cells were identified in vitro following in vivo retrograde labeling with rhodamine "beads". During brief (125 ms) depolarizations from a HP of -80 mV, I_{Ba} in CP cells begins to activate at ~-20 mV, peaks at ~+10 mV and reverses positive to +50 mV. The time- and voltage-dependent properties of I_{Ba} are consistent with the presence of High Voltage Activated ("HVA") channels. There is no evidence for Low Voltage Activated ("LVA") channels in CP cells. In SCP neurons, in contrast, an "LVA" current, which begins to activate at ~-40 mV and peaks at ~-20 mV, is evident. In SCP cells, therefore, both "LVA" and "HVA" channels are present. These results reveal that the intrinsic membrane properties of SCP and CP visual cortical neurons are distinct. Ongoing studies are aimed at determining the role of "LVA" currents in controlling the firing properties of SCP cells. (Support: NSF #BNS8809823 and NIH #T32-HL07275).

W-Pos416

INHIBITORY EFFECTS OF FORSKOLIN ON L-TYPE Ca^{2+} CHANNEL CURRENT IN FROG CARDIAC CELLS. M. Boutjdir, P.-F. Méry, A. Shrier & R. Fischmeister. INSERM U-241, Université de Paris-Sud, F-91405 Orsay, France.

Forskolin (Fo) is a potent activator of adenylate cyclase (AC), which enhances L-type cardiac Ca^{2+} current (I_{Ca}) when applied externally at micromolar concentrations. Here we found that, in the nanomolar concentration range (0.1-100 nM), Fo exerts an inhibitory effect on whole-cell I_{Ca} in frog isolated ventricular cells. Maximal inhibition (30-40%) was observed around 10 nM Fo. At similar concentrations, the Fo-analogue 1,9-dideoxy-forskolin, which does not activate AC, mimicked neither the stimulatory nor the inhibitory effects of Fo on I_{Ca}. Inhibition of I_{Ca} by Fo was more pronounced when I_{Ca} had been previously enhanced by cyclic AMP-dependent mechanisms, as compared to basal or Bay K 8644-stimulated I_{Ca}. Inhibitory effects of Fo were observed when I_{Ca} was stimulated either by isoprenaline (0.05-10 μM), or methylisobutylxanthine (270 μM), or intracellular cyclic AMP (2-5 μM), and persisted when ATP-gamma-S was substituted for intracellular ATP (3 mM). Therefore, the Fo inhibition of I_{Ca} is likely to take place at the level of the Ca^{2+} channel.

W-Pos417

INTRACELLULAR CALCIUM IN SYNAPTOSOMES MEASURED WITH FURA-2 AND STOPPED-FLOW SPECTROSCOPY. D.K. Bartschat, Dept. Physiology, Eastern Virginia Medical School, Norfolk, VA.

Rat brain synaptosomes were purified by Percoll gradient methods, were loaded with Fura-2 AM, and were depolarized by rapid (< 1 ms) mixing with elevated K^+. Fluorescence of intracellular Fura-2 (500 nm) was measured by alternating the excitation light between 340 and 380 nm between successive "shots" in the stopped-flow spectrophotometer, averaging 5-10 "shots" of each wavelength, followed by calculating the 340/380 ratio. In the absence of added Ca^{2+}, no changes in Fura-2 fluorescence were seen. When Ca^{2+} was added and the synaptosomes were depolarized with elevated K^+, the $[Ca^{2+}]_i$ rose in two phases: a fast (C_f) and a slow (C_s) component.

C_f: This component probably reflects Ca^{2+} influx through voltage-activated Ca^{2+} channels since: i) C_f is fast ($T_{1/2}$ ~50-150 ms) and apparently inactivates; ii) C_f increases with increasing depolarization; iii) C_f is blocked by cobalt, cadmium, and lanthanum; iv) C_f is not sensitive to dihydropyridines; and, v) C_f is blocked by omega-conotoxin.

C_s: This component probably reflects Ca^{2+} influx through "reverse mode" Na/Ca exchange: i) C_s is slow ($T_{1/2}$ ~1 sec); and, ii) Na^+-depletion largely eliminates C_s. Interestingly, while C_s is not voltage sensitive, Na^+-stimulated Ca^{2+} efflux, measured in other experiments, is decreased in depolarizing media.

These methods should allow the regulation of presynaptic $[Ca^{2+}]_i$ to be studied on a physiological time scale.

Supported by NS 21758.

W-Pos418

CALCIUM CURRENTS AND CHARGE MOVEMENT IN NORMAL HUMAN SKELETAL MUSCLE. J. García, K. McKinley*, S. Appel* & E. Stefani. Dept. Molecular Physiology & Biophysics and *Dept. Neurology, Baylor College of Medicine, Houston, TX, 77030.

Calcium currents and charge movement were recorded with the Vaseline gap voltage clamp technique. Single fibers were isolated from normal human muscle (*vastus lateralis*) obtained during biopsies. The solutions contained (mM): extracellular, TEA-methanesulfonate 150, $CaCl_2$ 10, $MgCl_2$ 2, TEA-HEPES 5, TTX 0.005; intracellular, Na-glutamate 120, Na_2-EGTA 10, Mg-ATP 3, Na_2-phosphocreatine 5, Na-HEPES 10, glucose 10; pH was 7.4 Temperature 17°C. Calcium currents were detected at -30 mV, the maximum amplitude was -2.5 μA/μF at +10 to +20 mV. The activation time constants were 164 ms and 133 ms at those membrane potentials. Two pulse experiments showed inactivation with a mid-point of -27 mV and a slope factor of 3 mV. Charge movement was detected at -70 mV and reached a plateau at 0-+10 mV with about 15 nC/μF; its activation curve had a mid-point of about -30 mV and a slope of 16 mV. The calcium currents recorded at this temperature are very slow to be activated during an action potential and thus they do not seem to directly participate in the excitation-contraction coupling mechanism. On the contrary, the charge movement may be fully activated under these conditions. Supported by NIH and MDA.

W-Pos419

INACTIVATION OF CALCIUM CURRENT IN THE A7r5 SMOOTH MUSCLE CELL LINE IS CALCIUM- AND VOLTAGE-DEPENDENT.

B. Giannattasio, S. W. Jones, T. N. Marks and A. Scarpa, Department of Physiology and Biophysics, Case Western Reserve University, Cleveland, OH 44106.

Inactivation of a dihydropyridine sensitive calcium current in A7r5 cells is faster with extracellular Ca^{2+} than with Ba^{2+}. In Ba^{2+} inactivation increases monotonically with depolarization. A 60 ms step in Ca^{2+} generates inactivation (τ~20 ms) that parallels the current, so there is little inactivation for steps approaching the reversal potential. Longer pulses in Ca^{2+} generate a slower inactivation process (τ~200 ms) resembling that occuring in Ba^{2+}. Lowering extracellular Ca^{2+}, but not Ba^{2+}, slowed inactivation. The results are consistent with the coexistence of a slow voltage-dependent inactivation, and a more rapid current-dependent inactivation observable only in Ca^{2+}. We studied recovery from inactivation following either a short pulse (producing significant inactivation only in Ca^{2+}) or a long pulse (giving inactivation with either cation). Recovery from inactivation was voltage dependent even from Ca^{2+} dependent inactivation. We propose a four state model with separate but interconvertable voltage- and Ca^{2+}-dependent inactivated states.

This work was supported by NIH grant HL 41206.

W-Pos420

KINETICS OF ACTIVATION OF L-TYPE CALCIUM CHANNELS IN THE A7r5 SMOOTH MUSCLE CELL LINE.

C. Obejero-Paz, T. N. Marks, S. W. Jones, G. R. Dubyak, and A. Scarpa. Dept. Physiol. and Biophys., Case Western Reserve Univ. School of Medicine, Cleveland, OH 44106.

Currents through L-type calcium channels were studied by whole cell and single channel (cell attached) patch clamp techniques with isotonic Ba^{2+}. Tail currents at -40 mV following 10 msec pulses to different potentials showed no saturation up to 100 mV. Tail currents were fairly well fit by the sum of two exponentials. A linear three state model was used to fit the whole cell data. Most of the voltage dependence is in the forward rate constant for the transition from state C_1 to C_2.

The mean open time for single channels was relatively constant between -10 and +10 mV (~25 msec). At least two classes of closed times were observed. The longer closed time was voltage dependent.

Rate constants from the three state model predict several features of the single channel behavior, including the mean open time, and long closed times which become shorter at positive potentials. The first latency distribution was less well fit, possibly indicating a third closed state.

$$C_1 \underset{1800}{\overset{60}{\rightleftharpoons}} C_2 \underset{2400}{\overset{1600}{\rightleftharpoons}} O$$

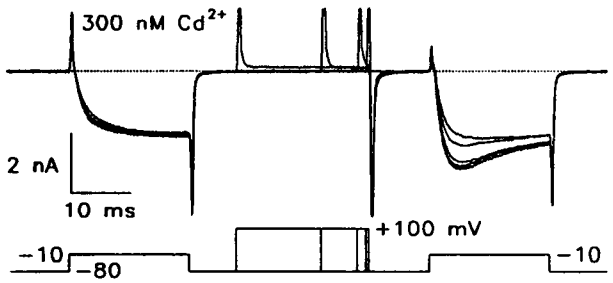
Rate constants (s^{-1}) at 0 mV.

W-Pos421

CADMIUM BLOCK OF CALCIUM CURRENT IN FROG SYMPATHETIC NEURONS.

F. Thevenod & S. W. Jones, Dept. Physiology & Biophysics, Case Western Reserve University, Cleveland, OH 44106.

Cadmium is driven out of calcium channels at extreme depolarized voltages (*J. Gen. Physiol.* 94:151, 1989). Reblocking is rapid and concentration-dependent at +10 to +30 mV, ~10^8 $M^{-1}s^{-1}$, with an unblocking rate of ~50 s^{-1}, in rough agreement with the dissociation constant of ~250 nM measured from steady-state block. Unblocking becomes more rapid at more positive voltages, (τ ~ 1.5 ms at +100 mV in 300 nM Cd^{2+}, compared to ~15 ms at +10 to +30 mV). Reblocking is slow (~seconds) at -80 mV, suggesting open channel block. However, steady-state block at -80 mV is strong, so closed channels can also be blocked.



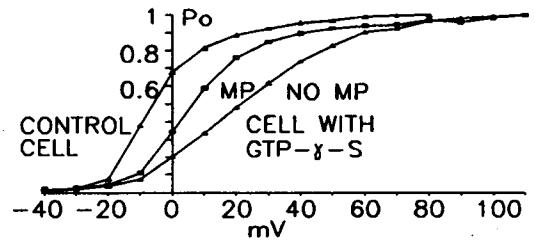
Unblocking during 0, 0.5, 2, 8, and 22 ms pulses to +100 mV. Most unblocking occurs by 2 ms, and the current reblocks during the postpulse.

W-Pos422

KINETIC ANALYSIS OF GTP-γ-S MODIFIED CALCIUM CURRENT IN FROG SYMPATHETIC NEURONS.

Keith S. Elmslie & Stephen W. Jones (Intro. by T. Hoshiko). Physiology & Biophysics, Case Western Reserve Univ., Cleveland, OH, 44106.

GTP-γ-S reduces the peak current and induces two components of activation of voltage dependent Ca current. These effects are rapidly removed (recovered) by strong depolarization. We believe GTP-γ-S modifies a portion of N-type Ca channels to occupy a new closed state. In GTP-γ-S, the slope of the activation curve is reduced and the 1/2 point is shifted right, but the "recovered" current has an activation curve with normal slope. The "recovered" current is shifted 10-30 mV to the right from control (no GTP-γ-S), but was similar in activation & inactivation.



Activation with & without GTP-γ-S and the effect of a 30 msec modifying pulse (MP) to 70 mV.

W-Pos423

CHOLESTEROL/DRUG MOLECULAR INTERACTIONS WITH MODEL AND NATIVE MEMBRANES. R.P. Mason, J. Moring and L.G. Herbette. - Biomolecular Structure Analysis Center, Univ. of Conn. Health Center, Farmington, CT. 06032

We used x-ray scattering and radioligand experiments to study the nonspecific interactions of cholesterol, 1,4-dihydropyridines and phenylalkylamines with model and native membranes. In dioleoyl phosphatidylcholine (DOPC) membranes, cholesterol and these drugs were shown to occupy well defined, time-averaged locations in the membrane bilayer. The resolution of these experiments was 8 Å. Membrane-based partition coefficient (K_p) experiments showed that the K_p values of these molecules was significantly modulated by DOPC cholesterol content. For example, ^{3}H-nimodipine showed a five-fold decrease in its K_p value as cholesterol content was raised from 0 to 60 mol% of total phospholipid. K_p measurements for ^{3}H-nimodipine in native cardiac and brain membranes with significantly different cholesterol contents were qualitatively and quantitatively consistent with the DOPC results. These data suggest membrane cholesterol content plays an important role in these drugs' interaction with their target membranes. (Supported by AHA; American Health Assistance Foundation; HL-33026; NIAAA-03510)

W-Pos424

MOLECULAR BASIS FOR DRUG-DRUG INTERACTIONS IN CARDIAC SARCOLEMMAL MEMBRANES. H.S. Young, R.P. Mason and L.G. Herbette - Biomolecular Structure Analysis Center, University of Connecticut Health Center, Farmington, CT 06032

The Class III antiarrhythmic agent clofilium noncompetitively inhibited the binding of the DHP calcium channel antagonist nimodipine to receptor sites in canine cardiac sarcolemmal (CSL) membranes. A structurally similar Class III compound, bretylium, lacked the ability to non-competitively inhibit this DHP binding. The time-averaged location of bretylium was found to be nearer the phospholipid headgroup region at ±17Å from the hydro-carbon core center of bovine cardiac sarcolemmal phosphotidylcholine (BPC) bilayers, in contrast to clofilium and nimodipine (±11Å). We suggest that the membrane location of clofilium, but not bretylium, defines the "membrane bilayer pathway" for DHP access to its receptor site, suggesting a molecular model for noncompetitive inhibition of DHP-receptor binding by drugs which do not specifically interact with the DHP-receptor site. (Supported by AHA; American Health Assistance Foundation; HL-33026)

W-Pos425

SINGLE Ca^{2+} CHANNELS IN PATCHES OF AXOSOMES FROM TRANSECTED SQUID AXON. Harvey M. Fishman & Kirti P. Tewari, Department of Physiology & Biophysics, University of Texas Medical Branch, Galveston, TX 77550-2779.

Axosomes (ca. 100 μm diam.) prepared from transected axons of squid (Tewari & Fishman, these abstrs.) were patch clamped. Solutions in both bath and pipet were identical, except for $CaCl_2$, and consisted of (in mM): 494 TMACl, x $CaCl_2$, 5 TrisCl, pH - 7.4 @ 24°C. TTX (1 μM) and 3,4, DAP (1 mM) were added to both solutions to block Na and K channels. Single-channel, steady-state currents were recorded in excised (inside-out) patches over a voltage range of ± 100 mV and amplitude histograms were produced at each voltage. Single-channel, current-voltage plots, derived from histogram data, yielded nonlinear curves. Reversal potentials for changes in Ca^{2+} gradient followed E_{Ca} and the slope conductance at large depolarization was 24 pS. Addition of Ni^{2+} (1 mM) or diltiazem (1 mM) to the pipet solution eliminated conduction in this channel. These results indicate a Ca^{2+} channel, which is consistent with our assumption that endoplasmic reticulum is a source of axosomal membrane. [Aided by ONR contract N000-14-87-K-0055]

W-Pos426

EXTRACTS OF Ryania CONTAIN AN INHIBITOR OF DIHYDROPYRIDINE BINDING THAT IS NOT RYANODINE. *J.L. Sutko, L. Ruest*[*], *J.A. Nichol, K. Gerzon*[+], *and J.A. Talvenheimo*[!]. Depts. of Pharmacology, Univ. Nevada, Reno, NV; [+]Univ. Indiana, Indianapolis, IN; [!]Univ. Miami, Miami, FL; and Dept. of Chemistry, [*]Univ. Sherbrooke, Sherbrooke, CAN. (Intro. by N. Brandt).

Crude extracts of Ryania wood affect the binding of [^{3}H]PN200-110 (PN) and [^{3}H]ryanodine (RY) to skeletal muscle transverse tubular (t-t) and sarcoplasmic reticulum (SR) membranes, respectively. Purified ryanodine affected only RY binding. The active compounds in crude extracts were isolated chromatographically. At concentrations of 5 and 50 μM a nonpolar fraction inhibited PN binding to t-t by 65 and 100%, and RY binding to SR by 14 and 57%. The latter effects are comparable to those of 10 nM ryanodine. At similar concentrations, a more polar fraction containing primarily ryanodine did not affect PN binding, but abolished RY binding. The nonpolar fraction did not affect [^{125}I]cyanopindolol binding to t-t, indicating that it does not have nonspecific effects on membrane proteins. In conclusion, an inhibitor of PN binding to t-t that is not ryanodine is present in extracts of Ryania wood.

Supported by NIH HL27470, HL36029; NSF DCB8811713.

W-Pos427

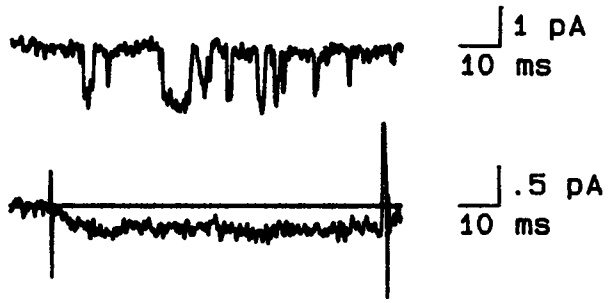
APAMINE, BLOCKS ONE TYPE OF Ca^{2+} CURRENT IN HEART CELLS. **Sculptoreanu, A., C. St-Pierre and G. Bkaily. Dept. of Physiology and Biophysics, Fac. of Medicine, Univ. of Sherbrooke, Sherbrooke, Que. Canada. J1H 5N4.**

Apamin, a bee venom polypeptide, was reported by our group to block the native Ca^{2+} slow action potential (APs) in culture embryonic chick heart cells. Using the whole-cell clamp technique in single ventricular cells from 10 days old chick embryo, we could demonstrate two types of slow Ca^{2+} currents. A low threshold and a high threshold type. Low concentration of apamin (10^{-10}M) was found to decrease the slow Ca^{2+} currents (I_{Ca}, I_{Ba}, I_{Sr}) activated from holding potential (HP) of -50 mV. However, apamin did not affected the calcium current activated from HP of -80 mV even at high concentrations. Also, apamin was found to have no effect on the TTX-sensitive fast Na^+ current of these cells. Quinidine, reversed the decrease of I_{Ca} by apamin. Therefore, apamin seems to be a selective blocker of one type Ca^{2+} current in heart muscle. This work was supported by MRCC No. MT 9816 to Dr Bkaily who is a scholar of CHF and A. Sculptoreanu a Ph.D. fellow of CHF.

W-Pos428

UNITARY Ca^{2+} CURRENTS IN A SMALL-CELL LUNG CANCER CELL LINE. J.J. Pancrazio and Yong I. Kim, Depts. of Biomed. Engin. and Neurology, University of Virginia Health Sciences Center, Charlottesville, VA 22908.

The small-cell lung cancer cell line NCI-H146 possesses a voltage-dependent Ca^{2+} current which (1) shows little or no inactivation for test potentials less than 400 msec in length, and (2) is not inactivated by adjustment of the holding potential from -80 to -40 mV (*Cancer Res. 49*:5901,1989). We now present a preliminary description of the properties of a single Ca^{2+} channel type underlying the whole-cell Ca^{2+} current in NCI-H146 tumor cells. With 110 mM Ba^{2+} in the pipette, single channel openings were resolved using the cell-attached patch-clamp configuration. As shown below, test potentials to -30 mV from a holding potential of -80 mV evoked openings averaging about 1.1 pA and 1.3 msec in duration. Long term openings (>5 msec) were occasionally present. Ensemble averaging indicates that this channel type, possibly "L", can account for the whole-cell Ca^{2+} current found in the NCI-H146 cell line.



W-Pos429

JUST ONE TYPE OF Ca^{2+} CHANNEL UNDERLIES THE MACROSCOPIC Ca^{2+} CURRENT IN CEREBELLAR GRANULE CELLS. Paul A. Slesinger & Jeffry B. Lansman, Department of Pharmacology, School of Medicine, University of California, San Francisco, CA 94143

Macroscopic Ca currents in granule cells decay to a non-zero level with strong depolarizations from negative holding potentials (V_h), but do not decay with identical depolarizations from more positive V_h. We proposed a single class of Ca channels underlies the total macroscopic current. Recordings of single Ca channel activity from cell-attached patches on granule cells *in vitro* with 110 mM $BaCl_2$ in the pipet showed that strong depolarizations produce mostly brief channel openings but occasionally produce long openings. The mean current from ensemble averages decays during strong depolarizations similar to whole-cell currents. The number of long channel openings elicited with strong depolarizations from all V_h's was increased in the presence of the DHP agonist +202-791, while the number of brief openings elicited with depolarizations from more positive V_h's was substantially reduced. In the presence of the DHP antagonist -202-791, brief channel openings occurred only at the beginning of the depolarization, enhancing the decay of the mean current. In some patches, we observed a smaller conductance channel, however its contribution to total current is small. We conclude that a Ca channel of ~25 pS gives rise to the macroscopic Ca current; an enhanced rate of inactivation from the open state in a sub-population may account for the decay of current.

W-Pos430

GTP-γ-S INHIBITS BOTH DECAYING AND SUSTAINED COMPONENTS OF Ca CURRENT IN CEREBELLAR GRANULE CELLS

Christine M. Haws and Jeffry B. Lansman
Department of Pharmacology, School of Medicine, University of California, San Francisco, CA 94143

We recorded whole-cell Ca channel currents from dissociated cerebellar granule cells maintained in culture. With 20 mM $BaCl_2$ in the bath and 500 μM GTP in the patch electrode, current elicited by voltage steps from -90 mV to positive potentials turned on rapidly and decayed to a non-zero level. When cells were internally dialysed with 500 μM GTP-γ-S, current activation was slowed, although steady-state activation measured from the amplitude of tail currents was unaffected. Within ~5 minutes of breaking into the cell, both the decaying and sustained components of current decreased to zero in parallel even though the electrode contained BAPTA, ATP, cAMP, catalytic subunit, and leupeptin. Adding Bay K 8644 to the bath immediately increased the current by 50-300%. The current decreased once again along a time course similar to that before adding agonist. The results suggest GTP-γ-S regulates the kinetics of Ca channel opening, but also produces a form of rundown that can be reversed by dihydropyridine agonists. The rundown appears to act independent of kinase A or leupeptin-sensitive proteases. The results also suggest the decaying and sustained components of macroscopic current are carried by similar types of channels.

W-Pos431

BLOCK OF SINGLE CALCIUM CHANNELS IN C2 MYOTUBES BY Zn, Fe, Co, and Ni.

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The blocking actions of the transition metals, Zn, Fe, Co, and Ni, on unitary Ca channel currents were recorded from cell-attached patches on the surface of C2 myotubes with 110 mM $BaCl_2$ in the patch pipet. Dihydropyridine agonist was used to prolong the duration of the single channel openings for observing blocking transitions. The kinetics of block were studied by analyzing the lifetimes of discrete blocking events for the slow blockers (Ni) and by spectral and amplitude distribution analysis for the fast blockers (Zn, Fe, and Co). The kinetics followed a simple open channel block model: entry rates depended linearly on blocker concentration; exit rates were independent of concentration. Entry rates decreased with decreasing ionic radius following the series Zn>Fe>Co>Ni, consistent with the Eigen-Diebler mechanism in which removing water from the inner coordination sphere is the rate limiting step for complex formation. Exit rates followed the series Zn (~15,000 s^{-1})>>Fe≈Co (~5000 s^{-1})>>Ni (~100 s^{-1}), inconsistent with simple coulombic effects arising from differences in ion size determining site affinity. Both entry and exit rates increased with hyperpolarization, but exit rates increased more steeply so that steady-state block was reduced at negative potentials.

W-Pos432

REGULATION OF Ca CHANNEL TYPES AND SPATIAL DISTRIBUTION IN C2 MYOTUBES BY CO-CULTURE WITH SPINAL NEURONS

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We have studied the influence of spinal neurons on the properties of single Ca channels in myotubes from the mouse C2 cell line. Recordings of single channel activity from cell-attached patches with 110 mM $BaCl_2$ in the patch electrode on C2 myotubes grown in the absence of neurons showed primarily a single type of dihydropyridine-sensitive Ca channel of ~15 pS. Currents turn on near 0 mV and mean currents obtained by averaging single channel records show that activation is slow. Co-culturing myotubes with spinal neurons dissociated from embryonic mice was associated with the appearance of rapidly inactivating T-type channels, often clustered in hot spots. In a small fraction of patches, we also observed a 25 pS dihydropyridine-sensitive channel, similar to that observed in nerve and cardiac muscle. These changes were detected within approximately one week of co-culturing neurons and myotubes, during which time the extension of neuronal processes could be observed. We suggest that neurons regulate Ca channel expression during the development of adult skeletal muscle.

W-Pos433

SINGLE CHANNEL RECORDINGS OF CALCIUM CHANNELS IN PANCREATIC β-CELLS

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The cell-attached configuration of the patch clamp technique was used to study single calcium channel currents in rat pancreatic β-cells. The patch electrode solution was (in mM): 100 $BaCl_2$, 10 TEA, 10 Hepes(NaOH), pH 7.4. The bath solution was (in mM): 135 KCl, 1 $MgCl_2$, 10 K-EGTA, 10 Hepes(KOH), pH 7.4. Under these ionic conditions, two distinct calcium channel conductances have been observed: one of 21.8 pS, with an ionic current of about 1 pA at 0 mV; and another of 6.4 pS conductance, with an ionic current of about 0.3 pA at 0 mV. Because we have observed the small conductance channel infrequently, we have not, as yet, studied its properties in detail. The following properties of the 21.8 pS channel are similar to L or FD type calcium channels described in other preparations. It can be activated from a holding potential of -40 mV. 1 μM BAY K 8644 increases the average current. Channel open probability increases with depolarization, and there is little inactivation with depolarizing pulses to 0 mV for 50 ms. Supported by NIDDK grant DK33212 and the Generalitat Valenciana.

W-Pos434

FUNCTIONAL SYMMETRY OF L-TYPE CALCIUM CHANNELS.

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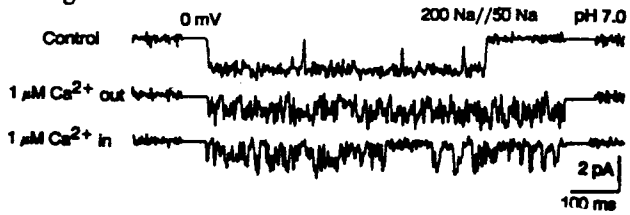
I-V curves for cardiac L-type Ca channels are linear when equal [Ba^{2+}] is present on both sides of the channels (R.L.R. et al., 1986, 1988). This apparent functional symmetry of the pore has now been observed for Ca^{2+} and Na^+ permeation, and for Ca-block of Na^+ currents (see also Hess et al., 1989). L-type Ca channels from porcine cardiac sarcolemma were incorporated into lipid bilayers (PE/PS 75/25) and single-channel currents were studied in the presence of 1 μM DHP agonist (+)-(S)-202-791 (Sandoz). With 100 mM $CaCl_2$ on both sides, the conductance for outward currents was 7-10 pS, similar to that found for inward Ca^{2+} currents. With symmetrical 200 mM NaCl, pH 7.0, free Ca^{2+} buffered to <40 nM with HEDTA, the I-V was symmetrical about the origin, and channel conductance was ~60 pS. At pH 7.5, conductance increased to ~90 pS, due to relief of rapid proton block (Hess et al., 1986, 1989). With 200//50 mM Na^+, currents at 0 mV were blocked by 0.5-1 μM Ca^{2+} from either side (Fig). Rapid blocking transitions were partially resolved at 200 Hz filtering (8-pole Bessel). The extent of block by 1 μM Ca^{2+} either inside or outside was about the same, suggesting that Ca^{2+} gains access to similar high-affinity sites from either side of the pore. We are examining the voltage-dependence of block from each side of the channel in attempts to locate Ca-binding sites within the membrane electric field.



W-Pos435

I_L IS THE DOMINANT I_{Ca} COMPONENT IN EMBRYONIC CHICK VENTRICULAR HEART CELLS

A. S. Kristof*, A Shrier*,and J.R. Clay+. *Dept. of Physiol., McGill, Montreal, Canada, H3G 1Y6 and +NINDS, NIH, Bethesda, MD 20892.

We have examined the I_{Ca} component in 6-day-old embryonic chick ventricular heart cells. Our pipette solution contained (in mM) 120 CsCl, 4 $MgCl_2$, 5 EGTA, 5 glucose, 3 Na_2ATP, 5 Na_2CP, 1 phosphate, 0.06 Na_2GTP, and 10 HEPES (pH 7.1). The bath contained 140 NaCl, 1.3 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 5 glucose, and 10 HEPES (pH 7.4). T=22°C. In 11 out of 12 preparations we found a single I_{Ca} component activated at V > -40 mV with a peak amplitude of -3.2 ± 1.6 pA/pF (n=6). Time to peak at 0 mV was 16.9 ± 4.0 ms. The current completely inactivated at all potentials within 200 ms. These results were independent of holding potential from -100 to -60 mV. The inactivation curve had a U-shaped voltage dependence which is characteristic of Ca_i-dependent inactivation of I_{Ca}. These results are consistent in every way with the classical I_L. They are also well described by our model (Shrier and Clay, Biophys. J., 50:861, 1986), which predicts that inactivation of I_{Ca} is due predominantly to the Ca_i mechanism. Our analysis differs from that of Kawano and DeHaan (Am. J. Physiol. 256:H1505, 1989), who attributed results similar to ours to the I_T component.

W-Pos436

CHARACTERISTICS OF CALCIUM CURRENT IN SINGLE ISOLATED RABBIT PORTAL VEIN MYOCYTES.

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Calcium currents (i_{Ca}) were measured in whole-cell clamped, enzymatically dispersed portal vein myocytes. With an internal solution containing (in mM): 120 Cs^+, 20 TEA^+, 14 EGTA, 10 HEPES, 10μM cAMP, and 5 MgATP, i_{Ca} was strongly modulated by holding potential (HP) and $[Ca^{2+}]_o$ and blocked by organic and inorganic Ca^{2+} antagonists Increasing HP from -40 to -90mV increased the magnitude of i_{Ca} but caused no signficiant shift in its voltage dependence. Normalized current-voltage (IV) curves (I/I_{max} vs V) for HP of -40 and -90mV measured 50 and 100 msec after the voltage steps were nearly superimposable failing to reveal more than one Ca^{2+} channel type. At all HPs, doubling $[Ca^{2+}]_o$ shifted the (IV) curve and the inactivation to the right by about 10mV. Substitution of Ca^{2+} with Ba^{2+} or Sr^{2+} shifted the IV curves and inactivation to the left, and decreased the maximum inward. Analysis of the i_{Ca} inactivation time course generally revealed two time constants (τ). The τ_{fast} decreased with increasing $[Ca^{2+}]_o$ and more negative values of HP and increased markedly when Ba^{2+} was the charge carrier suggesting the presence of Ca^{2+}-induced inactivation of the Ca^{2+} channel. Our results show that i_{Ca} in rabbit portal vein myocytes is similar to that described in other muscle cells but suggest the presence of only one type of Ca^{2+} channel.

W-Pos437

EFFECT OF AMPHIPHILES ON CALCIUM CURRENTS IN RABBIT VENTRICULAR MYOCYTES

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Fixed negative charges at membrane surfaces, mainly carried by phospholipids, are thought to produce local potentials which determine intramembrane electrical field and consequently affect ionic movement across the membrane. We used charged amphiphiles sodium dodecylsulfate (SDS, negatively charged at its head group) and dodecyltrimethylamine (DDTMA, positively charged at its head group) as phospholipid analogues. The whole cell recording configuration of patch-clamp technique was employed to observe the effect of these agents on the isolated voltage-gated calcium currents (I_{Ca}) in rabbit ventricular myocytes which were incubated overnight in MEM Eagle culture medium. After exposure to SDS (20 uM) containing solution, peak L-type I_{Ca} increased by 20-30% in 2-3 min; I-V relation and steady-state inactivation curves shifted in the negative direction. In the myocytes which showed T-type I_{Ca} in control conditions, SDS suppressed T-type I_{Ca} elicited from a holding potential of -80 mV. These results could be explained by a reduction of the intramembrane electrical field due to intercalation of the amphiphile into the membrane matrix, predominantly in the outer monolayer. In contrast, positively charged DDTMA (20 uM) showed an opposite effect on these parameters. Wash-out of SDS or DDTMA reversed the augmentation or suppression of L-type I_{Ca}, and partially reversed the change of gating properties of L-type I_{Ca}. These results indicate that the fixed charges on the outer monolayer play a significant role in modulating the properties of voltage-gated calcium currents. (This work is supported by NIH-HL-31253 & Castera Endowment.)

W-Pos438

LOW VOLTAGE ACTIVATED, 'T'-TYPE, CALCIUM CHANNELS IN HIPPOCAMPAL NEURONS

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T-type Ca current is important in the rhythmic firing behavior of many central neurons, but there are few reports of single T channels in CNS. On-cell patch recordings from tissue-cultured rat hippocampal neurons (in 120 mM K/10 mM EGTA saline) with 150 mM Li/0 mM Ca in the pipette revealed two types of channels (13 pS and 35 pS). Large channels could be activated with voltage steps from -40 mV, remained active throughout the steps and were affected by dihydropyridines. These seemed to be 'L' channels.

The small channels were activated by modest voltage steps from negative (-90 to -120 mV) holding potentials. Small channel activity rapidly (≤ 90 ms) ceased during steps to potentials less negative than -60 mV ($V_{1/2}$ for inactivation is -82 mV). Like L channels, small channels were insensitive to 5 μM TTX, but were blocked by including 2 mm Ca in the pipette with Li, indicating that they are not Na channels. Kinetics of ensemble currents obtained by averaging many sweeps closely resembled those of T current in whole-cell recordings. We conclude that the small single channels are T channels, the first such demonstration in these cells.

W-Pos439

REDUCTION IN NITRENDIPINE BINDING TO CARDIAC SARCOLEMMA AFTER 1 HOUR OF ISCHEMIA. Malcolm M. Bersohn, Anjali K. Morey. VA Med. Ctrs., Sepulveda and West Los Angeles, and Univ. California, Los Angeles, CA.

To investigate the effect of myocardial ischemia on the number of sarcolemmal Ca^{2+} channels, we purified sarcolemmal vesicles from rabbit hearts made globally ischemic for 1 hour. Purification of K^+-*p*nitrophenylphosphatase activity was the same for control (45 ± 7 fold) and ischemic (52 ± 5 fold) preparations (n=5). We measured specific ^{3}H-nitrendipine binding at 37° under equilibrium conditions. By Scatchard analysis (r=0.96 for control and 0.97 for ischemic), Kd was 830 pM for both groups. Bmax was higher for control sarcolemma (820 fmol·mg^{-1}) than for ischemic (590 fmol·mg^{-1}). Thus 1 hr of ischemia did not affect the affinity of the sarcolemmal Ca^{2+} channel for nitrendipine, but it did reduce the number of binding sites by 28%.

W-Pos440

CALCIUM CURRENTS IN RABBIT CORONARY ARTERY SMOOTH MUSCLE CELLS. Shibata, E.F., Matsuda, J.J. & Volk, K.A. Dept. of Physiology & Biophysics, Univ. of Iowa, Iowa City, IA 52242

Calcium currents were recorded from enzymatically isolated smooth muscle cells from rabbit epicardial coronary artery using a single pipette patch-clamp technique.

In 2.5 mM $[Ca^{2+}]_o$, depolarizing steps from a holding potential of -80 mV elicited a single time- and voltage inward current which was $[Ca^{2+}]_o$-dependent. The apparent E_{rev} in 2.5 mM $[Ca^{2+}]_o$ was +70 mV and shifted by 37 mV per tenfold increase in $[Ca^{2+}]_o$. This current was inhibited by 0.5 mM $CdCl_2$ and 1 μM nifedipine and was enhanced by 1 μM BAY K 8644. I_{Ca} was activated at a potential near -40 mV and peaked at +10 mV. No detectable low-threshold, rapidly inactivating T-type I_{Ca} was observed.

I_{Ca} exhibited a strong voltage-dependent inactivation process. However. the f_∞-curve displayed a slight non-monotonic, U-shaped voltage dependence. The half inactivation potential was -30 mV with a slope factor of 6.9 mV. The d_∞ curve has a half-activation potential at -4.4 mV and a slope factor of -6.3 mV. I_{Ca} was fully activated at approx. +20 mV.

Bath application of 0.2 μM ACh increased I_{Ca} by 40% of control. This effect was blocked by 1 μM atropine.

Supported by NIH HL 41031 and HL 14388.

W-Pos441

CALCIUM CHANNELS IN *DROSOPHILA* NERVE AND MUSCLE CELLS. Hung-Tat Leung and Lou Byerly. Dept. of Biological Sciences, U.S.C., Los Angeles, CA 90089-0371.

Voltage activated Ca channels of neurons and muscle cells in embryonic *Drosophila* cultures were studied in cell-attached patches using Ba^{2+} as the permeant ion. All the Ca channels studied have similar voltage dependence and similar slope conductance (~15pS). However, the kinetics of the Ca channels vary considerably from one patch to another. We have characterized kinetics by determining for each channel mean open time, fraction of active sweeps, inactivation during a sweep, and open probability for active sweeps. These parameters are calculated from the activity of 90ms sweeps recorded at 20mV every 5s. Scatter plots of these parameters for 42 individual channels suggest two types of Ca channels, an inactivating neuronal channel of relatively long open time (type I) and a non-inactivating channel found both in nerve and muscle (type II). The type I channel is active in only 20% of the sweeps, while the type II channel is active in over 60% of the sweeps. We intend to use spider toxins to further distinguish channel types.

W-Pos442

ω-CONOTOXIN GIVA BLOCKS THE CALCIUM CURRENT AT THE PRESYNAPTIC NERVE TERMINAL OF THE CHICK GIANT SYNAPSE. E.F. Stanley LB, NINCDS, NIH, Bethesda MD 20892.

There is considerable interest in the characterization of calcium channel types in vertebrate presynaptic nerve terminals. Most studies on this question have had to resort to indirect techniques, due to the small size of these structures. The calyx of the chick ciliary ganglion (CG) synapse is an exception in that it is possible to detect inward calcium currents with the patch-clamp technique in the whole cell mode (Stanley, Brain Res. *In press*). We have begun to characterize these calcium channels by defining their sensitivity to pharmacological agents and report here that they are blocked by ω-conotoxin.

CG neurons were isolated from chick embryos with intact presynaptic calyces by enzymatic dissociation. Standard patch clamp techniques were used to record from the presynaptic nerve terminal. The main component of the calcium current had a high threshold, showed little inactivation during a 25 ms pulse, and deactivated rapidly. ω-conotoxin GIVA was applied to the calyx from a closely apposed pipette during a train of voltage steps from -70 to +30 mV at 2 second intervals. A block of the inward calcium current was detected at 10 nM toxin and this block was virtually instantaneous at 10 μM.

These results indicate that the calcium current in this presynaptic nerve terminal is similar to high voltage activated calcium channels observed in other chick and non-chick neuronal cells.

W-Pos443

EFFECT OF DIHYDROPYRIDINES ON CA CHANNEL GATING CURRENT IN GUINEA-PIG VENTRICULAR MYOCYTES. Robert W. Hadley & W. J. Lederer, Dept. of Physiology, School of Medicine, Univ. of Maryland, 655 W. Baltimore St., Baltimore, MD 21201

Nonlinear charge movement in isolated guinea-pig ventricular myocytes seems to consist of two major components, which can be separated by holding potential. The second component seems to arise from L-type Ca channel gating, as its voltage-dependence is well-correlated with Ca channel activation and inactivation. This hypothesis was further tested by examining the effects of dihydropyridines on the gating current. Nitrendipine was found to inhibit the gating current in a voltage-dependent manner. The K_D for nitrendipine inhibition was 200 nM at -40 mV, which is in agreement with the known effects of the drug on the Ca channel. (-) Bay K 8644 was also studied, and was found to have complicated effects on the gating current. First, the drug had a voltage-dependent inhibitory effect, similar to its effects on I_{ca}. Second, it also slowed Q_{off} kinetics, which may underlie the drug's effects on Ca channel deactivation. Bay K 8644 also sometimes shifted the charge-voltage relationship negative, although this was sometimes obscured by the inhibitory effects of the drug. In summary, the effects of dihydropyridines on the purported Ca channel gating current correspond to their known effects on I_{ca}.

W-Pos444

DEPENDENCE OF Ca^{2+} CURRENT DEVELOPMENT ON RNA SYNTHESIS IN MUSCLE-LINEAGE CELLS OF THE ASCIDIAN *BOLTENIA VILLOSA*. L. Simoncini and W. J.Moody. Department of Zoology, University of Washington, Seattle, WA 98195.

In previous experiments, we have studied the ontogeny of voltage-dependent currents in embryos of the ascidian *Boltenia villosa*, using the whole-cell clamp on blastomeres isolated from various embryonic stages. All blastomeres at the gastrula stage (14 hrs after fertilization @ 8°C) show very similar electrical properties: they have a large inwardly rectifying K^+ current, but neither Ca^{2+} nor delayed K^+ currents. During the approximately 4 hours in which the gastrula develops into the neurula, several changes take place: 1) The inward rectifier is greatly reduced in all neurula stage cells irrespective of lineage; 2) Delayed outward K^+ currents appear predominantly, but not exclusively, in muscle-lineage cells; and 3) Large Ca^{2+} currents appear in the muscle-lineage cells but not in cells of any other lineages.

In the present experiments, actinomycin D has been used to examine the transcription-dependent period necessary for the development of Ca^{2+} channels in the muscle-lineage cells. The Ca^{2+} current fails to develop when mRNA synthesis is blocked before the 64-cell stage, while about 50% of the current develops when mRNA synthesis is blocked at gastrula. These results indicate that mRNA involved in the appearance of Ca^{2+} channels at neurula stage is first synthesized at the 64-cell stage about 8 hrs before Ca^{2+} currents appear.

W-Pos445

CA CHANNEL INACTIVATION INDUCED BY DHP AGONISTS IS NOT PREVENTED BY BLOCKING THE CURRENT ENTRY WITH CADMIUM. Wattana Bamrungphol-Watanapa and Clay M. Armstrong, Department of Physiology, University of Pennsylvania, PA 19104-6085.

We tested if a current-dependent mechanism (e.g. Markwardt & Nilius, 1988) could explain Ca channel inactivation induced by dihydropyridine (DHP) agonists in chick embryo ventricular myocytes. We exploited the fact that Cd can block Ca current more effectively at strong depolarization, but is driven out of the channel at repolarization, when the membrane potential becomes more negative (Chow & Armstrong, 1988; Swandulla & Armstrong, 1989). Therefore, we can follow the time course of inactivation, using tail current amplitude, while divalent entry during the pulse is blocked by Cd. With 10 or 20 mM Ba externally, Ca channels inactivate faster in the presence of 200-500 nM (±)Bay K 8644 or (+)202-791. Addition of 20-60 μM Cd does not change the time course of inactivation, even though the pulse current is blocked by more than 90%, and the tail current is reduced by 40-60% (in Bay K). Moreover, with 50 ms variable amplitude prepulses, similar bell-shaped dependency of the tail amplitude on the prepulse voltage is obtained whether 20 mM Ba or 20 mM Ca is the current carrier. Thus, DHP agonist-induced inactivation in chick embryo ventricular myocytes depends neither on the current density nor, at least for the rapidly inactivated component, on the species of the divalent permeant.

[Supported by NIH grant NS 12543 to CMA and a grant from the Royal Thai Government to WBW.]

W-Pos446

ASTROCYTES FROM KAINIC ACID LESIONED ADULT RAT HIPPOCAMPUS. Charles L. Bowman[1], John W. Swann[2] and Charles M. Severin[1]. School of Medicine, University at Buffalo, Buffalo, NY 14214. 2. Wadsworth Center for Laboratories and Research, Albany, NY 12203.

The properties of astrocytes in lesioned areas of the brain may be of general interest because reactive gliosis is a common end point of many neuropathies. We report that astrocytes can be isolated from the lesioned CA3 region of the rat hippocampus, grown in primary cell culture and studied using the patch clamp technique. The technique we use is a modification of Kay and Wong (J.Neurosci.Meth. 16:227-239 1986).

The CA3 region of the hippocampus of anesthetized adult Holtzmann rats was injected with 4nmol of kainic acid. After a minimum of one month, the animal was sacrificed and the hippocampus removed. Mini-slices of the CA3 region were exposed to trypsin (4-8 mg/ml) digestion for 1.5 hours at room temperature in a PIPES buffered Ringer's solution. The trypsin was removed and the slices were placed in MEM growth medium and titurated with about 40 passes through a glass pipette having a 500 micron diameter tip. Several drops of the dissociated tissue were placed on a glass coverslip for 2 to 24 hours prior to flooding the dish with 2 ml of additional growth medium. The coverslips were kept in a 5%CO_2 incubator at 37°C for several weeks prior to study.

Staining for glial fibrillary acidic protein (GFAP) confirmed the presence of astrocytes. In addition, a small round GFAP negative cell was present. The latter cells do not round up in response to dibutyrl-cAMP, in contrast to the GFAP staining cells. Patch clamp studies reveal membrane potentials of -60 to -70 mV, at room temperature. In contrast to neonatal astrocytes, the astrocytes from the lesioned areas appear to be without stretch receptors. Supported by NIH grants NS24891 to CLB, and NS18309 to JWS.

W-Pos447

CHEMORECEPTION IN THE CAT CAROTID BODY PERFUSED-SUPERFUSED IN VITRO. R. Iturriaga, S. Lahiri, D. Spergel, and W.L. Rumsey *. (Intro. by B. Storey). Dept. of Physiology, and Dept. of Biochemistry and Biophysics *. University of Pennsylvania. School of Medicine. Philadelphia, PA 19104.

We developed a perfused-superfused in vitro cat carotid body preparation to study chemoreceptor responses to natural and chemical stimuli. Carotid bifurcations were excised from 15 anesthetized cats and each placed in a chamber for perfusion (80 torr) and superfusion with Tyrode (buffered with HEPES-NaOH to pH 7.4 at 36 °C), equilibrated with 20 or 100% O_2 and 100% N_2, respectively. Carotid chemoreceptor discharges were recorded from the whole carotid sinus nerve. Interruption of perfusion increased chemoreceptor activity. Restoration of flow returned the activity to basal values in 15 s. Low pH and 5% CO_2 augmented chemoreceptor activity. Nicotine and NaCN increased chemoreceptor activity in a dose-dependent manner. Natural and chemical stimuli increased chemoreceptor activity 10-20 fold. The preparation retained its responsiveness for 2-3 hrs, enabling protocols of considerable duration. Supported by HL 19737-14 and HL 43413.

W-Pos448

OXYGEN DISTRIBUTION AND CHEMORECEPTION IN THE IN VITRO PERFUSED-SUPERFUSED CAROTID BODY. W.L. Rumsey*, R. Iturriaga, D.Spergel, S. Lahiri, and D.F. Wilson*. Dept. of Biochemistry & Biophysics*, and Dept. of Physiology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Chemoreceptor activity (CA) and intravascular PO_2 relationship was evaluated in a perfused-superfused cat carotid body preparation. The carotid bifurcation, placed in a chamber, was perfused (80 torr) and superfused with Tyrodes (pH 7.40, at 36 °C) equilibrated with 100% O_2 and 100% N_2, respectively. The O_2 distribution was determined by adding Pd-coproporphyrin and bovine serum albumin to the perfusate and illuminating the tissue (540-560 nm). Phosphorescence (Phos) emission (>665 nm) was detected by an intensified CCD camera while chemoreceptor discharges were recorded from the sinus nerve. When perfusion was stopped, Phos and CA rose concomitantly by several fold. Reperfusion returned Phos intensity and CA to basal values. Lowering the perfusate PO_2 to normoxic levels increased Phos intensity and hastened the onset of both CA and Phos responses. There is a strong correlation between PO_2 and CA. Supported by HL 07027 and HL 43413.

W-Pos449

IDENTIFICATION OF A RYANODINE RECEPTOR IN RAT AND BOVINE BRAIN. F.A. Lai, L. Xu and G. Meissner. (Intro. by G. Scarborough) Dept. of Biochemistry, University of North Carolina, Chapel Hill, NC 27599.

The alkaloid ryanodine is a specific ligand for the Ca^{2+} release channel complex of skeletal and cardiac muscle sarcoplasmic reticulum. Using microsomes isolated from rat and bovine whole brain, specific [^{3}H]ryanodine binding was observed with a nanomolar K_D and B_{max} of ~1 pmol/mg of protein. Brain microsomal proteins were solubilized in CHAPS and centrifuged through linear sucrose gradients. As has been observed for the skeletal and cardiac muscle ryanodine receptors (Lai *et al*, *Nature* 331, 315, 1988; *BBRC* 151, 441, 1988) a single [^{3}H]ryanodine receptor peak with apparent sedimentation coefficient of ~30S was obtained, which comigrated with high molecular weight polypeptides upon SDS gel electrophoresis. Incorporation of the solubilized 30S peak fractions into planar lipid bilayers induced single channel currents with properties similar to those observed for the skeletal and cardiac muscle ryanodine receptor-Ca^{2+} release channel complex (Liu *et al*, *Biophys. J.* 55, 415, 1989). Supported by NIH and MDA.

W-Pos450

CALCIUM INFLUX INTO HAIR CELL STEREOCILIA: FURTHER EVIDENCE FOR TRANSDUCTION CHANNELS AT THE TIPS. P.L.Huang and D.P. Corey. Dept of Neurology and Howard Hughes Medical Institute, Mass. Gen. Hospital; Program in Cell and Developmental Biology, Harvard Medical School, Boston, MA

An attractive model for hair cell transduction is that fine filaments linking the tips of stereocilia pull directly on mechanically sensitive transduction channels when the hair bundle is moved in the excitatory direction (Corey & Hudspeth, 1983; Hudspeth, 1983; Pickles et al, 1984). This model is supported by measurements of extracellular current flow (Hudspeth, 1982), but is challenged by fura-2 measurements suggesting calcium entry at the bases of stereocilia during transduction (Ohmori, 1988).

We have re-examined the site of calcium entry into bundles with the calcium indicator dyes fluo-3 and rhod-2, since transduction channels are permeable to calcium.

A proportion of transduction channels is open at rest; if transduction channels are at the tips of stereocilia, this predicts a standing gradient of calcium from tip to base. Dissociated hair cells, loaded with rhod-2/AM or fluo-3/AM, were examined with a confocal microscope in order to reject out-of-plane fluorescence. In each cell examined, the fluorescence signal was brightest at the tip of the bundle.

To rule out geometrical artifacts, and to avoid motion artifacts, calcium influx through transduction channels was modulated by patch-clamping the cells and changing the membrane potential. Fluo-3 (free acid) was loaded through the patch pipette. The brightest calcium signal occurred at the tips, and occurred at the most negative potentials (-80 mV). Following hyperpolarization, a wave of fluorescence moved from tips to base. The only channels in hair cells known to pass calcium at these negative potentials are the transduction channels, so these data are consistent with their localization at the tips of stereocilia.

W-Pos451

FACTORS AFFECTING Ca BINDING BY MYXICOLA AXOPLASMIC PROTEIN. Nabil F. Al-Baldawi and Ronald F. Abercrombie. Department of Physiology, Emory University School of Medicine, Atlanta, Georgia 30322.

The Ca-binding properties of axoplasmic proteins from the Myxicola giant axon have been investigated using a centrifugal/concentration-dialysis technique. Protein extracts of Myxicola axoplasm contain greater than 50% neurofilament subunits that bind calcium. Previous work has suggested that neurofilaments are a major calcium binding protein in this preparation. Scatchard plot analysis of the binding data suggests that most Ca is attached to a site with an equilibrium dissociation constant of ~8 μM and a capacity of 2.5 to 4.0 μmol/g axoplasmic protein. Other divalent cations--Cd, Mn, Al, Cu, Ba, and Zn--did not displace ^{45}Ca from its binding site. The protein extract could be stored at 4°C for up to 16 days with no appreciable change in its capacity or affinity for calcium. Ca binding equilibration took place in less than 30 min of incubation. Increasing the temperature from 4°C to 37°C or dialyzing the protein with high ionic strength KCl (2 M) reduced the Ca binding capacity by one-half; this latter effect was reversible. Calcium binding has a pH maximum near 7. The histidine-specific reagent diethyl pyrocarbonate reduced the calcium binding in a dose-dependent manner.

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SYNAPTIC CONNECTIVITY TO A MOTORNEURON POOL STUDIED BY COMPUTER SIMULATION. F.A. Dodge, IBM Research, T.J. Watson Center, Yorktown Heights, NY 10598.

A mathematical model of a single pool, in which the isometric twitch kinetics, intrinsic motorneuron properties, and Renshaw feedback parameters could be matched to empirical distributions, was used to estimate how the excitatory synaptic drive projects to motorneurons of different size during simple reflexes. Comparing the theoretical relation of firing rate to size and to tension with experimental data excludes the idea that excitation projects nonselectively according to surface area, but strongly supports the hypothesis that a common set of excitatory interneurons that project with the same number of synapses to both small and large motorneurons underlies the orderly recruitment typically observed in tonic reflexes and in human voluntary tension measurements. (Such a projection rule matches that of the monosynaptic Ia pathway.) A second, comparably numerous, subset of excitatory interneurons that project only to faster, larger motor-neurons is required to simulate ballistic movements that occur in some cutaneous reflexes and in paw-shaking.

W-Pos453

ULTRASTRUCTURAL LOCALIZATION OF MYOSIN IN NEURONS OF THE CENTRAL NERVOUS SYSTEM. Miller, M., Levitt, P. & Chantler, P.D. Dept. Anatomy, Medical College of Pennsylvania. PA. 19129.

We are interested in the functional role of myosin in neuronal cells. Using an antibody specifically raised against myosin purified from mouse neuroblastoma N2A cells, we were able to demonstrate that the localization of myosin was coincident with synapsin-1 in spinal cord tissue sections, at the light microscopic level (Miller et al,1988. J. Cell Biol. 107. p733a). We have now examined adult brain and spinal cord sections at the ultrastructural level by electron microscopy, using pre-embeddment staining techniques. In contrast to the pre-synaptic location of synapsin-1, myosin was principally confined to post-synaptic structures, including the post-synaptic density and regions of dendrites. Additional myosin staining was found within neuronal cell bodies. These ultrastructural results are consistent with, and clarify, our light-microscopic studies. They lend support to an active role for myosin during synaptic transmission.

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TWO POPULATIONS OF OLFACTORY NEURONS CAN BE DISTINGUISHED BY AMILORIDE BLOCKADE OF VOLTAGE-GATED INWARD CURRENTS. Gonzalo Ugarte, Bernardo Morales, Juan Bacigalupo and Pedro Labarca. Departamento de Biologia, Facultad de Ciencias, Universidad de Chile and Centro de Estudios Cientificos de Santiago.

In isolated frog olfactory neurons the frequency of action potentials can be increased by depolarizing the cell through a cell-attached pipette. This depolarization-evoked rate of action potentials is reversibly reduced to resting levels in 60% of cases upon exposure to 50 μM Amiloride in the bath, whereas 4 mM Co^{2+} reduces it in 100% of cases. Whole-cell recording studies, under conditions in which internal K^+ has been replaced by Cs^+, reveal that 50 μM Amiloride partially blocks the inward currents in about 50% of cells. Thus, Amiloride blockade allows to discriminate two populations of olfactory neurons in frog olfactory epithelium. Blockade is dose-dependent and observed in the 10-100 μM range. In those cells in which Amiloride partially blocks the inward currents, in a Na^+-free, 20 mM Ca^{2+} solution a small component of the inward current can be recorded which is completely and reversibly abolished by 50 μM Amiloride. (Supported by NIH GM-35981 and FONDECYT 1167/88).

W-Pos455

THE ELECTROPHYSIOLOGY OF A CRUSHED NERVE AXON. J.M. van Egeraat, R. Stasaski, J.P. Wikswo, Jr. Vanderbilt University, Department of Physics and Astronomy, Nashville, TN 37235.

The sequence of electrophysiological events following injury to a nerve axon is poorly understood, in part because of the limitations of existing electrical techniques and mathematical models. We recorded magnetic and electric signals from crayfish giant axons when action signals terminated in the vicinity of a crush that connected intracellular and extracellular space. In order to explain the data, we developed a model based on principles of mass transport and the Hodgin/Huxley model, modified for crayfish physiology. The model can accurately reproduce the measured data. The effect of the crush is evident up to 10 mm from the crush: statically as a gradual increase of the resting potential towards zero; dynamically, as a decline in amplitude of action current and potential. In addition, the biphasic action current becomes monophasic near the crush. The model predicts steady axial, intracellular currents near the crush on the order of 1 μA, which should be detectable with newly-developed SQUID magnetometers.

W-Pos456

QUANTAL TRANSMISSION INDEPENDENT OF PRESYNAPTIC FREE CALCIUM IN AVIAN CILIARY GANGLION NEURONS. D.C. Brosius, J.T. Hackett, and J.B. Tuttle, (Intro. by B.R. Duling) Depts. of Physiol. and Neurosci., Univ. of Virginia Health Science Center, Charlottesville, VA 22908

Depolarization is coupled to transmitter release by the second messenger Ca^{++}, but ethanol and hypertonic solutions evoke secretion independent of extracellular Ca^{++}. We checked whether these agents released cytosolic Ca^{++} in ciliary ganglion neurons maintained in tissue culture with a Meridian ACAS 470. Neurons were loaded with 5 μM indo-1-AM ester, washed and mounted for experimentation. Whole cell scans were taken of fluorescence excited by 380 nm incident laser illumination, and the ratio of peak emission at 405 and 485 nm taken. We compared the effects of high K^+, ethanol, and hypertonic solutions upon neuronal $[Ca^{++}]_i$. As expected 30 mM K^+ increased $[Ca^{++}]_i$. Ethanol (0.4 M) had no apparent effect upon $[Ca^{++}]_i$. In contrast, a 2-fold increase in osmotic pressure produced a significant rise in $[Ca^{++}]_i$. The effects of high K^+ and osmotic pressure were occlusive. The inhibitory action of hypertonic saline can be partly explained by its effect to block Ca^{++} current, but an action on Ca^{++} stores is also likely.
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W-Pos457

WITHDRAWN

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EFFECT OF IONOMYCIN ON THE FAST MECHANICAL RESPONSE OF OUTER HAIR CELLS. K. H. Iwasa and B. Kachar, Lab. Biophys. NINDS and Lab. Mol. Otol. NIDCD, NIH Bethesda, Md. 20892

We examined the effect of ionomycin, a calcium ionophore, on the fast mechanical response of the outer hair cells to an externally applied ac electric field to clarify the mechanism of the response. When the external medium contained more than 1 μM Ca^{2+}, the application of 2 μM ionomycin initially increased (up to 2 min. ar 1.5 mM Ca^{2+}; 5 min. at 1 μM Ca^{2+}) and then abolished (after 5 min. at 1.5 mM Ca^{2+}; 20 min. at 1 μM Ca^{2+}) the mechanical response. When the external medium contained 6 nM Ca^{2+}, no enhancement of the mechanical response was observed. Instead, the response reduced gradually over a period to 20 to 30 min., without a clear abolition of the movement. Fluorescence measurement with Fluo-3, a calcium indicator dye, revealed that the time course of the calcium elevation after the application of ionomycin in the regular external medium. At 5 min. after the application, when the mechanical response ceased, the fluorescence signal was only marginally increased at the basolateral membrane area. It took about 20 to 30 min. for the fluorescence signal to reach a steady level.

Our observation leads to two alternative interpretations. One is that calcium ions serve as a modulator of the mechanical response and the other is that calcium ions released voltage dependent manner cause the mechanical response.

W-Pos459

NEURAL MODEL OF ULTRASONIC CANINE BIOSENSOR. E.Fink,Command K-9,Box 435,Inyokern,CA.93527

A neural model of ultrasonic signal processing in dogs has been programmed which is adaptive. Mechanisms are treated for transfer of action signals between different levels of locomotor area of temporary memory (written to by unconditioning stimulus from another biosensor) and interaction between temporary memory and instinctive limbic memory. Ultrasonic conditioning stimulus indirectly addresses(or connects) oscillator neurons, via decoder neurons latched by a coincidence gate, and directly addresses permanent memory neurons. Summing the two types of neurons produces reinforcing or inhibiting effects, which explain the peaks observed in the hearing threshold curve. The level of temporary memory addressed by ultrasonic stimulus depends on the weighted sum of amplitudes of its spectral components. The lower levels of temporary memory have higher damping rates and are related to short-term memory; the upper levels of temporary memory have lower damping rates and are related to long-term memory. Hearing threshold, learning and forgetting curves are calculated for 32 behavior paradigms and compared with experimental results previously acquired. The theoretical curves fit the experimental curves within ±5% by adjusting the damping rates of oscillator neurons and constructing a minimum of 16 levels of memory

W-Pos460

INTRACELLULAR FREE MG^{2+} CONCENTRATIONS IN THE HUMAN BRAIN. J.S. Taylor, D.B. Vigneron, S.J. Nelson, J. Murphy-Boesch, T.R. Brown. Dept. of NMR and Medical Spectroscopy, Fox Chase Cancer Center, Phila., PA

Initial observations with a circularly polarized birdcage resonator constructed to carry out ^{31}P NMR studies of the head show an upfield shift of the β-ATP peak in human brain relative to its position in muscle, as well as a significant difference in the β-ATP peak positions for gray and white matter. The β-ATP shifts from the central core of the cerebrum (white matter) in 3 volunteers, obtained by averaging voxels of 27 cc located in white matter were: -18.99 ± 0.06 (n=6), -18.90 ± 0.21 (n=7), and -18.97 ± 0.17 (n=7) ppm. The most straightforward explanation for the data is that free Mg^{2+} concentrations in this region of human brain is 0.2 mM. Spectra from regions of muscle in the same 3D datasets have β-ATP peak positions corresponding to Mg^{2+} concentrations greater than 1 mM. Studies of the variation in spatial distribution of free Mg^{2+} and other ^{31}P observable brain metabolites are in progress.

W-Pos461

LONG TERM RECORDINGS IN SCHWANN CELLS SURROUNDING SQUID AXONS REVEAL MULTIPLE RESTING STATES. Y.Pichon, N.J.Abbott, E.R. Brown. M.B.A. Laboratory, Plymouth, UK.

Previous experiments on the squid axon-schwann cell preparation suggest that the latter are involved in K^+ clearance from the surface of the giant axons. Using high resistance (30 to 40 Mohms) microelectrodes, we were able to simultaneously record the membrane potential of a Schwann cell and that of the giant axon of the small squid species, Alloteuthis subulata during extended periods (up to 4 hours). Whereas the resting potential of the axon remained almost constant in the presence or the absence of electrical stimulation (around 65 mV), Schwann cells exhibited typically three different potential levels: a low level of about 17 mV, a medium level of 35 to 45 mV reminiscent of that obtained with other microelectrode techniques and a high level of 75-85 mV. Furthermore, even in the absence of stimulation of the giant axons, the resting potential of the Schwann cell was found to change slowly from - 40 mV to - 70 mV or more during the first minutes of the recording. This increase was accompanied by distinct fluctuations of the membrane potential, suggestive of the opening of Ca^{++} activated potassium channels.